PureExtreme™

Highest quality & performance

We guarantee that these products are free of contaminating activities. Our stringent quality control with the most advanced tests guarantees you pure products for your experiments. ISO9001 and ISO14001 is your assurance of con-

PureExtreme[™] Quality will provide the performance you need for your most demanding experiments.

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Introduction

Restriction endonucleases recognize specific nucleotide sequences and cleave DNA molecules at a position either within or outside their recognition site. These enzymes are important tools in numerous applications, including studies of DNA primary structure and recombinant DNA technology. More than 3600 restriction enzymes, exhibiting ~260 different specificities, have been isolated. They are described in the Restriction Enzyme database (REBASE).

Since 1977, Fermentas has discovered approxi-

mately 30% of all known restriction endonucleases. We are a leading global manufacturer of enzymes, offering 188 commercial restriction endonucleases. We actively screen for new restriction endonucleases and are continuously discovering new restriction enzyme specificities. Fermentas is the supplier of choice both for classic restriction enzymes and for new unique enzymes, which are not supplied by other companies.

Fermentas restriction endonucleases are produced under the ISO9001:2000 quality management system, which combined with our extensive quality control tests, guarantees consistent PureExtreme[™] Quality – the highest quality and performance. All Fermentas restriction endonucleases are tested using the rigorous Labeled Oligonucleotide (LO) test to ensure the absence of even trace activities of endodeoxyribonucleases, exodeoxyribonucleases and phosphatases. The high quality of Fermentas endonucleases makes them suitable for even the most demanding applications.

NEW An Innovation from Fermentas: FastDigest[™] Restriction Endonucleases

Digestion of DNA with restriction endonucleases can be a time consuming step in cloning and clone analysis. DNA digestions typically last for one hour and often DNA is incubated with the enzymes overnight. The PureExtreme[™] Quality of our enzymes has enabled us to develop a new line of products – the Fermentas FastDigest[™] Restriction Endonucleases, which are specifically formulated to cleave DNA in just 5 minutes.

High quality DNA is crucial for efficient DNA digestion in 5 minutes. We recommend the Fermentas GeneJET[™] Plasmid Miniprep Kit (#K0501) to purify DNA for FastDigest[™]. While FastDigest[™] is compatible with DNA purified using kits from other suppliers, Fermentas guarantees the performance of our products, which are tested under the most rigorous and demanding conditions.

We offer the following FastDigest[™] Restriction Endonucleases:

Table 1 1 EastDigest[™] Restriction Endonucleases

Table 1.1. FastDigest Restriction Endonuclease			
FastDigest [™] Enzyme	$\begin{array}{l} \text{Specificity} \\ 5' \rightarrow 3' \end{array}$	Catalog #	Page #
Apal	GGGCC↓C	ER1414	30
BamHI	GUGATCC	ER0054	31
Bcul (Spel)	A CTAGT	ER1254	33
BgIII	AJGATCT	ER0084	37
BstXI	CCANNNNN↓NTGG	ER1024	52
Bsu15I (Clal)	ATCGAT	ER0144	53
Eco32I (EcoRV)	GAT	ER0304	62
EcoRI	GJAATTC	ER0274	69
HindIII	AJAGCTT	ER0504	76
Kpnl	GGTAC↓C	ER0524	80
Ncol	C↓CATGG	ER0574	88
Ndel	CAJTATG	ER0584	89
Notl	GC↓GGCCGC	ER0594	90
Pael (Sphl)	GCATG↓C	ER0604	92
Pstl	CTGCA	ER0614	99
Sacl	GAGCT	ER1134	102
Smal	CCC↓GGG	ER0664	106
Xbal	T CTAGA	ER0684	116
Xhol	CUTCGAG	ER0694	117
	N = G, A, T or C		

Features

- Single and double digestions of plasmid DNA in just 5 minutes.
- Enhanced performance in one hour DNA cleavage reactions.
- A single reaction buffer for all FastDigest[™] enzymes.

Save Time – Digest DNA in Just 5min!

Applications

- · Fast clone analysis.
- Fast preparation of vectors for cloning.
- Standard DNA cleavage reactions.

Note

- Low quality plasmid DNA may require longer incubation times.
- Optimal results require gel purification of the digested DNA prior to ligation.



- 1-6
- M₁, M₂- ZipRuler[™] Express DNA Ladder Set (#SM1373) С - control pUC19 DNA digested with
- FastDigest[™] EcoRI and HindIII
 - miniprep DNA from recombinant clones, double digested with FastDigest[™] EcoRI and HindIII

Figure 1.1. Fast clone analysis.

A 2.3 kb PCR fragment was cloned into pUC19 vector. Plasmid DNA from overnight bacterial cultures of recombinant clones was purified using the GeneJET[™] Plasmid Miniprep Kit (#K0501) and analyzed by double digestion with FastDigest[™] EcoRI and FastDigest[™] HindIII (see the protocol for fast clone analysis below).

Analyze Your Clones 3X Faster with Fermentas

Protocol for Fast Clone Analysis				
 Purify DNA from 1.5ml of overnight cultures using GeneJET[™] Plasmid Miniprep Kit (#K0501). Pipette 2µl (~0.2µg) of each miniprep DNA into thin-wall tubes. Prepare the following reaction master mix: 			13min 2min 3min	
	Water, nuclease-free (#R0581)	(number of samples + 1) x 15µl		
	10X FastDigest [™] Buffer	(number of samples + 1) x 2µl		
	FastDigest [™] Restriction Endonuclease (number of samples + 1) µl			
 Add 18µl of the master mix into each tube with plasmid DNA, mix and spin down. Incubate at 37°C for 5 minutes to digest DNA. Add 4µl of an appropriate 6X loading dye solution into each tube and mix. Load on a 0.8-1% agarose gel and run electrophoresis for 10-20min using the ZipRuler[™] Express DNA Ladder Set (#SM1373). 		3min <mark>5min</mark> 2min 15min		
Total time: 4			43min	
	Note			

For double digestion, use 1µl/sample of each enzyme and correct the water volume appropriately.

www.fermentas.com/doubledigest www.fermentas.com/research

Fermentas Restriction Endonucleases

Formentee	Drototyne	Table 1.2. Fermentas		
Fermentas Enzyme	Prototype	Specificity $5' \rightarrow 3'$	Catalog #	Paç #
	Aarl	CACCTGC(4/8)		
Aarl Aasl	Aarl Drdl	GACNNNN INNGTC	ER1581/2 ER1721/2	24 24
Aatli	Aatll	GACGT		
Acc65I	Kpnl (GGTAC↓C)	GUGTACC	ER0991/2 ER0901/2	25 25
Adel	Dralll	CACNNNJGTG	ER1231/2	26
Adel	Btrl	CACUMNUGIG	ER1231/2 ER1941	26
Ajul	Ajul	\downarrow (7/12)GAA(N),TTGG(11/6) \downarrow	ER1941 ER1951	27
Alfi	Alfl	\downarrow (10/12)GCA(N), TGC(12/10) \downarrow	ER1801	27
Alol	Alol	\downarrow (7/12-13)GAAC(N), TCC(12-13/7) \downarrow	ER1491/2	27
Alul	Alul	AG ¹ CT	ER0011/2	28
Alw21I	HgiAl	GWGCWUC	ER0021/2	20
Alw26I	BsmAl	GTCTC(1/5)	ER0021/2 ER0031/2	29
		GUTGCAC		
Alw44I	ApaLl		ER0041/2	30
Apal	Apal	GGGCC↓C	ER1411/2/4	30
BamHI	BamHI	GJGATCC	ER0051/2/3/4	31
Baul	Bsil	CACGAG(-5/-1)↓	ER1841	32
Bcll	Bcll	TJGATCA	ER0721/2	32
Bcnl	Caull	CC↓SGG	ER0061/2	33
Bcul	Spel	AJCTAGT	ER1251/2/4	33
Bdal	Bdal	↓(10/12)TGA(N),TCA(12/10)↓	ER1961	34
Bfil	Bfil	ACTGGG(5/4)↓	ER1591/2	35
Bfml	Sfel	C↓TRYAG	ER1161/2	35
Bful	BciVI	GTATCC(6/5)↓	ER1501/2	30
Bgll	Bgll	GCCNNNN↓NGGC	ER0071/2	30
BgIII	BgIII	A↓GATCT	ER0081/2/4	3
Bme13901	ScrFI	CC↓NGG	ER1421/2	3
Boxl	PshAl	GACNN↓NNGTC	ER1431	38
Bpil	BbvII	GAAGAC(2/6)↓	ER1011/2	38
Bpll	Bpll	↓(8/13)GAG(N) ₅ CTC(13/8)↓	ER1311/2	30
Bpu10I	Bpu10I	CCTNAGC(-5/-2)↓	ER1181/2	30
Bpu1102I	Espl	GCJTNAGC	ER0091/2	4(
BseDI	Secl	C↓CNNGG	ER1081/2	4(
BseGI	FokI (GGATG(9/13)↓)	GGATG(2/0)↓	ER0871/2	4
BseJI	BsaBl	GATNN UNNATC	ER1711	4
BseLI	BsiYI		ER1201/2	42
BseMI	BsrDI	GCAATG(2/0)↓	ER1261/2	4
BseMII	BseMII	CTCAG(10/8)	ER1401/2	4.
BseNI	Bsrl	ACTGG(1/-1)↓ GKGCM↓C	ER0881/2	4
BseSI	BseSI		ER1441/2	4
BseXI	Bbvl	GCAGC(8/12)↓	ER1451/2	4
Bsh1236l	FnuDII	CGUCG	ER0921/2	4
Bsh12851	Mcrl	CGRY↓CG	ER0891	4
BshNI	HgiCl	GJGYRCC	ER1001	40
BshTl	Agel	AJCCGGT	ER1461/2	40
Bsp68l	Nrul	TCG↓CGA	ER0111/2	4
Bsp119I	Asull	TTJCGAA	ER0121	4
Bsp120I	Apal (GGGCC↓C)	G↓GGCCC	ER0131/2/3	48
Bsp143I	Mbol	↓GATC	ER0781/2	48
Bsp143II	Haell	RGCGC↓Y	ER0791/2	4
Bsp1407I	Bsp1407I	T↓GTACA	ER0931/2	4
BspLI	NIaIV	GGN↓NCC	ER1151/2	50
BspPI	Binl	GGATC(4/5)↓	ER1321/2	50
BspTI	AfIII	C TTAAG	ER0831/2	5
Bst1107I	Snal	GTAJIAC	ER0701/2	5
BstXI	BstXI	CCANNNNJNTGG	ER1021/2/4	52
Bsu15I	Clal	AT↓CGAT	ER0141/2/4	5
BsuRI	Haelli	GGJCC	ER0151/2	5
Bvel	BspMI	ACCTGC(4/8)↓	ER1741	5
Cail	AlwNI	CAGNNN CTG	ER1391/2	54
Cfrl	Cfrl	Y GGCCR	ER0161/2	55
Cfr9I	Smal (CCC↓GGG)	C CCGGG	ER0171/2	5
Cfr10I	Cfr10I	RJCCGGY	ER0181/2	50
Cfr13I	Asul	G↓GNCC	ER0191/2	50

Sinale letter code

ingle letter coue	
R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or C.
S = C or G;	

(continued on next page) Bulk quantities & custom formulations available on request 10-201

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Table 1.2. Fermentas Restriction Endonucleases.

(continued on next page)

Ferrentas Prototype Specificity Catalog Page Cref Scall CCCCLC ER0201/2 57 Copi High CACCCLCC ER021/2 57 Copi High CACCCLCC ER021/2 58 Copi Barl (CLC) GLACCC ER021/2 58 Dani Dani GmAATC ER1071/2 59 Dani Analit TTTLAAA ER0221/2 60 Earn1051 Earn1056 CACTTCL(1/4)L ER0231/2 60 Eco311 Eco11 GCCCC ER0231/2 61 Eco311 Eco11 Aduit GLOWCC ER0231/2 62 Eco311 Eco11 Aduit GLOWCC ER031/2 64 Eco571 Eco57M CTCAAC(1/4)L ER031/2 64 Eco711 Aduit CLCWCGC ER031/2 65 Eco711 Aduit CLCWCGC ER031/2 66 Eco811 Sauit CLCC				Table 1.2. Fermentas		
Cré2i Sacil CCCC.LGC FR021/12 57 Csel Hgal GAC6C(S/10)L ER074/12 57 Cspli Hgal GAC6C(S/10)L ER1901/2 58 Opni Dpni GmA17C ER1901/2 58 Opni Analil TTEAAA ER0231/2 59 Eam1105I Fam105I GACCMC/LQ ER0231/2 60 Eam1105I Fam106I GACCMC/LC ER0231/2 60 Ec0321 Eco311 GACCTC/L ER0231/2 62 Eco4711 AcdCATC/L CACLCC ER0311/2 63 Eco4711 AcdCATC/L ER0311/2 64 Eco571 Ec		Fermentas	Prototype	Specificity	Catalog	Page
Cré2i Sacil CCCC.LGC FR021/12 57 Csel Hgal GAC6C(S/10)L ER074/12 57 Cspli Hgal GAC6C(S/10)L ER1901/2 58 Opni Dpni GmA17C ER1901/2 58 Opni Analil TTEAAA ER0231/2 59 Eam1105I Fam105I GACCMC/LQ ER0231/2 60 Eam1105I Fam106I GACCMC/LC ER0231/2 60 Ec0321 Eco311 GACCTC/L ER0231/2 62 Eco4711 AcdCATC/L CACLCC ER0311/2 63 Eco4711 AcdCATC/L ER0311/2 64 Eco571 Ec		Enzyme		$5' \rightarrow 3'$	#	#
Cool Rerit C.G.J.GWCCC FR071/2 S7 Copbil Hgal CAGC(5/10).L FR1071/2 S8 Copbil Real (GTLAC) C.TAC FR0211 S8 Dpril Analit TTTLAAA FR02217/23 S9 Drai Analit TTTLAAA FR02217/23 S9 EamT105I EamT105I CACINNI, NARGTC ER02417/2 60 Ec1356I Sacl (GAGCTLC) GAGC/LC ER02417/2 62 Eco321 Eco311 GGTC1(T/S)L ER02971/2 62 Eco321 Eco471 Arali GCLACTC FR03117/2 63 Eco471 Arali C.LGWCC FR03117/2 63 Eco571 Eco5711 CGAAG(16/14).L ER0311/2 66 Eco571 Eco5711 CGAAG(16/14).L ER0311/2 66 Eco711 Sud CCL1ACT ER0311/2 66 Eco711 Sud CCL1ACT ER0311/2 66 Eco711 <t< th=""><th></th><th></th><th>Sacli</th><th></th><th>FR0201/2</th><th>57</th></t<>			Sacli		FR0201/2	57
Cscl Hgal GACGC(SPI0)L ER1901/2 S8 Dpn1 Dpn1 GmAATC ER0211 S8 Dpn1 Analii TTELAAA ER0221/2/3 S9 Eam11051 Eam1051 GACNINALNINGIC ER0221/2/3 S9 Eam11051 Sact (AcGCLC) GACCICC ER0231/2 60 Eco321 Eco311 GCTCT(1/9)_L ER0291/2 60 Eco321 Eco311 GCTCT(1/9)_L ER0291/2 62 Eco47111 Eco47111 AGCGCC ER0311/2 63 Eco571 Eco5711 CaGCCC ER0311/2 64 Eco5711 Eco5711 CaGCAC ER0311/2 64 Eco5711 Saul CaCCACCIGT ER0311/2 65 Eco6111 Saul CaCCACCIG						
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Dpni Dpni CmoA.HZC ER0221/2/2 59 Eam 11041 Ksp5321 CTCTTC(1/A)L ER0221/2 60 Eam 11051 Sacl (GAGCTLC) GACNNALNNGTC ER0231/2 61 Eco241 HqUI GRCCVLC ER0231/2 62 Eco311 Eco311 GCTCTC(1/5)_L ER0231/2 63 Eco321 Eco87 GRCCVLC ER0311/2/3/4 62 Eco47111 ACCLOCT ER0311/2 64 Eco5711 C16AAG(16/14)_L ER0311/2 64 Eco5711 Eco5711 C16AAG(16/14)_L ER0311/2 64 Eco5711 Eco5711 C16AAG(16/14)_L ER0311/2 64 Eco5711 Eco5711 C16AAG(16/14)_L ER0311/2 65 Eco711 Eco5711 C16AAG(16/14)_L ER0311/2 64 Eco8711 Eco5711 C16AAG(16/14)_L ER0311/2 64 Eco1701 Exo5711 C16AAG(16/14)_L ER0311/2 64 Eco1711 Eab11						
Drai Ahali TTLAAA ER0231/2/3 59 Eam1104 Ksp632 CTCTTC(1/4)						
Eam1041 Ksp6321 CTCTTC(1/4) ER0231/2 60 Ecn13611 Sacl (GAGCTLC) GACNNNLNNCTC ER0251 61 Eco241 HgUII GRCVLC ER0251 61 Eco311 EGCTC(1/5) ER0251 61 Eco321 Eco311 GCTCT(1/5) ER0251/2 62 Eco471 Avall GLGVCC ER031/2 63 Eco521 Kmall CLGCCC ER031/2 64 Eco571 Eco671 CTGRAG(16/14) ER031/2 64 Eco571 Eco671 CTGRAG(16/14) ER031/2 65 Eco711 Saul CC.L'INAGG ER031/2 66 Eco811 Saul CC.L'INAGG ER031/2 67 Eco710 BstEll G.LGCTA ER041/2 67 Eco711 BstEll G.LGCTA ER041/2 67 Eco711 Stu1 AGLCT ER031/2 67 Eco711 Stu1 AGLCT ER031/2						-
Eam 11051 Eam 11051 GACLONC ER0241/2 60 Ect3611 Scal (GACCLC) GACLONC ER0291/2 61 Eco311 Eco111 GRCVLC ER0291/2 62 Eco312 EcoRV GATLATC ER0291/2 63 Eco471 Avail GLCVCC ER0311/2 63 Eco571 Eco571 C1GAAG(fA/14)_1 ER0311/2 64 Eco571 Eco571 C1GAAG(fA/14)_1 ER0311/2 64 Eco571 Eco571 C1GAAG(fA/14)_1 ER0361/2 65 Eco712 PmaC1 CACLGTG ER0361/2 65 Eco713 Stal CLYCGRG ER0361/2 67 Eco1051 SnaB1 TACLGTA ER0401/2 67 Eco1051 SnaB1 TACLGTA ER041/2 67 Eco1051 SnaB1 TACLGTA ER041/2 67 Eco1051 SnaB1 TACLGTA ER041/2 72 Eco1051 SnaB1 TACLGTA						
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Eco241 FqUII GRCCVLC FR0281 61 Eco311 Eco311 GGTCTC(1/5)_ FR0291/2 62 Eco471 Avail GLGVCC FR0311/2 63 Eco4711 Eco4711 AcclaCT FR0311/2 63 Eco571 Eco571 CTGAAG(16/14)_1 FR0311/2 64 Eco571 Eco571 CTGAAG(16/14)_1 FR0311/2 64 Eco571 Eco571 CTGAAG(16/14)_1 FR0361/2 64 Eco5711 Saul CC11NAGG FR0361/2 65 Eco5111 Saul CC1TAGG FR0361/2 66 Eco711 Pmc1 CACLGTA FR0361/2 66 Eco711 Stul AGGLCT FR0361/2 67 Eco181 Avail CLCGTA FR041/2 68 Eco191 Drail RGLGTA FR041/2 69 Eco191 Styl CLGTACC FR021/2 70 Eco191 Drall RGGCCCC FR041 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>						
Eco311 ECo311 GGTCT(1/5)_L ER0291/2 6.2 Eco321 Eco71 Avall GLATC ER0301/2/3/4 6.2 Eco4711 Avall GLATC ER0301/2/3/4 6.2 Eco4711 Accl GCC ER031/2 6.3 Eco571 Eco571 CTGAAG(16/14)_L ER031/2 6.4 Eco571 Eco571 CTGAAG(16/14)_L ER031/2 6.6 Eco571 Eco571 CTGAAG(16/14)_L ER031/2 6.6 Eco511 Saul CCTINACG ER031/2 6.6 Eco611 Saul CCTINACG ER031/2 6.7 Eco101 Stit/ CLGWNGG ER0411 6.8 Eco111 Stit/ AGGLCCT ER0421/2 6.8 Eco1191 Drail RGLACCY ER021/2/3/4 69 Eco1191 EcoRII GCCGCC ER041/2 70 Ep31 CGTCT(1/5)_L ER0451/2 71 Esp31 CGTCTC(1/5)_L ER0451/2 <t< td=""><td></td><td>Ecl136II</td><td></td><td></td><td>ER0251</td><td>61</td></t<>		Ecl136II			ER0251	61
Eco321 EcoRV GATLATC ER0301/2/14 62 Eco4711 Eco47111 AGC.JGCT ER0321/2 63 Eco521 Xmall C.GGCG ER0331/2 64 Eco571 Eco161 Eco361/2 65 Eco81 Aval C.YCGRG ER0371/2 66 Eco391/2 67 Eco1051 SnaB1 TACLGTAC ER0371/2 67 Eco1051 SnaB1 TACLGTAC ER0401/2 67 Eco1101 Stul AGGLCT ER0401/2 67 Eco111 Saul CGCCC ER0411 68 Eco1471 Stul AGGLCT ER0401/2 70 Eco141 CGCCC ER0411 67 Eco141 70 Eco141 CGCCCC Eco141		Eco24I	HgiJII		ER0281	-
Eco471 Avail GLOWCC FR0311/2 63 Eco47111 Eco47111 AGCLGCT ER0321/2 64 Eco571 Eco5711 CTGAAG(16/14)J ER0331/2 64 Eco5711 Eco5711 CTGAAG(16/14)J ER0331/2 64 Eco5711 Eco5711 CTGRAG(16/14)J ER0331/2 64 Eco512 PmaC1 CALCTGR ER0331/2 65 Eco721 PmaC1 CALCTGR ER0331/2 66 Eco811 Saul CCUTNAGC ER0371/2 67 Eco111 BstEll GLOWCG ER0411 68 Eco111 Stul AGGLCT ER0411/2 68 Eco111 Stul AGGLCT ER0411/2 71 Eco111 Stul AGGLCT ER0411/2 71 Eco111 Stul AGGLCT ER0411/2 71 Eco111 Stul AGGLCT ER0411/2 72 Eco111 Stul CGCCCC ECo11/2		Eco31I	Eco31I	GGTCTC(1/5)↓	ER0291/2	62
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Eco4711 Eco4711 AGC.LGCT ER0321/2 63 Eco571 Eco71 Eco71 Eco71 Eco71 Eco71 Eco71 Eco71 Eco71 Eco71 Eco710 Eco711 Stu AGC.LGCA Eco711 Eco711 Stu AGG.LGCA Eco711 <		Eco47I	Avall	G↓GWCC	ER0311/2	63
Eco521 Xmall CLGCCCC FE0331/2 64 Eco5711 Eco5711 CTGAAG(16/14)1 ER0331/2 64 Eco5711 Eco5711 CTGAAG(16/14)1 ER0331/2 65 Eco721 PmaC1 CACLCTA ER0331/2 65 Eco811 Saul CLTVAGG ER0331/2 66 Eco811 Saul CLTVAGG ER031/2 67 Eco1301 Styl CLOWWCG ER0411 68 Eco1711 Stul AGG/CC1 ER0411 68 Eco1701 Drall RGLONCY ER021/2/3/4 69 Eco181 EcoR1 GLANTC ER021/2/3/4 69 EcoR1 EcoR1 GLANTC ER0411 70 Esp31 EcoR1 CGCCCC GCGAC(10/14)1 ER0451/2 73 Fsp31 Esp34 RIGC/GCC GCGAC(10/14)1 ER1811 71 Fsp31 Sujd GCGCC ECoR11/2 72 75 Fsp31		Eco47III	Eco47III	AGCJGCT	ER0321/2	63
Eco571 Eco571 CTGAAG(16/14)↓ ER0341/2 64 Eco57MI Eco57MI CTGRAG(16/14)↓ ER1671 65 Eco712 PmaCl CAC1GTG ER0351/2 65 Eco811 Saul CC1TNAGG ER0351/2 66 Eco811 BstEll G.JGTNAGG ER0371/2 67 Eco1051 SnaBl TAC1GTA ER0401/2 67 Eco1051 SnaBl TAC1GTA ER0401/2 67 Eco1101 Stul AGGLCT ER0421/2 68 Eco111 EcoR1 ELORI GLANCCY ER0421/2 69 EcoR1 EcoR1 GCACC ER041/1 68 EcoR1 EcoR1 GCACC ER041/2 70 Esp31 EcoR1 EcoR1 EcoR1 GLACCY ER0451/2 71 Faql FspA1 Er0ACC ER0451/2 72 72 Gsu1 CGCCCC ER0451/2 73 74 Hin1						
Eco57MI Eco57MI CGRAG(16/14)-L ER1671 65 Eco721 PmaCl CACLGTG ER0361/2 65 Eco811 Saul CLTNAGG ER0371/2 66 Eco811 Saul CLTVCGRG ER0391/2 67 Eco1301 SnaBl TACLGTA ER0401/2 67 Eco1301 Styl CLCWWGG ER0411/2 68 Eco1711 Stul AGGLCCT ER0421/2 68 Eco17091 Drail RGLGNCCY ER0251 69 EcoR1 EcoR1 GLANTC ER0421/2 70 Ebel Narl (GGLGCC) GGCLCC/L/5)-L ER0451/2 71 Fspal Fspal RTGCLGCAY ER1611 71 Fspal Fspal RTGCLGCAY ER1611/2 72 Gsul Gsul CTGAG(16/14)-L ER0471/2 74 Hin11 Acyl GRCCCYC ER0471/2 74 Hin11 Hin12 GGCCCYC ER0471/2						
Eco721 PmaCl CAC\LCTG ER0311/2 66 Eco811 Saul CC\LTNAGG ER0311/2 66 Eco811 Aval C.\LTCGRG ER0311/2 67 Eco151 SnaBI TAC\LCTA ER0401/2 67 Eco1301 Styl C.\LTCA ER0401/2 67 Eco1001 Drall RG\LGNCCY ER0261/2 69 Eco10191 Drall RG\LGNCCY ER0261/2 69 EcoRI EcoRI GGAC(C) GGCLCC ER0411 70 Esp31 EcoTCTC(1/5).L ER0451/2 71 72 Fsp81 Fsp1 RTGC\GCAY ER1811 71 Fsp31 Esp31 CGTCTC(1/5).L ER0451/2 72 Gsul Gsul CGGAC(GC1/14).L ER0451/2 73 Hhal Hhal CGCAC ER0471/2 74 Hin11 Aval GGCAC(C) ER0471/2 75 Hin11 Aval GGCAC <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td></td<>						
Eco811 Saul CC-LTNAGG ER0311/12 66 Eco881 Aval C-LYCGRG ER0381/2 67 Eco1911 BstEll G-LTNACC ER0391/2 67 Eco1051 SnaB1 TAC-LGTA ER0401/2 67 Eco1051 SnaB1 TAC-LGTA ER0401/2 67 Eco1051 SnaB1 TAC-LGTA ER0401/2 67 Eco1071 Stul AGG-LCCT ER0421/2 68 Eco1071 Drall RG-LGNCY ER0271/2/3/4 69 EcoR1 EcoR1 JCCWGG ER0411 70 Esp31 EcoR1 EcoR1 JCCWGG ER0451/2 71 Faql Fin1 GGGAC(10/14)L ER0451/2 72 FspB1 Mael C-LTGCAG(16/14)L ER0451/2 72 FspB1 Mael C-LTGCAG(16/14)L ER0451/2 74 Hin11 Acyl GR-LGCYC ER041/2 75 Hin11 Acyl GR-LGCYC </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>						
Eco881 Aval C./YGCRG ER03811 66 Eco911 BstEll G.JGTNACC ER0391/2 67 Eco1051 SnaB1 TAC.JGTA ER0401/2 67 Eco1001 Styl C.JCWWGG ER0411 68 Eco1001 Drall RGJGNCY ER0211/2 68 Eco01091 Drall RGJGNCY ER0211/2 69 EcoR1 EcoR1 G.JAATTC ER0211/2 70 Ehel Narl (GGJCGCC) GCCLCC ER0411 71 Esp31 EcoR1 GGAC(07/14)L ER0451/2 71 Faq1 FGGAG(07/14)L ER0451/2 73 Hal Hal GGAC ER1811 71 FspB1 Mael C.TAG ER161/2 72 Gsu1 GCGAC ER041/2 73 Hhal Hhal Hal Hal ER041/2 75 Hinf1 Hal GGAC ER041/2 76 Hinf1 Hinf1 C.						
Eco911 BstEll G.J.GTNACC ER0391/2 67 Eco1051 SnaBl TAC.LGTA ER0411/2 67 Eco1001 Styl C.LCWWGG ER0411 68 Eco1001 Drall RGJGNCY ER0421/2 68 Eco1001 Drall RGJGNCY ER0271/2/3/4 69 EcoRI EcoRI G.GACTC ER0271/2/3/4 69 EcoRI EcoRI G.GCCCC ER0411 70 Esp31 EcoRI EcoRI EcoRI EcoRI Fsp1 Fsp31 CGTCTC(1/5).1 ER0451/2 71 Faql Fini GGGAC(10/14).1 ER0451/2 72 Gsul Gsul CTGCAG(16/14).1 ER0451/2 73 Hin11 Alal GCGCCC ER0471/2 74 Hin11 Alal GCGCCC ER0471/2 74 Hin11 Alal GGGCGC ER0471/2 74 Hin11 Alal GGCCCCC ER0471/2 <						
Eco1051 Sna8l TAC.JGTA ER0401/2 67 Eco1301 Styl C.JCWWGG ER0411/2 68 Eco1711 Stul AGG.LCCT ER0421/2 68 Eco01091 Drall RGJGNCCY ER021/2 68 EcoRI EcoRI G.CANTC ER021/2 70 Echel Nart (GGJCGCC) GGC.LCC ER0411 70 Esp31 Esp31 CGTCTC(1/5).J ER0451/2 71 FspA1 FspA1 RTGC.LGCAY ER1661/2 72 FspB1 Mael C.JTAG ER1671/2 73 Hhal Hal GGC.LC ER1851 73 Hhal Hal GCGC.C ER0471/2 74 Hin11 Na181 CATG.J ER1851 73 Hhal Hal GCG.C.C ER0471/2 74 Hin11 Na181 CATG.J ER1851 73 Hin11 Na181 CATG.J ER0471/2 75						
Eco1301 Styl C.JCWWGG ER0411 68 Eco171 Stul AGGLCCT ER021/2 68 Eco01091 Drall RGJGNCCY ER021/2 69 EcoRI EcoRI GLAATTC ER021/2/3/4 69 EcoRI EcoRI GCWGG ER1921/2 70 EcoRI EcoRI GCCGC ER0411 70 Esp31 Esp31 CGTCTC(1/5).L ER0451/2 71 Faql Fin1 GGGAC(10/14).L ER161/2 72 Fsp81 Mael CJTAG ER161/2 72 Fsp81 Mael CJTAG ER161/2 73 Hin11 Acyl GRJCGYC ER0471/2 74 Hin11 Mall GGCGC ER0471/2 75						
Eco1471 Stul AGG ↓CCT ER0421/2 68 Eco71091 Drall RG↓CNCCY ER0271/2/3/4 69 EcoRI EcoRI GCATTC ER0271/2/3/4 69 EcoRI EcoRI ↓CCWGG ER0271/2/3/4 69 EcoRI EcoRI ↓CCWGG ER0271/2/3/4 69 EcoRI EcoRI ↓CCWGG ER0271/2/3/4 69 Esp31 Esp31 CGTCTC(1/5)↓ ER0451/2 71 Faq1 Fin1 GGG ↓CCACY ER161/2 72 Fsp81 Mael CJTAG ER161/2 73 Hhal Hal GCG ↓CCAY ER161/2 73 Hhal Hal GCG ↓CCAY ER1851 73 Hin11 Nalii CATG ↓ ER1851 73 Hin11 Nalii CATG ↓ ER1851 74 Hin11 Nalii CATG ↓ ER1851 74 Hin11 Hin14 ↓(8/13.14)GAY(N)_VTC(13-14/8)↓ ER1601/2						
Eco01091 Drall RGJGNCCY ER0261 69 EcoRI EcoRI GJAATTC ER0271/2/3/4 69 EcoRI EcoRII JCCWGG ER1921/2 70 Ehel Narl (GG JCGCC) GGC JCC ER0411 70 Esp31 Esp31 CCTCTC(1/5)J ER0451/2 71 Faql Finl GGCAC(10/14)J ER1811 71 FspB1 Mael CJTAG ER1751/2 72 Gsu1 Gsu1 CGGA(16/14)J ER0461/2 73 Hha1 Hha1 CGG GC ER1851 73 Hin11 Acyl GRJCGYC ER0471/2 74 Hin11 Miall CATC ER0471/2 75 Hin61 Hin41 GGU GGU ER0471/2 76 Hin11 Nall CATC ER0471/2 76 Hin11 Hin11 GGU GGU ER0471/2 77 Hin11 Hin11 GGTACGC ER0491		Eco130I			ER0411	68
EcoRI EcoRI GJATTC ER0271/2/3/4 69 EcoRII EcoRII LCCWGG ER021/2 70 Ehel Narl (GG LGCC) GCC LGCC ER0411 70 Esp31 Esp31 CGTCTC(1/5).J ER0451/2 71 Fagl Finl GGGAC(10/14)J ER1811 71 FspB1 Mael C.1TAG ER1761/2 72 Gsul Gsul CTGGAG(16/14)J ER0461/2 73 Hhal Hal GCGCQC ER0471/2 74 Hin11 Acyl GRJCCYC ER0471/2 74 Hin11 Acyl GCGCGC ER0481/2 73 Hin11 Nacl GCGCGC ER0481/2 75 Hin11 Hal (GCGCGC) G.CGCGC ER0481/2 76 Hin11 Hal (GCGCGC) G.CGCGC ER0491/2 76 Hin11 Hin11 GTVARAC ER0511/2 77 Hpa1 Hal (GCGCGC ER0511/2 77 <td></td> <td>Eco147I</td> <td>Stul</td> <td>AGGJCCT</td> <td>ER0421/2</td> <td>68</td>		Eco147I	Stul	AGGJCCT	ER0421/2	68
EcoRII EcoRII ↓CCWGG ER1921/2 70 Ehel Narl (GG↓CCC) GCC1CCC ER0441 70 Esp31 Esp31 CCTCTC(1/5)↓ ER0451/2 71 Faql Finl GGGAC(10/14)↓ ER1811 71 Fsp81 Mael C/TCTC(1/5)↓ ER0451/2 73 Hal Fsp81 Rel6/1/2 73 ER0451/2 73 Hal Hal C/GC4/C ER0451/2 73 Hhal Hal GCG4/C ER0451/2 73 Hin11 Acyl GR↓CGYC ER0471/2 74 Hin11 Acyl GG4/CGYC ER0471/2 74 Hin11 Acyl GG4/CGYC ER0471/2 75 Hind1 Hin11 C/GGYC ER0471/2 76 Hinc11 Hind1 G/GYA ER041/2 76 Hin11 Hin11 G/GYA ER041/2 77 Hp11 Hin16 G/LACG ER041/2 77<		Eco0109I	Drall	RGJGNCCY	ER0261	69
EcoRII EcoRII ↓CCWGG ER1921/2 70 Ehel Narl (GG↓CCC) GCC1CCC ER0441 70 Esp31 Esp31 CCTCTC(1/5)↓ ER0451/2 71 Faql Finl GGGAC(10/14)↓ ER1811 71 Fsp81 Mael C/TCTC(1/5)↓ ER0451/2 73 Hal Fsp81 Rel6/1/2 73 ER0451/2 73 Hal Hal C/GC4/C ER0451/2 73 Hhal Hal GCG4/C ER0451/2 73 Hin11 Acyl GR↓CGYC ER0471/2 74 Hin11 Acyl GG4/CGYC ER0471/2 74 Hin11 Acyl GG4/CGYC ER0471/2 75 Hind1 Hin11 C/GGYC ER0471/2 76 Hinc11 Hind1 G/GYA ER041/2 76 Hin11 Hin11 G/GYA ER041/2 77 Hp11 Hin16 G/LACG ER041/2 77<		EcoRI	EcoRI	G↓AATTC	ER0271/2/3/4	69
Ehel Narl (GG.LGCC) GGC.LGCC ER0441 70 Esp31 Esp31 CGTCTC(1/5).↓ ER0451/2 71 Faql Finl GGGAC(10/14).↓ ER1811 71 FspAl FspAl RTGC.LGCAY ER1661/2 72 Gsul Gsul CJTAG ER1761/2 73 Hha1 Hha1 GCG.LGC ER1851 73 Hin1 Acyl GRJCGYC ER0471/2 74 Hin1 Acyl GRJCGYC ER0471/2 74 Hin41 Hin41 GRJ/GYL ER181 73 Hin61 Hha4 GGGACC ER0471/2 75 Hin61 Hha4 GGCG.C ER0471/2 76 Hin61 Hha4 GCGC ER0471/2 76 Hin61 Hha4 GCGC ER0471/2 77 Hin61 Ha1 (GCGCJ.C) GLCGC ER0471/2 76 Hin61 Ha2 GATY GCGGA ER0511/2 77 <td>NEV</td> <td></td> <td>EcoRII</td> <td>↓CCWGG</td> <td>ER1921/2</td> <td>70</td>	NEV		EcoRII	↓CCWGG	ER1921/2	70
Esp3l Esp3l CGTCTC(1/5)↓ ER0451/2 71 Faqi Fin1 GGGAC(10/14)↓ ER1811 71 FspAl FspAl RTGCJGCAY ER16112 72 FspBl Mael CJTAG ER1751/2 72 Gsul Gsul CTGGAG(16/14)↓ ER0451/2 73 Hha1 Hha1 GCGJC ER1851 73 Hin1 Acyl GR4CGYC ER0471/2 74 Hin1 Naul CATG_ ER1851 73 Hin61 Hha1 CATG_ ER0471/2 74 Hin61 Hha1 (GCGJC) GJCGC ER0471/2 75 Hincil Hindil CATGAC ER0491/2 76 Hincil Hindil ALAGCTT ER0501/2/3/4 76 Hindil Hindil CLGGG ER0511/2 77 Hph1 Hpall CLGGG ER0511/2 77 Hph1 HpdGTGCA(8/7)↓ ER101/2 78 <td< td=""><td></td><td></td><td>Narl (GGLCGCC)</td><td></td><td></td><td></td></td<>			Narl (GGLCGCC)			
Faq Fin GGGAC(10/14) ER1811 71 FspAl FspAl RTGCJGCAY ER1661/2 72 FspBl Mael CJTAG ER1611/2 72 Gsul Gsul CTGGAG(16/14) ER0461/2 73 Hhal Hhal GGJCG ER1851 73 Hin1i Acyl GRJCGYC ER0471/2 74 Hin1i Nalail CATGJ ER1831 74 Hin41 Hin41 J(8/13-14)GAY(N) _b VTC(13-14/8) ER1601/2 75 Hin61 Hha1 GGGACC ER0481/2 75 Hin61 Hha1 GTGAC ER0491/2 76 Hin61 Ha1 GTGAC ER0501/2/3/ 77 Hp11 Ho11 GJACG ER0501/2/3/ 77 Hp11 Hp11 GGTGA(8/7) ER1511/2 78 Hp11 Hp11 GGTGA(8/7) ER1511/2 78 Hp14 Hp11 GCTGA(8/7) ER1511/2 79						-
FspAl FspAl RTGC JGCAY ER1661/2 72 FspBl Mael CJTAG ER1761/2 72 Gsul Gsul CTGGAG(16/14)↓ ER0461/2 73 Hhal Hhal Hal GCGLC ER1851 73 Hin1 Acyl GR↓CGYC ER0471/2 74 Hin11 Acyl GR↓CGYC ER0481/2 75 Hin61 Hhal (GCG↓C) GL\CCC ER0481/2 75 Hin61 Hal (GCG↓C) GL\CCC ER0481/2 75 Hin61 Hind11 GT\LACC ER0481/2 75 Hin61 Hind11 GCGCGC GL\CCC ER0491/2 76 Hin71 Hin71 GGCGLC) GL\CCC ER0491/2 77 Hpall Hpall CJGGG ER0491/2 77 Hpall Hpall GGCGA(8/7)↓ ER1001/2 78 Hpy81 MgalV GTN↓NAC ER1731/2 79 Hpy81 MgalV						
FspBl Mael C↓TAG ER1761/2 72 Gsul Gsul CTGGAG(16/14)↓ ER0461/2 73 Hhal Hhal GCG↓C ER0461/2 73 Hin1 Acyl GR↓CGYC ER0471/2 74 Hin11 Acyl GR↓CGYC ER0471/2 74 Hin11 Nall CATG↓ ER1831 74 Hin41 Hin41 ↓(8/13-14)GAY(N)₅VTC(13-14/8)↓ ER1601/2 75 Hin61 Hhal (GCG↓C) G↓CGC ER0481/2 75 Hin61 Hin61 GT√LRAC ER0491/2 76 Hin11 Hin61 G↓AGCTT ER0501/2/3/4 76 Hin11 Hin61 G↓AGCT ER0511/2 77 Hpall Hph1 GGGA(8/7)↓ ER1571/2 78 Hpy81 Mja1V GTN-LNAC ER1571/2 79 HpyF10V1 Mvol GCNNNNJANGC ER1571/2 79 Kpn1 Kpn1 GGTAC↓C ER0531/2						
Gsul Gsul CTGGAG(16/14)↓ ER0461/2 73 Hhal Hhal GCGJC ER1851 73 Hin1 Acyl GRJCGYC ER1851 73 Hin11 Acyl GRJCGYC ER0471/2 74 Hin11 Acyl GRJCGYC ER0471/2 74 Hin11 Acyl GGJCGC ER0471/2 74 Hin11 Hiadl J(8/13-14)GAY(N) ₅ VTC(13-14/8)↓ ER1601/2 75 Hin61 Hind(I) GTGAGRO ER0491/2 76 Hin11 Hind(II) GTYJRAC ER0511/2/3 77 Hpall Hinf(I) GJAGGG ER0511/2 77 Hpy81 MjalV GTNJNAC ER1571/2 78 HpyF10V1 Mvol GCTCTC(1/4)↓ ER1621/2						
Hhal Hhal GCGJC ER1851 73 Hin11 Acyl GRJCGYC ER0471/2 74 Hin11 Nialli CATGJ ER1831 74 Hin41 Hin41 J(8/13.14)GAY(N)_VTC(13-14/8)J ER1801 74 Hin61 Hnal (GCGJC) GJCGC ER0801/2 75 Hin61 Hnal (GCGJC) GJCGC ER0481/2 75 Hin61 Hal (GCGJC) GJCGC ER0481/2 76 Hinfi Hindlii GJANTC ER0511/2 77 Hpali Hpali CJCGG ER0511/2 77 Hph1 Hpali GGTGA(8/7)J ER101/2 78 Hpy81 Mjalv GTNJNAC ER1731/2 79 HpvF10VI Mwol GCNNNNNJNNGC ER1731/2 79 HpyF31 Ddel CJTNAG ER1731/2 80 Kpn1 Kpn1 GCTCTCT(1/4)J ER0531/2 80 Kpp1 Sapl GCTCTCT(1/4)J ER1621/						
Hin11 Acyl GR↓CGYC ER0471/2 74 Hin11 Ntalii CATG↓ ER1831 74 Hin41 Hin41 ↓(8/13-14)GAY(N) ₆ VTC(13-14/8)↓ ER1601/2 75 Hin61 Hhal (GCG↓C) G↓CGC ER0481/2 75 Hin61 Hold GT↓LRAC ER0491/2 76 Hin61 Hin61 GT↓LRAC ER0491/2 77 Hin61 Hinf1 GJANTC ER0491/2 77 Hin61 Hof1 GJANTC ER0491/2 77 Hp1 Hp1 GGTGA(8/7)↓ ER101/2 77 Hpy81 MjalV GTN↓NAC ER1571/2 78 HpyF31 Ddel C↓TNAG ER1571/2 79 Kpn1 Kpn1 GGTA↓AC ER1571/2 79 Kpn21 BspMil T↓CCGGA ER0531/2 80 KspA1 Hpa1 GTT↓AAC ER1031/2 81 LguI Sap1 GCTCTTC(1/4)↓ ER1031/2 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td></td<>						
Hin1ll Naill CATG↓ ER1831 74 Hin41 Hin41 ↓(8/13-14)GAY(N) ₈ VTC(13-14/8)↓ ER1601/2 75 Hin61 Hla1 (GCG↓C) G↓CCC ER0481/2 75 Hin61 Hla1 (GCG↓C) G↓CCC ER0491/2 76 Hin61 Hind11 GT¼RAC ER0491/2 76 Hin61 Hin61 G↓ANTC ER0501/2/3/4 77 Hp11 Hin61 G↓ANTC ER0501/2/3/4 76 Hin71 Hin71 GGTGA(8/7)↓ ER101/2 77 Hp11 Hp11 GGTGA(8/7)↓ ER1511/2 77 Hp11 Hp1 GGTA(8/7)↓ ER1812 79 Hp41 Hp1 GGTA(2/C ER1571/2 78 Hp513 Ddel C↓TNAG ER1881/2 79 Kpn1 Kpn1 GGTA(2/C ER0521/2/3/4 80 Kpn21 BspMil T↓CCGGA ER0511/2 81 Lgu1 Sap1 GCTCTTC(1/4)↓ ER1						
Hin41 Hin41 \downarrow (8/13-14)GAY(N) _b VTC(13-14/8) \downarrow ER1601/2 75 Hin61 Hhal (GCGJC) GJCGC ER0481/2 75 HincII HindII GTVLRAC ER0491/2 76 HindIII HindIII A_AGCTT ER0501/2/3/4 76 HinfI HinfI GJANTC ER0501/2/3/4 76 Hpall Hpall C_CGG ER0511/2 77 HphI HphI GGTGA(8/7).↓ ER101/2 78 Hpy81 MjalV GTN-NAC ER1571/2 78 HpyF31 Ddel C_TNAG ER181/2 79 HpyF10VI Mwol GGTNNNNJ.NNGC ER1731/2 78 KpnI KpnI GGTACLC ER0531/2 80 KspAI Hpal GTTJAAC ER1031/2 81 Lwel SfaNI GCATC(5/9).J ER1621/2 82 Mbol Mbol JGATC ER0811/2 83 Mbol Mbol GAGCGG(-3/-3).J						
Hin61 Hhal (GCG \downarrow C) G \downarrow CGC ER0481/2 75 Hincli Hindli GTV \downarrow RAC ER0491/2 76 Hincli Hindli A \downarrow AGCTT ER0511/2/3/4 76 Hindli Hindli G \downarrow AARC ER0511/2/3/4 76 Hpall Hpall C \downarrow CGG ER0511/2 77 Hph1 Hph1 GGTGA(8/7) \downarrow ER1011/2 78 Hpy81 MjalV GTN \downarrow NAC ER1571/2 78 Hpyf31 Ddel C \downarrow TNAG ER1881/2 79 HpyF31 Ddel C \downarrow TNAG ER1881/2 79 HpyF10VI Mwol GCNNNNN \downarrow NNGC ER1731/2 78 Kpn21 BspMII T \downarrow CCGGA ER0531/2 80 KspA1 Hpal GTT \downarrow AAC ER1031/2 81 Lwel SfaNI GCTCTTC(1/4) \downarrow ER1621/2 82 Mboi Mool \downarrow GACG ER0811/2 83 Mboi Ball TGG \downarrow CCA		Hin1II	NIaIII		ER1831	
HincllHindllGTYJRACER0491/276HindlllHindlllAJAGCTTER0501/2/3/476HinflHinflGJANTCER0501/2/377HpallHpallCJCGGER0511/277HpallHpallCGTGA(8/7)JER1101/278Hpy8lMjalVGTNJNACER1571/278HpyF3lDdelCJTNAGER1571/279HpyF10VIMwolGCNNNNJNNGCER1571/279KpnlKpnlGGTACJCER0521/2/3/480KpnlKpnlGGTACACER0521/2/3/480KpnlKpnlGCTCTTC(1/4)JER1031/281LwelSfaNIGCTCTTC(1/4)JER1031/281LwelSfaNIGCACGG(-3/-3)JER127182MbilBsrBlGAGCGG(-3/-3)JER127182MbolMbolJGATCER0811/283MbolMbolGCACCACER1211/284MulMlulAJCGCGTER051/284MulMulAJCGCGTER051/284MulMulAJCGCGER0511/285MsplHpallCJCGGER0511/286MssiPmelGTTJAAACER1341/286Mval EcoRII (JCCWGG)CCJWGGER0511/287Mval EcoRII (JCCWGG)CCJWGGER0511/287Mval EcoRII (JCCWGG)CCJWGGER0511/287Mval EcoRII (JCCWGG)CCJWGGER0511/288 <th></th> <th>Hin4I</th> <th></th> <th>↓(8/13-14)GAY(N),VTC(13-14/8)↓</th> <th>ER1601/2</th> <th>75</th>		Hin4I		↓(8/13-14)GAY(N),VTC(13-14/8)↓	ER1601/2	75
Hindili Hindili A \downarrow AGCTT ER0501/2/3/4 76 Hinfi Hinfi G \downarrow ANTC ER0801/2/3 77 Hpall Hpall C \downarrow CGG ER0511/2 77 Hphl Hphl GGTGA(8/7) \downarrow ER101/2 78 Hpy81 MjalV GTN \downarrow NAC ER1571/2 78 HpyF31 Ddel C \downarrow TNAG ER1571/2 79 HpyF31 Ddel C \downarrow TNAG ER1571/2 79 HpyF10VI Mwol GCTNNNN \downarrow NNGC ER1731/2 79 Kpn1 Kpn1 GGTAC \downarrow C ER0521/2/3/4 80 Kpn21 BspMII T \downarrow CCGGA ER0531/2 80 Kpn21 BspMII T \downarrow CCGGA ER1031/2 81 Lgui Sapl GCTCTTC(1/4) \downarrow ER1931/2 81 Lwel SfaNI GCACCG(-3/-3) \downarrow ER1271 82 Mbol Mbol GAGCGG(-3/-3) \downarrow ER0811/2 83 Mbol Mol		Hin6l	Hhal (GCG↓C)	GUCGC	ER0481/2	75
Hinfl Hinfl GJANTC ER0801/2/3 77 Hpall Hpall CJCGG ER0511/2 77 Hphl Hphl GGTGA(8/7) ER1101/2 78 Hpy81 MjalV GTNJNAC ER1571/2 78 HpyF31 Ddel CJTNAG ER1571/2 79 HpyF10VI Mwol GCNNNNNJNNCC ER1731/2 79 Kpn1 Kpn1 GGTACJC ER0521/2/3/4 80 Kpn21 BspMII TJCCGGA ER0521/2/3/4 80 KspA1 Hpal GTTJAAC ER1031/2 81 LguI Sap1 GCTCTTC(1/4) ER1931/2 81 Lwel SfaNI GCATC(5/9) ER1271 82 Mboil Mboil GAAGA(8/7) ER0821/2 83 Mboil Mboil GAAGA(8/7) ER0821/2 84 Mlui Mlui Alui AlcCCGT ER051/2 84 Mili Mali AcGCGGT ER0561/2<		Hincll	Hindll	GTY	ER0491/2	76
Hinfl Hinfl G↓ANTC ER0801/2/3 77 Hpall Hpall C↓CGG ER0511/2 77 Hphl Hphl GGTGA(8/7)↓ ER1101/2 78 Hpy8l MjalV GTN↓NAC ER1571/2 78 HpyF3l Ddel C↓TNAG ER1571/2 79 HpyF3l Ddel C↓TNAG ER1731/2 79 HpyF10VI Mwol GCNNNNN↓NNGC ER1731/2 79 Kpn1 Kpn1 GGTAC↓C ER0521/2/3/4 80 Kpn21 BspMII T↓CCGGA ER0531/2 80 KspAl Hpal GTT↓AAC ER1031/2 81 LguI Sap1 GCTCTTC(1/4)↓ ER1931/2 81 Lwel SfaNI GCACCG(-3/-3)↓ ER1621/2 82 Mboil Mboil GAAG(8/7)↓ ER0811/2 83 Mboil Mboil GAAG(8/7)↓ ER0821/2 84 Mlui Mulu A↓CGCGT ER071/2		HindIII	HindIII	AJAGCTT	ER0501/2/3/4	76
Hpail Hpail C \downarrow CGG ER0511/2 77 Hphl Hphl GGTGA(8/7) \downarrow ER1101/2 78 Hpy81 MjalV GTN \downarrow NAC ER1571/2 78 Hpy81 MjalV GTN \downarrow NAC ER1571/2 78 HpyF31 Ddel C \downarrow TNAG ER1571/2 78 HpyF10VI Mwol GCNNNN \downarrow NNGC ER1731/2 79 Kpn1 Kpn1 GGTAC \downarrow C ER0521/2/3/4 80 Kpn21 BspMII T \downarrow CCGGA ER0531/2 80 KspAl Hpal GTT \downarrow AAC ER1031/2 81 Lgul Sap1 GCTCTTC(1/4) \downarrow ER1931/2 81 Lwel SfaNI GCACC(5/9) \downarrow ER121/2 82 Mbil BsrB1 GAGCGG(-3/-3) \downarrow ER0811/2 83 Mbol Mbol \Box GG \downarrow CA ER0811/2 83 Mbol Mbol GAGCGGC-3/-3) \downarrow ER0821/2 83 Mbol Mbol GAGA(8/7) \downarrow			Hinfl	GJANTC	FR0801/2/3	77
HphlHphlGGTGA(8/7)ER1101/278Hpy81MjalVGTNJNACER1571/278Hpy81MjalVGTNJNACER1571/278HpyF31DdelCJTNAGER1881/279HpyF10V1MwolGCNNNNJNNGCER1731/279Kpn1Kpn1GGTACJCER0521/2/3/480Kpn21BspMilTJCCGGAER0531/280KspA1Hpa1GTTJAACER1031/281LgulSap1GCTCTTC(1/4)JER1621/282MbilBsrB1GAGCGG(-3/-3)JER121/283MbolMbolJGATCER0811/283MbolMbolGGTGCTER0811/283MbolMbolGAGCGGTER021/283MbolMbolGAGCGGTER021/283MbolMbolGAGCGGTER021/283MbolMbolGAAGA(8/7)JER021/283MbolMbolGGTGACJCER051/284MiulMlulAJCGCGTER0561/284MulMiulAJCGGGER051/285MsplHpallCJCGGER0511/286MunlMfelCJTJAAACER0511/287Mva12691BsmlGAATGC(1/-1)JER051/287Mva12691BsmlGAATGC(1/-1)JER051/288NcolNcolCJCATGGER051/2/488NdelNdelCAJTATGER051/2/489 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>						
Hpy8l MjalV GTN↓NAC ER1571/2 78 HpyF3l Ddel $C \downarrow TNAG$ ER1871/2 79 HpyF10VI Mwol GCNNNNNJNGC ER1731/2 79 Kpn1 Kpn1 GGTAC↓C ER0521/2/3/4 80 Kpn2l BspMII T↓CCGGA ER0531/2 80 KspAl Hpal GTT↓AAC ER1031/2 81 Lgui Sapl GCTCTTC(1/4)↓ ER1621/2 82 Mbil BsrBl GAGCGG(-3/-3)↓ ER1271 82 Mboil Mbol ↓GATC ER0811/2 83 Mboll Mbol GGACCGC ER0811/2 83 Mboll Mbol GAGCGG(-3/-3)↓ ER0821/2 83 Mboll Mbol GGACCGC ER0811/2 83 Mboll Mbol GAAGA(8/7)↓ ER0821/2 83 Mboll Mboll GAAGA(8/7)↓ ER0821/2 84 Mlu1 Mlu1 AlcGCGGT ER081/2						
HpyF31 Ddel C_ITNAG ER1881/2 79 HpyF10VI Mwol GCNNNNN_NGC ER1731/2 79 Kpn1 Kpn1 GGTAC_C ER0521/2/3/4 80 Kpn21 BspMII T_JCCGGA ER0531/2 80 KspA1 Hpal GTTJAAC ER1031/2 81 Lgul Sapl GCTCTTC(1/4)_J ER1931/2 81 Lwel SfaNI GCACCG(-3/-3)_J ER1271 82 Mbil BsrBI GAGCGG(-3/-3)_J ER1271 82 Mbol Mbol JGATC ER0811/2 83 Mbol Mbol GGACCGC ER0811/2 83 Mbol Mbol GAGCCGC-3/-3)_J ER0821/2 83 Mbol Mbol GGACCGC ER0811/2 84 Mlul Mbol GAAGA(8/7)_J ER0821/2 83 Mbol Mbol GAAGA(2/7)_J ER0821/2 84 Mlul Mlul AJCGCGT ER051/2						
HpyF10VI Mwol GCNNNNN\NGC ER1731/2 79 Kpn1 Kpn1 GGTAC\C ER0521/2/3/4 80 Kpn21 BspMII T\CCGGA ER0531/2 80 KspA1 Hpal GTT\AAC ER1031/2 81 Lgul Sapl GCTCTTC(1/4)\ ER1931/2 81 Lwel SfaNI GCACCGG(-3/-3)\ ER1271 82 Mbil BsrBI GAGCGG(-3/-3)\ ER1271 82 Mbol Mbol \GAGCCGC(-3/-3)\ ER0811/2 83 Mbol Mbol GGACCGC(-3/-3)\ ER0821/2 83 Mbol Mbol GGACCGC ER0811/2 84 Mul Mbol GAGCCGC ER0821/2 83 Mbol Mbol GGACCAC ER0811/2 84 Mlul Mlul A\LCGCCA ER0811/2 84 Mul Mul A\LCGCGG ER0541/2 84 MnII MnII C\LCGG ER0541/2 8	(TEM)	HnyF2I				
KpnlKpnlGGTAC↓CER0521/2/3/480Kpn2lBspMIIT↓CCGGAER0531/280KspAlHpalGTT↓AACER1031/281LgulSaplGCTCTTC(1/4)↓ER1931/281LwelSfaNIGCATC(5/9)↓ER1621/282MbilBsrBIGAGCGG(-3/-3)↓ER127182MbolMbol $\label{eq:starser}$ ER0811/283MbolMbol $\label{eq:starser}$ ER0821/283MbolMbolIGAAGA(8/7)↓ER0821/283MisiBallTGG↓CCAER1211/284MiulMlulA↓CGCGTER0561/284MnIIMnIICCTC(7/6)↓ER1071/285MsplHpaliC↓CGGER0541/286MsslPmelGTTT↓AAACER0541/286MuniMfelC↓AATTGER051/287MvalEcoRII (↓CCWGG)CC↓WGGER0551/287Mval269IBsmlGAATGC(1/-1)↓ER0961/288NcolNcolC↓CATGER0571/2/488NdelNdelCA↓TATGER0581/2/489						
Kpn2lBspMIIIT↓CCGGAER0531/280KspAlHpalGTT↓AACER1031/281LgulSaplGCTCTTC(1/4)↓ER1931/281LwelSfaNIGCATC(5/9)↓ER1621/282MbilBsrBIGAGCGG(-3/-3)↓ER127182MbolMbol $\carcleftal GAGCGG(-3/-3)↓$ ER0811/283MbolMbol $\carcleftal GAGCGG(-3/-3)↓$ ER0821/283MbolMbolGAAGA(8/7)↓ER0821/283MisiBallTGG↓CCAER1211/284MiulMlulA↓CGCGTER0561/284MnIIMnIICCTC(7/6)↓ER1071/285Mph1103IAvalllATGCA/TER0731/285MsplHpallC↓CGGER0541/286MsslPmelGTTT↓AAACER051/287MvalEcoRII (↓CCWGG)CC↓WGGER0551/287MvalEcoRII (↓CCWGG)CC↓WGGER0551/288NcolNcolC↓CATGER051/2/488NdelNdelCA↓TATGER051/2/489						
KspAlHpalGTT↓AACER1031/281LgulSaplGCTCTTC(1/4)↓ER1931/281LwelSfaNIGCATC(5/9)↓ER1621/282MbilBsrBIGAGCGG(-3/-3)↓ER127182MbolMbol \carclefta ER0811/283MbolMbol \carclefta ER0811/283MbolMbol \carclefta ER0821/283MbolMbolGAAGA(8/7)↓ER0821/283MisiBallTGG↓CCAER1211/284MiulMlulA↓CGCGTER0561/284MnIIMnIICCTC(7/6)↓ER1071/285Mph11031AvalllATGCA/TER0731/285MsplHpallC↓CGGER0541/286MunlMfelC↓AATTGER0551/287MvalEcoRII (↓CCWGG)CC↓WGGER0551/287Mval269IBsmlGAATGC(1/-1)↓ER0961/288NcolNcolC↓CATGER051/2/489						
LgulSaplGCTCTTC(1/4)ER1931/281LwelSfaNIGCATC(5/9)ER1621/282MbilBsrBIGAGCGG(-3/-3)ER127182MbolMbol \downarrow GATCER0811/283MbolMbol \downarrow GATCER0821/283MbolMbolGAAGA(8/7)ER0821/283MisiBallTGG-JCCAER1211/284MluiMluiALCGCGTER0561/284MnliMnliCCTC(7/6)ER0561/284MsiBallCCCGGER051/285Mph11031AvalliATGCALTER0731/285MsplHpallC-LCGGER0541/286MsslPmelGTTTJAAACER0541/286MuniMfelC-LAATTGER0751/287MvalEcoRil (JCCWGG)CC-WGGER0551/287Mval2691BsmlGAATGC(1/-1)ER0961/288NcolNcolC-LATGGER0571/2/488NdelNdelCAJTATGER0581/2/489						
LwelSfaNIGCATC(5/9)ER1621/282MbilBsrBIGAGCGG(-3/-3)ER127182MbolMbol \downarrow GATCER0811/283MbolIMbolIGAAGA(8/7)ER0821/283MisiBallTGG-JCCAER1211/284MiulMluiA \downarrow CGCGTER0561/284MnliMnliCCTC(7/6)ER0561/284MsiMallACGCGTER0541/285Mph1103IAvaillATGCAER0731/285MsplHpaliC \downarrow CGGER0541/286MsslPmelGTTTJAAACER0541/286MuniMfelC \downarrow AATTGER0751/287MvalEcoRII (\downarrow CCWGG)CC \downarrow WGGER0551/287Mval269IBsmlGAATGC(1/-1)ER0961/288NcolNcolC \downarrow CATGGER0571/2/488NdelNdelCA\TATGER0581/2/489						
MbilBsrBlGAGCGG(-3/-3) \downarrow ER127182MbolMbol \downarrow GATCER0811/283MbollMbollGAAGA(8/7) \downarrow ER0821/283MlsiBallTGG \downarrow CCAER1211/284MlulMlulA \downarrow CGCGTER0561/284MnllMnllCCTC(7/6) \downarrow ER1071/285Mph11031AvalllATGCA \downarrow TER0541/286MsslPmelC \downarrow CGGER0541/286MsslPmelGTT \downarrow AAACER1341/286MunlMfelC \downarrow AATTGER0551/287MvalEcoRli (\downarrow CCWGG)CC \downarrow WGGER0551/287Mva12691BsmlGAATGC(1/-1) \downarrow ER0961/288NcolNcolC \downarrow CATGGER0571/2/488NdelNdelCA \downarrow TATGER0581/2/489	WEW					
Mbol \downarrow GATCER0811/283MbollMbollGAAGA(8/7) ↓ER0821/283MisiBallTGG↓CCAER1211/284MiulMiulA↓CGCGTER0561/284MillMnllCCTC(7/6) ↓ER1071/285Mph11031AvallATGCA↓TER0731/285MsplHpallC↓CGGER0541/286MsslPmelGTTT↓AAACER1341/286MunlMfelC↓AATTGER0551/287MvalEcoRll (↓CCWGG)CC↓WGGER0551/287Mval2691BsmlGAATGC(1/-1) ↓ER0961/288NcolNcolC↓CATGGER0571/2/488NdelNdelCA↓TATGER0581/2/489						
Mboll Mboll GAAGA(8/7)↓ ER0821/2 83 Misi Ball TGG↓CCA ER1211/2 84 Miul Mlul A↓CGCGT ER0561/2 84 Mnli Mnli CCTC(7/6)↓ ER1071/2 85 Mph11031 Availl ATGCA↓T ER0731/2 85 Mspl Hpall C↓CGG ER0541/2 86 Mssl Pmel GTTT↓AAAC ER1341/2 86 Munl Mfel C↓AATTG ER0751/2 87 Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mva12691 Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89		Mbil	BsrBl	· · · ·	ER1271	82
MisiBallTGG JCCAER1211/284MiulMiulA JCGCGTER0561/284MnliMnliCCTC(7/6) JER1071/285Mph11031AvaillATGCA JTER0731/285MsplHpallC JCGGER0541/286MsslPmelGTTT JAAACER1341/286MunlMfelC JAATTGER0751/287MvalEcoRII (JCCWGG)CC JWGGER0551/287Mva12691BsmlGAATGC(1/-1) JER0961/288NcolNcolC JCATGGER0571/2/488NdelNdelCA JTATGER0581/2/489					ER0811/2	
MiulA \downarrow CGCGTER0561/284MnllMnllCCTC(7/6) \downarrow ER1071/285Mph1103IAvaillATGCA \downarrow TER0731/285MsplHpallC \downarrow CGGER0541/286MsslPmelGTTT \downarrow AAACER1341/286MunlMfelC \downarrow AATTGER0751/287MvalEcoRII (\downarrow CCWGG)CC \downarrow WGGER0551/287Mval269IBsmlGAATGC(1/-1) \downarrow ER0961/288NcolNcolC \downarrow CATGGER0571/2/488NdelNdelCA \downarrow TATGER0581/2/489		Mboll	Mboll	GAAGA(8/7)↓	ER0821/2	83
MiulA \downarrow CGCGTER0561/284MnliMnliCCTC(7/6) \downarrow ER1071/285Mph1103IAvailiATGCA \downarrow TER0731/285MsplHpallC \downarrow CGGER0541/286MsslPmelGTTT \downarrow AAACER1341/286MuniMfelC \downarrow AATTGER0751/287MvalEcoRII (\downarrow CCWGG)CC \downarrow WGGER0551/287Mval269IBsmlGAATGC(1/-1) \downarrow ER0961/288NcolNcolC \downarrow CATGGER0571/2/488NdelNdelCA \downarrow TATGER0581/2/489		MISI	Ball	TGG↓CCA	ER1211/2	84
Mnli CCTC(7/6)↓ ER1071/2 85 Mph1103I Availl ATGCA↓T ER0731/2 85 Mspl Hpall C↓CGG ER0541/2 86 Mssl Pmel GTTT↓AAAC ER1341/2 86 Munl Mfel C↓AATTG ER0751/2 87 Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mva1269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89		Mlul	Mlul	A↓CGCGT		84
Mph1103I Availi ATGCA↓T ER0731/2 85 Mspl Hpall C↓CGG ER0541/2 86 Mssl Pmel GTTT↓AAAC ER1341/2 86 Muni Mfel C↓AATTG ER0751/2 87 Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mva1269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
MsplHpallC↓CGGER0541/286MsslPmelGTTT↓AAACER1341/286MunlMfelC↓AATTGER0751/287MvalEcoRII (↓CCWGG)CC↓WGGER0551/287Mva1269IBsmlGAATGC(1/-1)↓ER0961/288NcolNcolC↓CATGGER0571/2/488NdelNdelCA↓TATGER0581/2/489						
Mssl Pmel GTTT↓AAAC ER1341/2 86 Munl Mfel C↓AATTG ER0751/2 87 Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mval269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
Muni Mfel C↓AATTG ER0751/2 87 Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mva1269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
Mval EcoRII (↓CCWGG) CC↓WGG ER0551/2 87 Mva1269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
Mva1269I Bsml GAATGC(1/-1)↓ ER0961/2 88 Ncol Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
Ncol C↓CATGG ER0571/2/4 88 Ndel Ndel CA↓TATG ER0581/2/4 89						
Ndel Ndel CAJTATG ER0581/2/4 89						
NhelG↓CTAGCER0971/289						
		Nhel	Nhel	G↓CTAGC	ER0971/2	89

Restriction Endonucleases Guide

ISO	ISO
9001	14001

	Fermentas	Prototype	Specificity	Catalog	Pag
	Enzyme		$5' \rightarrow 3'$	#	#
	NmuCl	Tsp45I	↓GTSAC	ER1511/2	90
	Notl	Notl	GC↓GGCCGC	ER0591/2/3/4	90
	Nsbl	Mstl	TGC↓GCA	ER1221/2	91
	Olil	Olil	CACNN↓NNGTG	ER1631/2	91
	Pael	Sphl	GCATG↓C	ER0601/2/4	92
	Pagl	BspHI	T↓CATGA	ER1281/2	92
	Pasl	Pasl	CC↓CWGGG	ER1861	93
	Paul	BsePl	G↓CGCGC	ER1091/2	93
	Pdil	Nael	GCC↓GGC	ER1521/2	94
	Pdml	Xmnl	GAANNUNNTTC	ER1531/2	94
	Pfel	Tfil	GJAWTC	ER1781	95
	Pfl23II	Spll	C↓GTACG	ER0851	95
	Pfol	Pfol	T↓CCNGGA	ER1751	96
	Ppil	Ppil	\downarrow (7/12)GAAC(N) ₅ CTC(13/8) \downarrow	ER1541/2	96
EV/	Ppu21I	BsaAl	YACJGTR	ER1971	97
E,	Pscl	BspLU11I	AUCATGT	ER1971 ER1871/2	97
	Psp5II	PpuMI	RGGWCCY	ER0761	98
	Psp1406l	Acli	AAJCGTT	ER0941/2	98
	Pstl	Pstl	CTGCAJG	ER0611/2/3/4	99
	Psul	Xholl	RJGATCY	ER1551	99
	Psyl	Tth1111	GACNUNNGTC	ER1331	100
	Pvul	Pvul	CGAT	ER0621/2	100
	Pvull	Pvull	CAG↓CTG	ER0631/2/3	101
	Rsal	Rsal	GT↓AC	ER1121/2	101
	Sacl	Sacl	GAGCT↓C	ER1131/2/3/4	102
	Sall	Sall	GUTCGAC	ER0641/2/3	102
	Satl	Fnu4HI	GC↓NGC	ER1641/2	103
	Scal	Scal	AGT	ER0431/2	103
	Schl	PleI (GAGTC(4/5)↓)	GAGTC(5/5)↓	ER1371	104
	Sdal	Sse8387I	CCTGCAJGG	ER1191/2	104
	Sdul	Sdul	GDGCHUC	ER0651	104
		Sfil	GGCCNNNN		
	Sfil			ER1821	105
EV/	Sgsl	Ascl	GGJCGCGCC	ER1891/2	106
	Smal	Smal	CCCJGGG	ER0661/2/3/4	106
-	Smil	Swal	ATTTJAAAT	ER1241/2	107
W	Smol	Smll	CJTYRAG	ER1981	107
	Smul	Faul	CCCGC(4/6)	ER1691/2	108
	Ssil	Acil	CCGC(-3/-1)↓	ER1791	108
	Sspl	Sspl	AATJATT	ER0771/2	109
	Taal	Tsp4Cl	ACN↓GT	ER1361/2	109
	Tail	Maell (A↓CGT)	ACGT	ER1141/2	110
	Taql	Tagl	T↓CGA	ER0671/2/3	110
	Tasl	TspEl	JAATT	ER1351/2	111
	Tatl	Tatl	WJGTACW	ER1291/2	111
	Taul	Taul	GCSGUC	ER1651/2	112
	Tru1I	Msel		ER0981/2/3	112
I			TARCCA(11/9)↓		
	Tsol	Tsol		ER1991	113
W	Tstl	Tstl	↓(8/13)CAC(N),TCC(12/7)↓	ER1911	113
	Van91I	PfIMI	CCANNNN VNTGG	ER0711/2	114
	Vspl	Vspl	AT	ER0911/2	114
	Xagl	EcoNI	CCTNN↓NNNAGG	ER1301/2	115
	Xapl	Apol	RJAATTY	ER1381/2	115
	Xbal	Xbal	T↓CTAGA	ER0681/2/3/4	116
	Xcel	Nspl	RCATG	ER1471/2	116
	Xhol	Xhol	C JTCGAG	ER0691/2/3/4	117
	XmaJI	Avrll	C↓CTAGG	ER1561/2	117
	Xmil	Accl	GTJMKAC	ER1481/2	118
	Nb.Bpu10I	Nb.Bpu10I	GCJTNAGG CG ANTCC	ER1681	119
	I-Scel	I-Scel	TAGGG ATAA CAGGGTAAT ATCCCTTATT GTCCCATTA	ER1771	120

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or C
S = C or G;	

Note

Alphabetic list of commercially available restriction endonucleases *see* on p.6.



REsearch[™] is a unique online tool designed to assist in the selection of a Fermentas restriction endonuclease(s) for your experiments using either the enzyme name or the recognition sequence. This tool also helps you to identify commercially available isoschizomers and choose the optimal buffer for double digestion. It also contains important information regarding restriction enzyme stability during prolonged incubations, conditions for their thermal inactivation, and guidelines on how to generate DNA ends, including cleavage close to the termini of PCR products. Information about new cleavage sites generated by ligation of blunt or compatible sticky ends is also presented together with data about the sensitivity of the restriction enzymes to DNA methylation. The REsearch[™] tool is regularly updated to include all neccessary information regarding the newly discovered restriction enzymes.

Use REsearch[™] at <u>www.fermentas.com/research</u>, DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> to plan your experiments.

Alphabetic List of Commercially Available Restriction Endonucleases

A number of restriction enzymes discovered by Fermentas are isoschizomers of commonly used prototype restriction enzymes. The following table will help you find the appropriate Fermentas enzymes for your experiments.

Note

- Enzymes in parentheses have different cleavage specificities (neoschizomers).
- Isoschizomers with different sensitivity to methylation are indicated by "m".
- Dpnl requires the presence of N6-methyladenine within the recognition sequence GATC.
- Enzymes produced by Fermentas are shown in orange.

Single letter code

R	= G or A:	Н	= A, C or T;
	= C or T:		= A, C or G:
W	= A or T;	В	= C, G or T;
M	= A or C;	D	= A, G or T;
К	= G or T;	Ν	= G, A, T or C
S	= C or G;		

Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity $5' \rightarrow 3'$	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
Aarl	CACCTGC(4/8)↓	Aarl (ER1581/2, p.24)	
Aasl	GACNNNN↓NNGTC	Aasl (ER1721/2, p.24)	Drdl, DseDl
Aatl	AGG↓CCT	Eco147I (ER0421/2, p.68)	Eco1471, Pcel, Stul
Aatll	GACGT↓C	Aatll (ER0991/2, p.25)	(Zral)
Accl	GT↓MKAC	Xmil (ER1481/2, p.118)	Fbll, Xmil
AccII	CG↓CG	Bsh1236I (ER0921/2, p.95)	Bsh1236I, BstFNI, BstUI, MvnI
AccIII	T↓CCGGA	Kpn2I ^m (ER0531/2, p.80)	Aor13HI, BseAI ^m , Bsp13I, BspEI ^m , Kpn2I ^m , MroI ^m
Acc16I	TGC↓GCA	Nsbl (ER1221/2, p.91)	Avill, Fspl, Nsbl
Acc36I	ACCTGC(4/8)↓	Bvel (ER1741, p.54)	BfuAl, BspMl, Bvel
Acc65I	G↓GTACC	Acc65I (ER0901/2/4, p.25),	Asp718I, (KpnI ^m)
		(Kpnl ^m) (GGTAC↓C) (ER0521/2/3/4, p.80)
AccB1I	G↓GYRCC	BshNI (ER1001, p.46)	Banl, BshNI, BspT107I
AccB7I	CCANNNN↓NTGG	Van911 (ER0711/2, p.114)	PfIMI, Van91I
AccBSI	CCGCTC(-3/-3)↓	Mbil (ER1271, p.82)	BsrBl, Mbil
Acil	CCGC(-3/-1)↓	Ssil (ER1791, p.108)	Ssil
Acll	AA↓CGTT	Psp1406I (ER0941/2, p.98)	Psp1406l
AcIWI	GGATC(4/5)↓	BspPI (ER1321/2, p.50)	Alwl, <mark>BspPl</mark>
Acsl	R↓AATTY	Xapl (ER1381/2/4, p.115)	Apol, Xapl
Acul	CTGAAG(16/14)↓	Eco57I (ER0341/2, p.64)	Eco57I
Acyl	GR↓CGYC	Hin1I (ER0471/2, p.74)	BsaHI, BstACI, Hin1I, Hsp92I
Adel	CACNNN↓GTG	Adel (ER1231/2, p.26)	Drall
Afal	GT↓AC	(<mark>Csp6I</mark> ™) (G↓TAC) (ER0211, p.58),	(Csp6l ^m), Rsal
		Rsal (ER1121/2, p.101)	
Afel	AGC↓GCT	Eco47III (ER0321/2, p.63)	Aor51HI, Eco47III
AfIII	C↓TTAAG	BspTI (ER0831/2, p.51)	Bfrl, BspTl, Bst98l, Vha464l
AfIII	A↓CRYGT		
Agel	A↓CCGGT	BshTI (ER1461/2, p.46)	AsiGI, <mark>BshTI</mark> , PinAl
Ahdl	GACNNN↓NNGTC	Eam1105I (ER0241/2, p.60)	AspEI, Dril, Eam1105I, EcIHKI
Ahll	A↓CTAGT	Bcul (ER1251/2, p.33)	Bcul, Spel
Ajil	CACGTC(-3/-3)↓	Ajil (ER1941, p.26)	BmgBl, Btrl
Ajnl	↓CCWGG	EcoRII ^m (ER1921/2, p.70),	(BstNI), (BstOI), (Bst2UI), EcoRII ^m , (Mval), Psp6I, PspGI ^m
		(Mval) (CC↓WGG) (ER0551/2, p.87)	
Ajul	↓(7/12)GAANNNNNNNTTGG(11/6)↓	Ajul (ER1951, p.27)	
Alel	CACNN↓NNGTG	Olil (ER1631/2, p.91)	Olil
Alfl	↓(10/12)GCANNNNNNTGC(12/10)↓	Alfl (ER1801, p.27)	

Restriction Endonucleases Guide

Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

		Ta	ble 1.3. Alphabetic List of Commercially Available Restriction Endonuclease
Enzyme	Specificity	Fermentas	Commercially available isoschizomers
	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
Alol	(7/12-13)GAACNNNNNTCC(12-13/7)		
Alul	AGJCT	Alul (ER0011/2, p.27)	
Alwi	GGATC(4/5)↓	BspPI (ER1321/2, p.50)	AcIWI, BspPI
Alw21I	GWGCW C	Alw211 (ER0021/2, p.29)	AspHI, Bbv12I, BsiHKAI
Alw26I	GTCTC(1/5)	Alw261 (ER0031/2, p.29)	BsmAl ^m , BstMAI
Alw44I	G↓TGCAC	Alw44I (ER0041/2, p.30)	ApaLI ^m , Vnel
lwNI	CAGNNN	Cail (ER1391/2, p.54)	Cail
ma87I	C↓YCGRG	Eco88I (ER0381/4, p.66)	Aval, BsoBl, Eco88l
Nor13HI	T↓CCGGA	Kpn2l (ER0531/2, p.80)	AccIII, BseAI, Bsp13I, BspEI, Kpn2I, Mrol
Nor51HI	AGC↓GCT	Eco47III (ER0321/2, p.63)	Afel, Eco47III
lpal	GGGCC↓C	Apal (ER1411/2, p.30),	(Bsp120I), (PspOMI)
		(Bsp120I) (G↓GGCCC) (ER0131/2/3, p.4	
\paLI	G↓TGCAC	Alw44I ^m (ER0041/2, p.30)	Alw44I ^m , Vnel
vpeKl	G↓CWGC		Tsel
pol	R↓AATTY	Xapl (ER1381/2/4, p.115)	Acsl, Xapl
lscl	GG↓CGCGCC	Sgsl (ER1891/2, p.106)	PalAl, <mark>Sgsl</mark>
sel	AT↓TAAT	Vspl (ER0911/2, p.114)	PshBl, Vspl
AsiGI	A↓CCGGT	BshTI (ER1461/2, p.46)	Agel, BshTI, PinAl
\siSI	GCGAT↓CGC		Rgal, Sgfl
spl	GACNUNNGTC	Psyl (ER1331, p.100)	PfIFI, Psyl, Tth1111
sp700l	GAANN	Pdml (ER1531/2, p.94)	MroXI, PdmI, XmnI ^m
Asp718I	G↓GTACC	Acc65I (ER0901/2/4, p.25),	Acc65I, (KpnI ^m)
007101	0.00	(KpnI ^m) (GGTAC↓C) (ER0521/2/3/4, p.8	
AspA2I	C↓CTAGG	XmaJI (ER1561/2, p.117)	Avril, Bini, XmaJi
AspEl	GACNNNUNNGTC	Eam1105I (ER0241/2, p.60)	Ahdi, Dril, Eam1105I, EcIHKI
AspHI	GWGCW C	Alw211 (ER0021/2, p.29)	Alw21I, Bbv12I, BsiHKAI
AspLEI	GCG ¹ C	Hhal (ER1851, p.73),	BstHHI, Cfol, Hhal, (Hin6I), (HinP1I), (HspAI)
ASPLEI	GCG√C		DSINNI, CIUI, NIIAI, (NIIIOI), (NIIIPTI), (NSPAI)
Nem COI	C CNCC	(Hin6I) (G↓CGC) (ER0481/2, p.75)	05-121 00-0/1
AspS9I	G↓GNCC	Cfr13I (ER0191/2, p.56)	Cfr13I, Sau96I
AsuC2I	CC↓SGG	Bcnl (ER0061/2, p.33)	Bcnl, Ncil
AsuHPI	GGTGA(8/7)↓	Hphl (ER1101/2, p.78)	Hphl
AsuNHI	G↓CTAGC	Nhel (ER0971/2, p.89)	(Bmtl), Nhel
Aval	C↓YCGRG	Eco881 ^m (ER0381/4, p.66)	Ama87I, BsoBI ^m , <mark>Eco88I</mark> ^m
Avall	G↓GWCC	Eco47I (ER0311/2, p.63)	Bme18I, Eco47I, Sinl, VpaK11BI
Avill	TGC↓GCA	Nsbl (ER1221/2, p.91)	Acc16l, Fspl, Nsbl
Avrll	C↓CTAGG	XmaJI (ER1561/2, p.117)	AspA2I, BInI, XmaJI
Axyl	CC↓TNAGG	Eco81I (ER0371/2, p.66)	Bse21I, Bsu36I, Eco81I
Bael	↓(10/15)ACNNNNGTAYC(12/7)↓		
Ball	TGG↓CCA	MISI (ER1211/2, p.84)	MISI, MIuNI, MscI, Msp20I
BamHI	G↓GATCC	BamHI (ER0051/2/3/4, p.31)	
Banl	G↓GYRCC	BshNI (ER1001, p.46)	AccB1I, BshNI, BspT107I
Banll	GRGCY↓C	Eco24I (ER0281/4, p.61)	Eco24I, EcoT38I, FriOI
Banlli	AT↓CGAT	Bsu15I (ER0141/2, p.53)	Bsa29I, Bsp106I, BspDI, BspXI, Bsu15I, Clal
Baul	CACGAG(-5/-1)↓	Baul (ER1841, p.32)	BssSI, Bst2Bl
Bbel	GGCGC↓C	(Ehel) (GGC↓GCC) (ER0441, p.70)	(Egel), (Ehel), (Kasl), (Mly113l), (Narl ^m), (Sfol ^m)
BbrPl	CAC↓GTG	Eco72I (ER0361/2, p.65)	Eco72I, PmaCI, PmII, PspCI
Bbsl	GAAGAC(2/6)	Bpil (ER1011/2, p.38)	Bpil, BpuAl, BstV2l
Bbul	GCATG C	Pael (ER0601/2/4, p.92)	Pael, Sphl
Bbvl	GCAGC(8/12)	BseXI (ER1451/2, p.44)	BseXI, BstV1
	GWGCW↓C		
Bbv12I BbvCl		Alw211 (ER0021/2, p.29)	Alw21I, AspHI, BsiHKAI
	CCTCAGC(-5/-2)		
Bccl	CCATC(4/5)		
BceAl	ACGGC(12/14)		
Bcgl	↓(10/12)CGANNNNNTGC(12/10)↓		
BciVI	GTATCC(6/5)	Bful (ER1501/2, p.36)	Bful
Bell	T↓GATCA	Bcll (ER0721/2, p.32)	Fbal, Ksp22l
Bonl	CC↓SGG	Bcnl (ER0061/2, p.33)	AsuC2I, Ncil
Bcul	A↓CTAGT	Bcul (ER1251/2, p.33)	Ahll, Spel
3dal 🛛	↓(10/12)TGANNNNNNTCA(12/10)↓	Bdal (ER1961, p.34)	
Bfal	C↓TAG	FspBI (ER1761/2, p.72)	FspBI, Mael, XspI
Bfil	ACTGGG(5/4)↓	Bfil (ER1591/2, p.35)	Bmrl
3fml	C↓TRYAG	Bfml (ER1161/2, p.35)	BstSFI, Sfcl
	C↓TTAAG	BspTI (ER0831/2, p.51)	AfIII, BspTI, Bst98I, Vha464I
31/1			
	ATG↓CAT	(Mph1103I) (ATGCA↓T) (ER0731/2, p.8	5) (EC0122I), (Mph1103I), (NSII), (ZSD2I)
Bfrl BfrBl Bful	ATG↓CAT GTATCC(6/5)↓	(Mph1103I) (ATGCA↓T) (ER0731/2, p.8 Bful (ER1501/2, p.36)	5) (ECOT22I), (Mph1103I), (NSII), (ZSp2I) BciVI

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity	Fermentas	Commercially available isoschizomers
	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
BfuCl	↓GATC	Bsp143I (ER0781/2, p.48), (DpnI™) (GA↓TC) (ER1701/2, p.59), MboI™ (ER0811/2, p.83)	Bsp143I, (BstKTI™), BstMBI, (DpnI™), DpnII™, Kzo9I, (MalI™), MboI™, Ndell' Sau3AI
Bgll	GCCNNNNUNGGC	Bgll (ER0071/2, p.36)	
BgIII	A↓GATCT	BgIII (ER0081/2, p.37)	
Bisl	GC↓NGC	Satl ^m (ER1641/2, p.103)	Fnu4HI ^m , Fsp4HI ^m , ItaI ^m , <mark>SatI</mark> ^m
Blnl	C↓CTAGG	XmaJI (ER1561/2, p.117)	AspA2I, AvrII, XmaJI
Blpl	GC↓TNAGC	Bpu1102I (ER0091/2, p.40)	Bpu1102I, Bsp1720I, Celll
Bme18I	G↓GWCC	Eco47I (ER0311/2, p.63)	Avall, Eco47I, Sinl, VpaK11BI
Bme13901	CC↓NGG	Bme1390I (ER1421/2, p.37)	(BssKI), (BstSCI), MspR9I, ScrFI, (StyD4I)
Bme1580I	GKGCM↓C	BseSI (ER1441/2, p.44)	BseSI
BmgBl	CACGTC(-3/-3)↓	Ajil (ER1941, p.26)	Ajil, Btrl
Bmrl	ACTGGG(5/4)↓	Bfil (ER1591/2, p.35)	Bfil
Bmtl	GCTAG	(Nhel ™) (G↓CTAGC) (ER0971/2, p.89)	(AsuNHI), (NheI ^m)
Bmyl	GDGCH↓C	Sdul (ER0651, p.105)	Bsp1286I, Mhll, Sdul
Boxl	GACNNUNNGTC	Boxl (ER1431, p.38)	BstPAI, PshAI
Bpil	GAAGAC(2/6)↓	Bpil (ER1011/2, p.38)	Bbsl, BpuAl, BstV2l
Bpll	↓(8/13)GAGNNNNNCTC(13/8)↓	Bpll (ER1311/2, p.39)	
Bpml	CTGGAG(16/14)↓	Gsul ^m (ER0461/2, p.73)	Gsul ^m
Bpu10I	CCTNAGC(-5/-2)↓	Bpu10I (ER1181/2, p.39)	
Bpu14l	TTUCGAA	Bsp119I (ER0121, p.47)	Bsp119I, BspT104I, BstBI, Csp45I, NspV, Sful
Bpu1102I	GC↓TNAGC	Bpu1102I (ER0091/2, p.40)	Blpl, Bsp1720l, Celll
BpuAl	GAAGAC(2/6)↓	Bpil (ER1011/2, p.38)	Bbsl, <mark>Bpil</mark> , BstV2I
BpuEl	CTTGAG(16/14)	F = 241 (ED0201/2 = (2)	D211 E241
Bsal	GGTCTC(1/5)↓	Eco31I (ER0291/2, p.62)	Bso31I, Eco31I
Bsa29I		Bsu15I (ER0141/2, p.53)	Banlli, Bsp106i, BspDi, BspXi, Bsu15i, Clai
BsaAl	YAC↓GTR GATNN↓NNATC	Ppu211 (ER1971, p.97)	BstBAI, Ppu211 Bse8I, BseJI, Maml
BsaBl BsaHl	GATNIN VINNATC GRUCGYC	BseJI (ER1711, p.41) Hin1I ^m (ER0471/2, p.74)	Acyl, BstACI, Hin1I ^m , Hsp92I
BsaJI	C↓CNNGG	BseDI (ER1081/2, p.40)	BseDI, BssECI
BsaMI	GAATGC(1/-1)↓	Mva1269I (ER0961/2, p.40)	Bsml, Mva1269I, Pctl
BsaWl	WVCCGGW	WVa 12091 (ER090 1/2, p.86)	DSIII, WV8 (207), I CU
BsaXI	↓(9/12)ACNNNNNCTCC(10/7)↓		
Bsc4l	CCNNNN NGG	BseLI (ER1201/2, p.42)	BseLI, BsiYI, Bsll
Bse1I	ACTGG(1/-1)↓	BseNI (ER0881/2, p.43)	BseNI, BsrI, BsrSI
Bse8l	GATNN VNATC	BseJI (ER1711, p.41)	BsaBl, BseJl, Maml
Bse211	CC TNAGG	Eco811 (ER0371/2, p.66)	Axyl, Bsu36l, Eco81l
Bse118I	R↓CCGGY	Cfr10I (ER0181/2, p.56)	BsrFI, Cfr10I
BseAl	T↓CCGGA	Kpn2I ^m (ER0531/2, p.80)	AccIII ^m , Aor13HI, Bsp13I, BspEI, Kpn2I ^m , MroI ^m
BseDI	C↓CNNGG	BseDI (ER1081/2, p.40)	BsaJI, BssECI
Bse3DI	GCAATG(2/0)↓	BseMI (ER1261/2, p.42)	BseMI, BsrDI
BseGI	GGATG(2/0)↓	BseGI (ER0871/2, p.41)	BstF5I, (Fokl)
BseJI	GATNNUNNATC	BseJI (ER1711, p.41)	BsaBl, Bse8l, Maml
BseLl	CCNNNNN↓NNGG	BseLI (ER1201/2, p.42)	Bsc4I, BsiYI, BsII
BseMI	GCAATG(2/0)↓	BseMI (ER1261/2, p.42)	Bse3DI, BsrDI
BseMII	CTCAG(10/8)↓	BseMII (ER1401/2, p.43)	(BspCNI)
BseNI	ACTGG(1/-1)↓	BseNI (ER0881/2, p.43)	Bse1I, BsrI, BsrSI
BsePl	G↓CGCGC	Paul (ER1091/2, p.93)	BssHll, Paul
BseRI	GAGGAG(10/8)↓		
BseSI	GKGCM↓C	BseSI (ER1441/2, p.44)	Bme1580I
BseXI	GCAGC(8/12)↓	BseXI (ER1451/2, p.52)	Bbvl, BstV1I
BseX3I	C↓GGCCG	Eco52I (ER0331/2, p.64)	BstZI, Eagl, EclXI, Eco52I
BseYI	CCCAGC(-5/-1)↓		
Bsgl	GTGCAG(16/14)↓		
Bsh1236l	CG↓CG	Bsh1236l (ER0921/2, p.45)	AccII, BstFNI, BstUI, MvnI
Bsh12851	CGRY↓CG	Bsh1285I (ER0891, p.45)	BsiEl, BstMCl
BshNI	G↓GYRCC	BshNI (ER1001, p.46)	AccB1I, Banl, BspT107I
BshTl	AUCCGGT	BshTI (ER1461/2, p.46)	Agel, AsiGI, PinAl
BsiEl	CGRY↓CG	Bsh1285I (ER0891, p.45)	Bsh1285I, BstMCI
BsiHKAI	GWGCW↓C	Alw211 (ER0021/2, p.29)	Alw21I, AspHI, Bbv12I
B. 0.1."	C↓GTACG	Pfl23II (ER0851, p.95)	Pfl23II, PspLI
BsiYl	CCNNNNNUNNGG	BseLI (ER1201/2, p.42)	Bsc4I, BseLI, BsII
BsiYI Bsll	CCNNNNN↓NNGG CCNNNNN↓NNGG	BseLI (ER1201/2, p.42)	Bsc4I, <mark>BseLI</mark> , BsiYI
BsiYI BsII BsIFI	CCNNNNN↓NNGG CCNNNNN↓NNGG GGGAC(10/14)↓	BseLI (ER1201/2, p.42) FaqI (ER1811, p.71)	Bsc4I, <mark>BseLI</mark> , BsiYI BsmFI, <mark>FaqI</mark>
BsiWI BsiYI BsII BsIFI Bsml BsmAI	CCNNNNN↓NNGG CCNNNNN↓NNGG	BseLI (ER1201/2, p.42)	Bsc4I, <mark>BseLI</mark> , BsiYI



Restriction Endonucleases Guide

Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

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nzyme	Specificity	Fermentas	Commercially available isoschizomers
Icm ^{DI}	$5' \rightarrow 3'$	enzyme (cat. #, page #)	Ecn21
smBl	CGTCTC(1/5)↓		Esp3I
smFl	GGGAC(10/14)↓	Faql (ER1811, p.71)	BsIFI, FaqI
so31I	GGTCTC(1/5)↓	Eco31I (ER0291/2, p.62)	Bsal, Eco31I
soBl	C↓YCGRG		Ama87I, Aval ^m , <mark>Eco88I</mark> ^m
sp13l	T↓CCGGA		AccIII, Aor13HI, BseAI, BspEI, Kpn2I, Mrol
sp19l	C↓CATGG	Ncol (ER0571/2, p.88)	Ncol
sp68l	TCG↓CGA	Bsp68I (ER0111/2, p.47)	Nrul
lsp106l	AT↓CGAT	Bsu15I (ER0141/2, p.53)	BanIII, Bsa29I, BspDI, BspXI, Bsu15I, Clal
sp1191	TT↓CGAA	Bsp119I (ER0121, p.47)	Bpu14I, BspT104I, BstBI, Csp45I, NspV, Sfulm
sp1201	G↓GGCCC	(Apal) (GGGCC↓C) (ER1411/2, p.30), Bsp120I (ER0131/2/3, p.48)	(Apal), PspOMI
3sp143I	↓GATC	Bsp143I (ER0781/2, p.48), (DpnI™) (GA↓TC) (ER1701/2, p.59), MboI™ (ER0811/2, p.83)	BfuCI, (BstKTI ^m), BstMBI, (DpnI^m), DpnII ^m , Kzo9I, (MaII ^m), MboI^m , NdeII ^m , Sau3AI
3sp143ll	RGCGC↓Y	Bsp143II (ER0791/2, p.49)	BstH2I, HaelI ^m
sp12861	GDGCH↓C	Sdul (ER0651, p.105)	Bmyl, Mhll, Sdul
sp14071	T↓GTACA	Bsp1407I (ER0931/2, p.49)	BsrGI, BstAUI, SspBI
sp17201	GC↓TNAGC	Bpu1102I (ER0091/2, p.40)	Blpl, Bpu1102I, Celli
SpCI 201	CGAT↓CG	Pvul (ER0621/2, p.100)	Ple19I, Pvul
SpCNI	CTCAG(9/7)↓	(BseMII) (CTCAG(10/8)) (ER1401/2, p.43)	
IspDI	ATUCGAT	Bsu15I (ER0141/2, p.53)	Banlli, Bsa29i, Bsp106i, BspXi, Bsu15i, Clai
	T↓CCGGA		AccIII, Aor13HI, BseAI, Bsp13I, Kpn2I ^m , Mrol ^m
IspEl			
IspHI			Pagl ^m , Rcal
SspLI	GGN↓NCC		NIalV ^m , PspN4I
spLU111	A↓CATGT		Pcil, Pscl
spMI	ACCTGC(4/8)↓		Acc36I, BfuAI, Bvel
3spPI	GGATC(4/5)↓	BspPI (ER1321/2, p.50)	AcIWI, Alwi
IspTI	C↓TTAAG	BspTI (ER0831/2, p.51)	AfIII, Bfrl, Bst98I, Vha464I
spT104I	TT↓CGAA	Bsp119I (ER0121, p.47)	Bpu14I, Bsp119I , BstBI, Csp45I, NspV, Sful [™]
spT107I	G↓GYRCC		AccB1I, Banl, BshNI
SpXI	AT↓CGAT		Banlll, Bsa29I, Bsp106I, BspDI, Bsu15I, Clal
Bsrl	ACTGG(1/-1)↓	BseNI (ER0881/2, p.43)	Bse1I, BseNI, BsrSI
BsrBl	CCGCTC(-3/-3)↓		AccBSI, Mbil ^m
BsrDI	GCAATG(2/0)↓	BseMI (ER1261/2, p.42)	Bse3DI, BseMI
BsrFI	R↓CCGGY	Cfr10I (ER0181/2, p.56)	Bse118I, Cfr10I
BsrGl	T↓GTACA	Bsp1407I (ER0931/2, p.49)	Bsp1407I, BstAUI, SspBI
BsrSl	ACTGG(1/-1)↓		Bse1I, BseNI, Bsrl
BssECI	C↓CNNGG		BsaJI, BseDI
BssHII	GUCGCGC		BsePI, Paul
BssKl	↓CCNGG	(Bme1390I) (CC↓NGG) (ER1421/2, p.37)	(Bme1390I), BstSCI, (MspR9I), (ScrFI), StyD4I
BssNAI	GTA↓TAC	Bst1107I (ER0701/2, p.52)	Bst1107I, BstZ17I
IssSI	CACGAG(-5/-1)↓		Baul, Bst2Bl
IssT11	C↓CWWGG		Eco130I, EcoT14I, Erhl, Styl
lst6l	CTCTTC(1/4)↓		Eam1104I, Earl, Ksp632I
Ist98I	C↓TTAAG		Afili, Bfri, BspTi, Vha464i
8st1107l	GTAJTAC	Bst1107I (ER0701/2, p.52)	BssNAI, BstZ17I
BstACI	GRUCGYC		Acyl, BsaHl, Hin1l, Hsp92l
	GCANNNNVNTGC	ιι (εκυ4/1/2, μ./4)	noyi, usulii, iiiiii, iispizi
BstAPI			Don1407I DerCl SenDI
IstAUI	TJGTACA	Bsp1407I (ER0931/2, p.49)	Bsp1407I, BsrGI, SspBI
IstBl	TTUCGAA	Bsp119I (ER0121, p.47)	Bpu14I, Bsp119I, BspT104I, Csp45I, NspV, Sful ^m
lst2Bl	CACGAG(-5/-1)↓	Baul (ER1841, p.32)	Baul, BssSI
IstBAI	YAC↓GTR	Ppu21I (ER1971, p.97)	BsaAI, Ppu21I
lst4Cl	ACNJGT	Taal (ER1361/2, p.109)	HpyCH4III, Taal
IstC8I	GCN↓NGC		Cac8I
stDEI	C↓TNAG	HpyF3I (ER1881/2, p.79)	Ddel, HpyF3I
stDSI	C↓CRYGG		Btgl
stEll	G↓GTNACC	Eco911 (ER0391/2, p.67)	BstPI, Eco91I, Eco065I, PspEl
stENI	CCTNNUNNAGG		EcoNI, Xaql
IstF5I	GGATG(2/0)↓	BseGI (ER0871/2, p.41)	BseGI, (Fokl)
IstFNI	CGUCG		Accli, Bsh1236I, BstUI, MvnI
	RGCGC↓Y		
IstH2I		Bsp143II (ER0791/2, p.48)	Bsp143II, Haell
BstHHI	GCG↓C	(Hin6I) (G↓CGC) (ER0481/2, p.75)	AspLEI, Cfol, Hhal, (Hinól), (HinP1l), (HspAl)
BstKTI	GAT↓C		(BfuCl ^m), (Bsp143I ^m), (BstMBI), (DpnI ^m), (DpnII), (Kzo9I ^m), (MalI ^m), (Mbol (Ndell), (Sau3AI ^m)

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity	Fermentas	le 1.3. Alphabetic List of Commercially Available Restriction Endonucleases Commercially available isoschizomers
Liizyiile	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
BstMAI	GTCTC(1/5)↓	Alw261 (ER0031/2, p.29)	Alw26I, BsmAl
BstMBI	↓GATC	Bsp143I (ER0781/2, p.48),	BfuCl, Bsp143I, (BstKTI), (DpnI ^m), DpnII, Kzo9I, (Mall), Mbol, Ndell,
Dottribl	V OI TI O	(DpnI ^m) (GA↓TC) (ER1701/2, p.59),	Sau3Al
		Mbol (ER0811/2, p.83)	300371
BstMCI	CGRY↓CG	Bsh1285I (ER0891, p.45)	Bsh1285I, BsiEl
BstMWI		HpyF10VI (ER1731/2, p.79)	HpyF10VI, Mwol
BstNI	CC↓WGG		(Ajnl), BstOI, Bst2UI, (EcoRII ^m), Mval, (Psp6l), (PspGI ^m)
DHNCI		Mval (ER0551/2, p.87)	March March
BstNSI	RCATG↓Y	Xcel (ER1471/2, p.116)	
BstOl	CC↓WGG		(Ajnl), BstNI, Bst2UI, (EcoRII ^m), Mval, (Psp6l), (PspGI ^m)
		Mval (ER0551/2, p.87)	
BstPl	GJGTNACC	Eco91I (ER0391/2, p.67)	BstEll, Eco91I, EcoO65I, PspEl
BstPAI	GACNNUNNGTC	Boxl (ER1431, p.38)	Boxl, PshAl
BstSCI	↓CCNGG		(Bme1390I), BssKI, (MspR9I), (ScrFI), StyD4I
BstSFI	C↓TRYAG	Bfml (ER1161/2, p.35)	Bfml, Sfcl
BstSNI	TAC↓GTA	Eco105I (ER0401/2, p.67)	Eco105I, SnaBl
BstUI	CG↓CG	Bsh1236I (ER0921/2, p.45)	AccII, Bsh1236I, BstFNI, MvnI
Bst2UI	CC↓WGG	(EcoRII ^m) (↓CCWGG) (ER1921/2, p.70),	(Ajnl), BstNl, BstOl, (EcoRII ^m), Mval, (Psp6l), (PspGI ^m)
		Mval (ER0551/2, p.87)	
BstV1I	GCAGC(8/12)↓	BseXI (ER1451/2, p.44)	Bbvl, BseXI
BstV2I	GAAGAC(2/6)↓	Bpil (ER1011/2, p.38)	Bbsl, Bpil, BpuAl
BstXI	CCANNNNN	BstXI (ER1021/2, p.44)	
BstX2I	R↓GATCY	Psul (ER1551, p.99)	BstYI, MfII, Psul, Xholl
BstYI	R↓GATCY	Psul (ER1551, p.99)	BstX2I, MfII ^m , Psul, Xholl
BstZl	CUGGCCG	Eco52I (ER0331/2, p.64)	BseX3I, Eagl, EcIXI, Eco52I
BstZ17I	GTAJTAC	Bst1107I (ER0701/2, p.52)	BssNAI, Bst1107I
	AT↓CGAT	· · · · · · · ·	
Bsu15I		Bsu15i (ER0141/2, p.53)	Banlli, Bsa29i, Bsp106i, BspDi, BspXi, Clai
Bsu36l		Eco81I (ER0371/2, p.66)	Axyl, Bse21l, Eco81l
BsuRI	GG↓CC	BsuRI (ER0151/2, p.53)	Haelli, Pali, Phol
Btgl	C↓CRYGG		BstDSI
BtgZl	GCGATG(10/14)↓		
Btrl	CACGTC(-3/-3)↓	Ajil (ER1941, p.26)	Ajil, BmgBl
Btsl	GCAGTG(2/0)↓		
Bvel	ACCTGC(4/8)↓	Bvel (ER1741, p.54)	Acc36I, BfuAI, BspMI
Cac8l	GCN NGC		BstC8I
Cail	CAGNNN↓CTG	Cail (ER1391/2, p.54)	AlwNI
CciNI	GC↓GGCCGC	Notl (ER0591/2/3, p.90)	Noti
Celll	GC↓TNAGC	Bpu1102I (ER0091/2, p.40)	Blpl, Bpu1102I , Bsp1720I
Cfol	GCG↓C	Hhal (ER1851, p.73),	AspLEI, BstHHI, Hhal, (Hin6I), (HinP1I), (HspAI)
		(Hin6I) (G↓CGC) (ER0481/2, p.75)	
Cfrl	Y↓GGCCR	Cfrl (ER0161/2, p.55)	Eael
Cfr9I	C↓CCGGG	Cfr9I (ER0171/2/4, p.55),	PspAI, (Smal ^m), XmaI, XmaCl
	0,00000	(Smal ^m) (CCC↓GGG) (ER0661/2/3/4, p.106	
Cfr10I	R↓CCGGY	Cfr10I (ER0181/2, p.56)	9 Bse118I, BsrFI
	GUGNCC	Cfr13I (ER0191/2, p.56)	AspS9I, Sau96I
Cfr13I Cfr42I	CCGC↓GG	Cfr42I (ER0201/2, p.57)	Kspl, Sacli, Sfr303l
		· · · · · ·	
Clal	AT CGAT	Bsu15I (ER0141/2, p.53)	BanIII, Bsa29I, Bsp106I, BspDI, BspXI, Bsu15I
Cpol	CG↓GWCCG	Cpol (ER0741/2, p.57)	Cspl, Rsrll, Rsr2l
Csel	GACGC(5/10)	Csel (ER1901/2, p.58)	Hgal
Cspl	CGUGWCCG	Cpol (ER0741/2, p.57)	Cpol, RsrII, Rsr2I
Csp6l	G↓TAC	Csp6I (ER0211, p.58),	(Afal ^m), (<mark>Rsal^m)</mark>
		(Rsal [™]) (GT↓AC) (ER1121/2, p.101)	
Csp45I	TT↓CGAA	Bsp119I (ER0121, p.47)	Bpu14I, Bsp119I, BspT104I, BstBI, NspV, Sful
CspCl	↓(11/13)CAANNNNNGTGG(12/10)↓		
CviAll	C↓ATG	(Hin1II) (CATG↓) (ER1831, p.74)	(Fatl), (Hin1II), (Hsp92II), (NIaIII ^m)
CviJI	RG↓CY		CviTI
Ddel	C↓TNAG	HpyF3I (ER1881/2, p.79)	BstDEI, HpyF3I
Dpnl	GAUTC	(Bsp143I ^m) (↓GATC) (ER0781/2, p.48),	(BfuCl ^m), (Bsp1431 ^m), (BstKTl ^m), (BstMBl ^m), (DpnII ^m), (Kzo91 ^m), Mall, (Mbol ^m)
- P		Dpnl (ER1701/2, p.59),	(Ndell ^m), (Sau3Al ^m)
		(Mbol ^m) (↓GATC) (ER0811/2, p.83)	
Dpnll	↓GATC	Bsp143I ^m (ER0781/2, p.48),	BfuCl ^m , Bsp143I ^m , (BstKTI), BstMBI, (DpnI ^m), Kzo9I ^m , (Mall), Mbol,
		$(DpnI^{m})$ (GA \downarrow TC) (ER1701/2, p.59),	Ndell, Sau3Al ^m
Dpilli			NUCH, JAUJAI'''
opini			
		Mbol (ER0811/2, p.83)	
Dral		Mbol (ER0811/2, p.83) Dral (ER0221/2/3, p.59)	F0400
	TTT↓AAA RG↓GNCCY CACNNN↓GTG	Mbol (ER0811/2, p.83)	Eco0109I Adel



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 Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity	Fermentas	Commercially available isoschizomers
Enzyme	$5' \rightarrow 3'$	enzyme (cat. #, page #)	commercially available isoschizomers
rdl	GACNNNN UNNGTC		
ril	GACNNNVNNGTC	Aasl (ER1721/2, p.24)	Aasl, DseDI Ahdl, AspEl, Eam1105I, EcIHKI
		Eam1105I (ER0241/2, p.60)	
seDI		Aasl (ER1721/2, p.24)	Aasi, Drdi
iel	Y↓GGCCR	Cfrl (ER0161/2, p.55)	Cfrl
ıgl	C↓GGCCG	Eco52I (ER0331/2, p.64)	BseX3I, BstZI, EclXI, Eco52I
m1104I	CTCTTC(1/4)↓	Eam1104I (ER0231/2, p.60)	Bst6l, Earl, Ksp632l
am11051	GACNNNUNNGTC	Eam1105I (ER0241/2, p.60)	Ahdl, AspEl, Dril, EcIHKI
arl	CTCTTC(1/4)↓	Eam1104I (ER0231/2, p.60)	Bst6l, Eam1104I, Ksp632I
cil	GGCGGA(11/9)↓		
: 136 	GAG↓CTC	Ecl136II (ER0251/4, p.61),	EcolCRI, (Psp124BI), (Sacl ^m)
		(Sacl [™]) (GAGCT↓C) (ER1131/2/3, p.102)	
:IHKI	GACNNN↓NNGTC	Eam1105I (ER0241/2, p.60)	Ahdl, AspEl, Dril, Eam11051
IXI	C↓GGCCG	Eco52I (ER0331/2, p.64)	BseX3I, BstZI, Eagl, Eco52I
co24l	GRGCY↓C	Eco24I (ER0281/4, p.61)	Banll, EcoT38I, FriOl
co31I	GGTCTC(1/5)↓	Eco31I (ER0291/2, p.62)	Bsal, Bso311
co32l	GAT	Eco32I (ER0301/2/3, p.62)	EcoRV
:0471	GUGWCC	Eco47I (ER0311/2, p.63)	Avall, Bme18I, Sinl, VpaK11BI
:047111	AGC↓GCT	Eco47III (ER0321/2, p.63)	Afel, Aor51HI
:047111 :0521	CUGGCCG	Eco52I (ERO331/2, p.64)	BseX3I, BstZI, Eagl, EclXI
			0
co57l	CTGAAG(16/14)↓ CAC↓GTG	Eco57I (ER0341/2, p.64)	Acul PhrDL DmacL DmlL DcnCL
co72l		Eco72I (ER0361/2, p.65)	BbrPI, PmaCI, PmII, PspCI
co81I	CC↓TNAGG	Eco811 (ER0371/2, p.66)	Axyl, Bse21I, Bsu36I
co88I	C↓YCGRG	Eco88I (ER0381/4, p.66)	Ama87I, Aval ^m , BsoBI ^m
co91I	GUGTNACC	Eco91I (ER0391/2, p.67)	BstEll, BstPl, EcoO65l, PspEl
co105l	TAC↓GTA	Eco105I (ER0401/2, p.67)	BstSNI, SnaBl
co130I	C↓CWWGG	Eco130I (ER0411, p.68)	BssT1I, EcoT14I, Erhl, Styl
co147l	AGG↓CCT	Eco147I (ER0421/2, p.68)	Aatl, Pcel, Stul
colCRI	GAG↓CTC	Ecl136II (ER0251/4, p.61),	Ecl136II, (Psp124BI), (SacI ^m)
		(Sacl ^m) (GAGCT↓C) (ER1131/2/3/4, p.102)	
co57MI	CTGRAG(16/14)↓	Eco57MI (ER1671, p.65)	
coNI	CCTNN VNNAGG	Xagl (ER1301/2, p.115)	BstENI, Xaql
200651	G↓GTNACC	Eco91I (ER0391/2, p.67)	BstEll, BstPl, Eco91I, PspEl
coO1091	RGJGNCCY		Drall
		Eco0109I (ER0261, p.69)	Diali
coP15I	CAGCAG(25/27)↓		
coRI	G AATTC	EcoRI (ER0271/2/3/4, p.69)	
coRII	↓CCWGG	EcoRII (ER1921/2, p.70),	AjnI ^m , (BstNI ^m), (BstOI ^m), (Bst2UI ^m), (MvaI ^m), Psp6I, PspGI
		(Mval ™) (CC↓WGG) (ER0551/2, p.87)	
coRV	GAT↓ATC	Eco32I (ER0301/2/3, p.62)	Eco32I
coT14I	C↓CWWGG	Eco130I (ER0411, p.68)	BssT1I, Eco130I, Erhl, Styl
coT22I	ATGCA↓T	Mph1103I (ER0731/2, p.85)	(BfrBl), Mph1103I, Nsil ^m , Zsp2I
coT38I	GRGCY↓C	Eco24I (ERO281/4, p.61)	Banll, Eco24I, FriOl
gel	GGC↓GCC	Ehel (ER0441, p.70)	(Bbel), Ehel, (Kasl), (Mly113l), (Narl), Sfol
hel	GGC↓GCC	Ehel (ER0441, p.70)	(Bbel), Egel, (Kasl ^m), (Mly113l), (Narl), Sfol ^m
hl	C↓CWWGG	Eco130I (ERO411, p.68)	BssT1I, Eco130I, EcoT14I, Styl
sp3l	CGTCTC(1/5)↓	Esp3I (ER0451/2, p.71)	BsmBl
all	↓(8/13)AAGNNNNNCTT(13/8)↓		Dombi
aql	GGGAC(10/14)↓	Faql (ER1811, p.71)	BsIFI, BsmFI
iqi itl			
	↓CATG	(Hin1II) (CATG↓) (ER1831, p.74)	(CviAll ^m), (Hin1II), (Hsp92II), (Nlall ^m)
aul		Smul (ER1691/2, p.108)	Smul
IUNDI	CAVTATG	Ndel (ER0581/2, p.89)	Ndel
bal	T↓GATCA	Bcll (ER0721/2, p.32)	Bcll, Ksp22l
oll	GTUMKAC	Xmil (ER1481/2, p.118)	Accl, Xmil
nu4HI	GC↓NGC	Satl (ER1641/2, p.103)	Bisl ^m , Fsp4HI, Ital ^m , <mark>Satl</mark>
okl	GGATG(9/13)↓	(BseGI) (GGATG(2/0)) (ER0871/2, p.41)	(BseGI), (BstF5I)
iOI	GRGCY↓C	Eco24I (ER0281/4, p.61)	Banll, Eco24I, EcoT38I
sel	GGCCGG↓CC	• •	
spl	TGC↓GCA	Nsbl (ER1221/2, p.91)	Acc16I, Avill, Nsbl
spAl	RTGC	FspAI (ER1661/2, p.72)	
spBl	C↓TAG	FspBI (ER1761/2, p.72)	Bfal, Mael, Xspl
	GCUNGC	Satl (ER1641/2, p.103)	Bisl [™] , Fnu4HI, Ital, Satl
sp4HI			
sul	CTGGAG(16/14)↓	Gsul (ER0461/2, p.73)	Bpml ^m
aell	RGCGCVY	Bsp143II ^m (ER0791/2, p.49)	Bsp143II ^m , BstH2I
		BsuRI (ER0151/2, p.54)	BsuRI, Pall, Phol
	GG↓CC		
	C↓CGG	Hpall (ER0511/2, p.77),	Hpall, Mspl ^m
aelli apli gal			

(continued on next page)



Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity	Fermentas	Ie 1.3. Alphabetic List of Commercially Available Restriction Endonucleases Commercially available isoschizomers
Elizyine	$5' \rightarrow 3'$		
		enzyme (cat. #, page #)	
Hhal	GCG↓C	Hhal (ER1851, p.73),	AspLEI, BstHHI, CfoI, (<mark>Hin6I</mark>), (HinP1I), (HspAI)
		(Hin6I) (G↓CGC) (ER0481/2, p.75)	
Hin1l	GR↓CGYC	Hin1I (ER0471/2, p.74)	Acyl, BsaHI ^m , BstACl, Hsp92l
Hin1II	CATG↓	Hin1II (ER1831, p.74)	(CviAII), (FatI), Hsp92II, NIaIII
Hin4I	↓(8/13)GAYNNNNNVTC(13/8)↓	Hin4I (ER1601/2, p.75)	
Hin6I	GUCGC	(Hhal) (GCG↓C) (ER1851, p.73),	(AspLEI), (BstHHI), (Cfol), (Hhal), HinP1I, HspAI
		Hin6I (ER0481/2, p.75)	$\chi + \Gamma = \rho \chi + \rho \chi + \rho \chi + \rho \chi + \rho \chi$
HinP1I	G↓CGC	(Hhal) (GCG↓C) (ER1851, p.73),	(AspLEI), (BstHHI), (CfoI), (Hhal), Hin6I, HspAI
	04000	Hin6I (ER0481/2, p.75)	(AspEEI), (Dstittil), (Cloi), (tittal), tittor, tispAi
112			18
Hincl	GTY RAC	Hincll (ER0491/2/4, p.76)	Hindll
Hindll	GTY	Hincll (ER0491/2/4, p.76)	Hincl
HindIII	AJAGCTT	HindIII (ER0501/2/3/4, p.76)	
Hinfl	G↓ANTC	Hinfl (ER0801/2/3, p.77)	
Hpal	GTT↓AAC	KspAI (ER1031/2, p.81)	KspAI
Hpall	C↓CGG	Hpall (ER0511/2, p.77),	Hapll, Mspl ^m
		Mspl ^m (ER0541/2, p.86)	
Hphl	GGTGA(8/7)↓	Hphi (ER1101/2, p.78)	AsuHPI
	GTN V NAC	Hpy8I (ER1571/2, p.78)	
Hpy8I		hpyoi (EK157172, p.76)	
Нру991			
Hpy188I	TCNJGA		
Hpy188III	TCUNNGA		
HpyCH4III	ACN GT	Taal (ER1361/2, p.109)	Bst4Cl, Taal
HpyCH4IV	A↓CGT	(Tail ^m) (ACGT↓) (ER1141/2, p.110)	Maell, (<mark>Tail</mark> ^m), (Tscl)
HpyCH4V	TG↓CA		
HpyF3I	C↓TNAG	HpyF3I (ER1881/2, p.79)	BstDEI, Ddel
HpyF10VI	GCNNNNNUNGC		BstMWI, Mwol
		HpyF10VI (ER1731/2, p.79)	
Hsp92I	GR↓CGYC	Hin1I (ER0471/2, p.74)	Acyl, BsaHl, BstACl, Hin1l
Hsp92II	CATG	Hin1II (ER1831, p.74)	(CviAll), (Fatl), Hin1II, NlallI
HspAl	G↓CGC	(Hhal) (GCG↓C) (ER1851, p.73),	(AspLEI), (BstHHI), (CfoI), (Hhal), Hin6I, HinP1I
		Hin6I (ER0481/2, p.75)	
Ital	GC↓NGC	Satl (ER1641/2, p.103)	Bisl ^m , Fnu4Hl ^m , Fsp4Hl ^m , <mark>Satl</mark>
Kasl	GJGCGCC	(Ehel ^m) (GGC↓GCC) (ER0441, p.70)	(Bbel), (Egel), (Ehel ^m), (Mly113I), (Narl ^m), (Sfol ^m)
Kpnl	GGTAC↓C	(Acc65I ^m) (G↓GTACC) (ER0901/2/4, p.25),	
крп	GGTAC↓C		(ACCODE), (ASP/TOE)
1/01	T 00000	Kpnl (ER0521/2/3/4, p.80)	
Kpn2l	T CCGGA	Kpn2l (ER0531/2, p.80)	AccIII ^m , Aor13HI, BseAI ^m , Bsp13I, BspEI ^m , Mrol
Kspl	cccc↓gg	Cfr42I (ER0201/2, p.57)	Cfr42I, SacII, Sfr303I
Ksp22I	T↓GATCA	Bcll (ER0721/2, p.32)	Bcll, Fbal
Ksp632I	CTCTTC(1/4)	Eam1104I (ER0231/2, p.60)	Bst6l, Eam1104I, Earl
KspAl	GTT↓AAC	KspAI (ER1031/2, p.81)	Hpal
Kzo9l	↓GATC	Bsp143I (ER0781/2, p.48),	BfuCI, Bsp143I, (BstKTI ^m), BstMBI, (DpnI ^m), DpnII ^m , (MaII ^m), MboI ^m , NdeII ^m
		(DpnI ^m) (GA↓TC) (ER1701/2, p.59),	Sau3Al
		Mbol [™] (ER0811/2, p.83)	
Laul	GCTCTTC(1/4)↓	Lgul (ER1931/2, p.81)	Conl
Lgul			Sapl
Lwel	GCATC(5/9)↓	Lwel (ER1621/2, p.82)	SfaNI
Mabl	AJCCWGGT		SexAI ^m
Mael	C↓TAG	FspBI (ER1761/2, p.72)	Bfal, FspBI, Xspl
Maell	A↓CGT	(Tail) (ACGT↓) (ER1141/2, p.110)	HpyCH4IV, (Tail), (Tscl)
Maelll	↓GTNAC		
Mall	GA↓TC	(Bsp143I ^m) (↓GATC) (ER0781/2, p.48),	(BfuCI ^m), (Bsp143I ^m), (BstKTI ^m), (BstMBI ^m), DpnI , (DpnII ^m), (Kzo9I ^m),
widii	0,10	Dpnl (ER1701/2, p.59),	(Mbol ^m), (Ndell ^m), (Sau3Al ^m)
		(Mbol ^m) (↓GATC) (ER0811/2, p.83)	
Maml	GATNN	BseJI (ER1711, p.41)	BsaBl, Bse8l, BseJl
Mbil	CCGCTC(-3/-3)↓	Mbil (ER1271, p.82)	AccBSI, BsrBI ^m
Mbol	↓GATC	Bsp143I ^m (ER0781/2, p.48),	BfuCl ^m , Bsp1431 ^m , (BstKTI), BstMBI, (DpnI ^m), DpnII, Kzo91 ^m , (MalI ^m),
		(DpnI ^m) (GA↓TC) (ER1701/2, p.59),	Ndell, Sau3Alm
		Mbol (ER0811/2, p.83)	
Mboll	GAAGA(8/7)↓	Mboll (ER0821/2, p.83)	
Mfel	C↓AATTG	Muni (ER0751/2, p.87)	Munl
Mfll	R↓GATCY	Psul [™] (ER1551, p.99)	BstX2I, BstYI ^m , PsuI ^m , Xholl ^m
171111			
Mali	GDGCH↓C	Sdul (ER0651, p.105)	Bmyl, Bsp1286l, Sdul
			Ball, MluNI, Mscl, Msp20I
MISI	TGG↓CCA	MISI (ER1211/2, p.84)	bail, marti, mool, mopzor
MISI		Misi (ER1211/2, p.84) Miui (ER0561/2, p.84)	
MISI	TGG↓CCA		Ball, MISI, Mscl, Msp201
Mlul MluNI	TGG↓CCA A↓CGCGT TGG↓CCA	Mlul (ER0561/2, p.84) Mlsl (ER1211/2, p.84)	Ball, MISI, MscI, Msp20I
Misi Miui	TGG↓CCA A↓CGCGT	Mlul (ER0561/2, p.84)	

(continued on next page)



Restriction Endonucleases Guide

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 Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity	Fermentas	Commercially available isoschizomers
	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
Vinli	CCTC(7/6)↓	MnII (ER1071/2, p.85)	
/ph11031	ATGCA	Mph1103I (ER0731/2, p.85)	(BfrBl), EcoT22I, Nsil, Zsp2I
Arol	T↓CCGGA	Kpn2l (ER0531/2, p.80)	Accill ^m , Aor13HI, BseAl ^m , Bsp13I, BspEl ^m , Kpn2I
/roNI	GUCCGGC	(Pdil) (GCC↓GGC) (ER1521/2, p.94)	(Nael), NgoMIV, (Pdil)
/iroXI	GAANNUNNTTC	Pdml (ER1531/2, p.94)	Asp700I, PdmI, XmnI
Ascl	TGGJCCA	MISI (ER1211/2, p.84)	Ball, Misi, MuNI, Msp201
Asel	TUTAA	Tru11 (ER0981/2/3, p.112)	Tru1I, Tru9I
		ITUTI (ER0981/2/3, p.112)	
VISII	CAYNN JNNRTG		SmiMI
Vispl	C↓CGG	Hpall ^m (ER0511/2, p.77),	Hapli ^m , <mark>Hpali</mark> ^m
1 001	700 000	Mspl (ER0541/2, p.86)	
Visp20I	TGGJCCA	MISI (ER1211/2, p.84)	Ball, <mark>MISI</mark> , MIuNI, Mscl
VispA11	CMGJCKG		
MspR9I	CC↓NGG	Bme1390I (ER1421/2, p.37)	Bme1390I, (BssKI), (BstSCI), ScrFI, (StyD4I)
Vissi	GTTTJAAAC	MssI (ER1341/2, p.86)	Pmel ^m
Munl	C↓AATTG	Munl (ER0751/2, p.87)	Mfel
Vival	CC↓WGG	(EcoRII [™]) (↓CCWGG) (ER1921/2, p.70),	(Ajnl), BstNI, BstOI, Bst2UI, (EcoRII ^m), (Psp6I), (PspGI ^m)
		Mval (ER0551/2, p.87)	
Viva12691	GAATGC(1/-1)↓	Mva1269I (ER0961/2, p.88)	BsaMI, BsmI, Pctl
/lvnl	CG↓CG	Bsh1236I (ER0921/2, p.45)	AccII, Bsh1236I, BstFNI, BstUI
Nwol	GCNNNNN↓NNGC	HpyF10VI (ER1731/2, p.79)	BstMWI, HpyF10VI
Vael	GCC↓GGC	Pdil (ER1521/2, p.94)	(MroNI), (NgoMIV), Pdil
Varl	GG↓CGCC	(Ehel) (GGC↓GCC) (ER0441, p.70)	(Bbel ^m), (Egel), (Ehel), (Kasl ^m), Mly113I, (Sfol ^m)
Ncil	CC↓SGG	Bcnl (ER0061/2, p.33)	AsuC2I, Bcnl
Vcol	C↓CATGG	Ncol (ER0571/2, p.88)	Bsp19l
Ndel	CAUTATG	Ndel (ER0581/2, p.89)	FauNDI
Ndell	↓GATC	Bsp143I ^m (ER0781/2, p.48),	BfuCl ^m , Bsp143I ^m , (BstKTI), BstMBI, (DpnI ^m), DpnII, Kzo9I ^m , (MalI ^m), Mbo
NUEII	VGAIC		
		(DpnI ^m) (GA↓TC) (ER1701/2, p.59),	Sau3Al ^m
		Mbol (ER0811/2, p.83)	
VgoMIV	GUCCGGC	(Pdil) (GCC↓GGC) (ER1521/2, p.94)	MroNI, (Nael), (Pdil)
Vhel	GUCTAGC	Nhel (ER0971/2, p.89)	AsuNHI, (Bmtl ^m)
Vlalli	CATG↓	Hin1II (ER1831, p.74)	(CviAll ^m), (Fatl ^m), Hin1II, Hsp92II
NIaIV	GGN↓NCC	BspLI ^m (ER1151/2, p.50)	BspLI ^m , PspN4I
NmuCl	↓GTSAC	NmuCI (ER1511/2, p.90)	Tsp45I
Notl	GC↓GGCCGC	Notl (ER0591/2/3, p.90)	CciNI
Nrul	TCG↓CGA	Bsp68I (ER0111/2, p.47)	Bsp68I
Nsbl	TGCJGCA	Nsbl (ER1221/2, p.91)	Acc16I, Avill, Fspl
Nsil	ATGCA↓T	Mph1103I (ER0731/2, p.85)	(BfrBl), EcoT22I ^m , Mph1103I, Zsp2I
Nspl	RCATG	Xcel (ER1471/2, p.116)	BstNSI, Xcel
NspV	TTUCGAA	Bsp119I (ER0121, p.47)	Bpu14I, Bsp119I, BspT104I, BstBI, Csp45I, Sful
Olil			Alel
		Olil (ER1631/2, p.91)	AIEI
Pacl		De el (ED0 (01 /0 / 4 - e 00)	Dhul Cali
Pael	GCATGUC	Pael (ER0601/2/4, p.92)	Bbul, Sphl
PaeR7I	C↓TCGAG	Xhol (ER0691/2/3, p.117)	Sfr274I, Tlil, Xhol
Pagl	TUCATGA	PagI (ER1281/2, p.92)	BspHI ^m , Rcal
Pall	GG↓CC	BsuRI (ER0151/2, p.53)	BsuRI, Haelli, Phol
PalAl	GG↓CGCGCC	Sgsl (ER1891/2, p.106)	Ascl, <mark>Sgsl</mark>
Pasl	CC↓CWGGG	Pasl (ER1861, p.93)	
Paul	G↓CGCGC	Paul (ER1091/2, p.93)	BsePI, BssHII
Pcel	AGG↓CCT	Eco147I (ER0421/2, p.68)	Aatl, Eco147I, Stul
Pcil	ALCATGT	Pscl (ER1871/2, p.97)	BspLU11I, Pscl
Pctl	GAATGC(1/-1)↓	Mva1269I (ER0961/2, p.88)	BsaMI, BsmI, Mva1269I
Pdil	GCCVGGC	Pdil (ER1521/2, p.94)	(MroNI), Nael, (NgoMIV)
Pdml	GAANN NNTTC	Pdml (ER1521/2, p.94)	Asp700I, MroXI, Xmnl ^m
Pfel	GLAWTC	Pfel (ER1781, p.95)	Tfil
Pfl23II		Pfl23II (ER0851, p.95)	BsiWI, PspLI
PfIFI		Psyl (ER1331, p.100)	Aspl, Psyl, Tth1111
PfIMI	CCANNNNUNTGG	Van911 (ER0711/2, p.114)	AccB7I, Van91I
Pfol	TUCCNGGA	Pfol (ER1751, p.96)	
Phol	GGUCC	BsuRI (ER0151/2, p.53)	BsuRI, Haelll, Pall
PinAl	A↓CCGGT	BshTI (ER1461/2, p.46)	Agel, AsiGl, <mark>BshTl</mark>
Plel	GAGTC(4/5)↓	(Schl ^m) (GAGTC(5/5)) (ER1371, p.104)	(Mlyl ^m), Ppsl, (Schl ^m)
Ple19I	CGATUCG	Pvul (ER0621/2, p.100)	BspCl, Pvul
PmaCl	CAC↓GTG	Eco72I (ER0361/2, p.65)	BbrPI, Eco72I, PmII, PspCI
Pmel	GTTTJAAAC	Mssl [™] (ER1341/2, p.86)	MssI ^m
		Eco72I (ER0361/2, p.65)	
Pmll	CAC↓GTG		BbrPI, Eco72I, PmaCI, PspCI

(continued on next page) Bulk quantities & custom formulations available on request



Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

			IC 1.3. Alphabetic Eist of commercially Available Restriction Endonacicases.
Enzyme	Specificity	Fermentas	Commercially available isoschizomers
	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
Docl	GAGTC(4/5)↓	(Schl) (GAGTC(5/5)) (ER1371, p.104)	
Ppsl			(Mlyl), Plel, (Schl)
Ppu21I	YAC↓GTR	Ppu21I (ER1971, p.97)	BsaAI, BstBAI
PpuMI	RG↓GWCCY	Psp5II (ER0761, p.98)	Psp5II, PspPPI
Pscl	A↓CATGT	Pscl (ER1871/2, p.97)	BspLU11I, Pcil
PshAl	GACNNUNNGTC	Boxl (ER1431, p.38)	BoxI, BstPAI
PshBl	AT	Vspl (ER0911/2, p.114)	Asel, <mark>Vspl</mark>
Psil	TTA↓TAA		
Psp5II	RG↓GWCCY	Psp5II (ER0761, p.98)	PpuMI, PspPPI
Psp6l	↓CCWGG	EcoRII (ER1921/2, p.70),	Ajnl ^m , (BstNI), (BstOI), (Bst2UI ^m), EcoRII, (Mval), PspGI
гэрог	<u>^</u> CCM00		Ajni , (Dstivi), (Dstoi), (Dstoi), ECOKII, (WVai), Espai
		(Mval) (CC↓WGG) (ER0551/2, p.87)	
Psp1406l	AA↓CGTT	Psp1406I (ER0941/2, p.98)	Acll
PspAl	C↓CCGGG	Cfr9I (ER0171/2/4, p.55),	Cfr9I, (Smal), XmaI, XmaCl
.1.		(Smal) (CCC↓GGG) (ER0661/2/3/4, p.106)	
Dem 10 (DI	CACCT		
Psp124BI	GAGCT↓C	(Ecl136II) (GAG↓CTC) (ER0251/4, p.61),	, (ECIT3011), (ECOIURI), SACI
		Sacl (ER1131/2/3/4, p.102)	
PspCl	CAC↓GTG	Eco72I (ER0361/2, p.65)	BbrPI, <mark>Eco72I</mark> , PmaCI, Pmll
PspEl	G↓GTNACC	Eco91I (ER0391/2, p.67)	BstEll, BstPl, Eco91l, Eco065l
PspGl	↓CCWGG	EcoRII (ER1921/2, p.70),	Ajnl, (BstNI ^m), (BstOI ^m), (Bst2UI), EcoRII, (MvaI ^m), Psp6I
		(Mval ^m) (CC↓WGG) (ER0551/2, p.87)	
PspLI	C↓GTACG	Pfl23II (ER0851, p.95)	BsiWI, Pf12311
PspN4I	GGN↓NCC	BspLI (ER1151/2, p.50)	BspLI, NlalV
PspOMI	G↓GGCCC	(Apal) (GGGCC↓C) (ER1411/2, p.30),	(Apal), Bsp120I
		Bsp120I (ER0131/2/3, p.48)	
PspPPI	RG↓GWCCY	Psp5II (ER0761, p.98)	PpuMI, Psp5II
PspXI	VCLTCGAGB	·····	n e e e e e e e e e e e e e e e e e e e
Psrl	↓(7/12)GAACNNNNNNTAC(12/7)↓		
Pstl	CTGCA	Pstl (ER0611/2/3/4, p.99)	
Psul	R↓GATCY	Psul (ER1551, p.99)	BstX2I, BstYI, MfII ^m , XhoII
Psyl	GACNUNNGTC	Psyl (ER1331, p.100)	Aspl, PfIFI, Tth1111
Pvul	CGAT↓CG	Pvul (ER0621/2, p.100)	BspCl, Ple19l
Pvull	CAG↓CTG	Pvull (ER0631/2/3, p.101)	
Rcal	T↓CATGA	Pagl (ER1281/2, p.92)	BspHI, PagI
Rgal	GCGAT↓CGC		AsiSI, Sgfl
Rsal	GT↓AC	(Csp6I ^m) (G↓TAC) (ER0211, p.58),	Afal, (Csp6I ^m)
KSdi	GIVAC		Aldi, (<mark>Ospoi</mark> ^m)
		Rsal (ER1121/2, p.101)	
Rsrll	CG↓GWCCG	Cpol (ER0741/2, p.58)	Cpol, Cspl, Rsr2l
Rsr2l	CG↓GWCCG	Cpol (ER0741/2, p.58)	Cpol, Cspl, Rsrll
Sacl	GAGCT↓C	(Ecl136II [™]) (GAG↓CTC) (ER0251/4, p.61),	
Jaci	GAGCIVC		, (ECITSUIT), (ECUICIN), ESPT24DI
		Sacl (ER1131/2/3/4, p.102)	
Sacll	CCGC↓GG	Cfr42I (ER0201/2, p.57)	Cfr42I, Kspl, Sfr303I
Sall	G↓TCGAC	Sall (ER0641/2/3/4, p.102)	
SanDI	GG↓GWCCC		
		(ED1021/2 = 01)	Land
Sapl	GCTCTTC(1/4)↓	Lgul (ER1931/2, p.81)	Lgul
Satl	GC↓NGC	Satl (ER1641/2, p.103)	Bisl ^m , Fnu4HI, Fsp4HI, Ital ^m
Sau96I	G↓GNCC	Cfr13I (ER0191/2, p.56)	AspS9I, Cfr13I
Sau3AI	↓ GATC	Bsp143I (ER0781/2, p.48),	BfuCl, Bsp1431 ^m , (BstKTI ^m), BstMBI, (DpnI ^m), DpnII ^m , Kzo9I, (MalI ^m),
		(DpnI ^m) (GA↓TC) (ER1701/2, p.59),	Mbol ^m . Ndell ^m
			INDUT, INUCII
:		Mbol ^m (ER0811/2, p.83)	
Sbfl	CCTGCA	Sdal (ER1191/2/4, p.104)	Sdal, Sse8387I
Scal	AGTJACT	Scal (ER0431/2, p.103)	Zrml
Schl	GAGTC(5/5)↓	Schl (ER1371, p.104)	MlyI, (PleI), (PpsI)
ScrFl	CC↓NGG	Bme1390I (ER1421/2, p.37)	Bme1390I, (BssKI), (BstSCI), MspR9I, (StyD4I)
Sdal	CCTGCA	Sdal (ER1191/2/4, p.104)	Sbfl, Sse8387I
Sdul	GDGCH↓C	Sdul (ER0651, p.105)	Bmyl, Bsp1286I, Mhll
SexAl	ALCCWGGT		Mabl
SfaNI	GCATC(5/9)↓	Lwel (ER1621/2, p.82)	Lwel
		· · · · · · · · · · · · · · · · · · ·	
Sfcl	C↓TRYAG	Bfml (ER1161/2, p.35)	Bfml, BstSFI
Sfil	GGCCNNNN↓NGGCC	Sfil (ER1821, p.105)	
Sfol	GGC↓GCC	Ehel ^m (ER0441, p.70)	(Bbel ^m), Egel, Ehel ^m , (Kasl ^m), (Mly113I), (Narl ^m)
Sfr274I	C↓TCGAG	Xhol (ER0691/2/3, p.117)	PaeR7I, Tiil, Xhol
Sfr303I	cccc↓gg	Cfr42I (ER0201/2, p.57)	Cfr42I, Kspl, Sacli
Sful	TT↓CGAA	Bsp119I [™] (ER0121, p.47)	Bpu14I, Bsp119I ^m , BspT104I ^m , BstBI ^m , Csp45I, NspV ^m
Sgfl	GCGAT↓CGC		AsiSI, Rgal
	CR↓CCGGYG		,, ,
SgrAl			
Sgsl	GGTCGCGCC	Sgsl (ER1891/2, p.106)	Asci, PalAl
Sinl	G↓GWCC	Eco47I (ER0311/2, p.63)	Avall, Bme18I, <mark>Eco47I</mark> , VpaK11BI
			(continued on next page



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 Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

			1e 1.3. Alphabetic List of Commercially Available Restriction Endonuclease
Enzyme	Specificity	Fermentas	Commercially available isoschizomers
	$5' \rightarrow 3'$	enzyme (cat. #, page #)	
Smal	CCC↓GGG	(Cfr9I ™) (C↓CCGGG) (ER0171/2, p.55),	(Cfr9I ^m), (PspAl), (XmaI ^m), (XmaCl)
		Smal (ER0661/2/3/4, p.106)	
imil	ATTTJAAAT	Smil (ER1241/2, p.107)	Swal
miMI	CAYNNUNRTG		MsII
mll	C TYRAG	Smol (ER1981, p.107)	Smol
mol	C↓TYRAG	Smol (ER1981, p.107)	Smll
mul	CCCGC(4/6)↓	Smul (ER1691/2, p.108)	Faul
naBl	TAC↓GTA	Eco105I (ER0401/2, p.67)	BstSNI, Eco105I
pel	A↓CTAGT	Bcul (ER1251/2, p.33)	Ahll, <mark>Bcul</mark>
phl	GCATG↓C	Pael (ER0601/2/4, p.92)	Bbul, Pael
rfl	GCCC↓GGGC		
se9l	↓AATT	Tasl (ER1351/2, p.111)	Tasl, Tsp509I, TspEl
se8387I	CCTGCA↓GG	Sdal (ER1191/2/4, p.104)	Sbfl, Sdal
sil	CCGC(-3/-1)↓	Ssil (ER1791, p.108)	Acil
spl	AAT↓ATT	Sspl (ER0771/2, p.109)	
spBl	T↓GTACA	Bsp1407I (ER0931/2, p.49)	Bsp1407I, BsrGI, BstAUI
tul	AGG↓CCT	Eco147I (ER0421/2, p.68)	Aatl, Eco147I, Pcel
tyl	C↓CWWGG	Eco130I (ER0411, p.68)	BssT1I, Eco130I, EcoT14I, Erhl
tyD4I	↓ CCNGG	(Bme1390I) (CCUNGG) (ER1421/2, p.37	7) (Bme1390I), BssKI, BstSCI, (MspR9I), (ScrFI)
wal	ATTT↓AAAT	Smil (ER1241/2, p.107)	Smil
aal	ACNJGT	Taal (ER1361/2, p.109)	Bst4Cl, HpyCH4III
ail	ACGT↓	Tail (ER1141/2, p.110)	(HpyCH4IV), (Maell), Tscl
aql	T↓CGA	Taql (ER0671/2/3, p.110)	
aqli	GACCGA(11/9)↓, CACCCA(11/9)↓		
asl		Tasl (ER1351/2, p.111)	Sse9I, Tsp509I, TspEI
atl	W↓GTACW	Tatl (ER1291/2, p.111)	330 //, 13p30 //, 13pEl
aul	GCSG↓C	Taul (ER1651/2, p.112)	
fil	G_AWTC	Pfel (ER1781, p.95)	Pfel
lil	C↓TCGAG	Xhol (ER0691/2/3, p.117)	PaeR7I, Sfr274I, Xhol
in ru1l	T↓TAA	Tru11 (ER0981/2/3, p.112)	Msel, Tru9l
ru9l	T↓TAA	Tru11 (ER0981/2/3, p.112)	Msel, Tru1i
scl	ACGT	Tail (ER1141/2, p.110)	(HpyCH4IV ^m), (MaeII), Tail
		Tall (ER 1 14 1/2, p. 1 10)	
sel	G CWGC	Teel (ED1001 - 110)	АреКІ
SOL	TARCCA(9/11)↓	Tsol (ER1991, p.113)	NexeOl
sp45l	↓GTSAC ↓AATT	NmuCl (ER1511/2, p.90)	NmuCl
sp5091		Tasl (ER1351/2, p.111)	Sse9I, Tasl, TspEI
spEl		Tasl (ER1351/2, p.111)	Sse9I, Tasl, Tsp509I
spGWI	ACGGA(11/9)↓		
spRI	CASTGNN(2/-7)↓		
stl	↓(8/13)CACNNNNNNTCC(12/7)↓	Tstl (ER1911, p.113)	
th1111	GACN	Psyl (ER1331, p.100)	Aspl, PfIFI, Psyl
lan911	CCANNNNUNTGG	Van911 (ER0711/2, p.114)	AccB7I, PfIMI
/ha464I	C↓TTAAG	BspTI (ER0831/2, p.51)	AfIII, BfrI, <mark>BspTI</mark> , Bst98I
nel	GUTGCAC	Alw44I (ER0041/2, p.30)	Alw44I, ApaLI
paK11BI	GUGWCC	Eco47I (ER0311/2, p.63)	Avall, Bme18I, Eco47I, Sinl
spl	AT↓TAAT	Vspl (ER0911/2, p.114)	Asel, PshBl
agl	CCTNN↓NNNAGG	Xagl (ER1301/2, p.115)	BstENI, EcoNI
apl	R↓AATTY	Xapl (ER1381/2/4, p.115)	Acsl, Apol
bal	T↓CTAGA	Xbal (ER0681/2/3/4, p.116)	
cel	RCATG↓Y	Xcel (ER1471/2, p.116)	BstNSI, Nspl
cml	CCANNNNN↓NNNNTGG		
hol	C↓TCGAG	Xhol (ER0691/2/3, p.117)	PaeR7I, Sfr274I, Tlil
holl	R↓GATCY	Psul (ER1551, p.99)	BstX2I, BstYI, MfII ^m , Psul
mal	C↓CCGGG	Cfr9I (ER0171/2/4, p.55),	Cfr91, PspAI, (Smal ^m), XmaCl
		(Smal ^m) (CCC↓GGG) (ER0661/2/3/4, p.106	
maCl	C↓CCGGG	Cfr9I (ER0171/2/4, p.55),	Cfr91, PspAI, (Smal), Xmal
		(Smal) (CCC\GGG) (ER0661/2/3/4, p.106	
(maJI	C↓CTAGG	XmaJI (ER1561/2, p.117)	AspA2I, Avril, Bini
mil	GTUMKAC	Xmil (ER1481/2, p.118)	Acci, Fbli
mnl	GAANNUNNTTC	Pdml ^m (ER1531/2, p.94)	Asp700I, MroXI, PdmI ^m
spl	C TAG	FspBI (ER1761/2, p.72)	Bfal, FspBl, Mael
ral		(AatII ^m)(GACGT↓C) (ER0991/2, p.25)	(Aatilm)
irml Ion 21		Scal (ER0431/2, p.103)	Scal
Zsp2l	ATGCA↓T	Mph1103I (ER0731/2, p.85)	(BfrBl), EcoT22I, Mph1103I, Nsil



Recognition Specificities

	_
Specificity $5' \rightarrow 3'$	Enzyme
A	
AAJCGTT	Psp1406l
AJAGCTT	HindIII
AAGGAG(20/18)	CstMI
\downarrow (8/13)AAG(N) ₅ CTT(13/8) \downarrow	Fall
AATJATT	Sspl
JAATT	Tasl
ACAYNNNNGTA	UbaF9I
AUCATGT	Pscl
A↓CCGGT	BshTl
ACCNNNNNGGT	HgiEll
ACCTGC(4/8)	Bvel
A↓CCWGGT	SexAl
A↓CGCGT	Mlul
ACGGA(11/9)↓	TspGWI
ACGGC(12/14)↓	BceAl
ACGGG	BscGI
A↓CGT	Maell
ACGT	Tail
↓(10/15)AC(N),GTAYC(12/7)↓	Bael
ACN GT	Taal
ACNNUNCTC	
	CjeNII
↓(9/12)AC(N) ₅ CTCC(10/7)↓	BsaXI
AUCRYGT	AfIII
AUCTAGT	Bcul
ACTGGG(5/4)↓	Bfil
ACTGG(1/-1)↓	BseNI
↓(9/15)AC(N) ₆ TGG(14/8)↓	Cjel
A↓GATCT	BgIII
AGC↓GCT	Eco47III
AG↓CT	Alul
AGG↓CCT	Eco147I
AG↓GWCCT	Sse86471
AGT	Scal
AT↓CGAT	Bsu15I
ATGAA(11/9)↓	TspDTI
ATG↓CAT	BfrBl
ATGCA	Mph1103I
ATTAAT	Vspl
ATTTJAAAT	Smil
C	Jiiii
CAACAC	Bsbl
↓(11/13)CAA(N) ₅ GTGG(12/10)↓	CspCl
CAARCA(11/9)↓	Tth1111
C AATTG	Munl
CACCCA(11/9)↓	Taqll
CACCTGC(4/8)↓	Aarl
CACGAG(-5/-1)	Baul
CACJGTC	Ajil
CAC↓GTG	Eco72I
CACNNNJGTG	Adel
CACNNUNGTG	Olil
\downarrow (8/13)CAC(N) ₆ TCC(12/7) \downarrow	Tstl
↓(0/2)CACTGC	Btsl
CAGCTC(7/11)↓	AceIII
CAG↓CTG	Pvull
CAGNNN↓CTG	Cail
CASTG(2/-7)↓	TspRI
CAYNNNNRTG	UbaF10I
CAYNN	MsII
CAJIATG	Ndel
CATCAG	Ssml
↓(0/2)CATCC	BseGI
	03001

Specificity $5' \rightarrow 3'$	Enzyme
(14/10)CATCC	Stsl
ĊATC↓G	Cdil
↓(14/10)CATCGC	BtgZl
↓CATG	Fatl
CATG	Hin1II
_C↓ATG	CviAll
↓(0/2)CATTGC	BseMI
\downarrow (6/11)CCAA(N) ₇ TTC(12/7) \downarrow	Ajul
$\downarrow (10/12) CCAC(N)_5 TTG(13/11) \downarrow$	CspCl
CCAGA	BspNCI
\downarrow (-1/1)CCAGT	BseNI
\downarrow (8/14)CCA(N) ₆ GT(15/9) \downarrow	Cjel
↓(7/12)CCA(N),GTC(13/8)↓ ↓(7/13)CCA(N),TC(14/8)↓	Bsp 241 Cje Pl
CCANNNN VNTGG	Van91I
	Xcml
CCANNNNN	BstXI
CCATC	Bccl
CUCATGG	Ncol
C↓CRYGG	Dsal
CCCACA(12/9)↓	RIEAI
\downarrow (4/5)CCCAGT	Bfil
C↓CCAGC	BseYI
CCCGC(4/6)↓	Smul
C↓CCGGG	Cfr9l
CCC↓GGG	Smal
CCCG(4/8)↓	Sth132I
CCCGT	BscGl
CC↓CWGGG	Pasl
CCGC(-3/-1)↓	Ssil
CCGC↓GG	Cfr42I
CCGCTC(-3/-3)↓	Mbil
<u>C</u> ↓CGG	Hpall,Mspl
↓CCNGG	BssKI
CC↓NGG	Bme1390I
C↓CNNGG	BseDI
	BseLI
<u>C↓CRYGG</u>	Dsal
	EcoHI
CC↓SGG	Bcnl
C CTAGG	XmaJI
CCTCAGC(-5/-2)↓	BbvCl
	Mnll
CCTGCAJGG CCJTNAGC	Sdal Routol
	Bpu10I
	Eco81I Xaql
CCTTC	Hin4II
↓CCWGG	EcoRII
CCJWGG	Mval
C↓CWWGG	Eco130I
CGAACG	UbaPI
CGATJCG	Pvul
↓(10/12)CGA(N),TGC(12/10)↓	Bcgl
CG ¹ CCGGCG	Sse 232I
↓CGCG	Sell
CGLCG	Bsh1236l
C↓GGCCG	Eco52I
↓(-1/-5)CGGCCR	Gdill
↓(8/4)CGGG	Sth132I
CGJGWCCG	Cpol
CGRY↓CG	Bsh12851
0 07100	Pfl23II
CJGTACG	FIIZJII
CGTCGACG	SgrDI

Specificity $5' \rightarrow 3'$ CGTCTC(1/5)↓ Esp3I CGTTCG UbaPI CGWCG Hpy99I CMG↓CKG MspA1I CR↓CCGGYG SgrAl CTACGA Pfl 11081 C↓TAG FspBI CTCAG(10/8)↓ BseMII ↓(14/16)CTCCAG Gsul ↓(8/10)CTCCTC BseRI ↓(18/20)CTCCTT CstMI C↓TCGAG Xhol Scil CTC↓GAG ↓(14/16)CTCAAG BpuEl CTCGTG(-5/-1)↓ Baul CTCTTC(1/4)↓ Eam1104I CTGAAG(16/14)↓ Eco57I CTGATG Ssml ↓(14/16)CTGCAC Bsql CTGCA↓G Pstl CTGGAC BspGI ↓(8/10)CTGAG **BseMII** CTGGAG(16/14) Gsul CTGRAG(16/14)↓ Eco57MI C**↓**TNAG HpyF3I C↓TRYAG Bfml C↓TTAAG **BspTI** ↓(14/16)CTTCAG Eco57I CTTGAG(16/14)↓ BpuEl C**↓**TYRAG Smol ↓(14/16)CTYCAG Eco57MI C↓YCGRG Eco88I CYCGR↓G NIi 38771 G GAACCA Drd II ↓(7/12)GAA(N)₇TTGG(11/6)↓ Ajul ↓(7/12)GAAC(N)₅CTC(13/8)↓ Ppil ↓(7/12)GAAC(N) TAC(12/7)↓ Psrl (7/12-13)GAAC(Ň) TCC(12-13/7) Alol GAAGAC(2/6)↓ Bpil GAAGAC(7/11)↓ Bbr7I ↓(4/1)GAAGAG Eam1104I ↓(4/1)GAAGAGC Lgul GAAGA(8/7)↓ Mboll GAANN↓NNTTC Pdml GAATGC(1/-1)↓ Mva1269I G↓AATTC **EcoRI** ↓(8/13-14)GAY(N)₅VTC(13-14/8)↓**Hin4**I ↓(0/-3)GACCC SimI GACCGA(11/9) Tagli GACGC(5/10) Csel GACGT↓C Aatll GAC↓GTG Ajil ↓(5/5)GACTC Schl ↓(5/4)GACTC Plel GACN↓NNGTC Psyl GACNN↓NNGTC Boxl GACNNN↓NNGTC Eam1105I GACNNNN↓NNGTC Aasl ↓(8/13) GAC(N),TGG(12/7)↓ Bsp24I ↓(5/1)GAGAC Alw26I ↓(5/1)GAGACC Eco31I ↓(5/1)GAGACG Esp3I ↓(-3/-3)GAGCGG Mbil (continued on next page)

Table 1.4. Recognition Specificities.

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↓(13/9)CATCC

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Fokl

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

Restriction Endonucleases Guide

Table 1.4. Recognition Specificities

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Specificity $5' \rightarrow 3'$	Enzyme
GAG↓CTC	Ecl136II
GAGCT	Sacl
↓(11/7)GAGCTG	AceIII
↓(6/7)GAGG	MnII
GAGNNNCTC	Tssl
GAGNNNNNGT	CjeNII
GAGTC(5/5)↓	Schl
GAGTC(4/5)↓	Plel
↓(8/13)GAG(N) ₅ CTC(13/8)↓	Bpll
GAGGAG(10/8)↓	BseRI
\downarrow (8/13)GAG(N) ₅ GTTC(12/7) \downarrow	Ppil
GJANTC	Hinfl
↓(7/13) GAY(N)₅RTC(14/9)↓	HaelV
GATLATC	Eco32I
JGATC	Bsp143I, Mbol
GAT	BstKTI
GATC	Chal
Gm ⁶ AJTC	Dpnl
↓(5/4)GATCC	BspPI
↓(9/5)GATGC	Lwel
\downarrow (6/4)GATGC	BscAl
GATGG	Bccl
\downarrow (8/14)GA(N) ₇ TGG(13/7) \downarrow	CjePI
	BseJI
GJAWTC	Pfel
GCAATG(2/0)↓	BseMI
GCAGC(8/12)↓	BseXI
GCAGGTG ↓(8/4)GCAGGT	Aarl Bvel
GCAGTG(2/0)↓	Btsl
GCANNNNUNTGC	BstAPI
GCANNNNNJTGC	ApaBl
↓(10/12)GCA(N),TGC(12/10)↓	Alfl
GCATC(5/9)↓	Lwel
GCATC(4/6)↓	BscAl
↓(10/12)GCA(N),TCG(12/10)↓	Bcgl
GCATG C	Pael
\downarrow (-1/1)GCATTC	Mva1269I
GCCC↓GGGC	Srfl
GCCGC	AspCNI
G↓CCGGC	NgoMIV
GCCJGGC	Pdil
↓(14/12)GCCGT	BceAl
GCCNNNNUNGGC	Bgll
GCGAC	Tsul
GCGAT↓CGC	Sgfl
GCGATG(10/14)↓	BtgZl
G↓CGC	Hin6I
GCGJC	Hhal
G ¹ CGCGC	Paul
GCGG(-3/-1)↓	Ssil
GCJGGCCGC	Notl
↓(6/4)GCGGG	Smul
GCJNGC	Satl
<u>GCN↓NGC</u>	Cac8l
GCNNNNN VNNGC	HpyF10VI
↓(10/5)GCGTC	Csel
GCSGUC	Taul
G CTAGC	Nhel
GCTAG C	Bmtl
GCTCTTC(1/4)↓	Lgul
GCTGAGG(-5/-2)↓	BbvCl
↓(12/8)GCTGC	BseXI
GUCTGGG	BseYI
GC↓TNAGC GC↓TNAGG	Bpu1102I
	Bpu10I

Specificity $5' \rightarrow 3'$	Enzyme
G↓CWGC	Tsel
GDGCH↓C	Sdul
↓(7/10)GGAG(N)₅GT(12/9)↓	BsaXI
G↓GATCC	BamHI
GGATC(4/5)↓	BspPI
↓(7/12)GGA(N) ₆ GTG(13/8)↓	Tstl
↓(7/12-13)GGA(N),GTTC(12-13/7)	↓Alol
↓(5/6)GGATAC	Bful
GGATG(2/0)↓	BseGI
GGATG(9/13)↓	Fokl
GGATG(10/14)	Stsl
GG↓CC	BsuRI
GGCCGG↓CC	Fsel
GGCCNNNN	Sfil
G↓GCGCC	Kasl
GGJCGCC	Narl
GGC↓GCC	Ehel
GGCGC↓C	Bbel
GG↓CGCGCC	Sgsl
GGCGGA(11/9)↓	Ecil
GGGAC(10/14)↓	Faql
GUGGCCC	Bsp120I
GGGCC↓C	Apal
GGGCMC	Bmgl
GGGTC(-3/0)↓	Siml
GG↓GWCCC	SanDI
↓GGNCC	Unbl
G↓GNCC	Cfr13I
GGNC↓C	Fmul
GGN↓NCC	BspLI
G↓GYRCC	BshNI
G↓GTACC	Acc65I
GGTAC↓C	Kpnl
GGTCTC(1/5)↓	Eco31I
GGTGA(8/7)↓	Hphl
GGTGA(8/8)↓	SspD5I
GUGTNACC	Eco91I
↓GGWCC	VpaK11AI
G↓GWCC	Eco47I
GKGCCC	Bmgl
GKGCMJC	BseSI
GRJCGYC	Hin1l
GRGCY	Eco24I
\downarrow (7/12)GRTAC(N) ₄ GT(15/10) \downarrow	Bael
GUTAC	Csp6l
GTLAC	Rsal
\downarrow (7/12)GTA(N) ₆ GTTC(12/7) \downarrow	Psrl
GTAJTAC	Bst11071
GTATCC(6/5)↓	Bful
GTCCAG	BspGI
↓(14/10)GTCCC	Faql
GJTCGAC	Sall
GTCGC	Tsul
GTCTC(1/5)↓	Alw26I
(6/2)GTCTTC	Bpil
↓(11/7)GTCTTC	Bbr7I
GJTGCAC	Alw44I
GTGCAG(16/14)↓	Bsgl
GTGCAG(16/14)↓ GTGTTG	Bsgl Bsb1
GTGCAG(16/14)↓ GTGTTG GT↓MKAC	0
GTGCAG(16/14)↓ GTGTTG GT↓MKAC ↓GTNAC	Bsbl
GTGCAG(16/14)↓ GTGTTG GT↓MKAC ↓GTNAC GTN↓NAC	Bsb1 Xmil
GTGCAG(16/14)↓ GTGTTG GT↓MKAC ↓GTNAC	Bsbl Xmil Maelll
GTGCAG(16/14)↓ GTGTTG GT↓MKAC ↓GTNAC GTN↓NAC	Bsbl Xmil Maelll Hpy8l
GTGCAG(16/14)↓ GTGTTG GT↓MKAC ↓GTNAC GTN↓NAC ↓GTSAC	Bsbl Xmil Maelll Hpy8l NmuCl

Table 1.4. Recogn	nition Specificities.
Specificity $5' \rightarrow 3'$	Enzyme
GTTT↓AAAC	Mssl
GWGCW↓C R	Alw21I
R↓AATTY	Xapl
RCATG	Xcel
RUCCGGY	Cfr10I
RJGATCY	Psul
RGCJGCY	Lpnl
RGCGCVY	Bsp143II
RGJCY	CviJI
RG GWCCY	Psp5II
RGJGNCCY RGGNCJCY	Eco0109I
	Pssl
RTGC↓GCAY Y	FspAl
YAC↓GTR	Ppu21I
YGGCCG(-5/-1)↓	Gdill
YUGGCCR	Cfrl
T	UIII
TACJGTA	Eco105I
TACNNNNRTGT	UbaF9I
TARCCA(9/11)↓	Tsol
↓(7/8)TCACC	Hphl
↓(8/8)TCACC	SspD5I
TLCATGA	Pagl
↓(9/11)TCCGCC	Ecil
TUCCGGA	Kpn2l
TUCCNGGA	Pfol
TCCRAC(20/18)↓	Mmel
T↓CGA	Taql
TCG↓CGA	Bsp68I
TCGTAG	Pfl 11081
TCNJGA	Hpy188I
TCUNNGA	Hpy188III
T↓CTAGA	Xbal
TCTGG	BspNCI
↓(7/8)TCTTC	Mboll
↓(10/12)TGA(N),TCA(12/10)↓	Bdal
TJGATCA	Bcll
TGUCA	CviRI
TGCJGCA	Nsbl
	MISI
↓(9/11)TTCGGTC ↓(9/11)TTGGGTG	TaqII
TGGTTC	Taq II Drd II
↓(9/11)TGYTTG	Tth111II
TJGTACA	Bsp1407I
↓(9/12)TGTGGG	RIEAI
	Tru1
TTAATTAA	Pacl
TTAJIAA	Psil
↓(9/11)TTCAT	TspDTI
TTJCGAA	Bsp119I
TTTJAAA	Dral
V	
VC↓TCGAGB	PspXI
W	
W↓CCGGW	BsaWI
WGGJCCW	Hael
W↓GTACW	Tatl
Nato	

- Note
 Enzymes produced by Fermentas are shown in orange.
 Enzymes discovered at Fermentas, but not yet commercially available are highlighted (Aaal).
 Restriction enzymes that are not currently commercially available are highlighted (Bbb).



Commercial Restriction Enzymes Sorted by the Type of DNA Ends Generated

Enzymes Generating 5'-protruding Ends

Recognition Enzyme 5'-protruding end (5' \rightarrow 3') 5nt sequence AAUCGTT Psp1406l CG ALAGCTT HindIII AGCT ↓AATT AATT Tasl A CATGT CATG Pscl CCGG ALCCGGT **BshTl** ACCTGC(4/8)↓* **Bvel** NNNN CCWGG A^LCCWGGT SexAl A↓CGCGT CGCG Mlul A CRYGT AfIIII CRYG A CTAG1 Bcul CTAG AUGATCT BgIII GATC CG ATCGAT Bsu15 **ATTAT** Vspl TA AATT C↓AATTG Munl CACCTGC(4/8)↓* NNNN Aarl CACGAG(-5/-1)↓* Baul ACGA CAUTATG Ndel TA ↓(13/9)CATCC* NNNN Fokl ↓(14/10)CATCGC BtgZI NNNN C↓CATGG CATG Ncol CLCCAGO CCAG BseYI CCCGC(4/6) NN Smul C↓CCGGG Cfr9l CCGG CC↓CWGGG CWG Pasl CCGC(-3/-1)↓* Ssil CG CG C↓CGG Hpall C↓CGG CG Mspl CCNGG ↓CCNGG BssKl CC↓NGG Bme1390I Ν C↓CNNGG CNNG **BseDI** C↓CRYGG CRYG Dsal CCUSGG S Bcnl C↓CTAGG **XmaJI** CTAG CCTCAGC(-5/-2)↓* BbvCI TCA CCTNAGC(-5/-2)↓* Bpu10I TNA CCUTNAGG Eco81I TNA CCTNN↓NNNAGG XagI Ν ↓CCWGG CCWGG EcoRII W CCUWGG Mval C↓CWWGG Eco130I CWWG C↓GGCCG GGCC Eco52I GWC CG↓GWCCG Cpol GTAC C↓GTACG Pfl23II CGTCTC(1/5)↓* NNNN Esp3I C VCGRG Eco88I YCGR CR↓CCGGYG SgrAl CCGG C TAG TA **FspBI** C↓TCGAG TCGA Xhol CTCGTG(-5/-1)↓* Baul TCGT CTCTTC(1/4)↓* Eam1104I NNN C TYRAG Smol TYRA C↓TNAG TNA HpyF3 C TRYAG TRYA Bfml C↓TTAAG TTAA **BspTI** GAAGAC(2/6)↓* Bpil NNNN NNN (4/1)GAAGAG Eam1104I ↓(4/1)GAAGAGC* Lgul NNN **G A A T C** EcoRI AATT GACGC(5/10)↓* NNNNN Csel **GACN** NNGTC Ν Psyl Ν ↓(5/4)GACTC Plel ↓(5/1)GAGAC* Alw26I NNNN ↓(5/1)GAGACC Eco31I NNNN ↓(5/1)GAGACG NNNN Esp3I Ν GAGTC(4/5)↓* Plel GJANTC Hinfl ANT GATC **↓**GATC Bsp143I GATC GATC Mbol ↓(5/4)GATCC* Ν **BspPl** NNNN ↓(9/5)GATGC* Lwel

GLCGC GCGLC GCGCCGC GCGC(-3/-1) GCLGCCCGC L(6/4)GCGGG* L(10/5)GCGTC* GCLGCC GLCTAGC GCTCTC(1/4) GCTGAGG(-5/-2) L(12/8)GCTGC*	Pfel BseXI Bvel Lwel BtgZI Hin6l Hhal Paul Ssil Notl Ssul Csel Satl Nhel Lgul DbuCl	1nt	CG CG CG NN	3nt AWT	4nt NNNN NNNN NNNN CGCG	5nt
GCAGC(8/12) J* J(8/4)GCAGGT* GCATC(5/9) J* GCGATG(10/14) J GLCGC GCGLC GCGCC GCGCC GCGCC GCGCCC GCGCCC J(6/4)GCGGG J(10/5)GCGTC* GCLNGC GLCTAGC GCTCTTC(1/4) J* GCTGAGG(-5/-2) J* J(12/8)GCTGC*	BseXI Bvel Lwel BtgZl Hin61 Hhal Paul Ssil Notl Smul Csel Satl Nhel Lqul	N	CG	AWT	NNNN NNNN NNNN CGCG	
↓(8/4)GCAGGT* GCATC(5/9)↓* GCGATG(10/14)↓ G↓CGC GCG↓C GCG↓C GCGC GCGGC-3/-1)↓* GC↓GGCGCGC ↓(6/4)GCGGGC* ↓(10/5)GCGTC* GC↓NGC G↓CTAGC GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Bvel Lwel BtgZl Hin61 Hha1 Paul Ssil Ssil Smul Csel Satl Satl Nhel Lqul	N	CG		NNNN NNNN NNNN CGCG	
GCATC(5/9) J* GCCATG(10/14) J GLCGC GCGLC GLCGCC GCGC-3/-1) J* GCJGCCCC U(6/4)GCGGG J(10/5)GCGTC* GCJNGC GLCTAGC GCTCTTC(1/4) J* GCTGAGG(-5/-2) J* J(12/8)GCTGC*	Lwel BtgZl Hin6l Hhal Paul Ssil Notl Smul Csel Satl Nhel Lqul	N	CG		NNNN NNNN CGCG	
GCGATG(10/14) J GLCGC GCGLC GCGCGC GCGC(-3/-1) J* GCLGGCCGC J(6/4)GCGGG* J(10/5)GCGTC* GCLGGCC GLCTAGC GCTCTTC(1/4) J* GCTGAGG(-5/-2) J* J(12/8)GCTGC*	BtgZl Hin61 Hhal Paul Ssil Not1 Smul Csel Sat1 Nhel Lqul	N	CG		NNNN CGCG	
GCGG(-3/-1)↓* GC_GGCCGC ↓(6/4)GCGGG* ↓(10/5)GCGTC* GC_NGC G_LCTAGC GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Hin6I Hhal Paul Ssil Notl Smul Csel Satl Nhel Lqul	N	CG		CGCG	
GCG↓C G↓CGCGC GCG(-3/-1)↓* GC↓GGCGCGC ↓(10/5)GCGTC* GC↓NGC G↓CTAGC GCTCTAGC GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Hhal Paul Ssil Notl Smul Csel Satl Nhel Lgul	N	CG			
GLCGCGC GCGG(-3/-1) J* GCLGGCCGC J(6/4)GCGGG* J(10/5)GCGTC* GCLNGC GLCTAGC GCTCTTC(1/4) J* GCTGAGG(-5/-2) J* J(12/8)GCTGC*	Paul Ssil Notl Smul Csel Satl Nhel Lgul	N	CG			
GCGG(-3/-1)↓* GC_GGCCGC ↓(6/4)GCGGG* ↓(10/5)GCGTC* GC_NGC G_LCTAGC GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Ssil Notl Smul Csel Satl Nhel Lgul	N				
GCUGGCCGC U(6/4)GCGGG* U(10/5)GCGTC* GCUNGC GUCTAGC GCTCTTC(1/4)U* GCTGAGG(-5/-2)U* U(12/8)GCTGC*	Notl Smul Csel Satl Nhel Lgul	N			0000	
↓(6/4)GCGGG* ↓(10/5)GCGTC* GC↓NGC G↓CTAGC GCTCTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Smul Csel Satl Nhel Lgul	N	NN			
↓(10/5)GCGTC* GC↓NGC G↓CTAGC GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Csel Satl Nhel Lgul	N	NN		GGCC	
GCJNGC GJCTAGC GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Satl Nhel Lgul	Ν				
$G \downarrow CTAGC$ $GCTCTTC(1/4) \downarrow^*$ $GCTGAGG(-5/-2) \downarrow^*$ $\downarrow(12/8)GCTGC^*$	Nhel Lgul	N				NNNN
GCTCTTC(1/4)↓* GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	Lgul				0710	
GCTGAGG(-5/-2)↓* ↓(12/8)GCTGC*	DhuCl		_	NININI	CTAG	
↓(12/8)GCTGC*			_	NNN		
				TGA	NINININI	
	BseXI				NNNN	
	BseYI			TNIA	CTGG	
GC TNAGC	Bpu1102I		-	TNA		
GCTNAGG(-5/-2)↓* G↓CWGC	_		-	TNA		
	Tsel	N	-	CWG		
GGATC(4/5)↓* G↓GATCC	BspPI BamHI	IN			GATC	
GGATG(9/13)↓*	Fokl				NNNN	
GGUCGCGCC	Sgsl				CGCG	
GGGAC(10/14)↓*	Faql		-	-	NNNN	
GUGAC(10/14)	Bsp120I			-	GGCC	
GGJGWCCC	SanDI			GWC	0000	
GJGYRCC	BshNI			000	GYRC	
GUGNCC	Cfr13I			GNC	UIIKU	
GUGTACC	Acc65I			0110	GTAC	
GGTCTC(1/5)↓*	Eco31I				NNNN	
GJGTNACC	Eco91I			-		GTNAC
GUGWCC	Eco47I			GWC		0111110
GRUCGYC	Hin1I		CG			
GUTAC	Csp6l		TA	-		
↓(14/10)GTCCC*	Fagl				NNNN	
GUTCGAC	Sall				TCGA	
GTCTC(1/5)↓*	Alw26I				NNNN	
↓(6/2)GTCTTC*	Bpil				NNNN	
GUTGCAC	Alw44I				TGCA	
GT MKAC	Xmil		MK			
↓GTNAC	MaeIII					GTNAC
↓GTSAC	NmuCl					GTSAC
Y↓GGCCR	Cfrl				GGCC	
R↓AATTY	Xapl				AATT	
RUCCGGY	Cfr10I				CCGG	
RJGATCY	Psul				GATC	
RGJGNCCY	Eco0109I			GNC		
RGJGWCCY	Psp5II		_	GWC		
TUCATGA	Pagl				CATG	
TUCCGGA	Kpn2l				CCGG	
TUCCNGGA	Pfol					CCNGC
TUCGA	Taql		CG			
TUCTAGA	Xbal				CTAG	
TUGATCA	Bcll		_		GATC	
TUGTACA	Bsp1407I				GTAC	
	Tru1l		TA			
	Bsp119I		CG		TOOL	
	PspXI				TCGA	
WLCCGGW	BsaWI				CCGG	
WJGTACW	Tatl				GTAC	
Single letter code				_		
	K = G or T;		= C, G or 1			
	S = C or G;		= A, G or T			
	H = A, C or T;		= G, A, T o	ru.		
M = A or C; Vote	V = A, C or G					

Table 1.5. Commercial Restriction Enzymes Sorted by the Types of DNA Ends Generated.

Asymmetric sequences are indicated by "*".

Enzymes produced by Fermentas are shown in orange.

(continued on next page)

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Restriction Endonucleases Guide

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Enzymes Generating 3'-protruding Ends

, ,	-				(5) 0)	
Recognition sequence	Enzyme	1nt	3'-protru 2nt	ding end 3nt	$(5' \rightarrow 3')$ 4nt	9nt
ACGGA(11/9)↓*	TspGWI	IIIL	NN	JII	4111	7111
ACGT	Tail		ININ		ACGT	
ACNJGT	Taal	N			1001	
ACTGG(1/-1)↓*	BseNI		GN			
ACTGGG(5/4)↓*	Bfil	Ν				
ATGAA(11/9)↓*	TspDTI		NN			
ATGCA	Mph1103I				TGCA	
CACNNNJGTG	Adel			NNN		
↓(0/2)CACTGC*	Btsl		NN	NININI		
CAGNNN↓CTG CASTG(2/-7)↓*	Cail TspRl			NNN		NNCASTGNN
↓(0/2)CATCC*	BseGI		NN			NINCASTONIN
CATG	Hin1II		ININ		CATG	
↓(0/2)CATTGC*	BseMI		NN		0/110	
↓(-1/1)CCAGT*	BseNI		NC			
$CCA(N)_{5} \downarrow (N)_{4} TGG$		Ν				
CCANNNNN					NNNN	
CCANNNNINTGG	Van91I			NNN		
↓(4/5)CCCAGT*	Bfil	N	00			
	Cfr42I		GC	NININI		
CCNNNNN↓NNGG CCTC(7/6)↓*	Mnll	N		NNN		
	Sdal	IN			TGCA	
CGATUCG	Pvul		AT		1004	
CGRY	Bsh1285I		RY			
	BseMII		NN			
↓(14/16)CTCCAG*	Gsul		NN			
↓(8/10)CTCCTC*	BseRI		NN			
CTGAAG(16/14)↓*			NN			
CTGRAG(16/14)↓*			NN			
(8/10)CTGAG*	BseMII		NN			
\downarrow (14/16)CTGCAC* CTGCA \downarrow G	Pstl		NN		TGCA	
CTGCAVG CTGGAG(16/14)			NN		TGCA	
↓(14/16)CTTCAG*			NN			
GAAGA(8/7)↓*	Mboll	N				
GAATGC(1/-1)↓*	Mva1269I		CN			
GACGTUC	Aatll				ACGT	
GACNNN↓NNGTC	Eam1105I	Ν				
GACNNNN↓NNGTC			NN			
GAGCTUC	Sacl				AGCT	
↓(6/7)GAGG*	Mnll	N	NINI			
GAGGAG(10/8)	BseRI BseMI		NN NN			
$GCAATG(2/0)\downarrow^*$ GCAGTG(2/0) \downarrow^*	Btsl		NN			
GCANNNN VNTGC	BstAPI		ININ	NNN		
GCATG↓C	Pael				CATG	
↓(-1/1)GCATTC*	Mva1269I		NG			
GCCNNNN↓NGGC	Bgll			NNN		
GCGAT	Sgfl		AT			
GCNNNNN↓NNGC				NNN		
GDGCH C	Sdul				DGCH	
↓(5/6)GGATAC*	Bful	N	NINI -			
GGATG(2/0)↓*	BseGI		NN		CCGG	
GGCCGG↓CC GGCC(N),↓NGGCC	Fsel Sfil			NNN	ււսն	
$GGCGGA(11/9)\downarrow^*$	Ecil		NN	INIVIN		
GGGCCVC	Apal		ININ		GGCC	
GCSG↓C	Taul			CSG	0000	
GGTAC↓C	Kpnl				GTAC	
GGTGA(8/7)↓*	Hphl	Ν				
GKGCMJC	BseSI				KGCM	
GRGCY↓C	Eco24I				RGCY	
GTATCC(6/5)↓*	Bful	Ν				
	Bsgl		NN		11/0 011	
	Alw21I				WGCW	
					CATG	
RCATG↓Y	Xcel				GCGC	
GWGCW↓C RCATG↓Y RGCGC↓Y	Bsp143II		NIN			
RCATG↓Y RGCGC↓Y TARCCA(11/9)↓*	Bsp143II Tsol	N	NN			
RCATG \downarrow Y RGCGC \downarrow Y TARCCA(11/9) \downarrow * \downarrow (7/8) TCACC*	Bsp143II Tsol Hphl	N				
RCATG JY RGCGC JY TARCCA(11/9) J^* $J(7/8)TCACC^*$ $J(9/11)TCCGCC^*$	Bsp143II Tsol Hphl Ecil	N	NN			
RCATG JY RGCGC JY TARCCA(11/9) J^* $J(7/8)TCACC^*$ $J(9/11)TCCGCC^*$ $J(9/11)TCCGT^*$	Bsp143II Tsol Hphl	N				
RCATG JY RGCGC JY TARCCA(11/9) J* J(7/8) TCACC* J(9/11) TCCGCC*	Bsp143II Tsol HphI Ecil TspGWI		NN			
RCATGJY RGCGCJY TARCCA(11/9)J* J(7/8)TCACC* J(9/11)TCCGCC* J(9/11)TCCGT* J(7/8)TCTTC*	Bsp143II Tsol HphI Ecil TspGWI MbolI		NN NN			

Enzymes Generating Blunt Ends

Recognition	Enzyme
sequence	
AATATT	Sspl
AGC	Eco47III
AGUCT	Alul
AGGUCCT	Eco147I
AGTACT	Scal
ATTTJAAAT	Smil
CACUGTC	Ajil
CACUGTC	Eco72I
	Olil
	Pvull
	Msll
CCCJGGG	Smal
CCGCTC(-3/-3)↓*	Mbil
CGUCG	Bsh1236l
CMGJCKG	NspBII
GAANNUNNTTC	Pdml
GACGTG(-3/-3)	Ajil
GACONO NNGTC	BoxI
↓(5/5)GACTC*	Schl Mbil
GAGCGG(-3/-3)↓* GAG↓CTC	
	Ecl136II
GAGTC(5/5)↓* GAT↓ATC	Schl
GATNNUNNATC	Eco32I
GATNN INNATC Gm ⁶ A TC	BseJI
	Dpnl
	Srfl
GCNUNGC	Pdil Cac8l
GG↓CC	BsuRI
GGCJGCC	Ehel
GGN↓NCC	BspLI
GTJAC	Rsal
GTAJTAC	Bst1107I
GTN NAC	Hpy8I
GTY	Hincll
GTTAAC	KspAl
GTTT AAAC	Mssl
YAC↓GTR	Ppu21I
RGJCY	CviJI
RTGCJGCAY	FspAl
TACUGTA	Eco105I
TCGUCGA	Bsp68I
TGJCA	CviRI
TGCJGCA	Nsbl
TGGJCCA	MISI
TTAJTAA	Psil
TTTJAAA	Dral

Enzymes Cleaving DNA on Both Sides of Their **Recognition Sequence**

	Recognition sequence	Enzyme
	↓(8/13)AAG(N) ₅ CTT(13/8)↓	Fall
	\downarrow (10/15)AC(N) GTAYC(12/7) \downarrow	Bael
	↓(11/13)CAA(N)₅GTGG(12/10)↓	CspCl
	↓(8/13)CAC(N),TCC(12/7)↓	Tstl
	↓(6/11)CCAA(N),TTC(12/7)↓	Ajul
	↓(10/12)CCAC(N) ₅ TTG(13/11)↓	CspCl
	↓(10/12)CGA(N), ŤGC(12/10)↓	Bcgl
	\downarrow (7/12)GAA(N) ₇ TTGG(11/6) \downarrow	Ajul
	$\downarrow (7/12) \text{GAAC(N)}_{5} \text{CTC(13/8)} \downarrow$ $\downarrow (7/12) \text{GAAC(N)}_{6} \text{TAC(12/7)} \downarrow$	Ppil Psrl
	↓(7/12-13)GAAC(N),TCC(12-13/7)↓	Alol
	↓(8/13)GAG(N) ₅ CTC(13/8)↓	Bpll
	↓(8/13)GAG(N),GTTC(12/7)↓	Ppil
	↓(8/13-14)GAY(N)_VTC(13-14/8)↓	Hin4I
	↓(10/12)GCA(N),TCG(12/10)↓	Bcgl
	↓(10/12)GCA(N),TGC(12/10)↓	Alfi
lote	↓(7/12)GGA(N),GTG(13/8)↓	Tstl
Asymmetric sequences	↓(7/12-13)GGA(N),GTTC(12-13/7)↓	Alol
are indicated by "*".	↓(7/12)GRTAC(N),GT(15/10)↓	Bael
Fermentas are shown	↓(7/12)GTA(N),GTTC(12/7)↓ ↓(10/12)TGA(N),TCA(12/10)↓	Psrl Bdal
	(10/12/10A(11) 10A(12/10)↓	Duai

Bulk quantities & custom formulations available on request

in orange.



PureExtreme[™] Quality Guarantee

Fermentas restriction endonucleases are produced under the ISO9001:2000 quality management system, which combined with our own extensive quality control tests, guarantees consistent **PureExtreme[™] Quality** – the highest quality and performance – for the entire Fermentas product line. Fermentas restriction endonucleases pass all standard quality control assays, as well as our unique **Labeled Oligonucleotide (LO) test** which is the most sensitive test for the detection of trace activities of endodeoxyribonucleases, exodeoxyribonucleases and phosphatases. We monitor all enzyme lots to ensure they meet these stringent quality control specifications right up to their expiry date.

The PureExtreme[™] Quality of restriction enzymes ensures that the integrity of your DNA is not compromised making them the enzymes of choice for even the most demanding applications.

Activity Assay

One unit of restriction endonuclease is the amount of enzyme required to hydrolyze 1µg of substrate DNA in 60min in 50µl of reaction mixture under recommended conditions. To determine restriction endonuclease activity, concentrated enzymes are first diluted to approximately 500-1000 units/ml with enzyme dilution buffer (20mM potassium phosphate (pH 7.4), 200mM KCI, 1mM EDTA, 7mM 2-mercaptoethanol, 10% glycerol and 0.2mg/ml BSA).

In general, enzymes are assayed with λ phage DNA at 37°C.

However, some exceptions apply:

Some Fermentas restriction endonucleases

Quality Control

Labeled Oligonucleotide Test (LO)

The Labeled Oligonucleotide (LO) test is the most sensitive assay for the purity of restriction endonucleases. The assay allows the identification of trace contaminants (endodeoxyribonucleases, exodeoxyribonucleases and phosphatases) in restriction enzyme preparations that are missed by other assays. The 5'-[32P]-labeled synthetic oligonucleotides (single-stranded and doublestranded) used as substrates in the LO test are designed without recognition sites for the restriction enzymes. After these labeled oligonucleotides are incubated with an enzyme, denatured reaction products are separated on a polyacrylamide gel and then analyzed by phospho-imaging. The presence of contaminating other endodeoxyribonucleases or exodeoxyribonuclease results in the degradation of the oligonucleotides (see Fig. 1.1).

A decrease in the specific radioactivity of the test oligonucleotides indicates the presence of contaminant phosphatases. The restriction enzyme conforms to this quality criterion if there is no degradation of both the single-stranded oligonucleotide and the double-stranded oligonucleotide, and if there is no decrease in band intensity. show optimum activity at temperatures other than 37°C. Therefore, the optimum incubation temperature for each restriction endonuclease is provided under "Conditions for 100% Activity" in the restriction endonuclease description in the catalog as well as in Table 1.8. "Reaction Conditions for Restriction Endonucleases" (*see* p.124) and in Table 1.10. "Activity of Mesophilic and Thermophilic Enzymes at 37°C" (*see* p.130).

- Restriction endonucleases without recognition sites on λ DNA are assayed with another specific DNA substrate.
- Restriction endonucleases with only a few recognition sites on the λ DNA are assayed using λ DNA previously hydrolyzed with another restriction endonuclease.
- Restriction endonucleases sensitive to Dam or Dcm methylation are assayed with Lambda DNA (*dam⁻*, *dcm⁻*), #SD0021. For more detailed information regarding methylation effects, *see* pp.132-138.

Most restriction endonucleases are supplied at a user-friendly concentration of $10u/\mu$ l; a number of enzymes are also available at high concentration (HC) – $50u/\mu$ l.

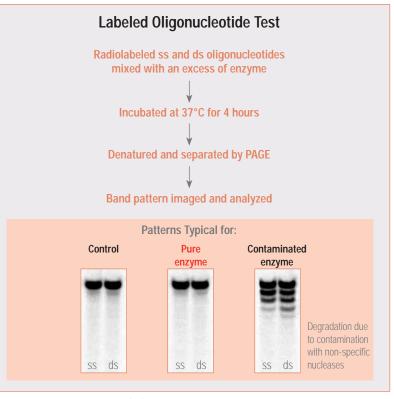


Figure 1.1. Labeled Oligonucleotide (LO) Test.

 $ss-{\rm single-stranded\ radiolabeled\ oligonucleotide}\quad ds-{\rm double-stranded\ radiolabeled\ oligonucleotide}\\ Pure\ enzyme-\ Fermentas\ Notl$

(continued on next page) Contaminated enzyme - competitor's Notl

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ISO ISO 9001 14001

Non-specific Nuclease and Cross-contamination Assay

Varying amounts of restriction endonuclease (2-20 units) are incubated for 16 hours with 1µg of substrate DNA under the recommended assay conditions. After electrophoretic separation of the DNA fragments, the characteristic banding patterns are examined for alterations. To pass the test, the restriction enzyme must yield an unaltered banding pattern under conditions of up to 160-fold overdigestion (10 units x 16 hours). For information regarding restriction enzyme star activity, *see* the product description or Certificate of Analysis supplied with each enzyme.

Ligation and Recleavage Assay

The ligation and recleavage assay tests the integrity of DNA ends. DNA fragments obtained after 2-, 10- and 50-fold overdigestion (units/µg of DNA x hours) are ligated with T4 DNA ligase and then recut with the same restriction endonuclease. Only DNA fragments with intact 5'- and 3'-termini are ligated by T4 DNA ligase, and only molecules with reconstructed recognition sites can then be cleaved by the same restriction

endonuclease. A restriction enzyme conforms to the quality criterion if the ligation efficiency of DNA fragments, generated by digestion with the restriction endonuclease, does not depend on the excess of enzyme used for the initial cleavage of DNA (*see* above).

The percentage of DNA that can be successfully ligated and then recleaved is presented for each restriction enzyme both in its catalog description and the Certificate of Analysis supplied with each enzyme.

Blue/White (B/W) Cloning Assay

The Blue/White cloning assay is designed to test the integrity of DNA ends. pUC57 DNA is digested at unique sites within the *lacZ* reporter gene with 10 units of a restriction enzyme in its optimal buffer. After a 16 hour incubation, the plasmid DNA is recircularized by ligation and transformed into *E.coli* XL1-Blue competent cells. The cells are then plated onto X-Gal/IPTG/Amp agar. An intact *lacZ* gene will give rise to a blue colony. If the termini of the linearized pUC57 are altered by contaminating exodeoxynucleases, the *lacZ* reading frame is interrupted, which results in the

1. RESTRICTION ENDONUCLEASES PureExtreme[™] Quality Guarantee

> appearance of white colonies. The higher the ratio of blue to white colonies, the higher the quality of restriction enzyme. An enzyme conforms to this quality criterion if the number of white colonies does not exceed 3%. Details of the assay are given in the Certificate of Analysis of each product. The test is applicable for enzymes recognizing unique sites within the *lacZ* reporter gene, and for those lacking recognition sites in pUC57. In the latter case, the assay is performed with the mixture of pUC57/HindIII, pUC57/Pstl and pUC57/Eco32I DNA fragments representing three different types of termini (3'-overhang, 5'-overhang and blunt ends).

All restriction endonucleases should be stored at -20°C. For Hin1II, storage at -70°C is recommended.

During shipment on dry ice, enzymes may freeze. This does not affect their quality because

all Fermentas enzymes are 100% active after at least three freeze-thaw cycles.

For 24-48 hour delivery, enzymes may be shipped on blue ice since their quality is not affected by short exposure to $+4^{\circ}$ C.



Guide to Properties of Restriction Endonucleases

Fermentas restriction enzyme icons are designed as a guide for the selection of optimal conditions for your restriction digestion.

B G O R Tango Unique	Five Buffer System. Letters in the buffer icon indicate the buffer recommended for each restriction enzyme. They correspond to the codes of the Five Buffer System: B (blue), G (green), O (orange), R (red), Tango [™] (yellow), respectively. Enzymes indicated by the "Unique" icon require a special buffer, which is supplied with the enzyme (<i>see</i> p.122).
DTT SAM Oligo	Additives. Indicated additives should be used in the 1X reaction mixture to obtain the stated activity. Solutions of S-adenosylmethionine and oligonucleotides are supplied in separate vials. DTT (#R0861) is available separately.
30° 37° 50° 55° 65°	Incubation Temperature. Indicates the optimal incubation temperature in °Celsius.
40% 60% 70% 80% 90% 95%	Ligation Efficiency. Indicates the ligation efficiency of DNA fragments generated by digestion with the restriction endonuclease (<i>see</i> conditions on p.21).
\bigstar	Star Activity. Restriction enzyme may exhibit star activity under the conditions described (<i>see</i> p.131).
Dam Dcm CG	Sensitive to Dam, Dcm or CpG Methylation. DNA cleavage by the restriction endonuclease is blocked or impaired by Dam, Dcm or CpG methylation of the target sequences (<i>see</i> pp.133-135).
Dam Dam CG	Sensitive to Overlapping Dam, Dcm or CpG Methylation. The target site may be methylated in certain sequence contexts (overlapping methylation). This will result in blocked or impaired DNA cleavage (<i>see</i> pp.133-138).
	Thermal Inactivation. Indicates thermal inactivation of the enzyme at 65°C or 80°C in 20min (<i>see</i> p. 123). Mindicates that only small amounts of restriction endonuclease (up to 10 units) can be inactivated at 80°C in 20min.
HC	High Concentration. Indicates that the enzyme is available at a high concentration (50 u/μ I).
X	Genome Qualified. Indicates that the restriction enzyme cleaves agarose-embedded DNA during megabase mapping of chromosomes. Handling of native DNA of this size in solution is often difficult due to double-stranded breaks introduced by mechanical DNA shearing. To avoid this problem, DNA is usually embedded into agarose plugs prior to digestion. Digested DNA is analyzed by pulsed field gel electrophoresis (PFGE).
	Recombinant Enzyme. Indicates that the restriction enzyme has been purified from recombinant <i>E.coli</i> designed to overexpress this enzyme.
	Blue/White Certified. Indicates that the restriction enzyme was tested by the Blue/White cloning assay (<i>see</i> p.21).
	FastDigest [™] Enzyme. Indicates the availability of a special formulation of the enzyme for fast digestion (<i>see</i> p.2).

Guide to Properties of Restriction Endonucleases

Classification of Restriction Endonucleases

Restriction endonucleases are enzymes that recognize specific nucleotide sequences and cleave DNA molecules precisely at a distinct position, either within or outside their recognition site, generating DNA with "sticky" ends (with 5'- or 3'-overhang) or "blunt" ends.

The phenomenon of host specificity was first observed by Luria and Human in the early 1950s (1). Nearly a decade later, Arber and Dussoix predicted its molecular basis (2). They proposed that host specificity is based on a two-enzyme system: a restriction enzyme, which recognizes specific DNA sequences and cleaves foreign DNA upon its entrance into the bacterial cell, and a modification enzyme (methyltransferase), which protects the host DNA from degradation by its own restriction endonuclease. Both restriction endonuclease and modification methyltransferase recognize the same nucleotide sequence and together they form a restriction-modification (R-M) system.

R-M systems have been classified into four types (I, II, III and IV), depending on the complexity of their structure, cofactor requirements and substrate specificity (3). Most characterized and frequently used restriction enzymes are type II. These restriction-modification systems are widespread among bacteria and have also been isolated from phages, Archaea and viruses of eukaryotic algae. These enzymes recognize specific 4-8 bp long DNA sequences. For most nucleotide sequences, more than one enzyme is known that recognizes that sequence. According to the nomenclature of restriction endonucleases, restriction enzymes with a unique specificity which have been discovered first are called prototypes. Subsequently discovered enzymes with the same specificity are called isoschizomers, which may differ in site preferences, reaction conditions, as well as in their sensitivity to methylation and star activity. To meet specific experimental goals, particular isoschizomers can be used. Restriction enzymes

that recognize the same nucleotide sequence, but cleave DNA at different positions, are called neoschizomers. Type II enzymes may cleave a DNA sequence either within the recognition site, or at a specified position up to 20 base pairs outside. Fermentas, a leading global manufacturer of restriction enzymes, currently offers 188 restriction enzymes.

References

4

- 1. Luria, S.E., Human, M.L., A nonhereditary, host-induced variation of bacterial viruses, J. Bacteriol., 64, 557-569, 1952
- 2. Arber, W., Dussoix, D., Host specificity of DNA producted by Escherichia Coli: I. Host controlled modification of bacteriophage lambda, J. Mol. Biol., 5, 18-36, 1962.
- 3 Roberts, R.J., et al., A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes, Nucleic Acids Res., 31, 1805-1812, 2003.

Product Entry Guide Bcul (Spel)

Tango 37º 80% 🗶 🔏 🕯 🕐 В G 0 R Tango 2X Tango 50-100 50-100 0-20 20-50 100 6 5'....**A C T A G T**....3' Concentration Ligation and Recleavage 3'...**T G A T C[^]A**...5' 10u/µl 8_{400u} 7 Conditions for 100% Activity #ER1251 1X Buffer Tango[™]. Supplied with: Methylation Effects 33mM Tris-acetate (pH 7.9 at 37°C), 10X Buffer Tango™ 10mM Mg-acetate, 66mM K-acetate and Dam: never overlaps - no effect. #ER1252 2000u 0.1mg/ml BSA. Dcm: never overlaps - no effect. Supplied with: Incubate at 37°C. CpG: never overlaps - no effect. 10X Buffer Tango™ Storage Buffer FastDigest[™] Bcul (see p.2) Bcul is supplied in storage buffer: #ER1254 50 reactions 10mM Tris-HCI (pH 7.5 at 25°C), 300mM KCI, Supplied with: 10X FastDigest[™] Buffer 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% glycerol. 10 11 Adenovirus-2 DNA in 16 hours. Ad2 DNA 0.7% agarose Note 12 Lambda ФХ174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 0 0 0 0 0 Restriction endonuclease. Its prototype. 7 Catalog number. 2 8 3 Icons. For more information see p.22. Relative enzyme activity (%) in Five Buffer System. 4

3

- Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour).
- NR buffer is not recommended, because of high star activity
- 5 Recognition sequence and the cleavage sites.

After 50-fold overdigestion with Bcul, more than 80% of the DNA fragments can be ligated and more than 90% of these can be recut.

0-20

Ad2

3

EcoKI: may overlap - effect not determined. EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 10 units of enzyme are required for complete digestion of 1µg of agarose-embedded

|--|

- 6 Electrophoretic pattern of cleavage products.
- Number of enzyme activity units in package.
- FastDigest[™] restriction endonuclease, see p.2. 9
- 10 DNA substrate and concentration of gel.
- Other important information about specific enzyme 11 features.
- 12 Number of recognition sites in the phage or plasmid DNA.

000000

O CO D



Aarl

Unique Oligo 37º 📆 😾 😋

В	G	0	R	Tango	2X Tango
NR	NR	0-20	0-20	NR	50-100

5'...C A C C T G C (N) \downarrow ...3' 3′...**G T** CCN #ER1581 Supplied 10X Buffe 10X Buffe 50X oligo #ER1582 Supplied 10X Buffe

GTGGA	CG(N) ₈ 15'	
ER1581 Supplied with:	25u	
10X Buffer Aarl 10X Buffer Tango™ 50X oligonucleotide	1ml 1ml 25µl	
ER1582	125u	
Supplied with: 10X Buffer Aarl 10X Buffer Tango [™] 50X oligonucleotide	1ml 1ml 2x25µl	

Concentration

1-3u/µl

λDNA

1.0% agarose

Conditions for 100% Activity

[1X Buffer Aarl] + oligonucleotide: [10mM Bis-Tris Propane-HCI (pH 6.5 at 37°C), 10mM MgCl₂, 100mM KCl, 0.1mg/ml BSA] + 1µM of oligonucleotide (see Note). Incubate at 37°C.

Storage Buffer

Aarl is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 100mM KCI, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 10-fold overdigestion with Aarl, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap – cleavage impaired (p.136).

EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

- For cleavage with Aarl at least two copies of its recognition sequence are required.
- Inclusion of 1µM oligonucleotide with the Aarl recognition sequence in the reaction mixture significantly improves cleavage of DNAs, especially of those with a single Aarl site. Still, a complete cleavage of some substrates with Aarl is difficult to achieve.
- Greater than 10-fold overdigestion with Aarl may result in star activity.
- Aarl may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
12	0	0	0	0	0	0	0	0	0	0

Aasl (Drdl)

B 37° 📆 🛧 🛯 🖉 🗡

5'...G A C N N N N N N G T C...3' 3'...**C T G N N¹N N N N C A G**...5'

Concentration

10u/µl

Conditions for 100% Activity

1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl₂ and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Aasl is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

370

After 10-fold overdigestion with Aasl, more than 95% of the DNA fragments can be ligated and recut

Activity in Five Buffer В 0 R Tango 2X Tango G 20-50 0-20 0-20 50-100* 100 0-20 appears at a greater than 5-fold overdigestion (5u x 1h). Methylation Effects

Dam: never overlaps – no effect. Dcm: never overlaps - no effect. CpG: may overlap – cleavage impaired (p.136). EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Greater than 10-fold overdigestion with AasI may result in star activity.

					roout.						
La	mbda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
	3	1	1	2	2	2	2	2	2	1	1

Recommended

DTT

Requires

DTT

www.fermentas.com/doubledigest

www.fermentas.com/research

Star

Activity



95% Ligation Efficiency

ISO ISO 9001 14001	1. RE	STRICTION ENDONUCLEASES Product Description
Aatll	Tango 37º 📆 CG 🏷 🗡	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 20-50 0-20 0-20 100 20-50
5'G A C G T↓C3' 3'C [↑] T G C A G5' #ER0991 300u <i>Supplied with:</i> 10X Buffer Tango [™] 1ml #ER0992 1500u <i>Supplied with:</i> 10X Buffer Tango [™] 1ml	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango™: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Aatll is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with AatII, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: may overlap – no effect (p.138). Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.
Lambda ΦX174 M13mp18/19 pBR32 10 1 0 1	22 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 1 1 0 0	pBluescriptIISK(-/+) pACYC177 pACYC184 0 1 0
Acci Fermentas enzy	yme <mark>Xmil</mark> , p.118	
AccII Fermentas enzy	/me <mark>Bsh1236I</mark> , p.45	
AccIII Fermentas enzy	yme Kpn2I (different sensitivity to methylation), p.80	
Acc65I (Kpnl*)	0 37° 📆 Den CG 🎘 🖌 🥯	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 20-50 100 20-50 20-50 50-100
* Unlike Kpnl, Acc65I produces DNA	Concentration	Ligation and Recleavage

* Unlike Kpnl, Acc65I fragments with a 4- $5'G \downarrow G T A C$ $3'C C A T G \uparrow$	base 5'-extension	5	ConcentrationLigation and Recleavage10u/µlAfter 50-fold overdigestion with AccConditions for 100% Activitymately 95% of the DNA fragments1X Buffer O:and recut.							
#ERO901 Supplied with: 10X Buffer O 10X Buffer Tango™	1000u 1ml 1ml		100mN Incubat		d 0.1mg/ml E).	°C), 10mM MgCl ₂ , 3SA.	Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – cleavage impaired (p.134). CpG: may overlap – cleavage impaired (p.136).			
#ER0902 Supplied with:	5000u		Acc65I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100				EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.			
10X Buffer O 10X Buffer Tango""	2x1ml 1ml	1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 0.7% agarose					Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours.			
$\begin{array}{c c} Lambda & \Phi X174 \\ \hline 2 & 0 \end{array}$	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
AccB7I	Fermen	-	Van91I , p.1	14				Ū		
Acil	Fermen	tas enzyme	Ssil , p.108							
Acll	Fermen	tas enzyme	Psp1406I,	p.98						
Acsl	Fermen	tas enzyme	Xapl , p.115	5						

Genome Qualified

Recombinant Enzyme

Fermentas enzyme Eco57I, p.64

Fermentas enzyme Hin1I, p.74

HC High Concentration

Thermal Inactivation

Acul

Acyl

CG Sensitivity to CpG Methylation

FastDigest" Enzyme

Bulk quantities & custom formulations available on request



Adel (Dral	/						B 0-20	G 0 100 20-50) 100 '	ango 2X Tai 100* 20-5
5' C A C N N 1 3' G T G^îN N 1			Сопсе 10и/µl	entration	1		<i>Meth</i> y Dam:	vity appears at a great /lation Effect never overlaps	ts – no effect.	digestion (5u x 1
#ER1231 Supplied with: 10X Buffer G 10X Buffer Tango™	500u 1ml 1ml		1X Buf 10mM	fer G: Tris-HCI (j	• 100% Act oH 7.5 at 37 0.1mg/ml B	°C), 10mM MgCl ₂ ,	<mark>CpG</mark> : EcoKI:	never overlaps may overlap – may overlap – may overlap –	cleavage imp effect not de	termined.
#ER1232 Supplied with: 10X Buffer G 10X Buffer Tango™	2500u 1ml 1ml λ DNA 0.7% agarose		Incuba Stora Adel is	te at 37°C ge Buffe supplied i	r in:	°C), 300mM KCl,	Minimu comple	tion of Agard um 10 units of t ete digestion of in 16 hours.	the enzyme a	re required
			1mM E 50% (\ <i>Ligati</i> After 1 80% o	1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. <i>Ligation and Recleavage</i> After 10-fold overdigestion with Adel, more than 80% of the DNA fragments can be ligated and more than 90% of these can be recut.				r than 20-fold sult in star acti		ion with <i>I</i>
Lambda ΦX174 10 1	M13mp18/19 1	pBR322 0	more ti pUC18/19 0	han 90% (pUC57 0	of these can pTZ19R/U 1	be recut. pBluescriptIIKS(-/+) 1	pBlue	escriptIISK(-/+) 1	pACYC177 1	pACYC18 0
Afal	Fermen	tas enzyme C	sp6l (diffe	erent cleav	/age positior) p.58 and <mark>Rsal</mark> , p.1	01			
Afel		tas enzyme E				<u>, 1 1</u>				
AfIII	Fermen	tas enzyme <mark>E</mark>	spTI , p.51	1						
Agel	Fermen	tas enzyme <mark>E</mark>	ishTi , p.46	6						
Ahalli	Fermen	tas enzyme E)ral , p.59							
Ahdl	Formon	tas enzyme E	am11051	n 60						

1 ///		
A	jil	(Btrl)

10X Buffer Ajil

10X Buffer Tango™

Unique 37º 7 CG 🏷 💰 🕷

Conditions for 100% Activity

Concentration

5u/µl

5'...C A C G T C...3' 3'...G T GC A G...5'

#ER1941	200u
Supplied with:	

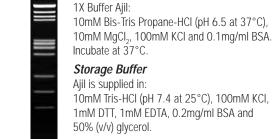
Recommended

DTT Requires

Tango Recons

1ml

1ml



 λ DNA

SAM Requires

0.7% agarose

Ligation and Recleavage

After 50-fold overdigestion with Ajil, approximately 80% of the DNA fragments can be ligated. No more than 50% of these can be recut due to the asymmetric recognition sequence of Ajil. The remaining uncleaved ligation products may be cut by Aatll and Eco72I (PmaCI).

D	G	0	ĸ	Tanyu	ZA Tanyu
NR	NR	20-50*		NR	20-50*
* Star ac	tivity appears a	it a greater th	nan 5-fold (overdigestio	n (5u x 1h).
Meth	ylation E	Effects			
Dam:	never over	erlaps –	no effec	ct.	
Dcm:	never over	erlaps –	no effec	ct.	
CpG:	complete	ly overla	ps – cle	eavage l	blocked
	(p.135).				
EcoKI:	may over	lap – eff	ect not	determi	ned.
EcoBI:	may over	lap – eff	ect not	determi	ned.
Minim compl	stion of a lum 5 uni ete digest A in 16 ho	ts of the ion of 1µ	enzym	ie is rec	quired for

0

R

Tango 2X Tango

G

Note

В

Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.

> Activity Star

Dam Sensitivity to Dam Methylation

				,	,		· · ·				
Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
17	0	Ō	0	0	0	0	0	0	0	0	
-											
 www.fer	mentas	.com www	v.termenta	s.com/doul	pledigest	www.ter	mentas.com/researd	ch			

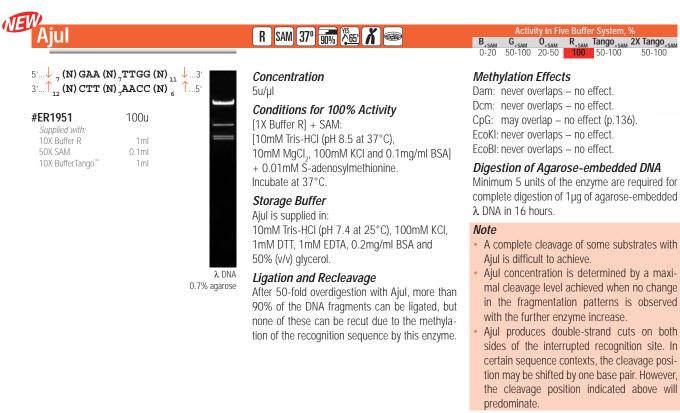
37⁰ Incubation

Temperature

95% Efficiency



Product Description



Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	1	0	0	0	0	0	0	0	1	0

Alel

Fermentas enzyme Olil, p.91

Alf

R SAM 37° 70% 555 X 🕯

$5'...\downarrow_{10}$ (N) GCA (N) ₆TGC (N) ₁₂ \downarrow ... 3' 3'...¹₁₂ (N) CGT (N) ACG (N) ¹⁰₁₀¹...5'

#	ER1801 Supplied win 10X Buffer F 50X SAM 10X BufferT	2	1ml 0.1ml 1ml	λ DNA 1.0% agarose	
	Lambda	ФХ174	M13mp18/19	pBR322	pUC18/
	22	0	1	3	0

Thermal

10 Inactivation

Sensitivity to

CG Sensitivity to CpG Methylation

		-

Concentration 1-3u/µl

Conditions for 100% Activity

X Buffer R] + SAM: 0mM Tris-HCI (pH 8.5 at 37°C), mM MgCl₂, 100mM KCl and 0.1mg/ml BSA] 0.01mM S-adenosylmethionine. cubate at 37°C.

orage Buffer

High

Concentration

HC

I is supplied in: mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, nM DTT, 0.1mM EDTA, 0.2mg/ml BSA and % (v/v) glycerol.

gation and Recleavage

ter 20-fold overdigestion with Alfl, approxiately 70% of the DNA fragments can be ated, but none of these can be recut due to e methylation of the recognition sequence by s enzyme.

B_{+SAM} G_{+SAM} 0_{+SAM} R_{+SAM} Tango_{+SAM} 2X Tango_{+SAM} 0-20 0-20 0-20 0-20 20-50

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap – no effect (p.136). EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

- A complete cleavage of some substrates with Alfl is difficult to achieve.
- Alfl concentration is determined by a maximal cleavage level achieved when no change in the fragmentation pattern is observed with the further enzyme increase.

pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 /19 1 1 3

Genome

Qualified

 \bigcirc

Enzyme

Certified



Alo

B 30° 🛲 😾 🕅 🏷 🥯

	Activit	y in Five	Buffer Sy	stem, %	
В	G	0	R	Tango	2X Tango
-20	0-20	0-20	100	20-50	100

Dam Sensitivity to Dam Methylation

5′↓ ₇ (N) G 3′↑ ₁₂₋₁₃ (N) C
#ER1491 Supplied with: 10X Buffer R 10X Buffer Tango
#ER1492 Supplied with: 10X Buffer R 10X Buffer Tango
Lambda ΦX

AIUI			K J	U° 70%			В	G	0	R	Tango	2X Tango		
5′↓ ₇ (N) GAAC 3′↑ ₁₂₋₁₃ (N) CTTC	C (N) ₆ TCC (N) ₁₂ S (N) ₆ AGG (N) ₇	-13↓3° ↑5	1-3u/µ		100% Acti	ivity	0-20 0-20 0-20 100 20-50 1 CpG: may overlap – cleavage impaired (p. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.							
#ER1491 Supplied with: 10X Buffer R 10X Buffer Tango™ #ER1492 Supplied with:	100u 1ml 1ml 500u		1X Buff 10mM 100mN Incubat	fer R: Tris-HCI (p	0H 8.5 at 37° 0.1mg/ml BS	°C), 10mM MgCl ₂ ,	 Alol side uniq 	bation at 3 produces s from the ue feature	s doub e interru e is a de	ole-strand upted reco legenerate	cuts ognitio e cleava	on both n site. Its age point		
10X Buffer R 10X Buffer Tango™	1ml 1ml	λ DNA	10mM 1mM D 50% (v <i>Ligati</i>)TT, 1mM I /v) glycero <i>on and R</i>	oH 7.4 at 25° EDTA, 0.2mg ol. Pecleavage	°C), 100mM KCI, /ml BSA and with Alol, approxi-	 on the 3' side of the recognition s or 13 nt away). The presence of SAM in the read results in incomplete cleavage with Greater than 10-fold overdigest may result in star activity. 					action mixture vith Alol. tion with Alol		
		0.7% agarose	mately and mo <i>Methy</i> Dam: 1	70% of the ore than 80 <i>Ilation Ef</i> may overla	e DNA fragm 0% of these (f fects	ents can be ligated can be recut. bt determined.	 Alol may remain associated with the cle DNA. This may cause DNA band sh during electrophoresis. To avoid an at DNA band pattern, use the 6X Loading I SDS Solution (#R1151) for sample prepa or heat the digested DNA in the preser SDS prior to electrophoresis. 				shifting atypical ng Dye & eparation			
Lambda ΦX174 7 0	M13mp18/19 1	pBR322 p 0	UC18/19 0	pUC57 0	pTZ19R/U 1	pBluescriptIIKS(-/+) 1	pBlue	e scriptIISK(·/+)	pACYC177 0	′рА	0 0		
Alul			Tango 3	70 95%	<u>\$</u> 2 65 '		В	Activity G	in Five I 0	Buffer Syst R		2X Tango		

				lange				В	G	0	R	Tango	2X Tango	
				_				50-100	0-20	0-20	0-20	100	20-50	
5′ A G C				Conce	entration	1		Ligation and Recleavage						
3′ ⊤ C[↑]G	A 5′			10u/µl				After 5	0-fold c	overdige	stion wit	h Alul,	approxi-	
#ER0011 Supplied with	h.	600u	_		i tions for fer Tango''	100% Act i ::	ivity	mately 95% of the DNA fragments ligated and recut.						
10X Buffer Ta		1ml				nte (pH 7.9 at	: 37°C),	Methy	lation	Effects				
#ER0012 Supplied with: 10X Buffer Tango™		3000u		-	10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA.				Dam: never overlaps – no effect. Dcm: never overlaps – no effect.					
		2x1ml	_	Incuba	Incubate at 37°C.					CpG: never overlaps – no effect.				
			Ē	Alul is 10mM 1mM [50% (v		n: pH 7.5 at 25 VI EDTA, 0.2r	°C), 50mM KCI, ng/ml BSA and	EcoKI: never overlaps – no effect. EcoBI: may overlap – blocked (p.138).						
			λ Di											
			1.4% agaro	26										
Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	scriptIISK	(-/+)	pACYC17	7 p/	ACYC184	
143	24	27	17	16	16	17	17		17		12		13	

Alwl Fermentas enzyme BspPI, p.50

DTT Requires DTT

Supporting ProductsDilution Buffer0.5M EDTA, pH 8.06X Loading Dye & SDS Solution	p.121 p.369 p.336	 Fermentas Restriction Endonucleases: PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com	rmentas co	m/doubledigest www.fermentas.com/research

37^o Incubation Temperature

95% Efficiency

Star Activity

SAM Requires SAM

1

Tango Recommended Buffer



Product Description

Altiv/211 (HgiAl)ImageAltivity in five buffer System. %AAAS.G.T.G.C.T.C. 3: 3:: CTA CGA G.G.S' T T TSourceConcentration 10u/µlUigition and Recleavage Altivity 1X Buffer 0: Some Mitch System. %Uigition and Recleavage Altivity in five Buffer System. %#ER0021500u Sogreted with 100 Buffer 3 and 100 Buffer 1 ango"Concentration 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoConcentration 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoConcentration 100 Buffer 1 ango 100 Buffer 1 angoUigit 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoUigit 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoUigit 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoSource 100 Buffer 1 ango 100 Buffer 1 ango 100 Buffer 1 angoUigit 100 Buffer 1 ango 100 Buffer 1 a	ISO ISO 9001 14001			1. RESTRICTION ENDONUCLEASE Product Descripti	
Since Trig C	Alw211 (HgiAI)		0 37º 📆 🎦	B G O R Tango 2X Tar	
Alw26l (BsmAl) Imp 37 mm 27 mm 20 mm 27 mm 20 mm	5' G T G C T ↓ C 3' 3' C ↑ A C G A G 5' T T *ER0021 5000 <i>Supplied with:</i> 10X Buffer O 1n 10X Buffer Tango™ 1n *ER0022 25000 <i>Supplied with:</i> 10X Buffer O 1n 10X Buffer O 1n 10X Buffer Tango™ 1n	nl nl nl nl λ DNA 1.0% agarose mp18/19 pBR322 p	10u/µl Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCI (pH 7.5 at 37°C), 10m 100mM NaCI and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Alw211 is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 300 1mM DTT, 0.1mM EDTA, 0.2mg/ml B 50% (v/v) glycerol. DUC18/19 pUC57 pTZ19R/U pBlue	After 50-fold overdigestion with Alw21I, me than 95% of the DNA fragments can be ligat and recut. nM MgCl ₂ , Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. DCpG: may overlap – no effect (p.136). EcoKI: may overlap – no effect (p.138). EcoBI: may overlap – effect not determined. SA and scriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184	ted 1
Concentration Ligation and Recleavage 10u/µl After 50-fold overdigestion with Alw26I, more than 95% of the DNA fragments can be ligated and recut. Supplied with: 1000u 10X Buffer Tango [™] 1ml ER0032 5000u Supplied with: 0.1mg/ml BSA. 10X Buffer Tango [™] 2x1ml Supplied with: 0.1mg/ml BSA. 10X Buffer Tango [™] 2x1ml				Activity in Five Buffer System, % B G O R Tango 2X Tar	
ER0031 1000u Supplied with: 1X Buffer Tango™: 10X Buffer Tango™ 1ml ER0032 5000u Supplied with: 10MM Mg-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and Dam: never overlaps – no effect. 0.1mg/ml BSA. Dcm: never overlaps – no effect. 10X Buffer Tango™ 2x1ml Storage Buffer EcoKI: never overlaps – no effect.				Ligation and Recleavage After 50-fold overdigestion with Alw26I, me	ore
10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 1.0% agarose	Supplied with: 10X Buffer Tango™ 1n ER0032 Supplied with:	nl u	1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Alw26I is supplied in:	and recut. Methylation Effects and Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.13 EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.	

Genome Qualified



Alw44I (<i>i</i>	чраці)		lango d	90%			B 50-100	G 100	0 0-20		ango 2X Tango 100 50-100	
5′ G↓T G C A 3′ C A C G T Î			Сопсе 10и/µl	entration			<i>Ligation and Recleavage</i> After 50-fold overdigestion with Alw44I, more than 90% of the DNA fragments can be ligated and more than 95% of these can be recut.					
#ER0041	1000u	-		itions for fer Tango™	100% Acti	ivity						
<i>Supplied with:</i> 10X Buffer Tango™	1ml	_	33mM	Tris-aceta	te (pH 7.9 at	,	Methylation Effects					
#ER0042			Mg-aceta /ml BSA.	te, 66mM K-	acetate and	d Dam: never overlaps – no effect. Dcm: never overlaps – no effect.						
Supplied with: 10X Buffer Tango™ 2x1ml			Incubate at 37°C.					CpG: may overlap – blocked (p.136).				
				ge Buffer is supplie				2		io effect (p.* - no effect.	138).	
		λ DI 0.7% agarc	10mM 1mM [50% (v	Tris-HCI (p	oH 7.5 at 25° VI EDTA, 0.2r	°C), 50mM KCI, ng/mI BSA and	Minimun	n 5 units e digesti	s of the		Ided DNA re required for ose-embedded	
Lambda ΦX174	4 M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	criptIISK(-/+)	pACYC177	pACYC184	
4 1	1*	3	3	3	2	2		2		1	0	

AlwNI	Fermentas enzyme Cail, p.54
Aor13HI	Fermentas enzyme Kpn2I, p.80
Aor51HI	Fermentas enzyme Eco47III, p.63

Ap	bal				B 3	7° 95% D	CG <u>765</u>	X 📾 🕐	B 100	Activity G 20-50	y in Five 0 0-20	Buffer Sys R 0-20	stem, % Tango 20-50	2X Tango 0-20
3'C ['] #ER1 <i>Supp</i> 10X	C C				10u/µl Condi 1X Bufi 10mM	fer B:	• 100% Acti oH 7.5 at 37°	vity °C), 10mM MgCl ₂	Dam: r Dcm: r CpG: r EcoKI: r		erlaps – lap – cl lap – cl erlaps –	- no effec leavage i leavage i - no effec	mpaireo mpaireo :t.	(p.134). (p.136).
<i>Sup</i> 10X	#ER1412 5x3000u Supplied with: 10X Buffer B 2x1ml 10X Buffer Tango™ 1ml				Incubat Storag Apal is	te at 37°C ge Buffei supplied i	;. r n:	°C), 50mM NaCl,	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.					
#ER1 Supp	FastDigest [™] Apal (see p.2) #ER1414 100 reactions Supplied with: 10X FastDigest [™] Buffer 1ml			λDN	1mM DTT, 1mM EDTA, 0.2mg/ml BSA and Note 50% (v/v) glycerol. Incubation at 30°C resulting activity. Ligation and Recleavage in activity. DNA After 50-fold overdigestion with Apal, more than					sults in a	2-fold	increase		
Lan	nbda	ФХ174	M13mp18/19	0.7% agarose	AILEI J			pBluescriptIIKS(-/+)	pBlue	escriptIISK	(-/+)	pACYC17	7 pA	CYC184
	1	0	0	0	0	1	0	1		1	<u>, , , , , , , , , , , , , , , , , , , </u>	0	I	0

DTT Requires DTT SAM Requires SAM **37**^o Incubation Temperature

www.fermentas.com/doubledigest www.fermentas.com/research



ISO ISO 9001 14001

Bulk quantities & custom formulations available on request

Blue/White Certified

FastDigest[™] Enzyme

Product Description	3

		6 9 9
ISO ISO 9001 14001	1. RESTRICTION ENDONUCLEASES	70
	Product Description	8. O
ApaLI	Fermentas enzyme Alw44I (different sensitivity to methylation), p.30	1
Apol	Fermentas enzyme Xapl, p.115	
Ascl	Fermentas enzyme SgsI, p.106	0
Asel	Fermentas enzyme Vspl, p.114	1
Aspl	Fermentas enzyme Psyl, p.100	
Asp700I	Fermentas enzyme PdmI, p.94	
Asp718I	Fermentas enzymes Acc651, p.25 and Kpn1 (different cleavage position and different sensitivity to methylation), p.80	
AspEl	Fermentas enzyme Eam1105I, p.60	
AspHI	Fermentas enzyme Alw21I, p.29	
Asul	Fermentas enzyme Cfr13I, p.56	
Asull	Fermentas enzyme Bsp119I, p.47	
Aval	Fermentas enzyme Eco881, p.66	
Avall	Fermentas enzyme Eco471, p.63	
Avall	Fermentas enzyme Mph1103I, p.85	
Avill	Fermentas enzyme Nsbl, p.91	
AvrII	Fermentas enzyme XmaJI, p.117	
Ball	Fermentas enzyme MISI, p.84	

BamHI		Unique 37º 痭 🔊 HC 🗡 📾 🕐	B G R Tango 2X Tango 20-50* 100 20-50 50-100* 100* 50-100
5′G↓G A T C C3′ 3′C C T A G [↑] G5′		<i>Concentration</i> 10u/µl	* Star activity appears at a greater than 5-fold overdigestion (5u x 1h). <i>Ligation and Recleavage</i> After 50-fold overdigestion with BamHI, more
#ER0051 4000u Supplied with: 2x1ml 10X Buffer BamHI 2x1ml 10X Buffer Tango™ 1ml #ER0052 5x4000u Supplied with: 10X Buffer BamHI 10X Buffer BamHI 4x1ml 10X Buffer BamHI 500000	à DNA	50u/µl, HC Conditions for 100% Activity 1X Buffer BamHI: 10mM Tris-HCl (pH 8.0 at 37°C), 5mM MgCl ₂ , 100mM KCl, 0.02% Triton X-100, 1mM 2-mercaptoethanol and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer BamHI is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 200mM NaCl, 1mM DTT, 0.1mM EDTA, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.	 than 95% of the DNA fragments can be ligated and recut. <i>Methylation Effects</i> Dam: completely overlaps – no effect (p.133). Dcm: may overlap – no effect (p.134). CpG: may overlap – no effect (p.136). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. <i>Digestion of Agarose-embedded DNA</i> Minimum 5 units of the enzyme are required for complete digestion of 1μg of agarose-embedded A DNA in 16 hours.
#ER0054 200 reactions Supplied with: 10X FastDigest [™] Buffer 1ml	0.7% agarose		<i>Note</i> Low salt, high glycerol (>5%) concentrations pH >8.0 or a large excess of enzyme may resu in star activity.
Lambda ΦX174 M13mp18/19	pBR322 p 1	UC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	pBluescriptIISK(-/+) pACYC177 pACYC184 1 1 1

Genome Qualified

HC High Concentration

Recombinant Enzyme

Thermal Inactivation

CG Sensitivity to CpG Methylation



Baul (Bsil)			Tango 3	7° 95% 2	<u>65</u> X		B 0-20	G	Five Buffer S 0 R 0-20 50-100	Tang	o 2X Tango
Y C IACGAG YGTGCTÎC F ER1841 Supplied with: 10X Buffer Tango™	-		10u/µl Condi 1X Bufi 33mM 10mM 0.1mg/ Incubat Storag Baul is	fer Tango [™] Tris-aceta Mg-aceta (mI BSA. te at 37°C ge Buffer supplied i	100% Acti ': te (pH 7.9 at te, 66mM K- n:	37°C), acetate and	After 50- 95% of recut. Dam: ne Dcm: ne CpG: cc EcoKI: m EcoBI: m	fold overo the DNA t ever overla ever overla ompletely ay overlap ay overlap	aps – no eff aps – no eff overlaps – r o – effect no o – effect no	ect. ect. ect. no effec ot deterr ot deterr	ligated and t. mined. mined.
			1mM D)TT, 1mM		°C), 100mM KCl, 6 Triton X-100, alvcerol	Minimun	n 5 units o	of the enzyn	ne are r	
		λ DNA 0.7% agarose	0		10 3070 (079)	9.900.01		16 hours		5	
<u>Lambda</u> Φ Χ174 8 2	M13mp18/19 0	0.7% agarose	0	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir		S.	0	p ACYC184 1
	0	0.7% agarose pBR322	р UC18/19 3	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	
8 2	o Ferment	0.7% agarose pBR322 3 as enzyme E	pUC18/19 3 hel (differe	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	
⁸ ²	0 Ferment Ferment	0.7% agarose pBR322 3 as enzyme E as enzyme E	pUC18/19 3 hel (differ co721, p.6	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	
8 2 Bbel BbrPl	0 Ferment Ferment Ferment	0.7% agarose pBR322 3 as enzyme E as enzyme E as enzyme B	pUC18/19 3 hel (differ co721, p.6 spil, p.38	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	
8 2 Bbel BbrPl Bbsl	0 Ferment Ferment Ferment Ferment	0.7% agarose pBR322 3 as enzyme E as enzyme E as enzyme B as enzyme P	pUC18/19 3 (hel (differ (co721, p.6 (co721, p.38) (co721, p.92)	pUC57 3 ent cleava	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	
8 2 Bbel BbrPl Bbsl Bbul	o Ferment Ferment Ferment Ferment Ferment	0.7% agarose pBR322 3 as enzyme E as enzyme E as enzyme B	pUC18/19 3 (hel (differentiation) (co72I, p.6 (co72I, p.38 (co72I, p.38) (co72I, p.38) (co72I, p.38) (co72I, p.44)	pUC57 3 ent cleava	pTZ19R/U 2	pBluescriptIIKS(-/+)	λ DNA ir	n 16 hours	5. +) pACYC	0	

Bcll			G S	5° 95%)	🛧 Dam 🔊		B G O		ingo 2X Tango
5′ T↓G A T C 3′ A C T A G ↑			Сопсе 10и/µl	entration			20-50 100 20-5 * Star activity appears at a grea Ligation and Rector After 10-fold overdig	ter than 5-fold overd eavage estion with Bo	cll, more than
#ER0721 Supplied with:	1000u		<i>Condi</i> 1X Buf		100% Act	ivity	95% of the DNA frag recut.	gments can b	e ligated and
10X Buffer G 10X Buffer Tango™	1ml 1ml		50mM	NaCl and	0.1mg/ml B	°C), 10mM MgCl ₂ , SA.	Methylation Effect Dam: completely over		<mark>ed</mark> (p.133).
#ER0722 Supplied with:	5000u			te at 55°C ge Buffe i			Dcm: never overlaps CpG: never overlaps		
10X Buffer G 10X Buffer Tango™	2x1ml 1ml		Bcll is	supplied ir	1:	°C), 100mM KCI,	EcoKI: never overlaps EcoBI: may overlap –		38).
		λ DNA (<i>dam⁻</i>) 0.7% agarose	1mM E 50% (v		EDTA, 0.2m	g/ml BSA and	 Note Incubation at 37°C Greater than 15-f may result in start Assayed using λ E 	old overdiges activity.	tion with Bcll
Lambda Φ X174 8 0	M13mp18/19 0	pBR322 0	pUC18/19 0	pUC57	pTZ19R/U 0	pBluescriptIIKS(-/+) 0	pBluescriptIISK(-/+) 0	pACYC177 0	pACYC184
www.fermenta	is.com www	v.fermentas	.com/dou	bledigest	www.fer	rmentas.com/resea	ırch		

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

SAM Requires SAM

DTT Requires DTT

1

Tango Recommended Buffer

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

		Product Description	A
Bcnl (Caull)	Tango 37º 80% CG) 555	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 50-100 50-100 50-100 100 50-100	1
G 5'C C↓C G G3' 3'G G G↑C C5' C #ER0061 1000u Supplied with: 10X Buffer Tango™ 1ml	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.	<i>Ligation and Recleavage</i> After 10-fold overdigestion with Bcnl, more than 80% of the DNA fragments can be ligated in a reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments. More than 95% of these can be recut. <i>Methylation Effects</i>	
#ER0062 5000u Supplied with: 10X Buffer Tango™ 2x1ml	Incubate at 37°C.Storage BufferBcnl is supplied in:10mM potassium phosphate (pH 7.4 at 25°C),200mM NaCl, 1mM EDTA,7mM 2-mercaptoethanol, 0.2mg/ml BSA andλ DNA50% (v/v) glycerol.	 Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – cleavage impaired (p.135). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. 	
Lambda ΦX174 M13mp18/19 114 1 4	x bitA 50.76 (V/V) grycerol. 1.4% agarose	+) pBluescriptIISK(-/+) pACYC177 pACYC184 6 6 10	
	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+	6 6 10 Activity in Five Buffer System, % B G O R Tango 2X Tango	
114 1 4 Bcul (Spel) 5'a↓c t a g t3' 3't g a t cîa5'	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 10 7 7 5 6 Tango 37° 80% ∑ ∑ ∞ ∑ Concentration 10u/µl Conditions for 100% Activity	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 50-100 0-20 20-50 100 0-20 Ligation and Recleavage After 50-fold overdigestion with Bcul, more than 80% of the DNA fragments can be ligated and Bcul Dual	
114 1 4 Bcul (Spel) 5'а↓стаст3'	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 10 7 7 5 6 Tango 37° 100 100 100 100 100 100 1000 1000 100	6610Activity in Five Buffer System, %BG0RTango2X Tango50-10050-1000-2020-501000-20Ligation and RecleavageAfter 50-fold overdigestion with Bcul, more than	







HC High Concentration

Genome Qualified





G SAM 30° 큤 났 Dam 🏂

 Activity in Five Buffer System, %

 B_{+SAM}
 G_{+SAM}
 O_{+SAM}
 R_{+SAM}
 Tango_{+SAM}
 2X Tango_{+SAM}

 NR
 100
 0-20
 20-50
 50-100*
 50-100

5′↓ ₁₀ (N) TGA (I 3′↑ ₁₂ (N) ACT (I #ER1961 <i>Supplied with:</i> 10X Buffer G 50X SAM 10X BufferTango™	x) ₆TCA (N) ₁ x) ₆AGT (N) ₁ 50u 1ml 0.1ml 1ml	. ² ↓3' 10↑5'	Concentration 1-3u/µl Conditions for 100% Activity [1X Buffer G] + SAM: [10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ , 50mM NaCI and 0.1mg/ml BSA] + 0.05mM S-adenosylmethionine. Incubate at 30°C.	NR 100 0-20 20-50 50-100* 50-100 * Star activity appears at a greater than 5-fold overdigestion (5u x 1h). Methylation Effects Dam: may overlap – blocked (p.133). Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Mote • Incubation at 37°C results in 30% activity.
		λ. DNA (<i>dam-</i>) 0.7% agarose	Storage Buffer Bdal is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 10-fold overdigestion with Bdal, more than 70% of the DNA fragments can be ligated, but none of these can be recut due to the methyla- tion of the recognition sequence by this enzyme.	 Requires S-adenosylmethionine for activity. A complete cleavage of some substrates with Bdal is difficult to achieve. Bdal concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase. Bdal produces double-strand cuts on both sides of the interrupted recognition site. In certain sequence contexts, the cleavage position may be shifted by one base pair. However, the cleavage position indicated above will predominate. Greater than 40-fold overdigestion with Bdal may result in star activity. Bdal may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis. Assayed using λ DNA (<i>dam</i>⁻) (#SD0021).
Lambda ΦΧ174 28 4	M13mp18/19 7	pBR322 pl 4	JC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 2 2 2 2 2) pBluescriptIISK(-/+) pACYC177 pACYC184 2 3 6

Bfal

Tango Recommended Buffer

Fermentas enzyme FspBI, p.72

SAM Requires SAM

DTT Requires DTT

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com www.f	ermentas.co	om/doubledigest www.fermentas.com/research

37^o Incubation Temperature

95% Ligation Efficiency

Star Activity

Dam Sensitivity to Dam Methylation

ISO ISO 9001 14001

CG Sensitivity to CpG Methylation

Thermal Inactivation

1. RESTRICTION ENDONUCLEASES

G

Bulk quantities & custom formulations available on request Blue/White

Certified

FastDigest[™] Enzyme

B

Activity in Five Buffer System, %

R

0

Product Description

Tango 2X Tango

0000

5' A C T G G G 3' T G A C C C #ER1591			1-3u/µ Cond		100% Acti	ivity	<i>Ligation and Recleavage</i> After 10-fold overdigestion with Bfil, more than 40% of the pBR322 DNA fragments can be ligated and more than 90% of these can be
Supplied with: 10X Buffer Tango™ 3X Bfil Stop Solution #ER1592 Supplied with	1ml 0.5ml 250u		33mM 10mM 0.1mg	Tris-aceta	te (pH 7.9 at te, 66mM K-		recut. <i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect.
Supplied with: 10X Buffer Tango™ 3X Bfil Stop Solution	1ml 0.5ml		Termi Do no	ination of t attempt	Digestion to stop a	Reaction digestion reaction If no further enzy-	CpG: never overlaps – no effect. EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined.
		λ DNA 0.7% agarose	require Bfil St incuba DNA is tions, 20min 3X Bf (Warm neous 0.6% S	d, use Sto op Solutior ting at 65 s to be us inactivate I il Stop So up, to ens before use	pp Solution to termina °C for 10m ed in down- Bfil by incut olution (sup ure the solut .)	digested DNA are containing SDS or te the reaction by in. If the digested -stream manipula- bating at 65°C for <i>applied with</i>) ion is homoge- nol blue and	 Note Bfil is the only known restriction endonuclease that cleaves DNA specifically both in the presence and absence of Mg²⁺ ions in the reaction mixture (Sapranauskas, R., et al., J.Biol.Chem., 275, 30878-30885, 2000). Chelating of Mg²⁺ ions by EDTA does not inhibit Bfil activity and may cause non-specific products. The non-specific cleavage increases at temperatures over 37°C. Greater than 40-fold overdigestion with Bfil may result in star activity.
			Bfil is s 10mM 1mM [: H 7.5 at 25' / EDTA, 0.2r	°C), 100mM KCI, ng/mI BSA and	
Lambda Φ X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+) pACYC177 pACYC184

Tango 37º 👬 😾 🏷

Bfml (Sfel) Img 37 m Img 37 m	Bfml (Sfel			Tanno	70 💻 🗏	<u>865'</u>					Buffer Sy		
5 [·] C↓T Pu Py A G3 [·] 3 [·] G A Py Pu T [·] C5 [·] #ER1161 2000 Supplied with: 10X Buffer Tango [™] 1ml #ER1162 10000 Supplied with: 10X Buffer Tango [™] 1ml ADNA 1.0% agarose A functions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37 °C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37 °C. Storage Buffer Bfml is supplied in: 10mM Tris-HCl (pH 7.5 at 25 °C), 100mM KCl, 1md DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. ADNA 1.0% agarose DCS7 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIIKS(-/+) pACYC177 pACYC184)			95%	00		_	-	-			
#ER1161 200u Supplied with: 1x Buffer Tango™: x3mM Tris-acetate (pH 7.9 at 37°C), Methylation Effects 10X Buffer Tango™ 1ml 33mM Tris-acetate (pH 7.9 at 37°C), Dam: never overlaps – no effect. Supplied with: 1000u 0.1mg/ml BSA. Dcm: never overlaps – no effect. 10X Buffer Tango™ 1ml Iml Dam: never overlaps – no effect. Supplied with: CpG: never overlaps – no effect. CpG: never overlaps – no effect. 10X Buffer Tango™ 1ml Iml Iml Note Incubate at 37°C. CpG: never overlaps – no effect. Storage Buffer Bfml is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, EcoBI: never overlaps – no effect. 10M Tris-HCl (pH 7.5 at 25°C), 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and Dow salt, high glycerol (>5%) concentrations, 50% (v/v) glycerol. Note Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity. 1.0% agarose pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184					entration			Ligation and Recleavage					
10X Buffer Tango [™] 1ml 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Methylation Effects Supplied with: 10X Buffer Tango [™] 1ml 1ml Iml John Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Dam: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. Storage Buffer Bfml is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Note Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity. Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIIKK(-/+) pACYC177 pACYC184		200u					ivity		of the DN	IA fragn	nents ca	in be liç	gated and
#ERT162 1000u Supplied with: 10X Buffer Tango [™] 10X Buffer Tango [™] 1ml 0.1 mg/ml BSA. Incubate at 37°C. Storage Buffer Bfml is supplied in: 10MM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, EcoBI: never overlaps – no effect. 10MM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, Note 10MM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, Note 10MM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. NDNA 1.0% agarose		1ml						-					
Storage banch Bfml is supplied in: EcoBl: never overlaps – no effect. Bfml is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, Note 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and Low salt, high glycerol (>5%) concentrations, 0% (v/v) glycerol. pH >8.0 or a large excess of enzyme may result 1.0% agarose in star activity.	Supplied with:			0.1mg Incuba	/ml BSA. te at 37°C		acetate and	Dcm: CpG:	never over over over	erlaps – erlaps –	- no effe - no effe	ct. ct.	
10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Note λ DNA 1.0% agarose 1.0mA Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Note Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184					-								
Lambda Φ X174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184	λ			10mM 1mM [50% (v	Tris-HCI (p)TT, 0.1mN	oH 7.5 at 25 VI EDTA, 0.21		Low s pH >8	.0 or a la				
			1.0% agaros	e									
38 6 / 4 4 6 6 6 1 1		M13mp18/19	pBR322					pBlue	escriptIISK	(-/+)	pACYC1	77 p/	ACYC184
	38 6	/	4	4	4	Ō	0		O		I		I

Genome Qualified

HC High Concentration

Recombinant Enzyme

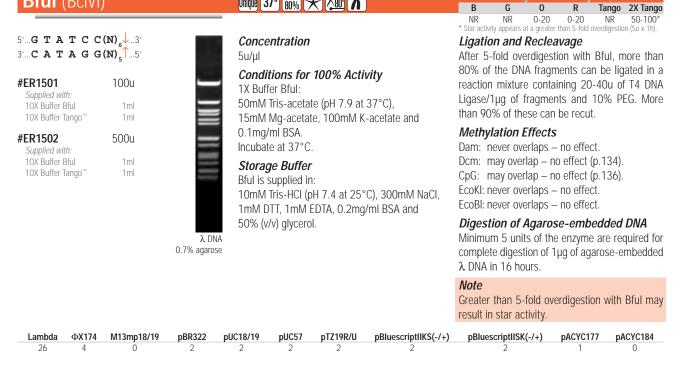
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Bful (BciVI)

Unique 37º 👬 🛧 🦉 🗡



Activity in Five Buffer

Tango 2X Tango

BfuAl	Fermentas enzyme Bvel, p.54
BfuCl	Fermentas enzymes Bsp143I , p.48, DpnI (different cleavage position and different sensitivity to methylation), p.59 and MboI (different sensitivity to methylation), p.83

Bgll			0 37	⁷⁰ 95% C	<u>165</u>		B 0-20	Activity in IGC50-10010		Tango 2X	(Tango 100
3′C G G N [↑] N #ER0071	n n↓n g g c n n n c c g 2000u	-	10u/µl		100% Acti	vity	After 5	ion and Rec 50-fold overdi of the DNA fra	gestion with		
Supplied with: 10X Buffer O 10X Buffer Tango [™] #ER0072	1ml 1ml 5x2000u		50mM 1 100mM	ris-HCI (p	0.1mg/ml B	C), 10mM MgCl ₂ , ISA.	Dam:	ylation Effe never overlap may overlap	s – no effec		
Supplied with: 10X Buffer O 10X Buffer Tango™	2x1ml 1ml		Bgll is s	e Buffer upplied in ris-HCI (p	:	C), 300mM NaCl,	EcoKI:	may overlap never overlap never overlap	os – no effec	t.	.136).
		λ DNA 0.7% agarose	1mM D		1 EDTA, 0.2m	ng/ml BSA and	Minim	stion of Aga um 5 units of ete digestion of in 16 hours.	the enzyme	are requir	red for
$\begin{array}{c c} Lambda & \Phi X12 \\ \hline 29 & 0 \end{array}$	14 M13mp18/19	pBR322 pU	C18/19	pUC57	pTZ19R/U 2	pBluescriptIIKS(-/+)	pBlu	escriptIISK(-/+)	pACYC17 1	7 pACYC	
www.ferment			om/doub equires	ledigest		mentas.com/resea		Star Activity	meni	Sensitivity to Dam Methyla	

ISO	ISO
9001	14001

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

1. RESTRICTION ENDONUCLEASES

Product Description

O e P

6 0 1 0	Dyili			UJJ	95%	2 X 📼		B 0-20	G 20-50	0 R 100 50-1		2X Tan g 100
Supplied with: 1X Buffer O: Inti 10X Buffer O 1ml 10X Buffer Tango ^m 1ml 10X Buffer Tango ^m 1ml #ER0082 2500u Supplied with: 10X Buffer Tango ^m 10X Buffer O 1ml 10X Buffer Tango ^m 1ml 10X Buffer O 1ml 10X Buffer Tango ^m 1ml 10X Buffer O 1ml 10X Buffer Tango ^m 1ml 10X FastDigest ^m Buffer 1ml 0.7% agarose 1ml 10X FastDigest ^m Buffer 1ml <	3' T C T A GA	5′	_	10u/µl <i>Condit</i>	ions for		vity	After 5 95% o	0-fold ove	erdigestion v	vith BgIII,	
Supplied with: 10X Buffer Tango™ 1ml Storage Buffer Bglll is supplied in: 10X Buffer Tango™ 1ml Storage Buffer Bglll is supplied in: 10mM Tris-HCl (pH 8.2 at 25°C), 200mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol. CpG: never overlaps – no effect. EcoBI: may overlap – cleavage impaired (p.* #ER0084 50 reactions Supplied with: 10X FastDigest™ Buffer 1ml A DNA 0.7% agarose A DNA DNA DNA Digestion of Agarose-embedded DN/ Minimum 5 units of the enzyme are require complete digestion of 1µg of agarose-embedded A DNA in 16 hours. Lambda ФX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptilKS(-/+) pBluescriptilKS(-/+) pACYC177 pA	Supplied with: 10X Buffer O 10X Buffer Tango™	1ml 1ml		50mM 1 100mM	ris-HCI (NaCI an	d 0.1mg/ml E		<i>Methy</i> Dam:	completel	y overlaps –		(p.133)
FastDigest™ BgIII (see p.2) Torini instrict (pri 0.2 at 2.3 c), Zoomin (ct, 10, 2 at	Supplied with: 10X Buffer O	1ml		Storag BgIII is s	e Buffe supplied i	r n:	C) 200mM KCl	CpG: EcoKI:	never over never over	rlaps – no e rlaps – no e	ffect. ffect.	ed (p.13
6 0 1 0	#ER0084 50 re Supplied with:	eactions		1mM D	 TT, 0.1ml	vi EDTA, 0.5r		Diges Minimu comple	<i>tion of A</i> um 5 units ete digestie	garose-ei s of the enzy on of 1µg of	nbedded /me are re	1 DNA equired 1
Bini Fermentas enzyme XmaJI, p.117		M13mp18/19 1						pBlue				ACYC184 0
	Binl	Ferment	as enzyme <mark>Bs</mark>	pPI , p.50								
	Blnl	Ferment	as enzyme Xn	naJI , p.11	7							
BIPI Fermentas enzyme Bpu1102I, p.40	Blpl	Ferment	as enzyme <mark>Bp</mark>	o u1102I , p	.40							
	Bme1390	(ScrFI)		0 37	7º 80%	CG A	0	B 20-50	Activity G 50-100	0 R	Tango	o 2X Tar
B G O R lango 2X	5′ C C↓N G G 3				ntration				on and F			

3'G G N C C #ER1421 Supplied with: 10X Buffer O 10X Buffer Tango™	5' 500u 1ml 1ml	1X Bu 50mN	litions for ffer O: 1 Tris-HCI (p	° 100% Acti oH 7.5 at 37° d 0.1mg/ml E	°C), 10mM MgCl ₂ ,	After 50-fold overd more than 80% of the ligated in a reaction of T4 DNA Ligase/1 PEG. More than 90%	he DNA fragr mixture conta ug of fragme	nents can be iining 20-40u nts and 10%
#ER1422 Supplied with: 10X Buffer O 10X Buffer Tango [™]	2500u 1ml 1ml	Incuba Stora Bme1 10mM	ate at 37°C I ge Buffer 3901 is sup 1 Tris-HCI (p	plied in: pH 7.5 at 25°	°C), 200mM KCl, ng/ml BSA and	Methylation Effect Dam: never overlaps Dcm: may overlap – CpG: may overlap – EcoKI: never overlaps	 no effect. blocked (p.13 blocked (p.13 no effect. 	•
Lambda Φ Χ174 184 3	M13mp18/19	50% (λ DNA (<i>dcm</i> ⁻) 1.4% agarose pBR322 pUC18/19 16 12	v/v) glycero pUC57	p TZ19R/U 10	pBluescriptIIKS(-/+)	EcoBI: never overlaps Note Assayed using λ DNA pBluescriptIISK(-/+)		00021). pACYC184 22

Bme1508I	Fermentas enzyme BseSI, p.44
BmgBl	Fermentas enzyme Ajil, p.26
Bmrl	Fermentas enzyme Bfil, p.35
Bmtl	Fermentas enzyme Nhel (different cleavage position), p.89
Bmyl	Fermentas enzyme Sdul, p.105
-	

Genome Qualified

Recombinant Enzyme

Bulk quantities & custom formulations available on request Blue/White Certified

FastDigest[™] Enzyme

Correct



5'...GACNNVNNGTC...3'

3'...C T G N N N N C A G...5'

500u

1ml

Boxl (PshAI)

#ER1431

Supplied with:

10X Buffer Tango™

Tango 37º 50% 🔀 🧏 🖉 🐼

	Activit	y in Five	Buffer Sys	stem, %	
В	G	0	R	Tango	2X Tango
0.20	0.20	0.20	20.50	100	20.50

Concentration

 λ DNA

1.0% agarose

10u/µl

Conditions for 100% Activity 1X Buffer Tango[™]:

33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Boxl is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 50-fold overdigestion with BoxI, more than 90% of the DNA fragments can be ligated and more than 95% of these can be recut.

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect.

CpG: may overlap - cleavage impaired (p.136). EcoKI: never overlaps - no effect.

EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Incubation at 30°C results in a 2.5-fold increase in activity.

 Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	1	0	1	0	0	0	0	0	0	2

Bpil (Bbvll)		G 3	7° 95% 2	<u>165</u> X @		Act B G 20-50 100	ivity in Five 0 50-100	R	item, % Tango 50-100	2X Tango 50-100
5' G A A G A C 3' C T T C T G #ER1011 <i>Supplied with:</i> 10X Buffer G			10u/µl Condi 1X Buff	er G:	⁻ 100% Acti	ivity °C), 10mM MgCl _a ,	<i>Methylatio</i> Dam: never Dcm: never CpG: may o EcoKI: never	overlaps - overlaps - verlap – n overlaps -	- no effec - no effec 10 effect (j - no effec	t. p.136). t.	
10X Buffer Tango [™] #ER1012 <i>Supplied with:</i> 10X Buffer G 10X Buffer Tango [™]	1ml 1000u 1ml 1ml		50mM Incubat Storag Bpil is s	NaCI and e at 37°C ge Buffei supplied in	0.1mg/ml BS 2. r n:		EcoBI: may o Digestion o Minimum 5 to complete dig λ DNA in 16	of Agaros units of the estion of 1	se-embe e enzyme	e dded are rec	DNA quired for
		λ DNA 0.7% agarose	50% (v. Ligatic After 50	/v) glycero on and R D-fold ove	ol. Recleavage erdigestion wi	I/ml BSA and ith Bpil, more than can be ligated and	<i>Note</i> Bpil cleaves and can ger hangs. This product cloni	erate any feature i	desired s useful	4 base	5'-over-
Lambda ΦX174 24 3	M13mp18/19 0	pBR322 p 3	recut. UC18/19 0	pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 0	pBluescriptl 0	ISK(-/+)	pACYC17 1	7 pA	CYC184 3

Supporting Products		Fermentas Restriction Endonucleases:
Dilution Buffer	p.121	 PureExtreme[™] Quality & Performance
0.5M EDTA, pH 8.0 (V Looding Due & SDS Solution	p.369	 Supplied with 10X concentrated buffers:
6X Loading Dye & SDS Solution	p.336	 – color-coded optimal buffer (one of B, G, O, R, Tango[™])
		 – universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer
		– REsearch [™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
	montoo	na (daubladigaatuuuu farmantaa aan (raaaarab

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www.fermentas.com Tango Buffer Recommended

DTT Requires

DTT

SAM

www.fermentas.com/doubledigest www.fermentas.com/research SAM Requires

37 ^o Incubation Temperature	95% Ligation Efficiency	Star Activity
l'emperature	(90%) Efficiency	



ISO ISO 9001 14001

Bpl

1. RESTRICTION ENDONUCLEASES

Activity in Five Bu

G_{+SAM} 20-50

В_{+SAM} 0-20 Product Description



5′↓ ₈ (N) G A 3′↑ ₁₃ (N) C T	G(N) ₅ CT C(N) ₅ GA	C (N) ¹³ ↓3′ G (N) ⁸ ↓5′
#ER1311 Supplied with: 10X Buffer Tango™ 50X SAM	100u 1ml 0.1ml	
#ER1312 Supplied with: 10X Buffer Tango™ 50X SAM	500u 1ml 2x0.1ml	
		λ DNA 0.7% agarose

Tango SAM 37º 7777 🎦 🔏 🐼

Concentration 5u/µl

Conditions for 100% Activity

[1X Buffer Tango[™]] + SAM: [33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA] + 0.05mM S-adenosylmethionine. Incubate at 37°C.

Storage Buffer

Bpll is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 10-fold overdigestion with Bpll, more than 70% of the DNA fragments can be ligated but, none of these can be recut due to the methylation of the recognition sequence by this enzyme.

Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.136). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.

Digestion of Agarose-embedded DNA

Minimum 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

- Bpll requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine.
 0.05mM S-adenosylmethionine gives more than a 100-fold increase in Bpll activity. Still, a complete cleavage of some substrates with Bpll is difficult to achieve.
- Bpll concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	Ö	0	0	0	0	0	0	0	0

Bpml

5′ 3′

#

#

Fermentas enzyme Gsul, p.73

Bpu10I			Unique 37	° 90% (В	G	0		Tango	2X Tango
5' C C↓ T N A G 3' G G A N T [↑] C #ER1181 Supplied with: 10X Buffer Bpu10I 10X Buffer Tango [™]			1X Buffe 10mM E 10mM N	ions for er Bpu10 lis-Tris P AgCl ₂ , 1(r 100% Activ II:	H 6.5 at 37°C),	Methy Dam: Dcm: CpG: EcoKI: EcoBI:	vity appears /lation never ov never ov may over never ov	Effects erlaps – erlaps – rlap – no erlaps –	than 5-fold ov	.136).	100* n (5u x 1h).
#ER1182 Supplied with: 10X Buffer Bpu10I 10X Buffer Tango [~] "	1000u 1ml 1ml	λ DNA 0.7% agarose	1mM DT 50% (v/v <i>Ligatio</i> After 50 than 90 be ligate 40u of 10% PE recut du of Bpu1	at 37°C e Buffe s supplie ris-HCl (j T, 0.1ml J) glycere m and K b-fold ov % of the ed in a r T4 DNA G. No m te to asy OI. The	r ed in: pH 7.5 at 25°(M EDTA, 0.2m ol. Recleavage verdigestion w e pBR322 DN reaction mixtuu . Ligase/1µg of nore than 50% ymmetric reco remaining u	C), 200mM KCI, g/ml BSA and ith Bpu10I, more A fragments can re containing 20- of fragments and of these can be gnition sequence ncleaved ligation co81I (Saul) and	resu we time • Low pH	ilt in inco recomm instead salt, hig	omplete nend ind of using h glycer a large	ryme (>4L DNA cleav creasing g an excess ol (>5%) excess of	age. T the ir s of B conce	herefore, ncubation pu101.
Lambda ΦΧ174 19 7	M13mp18/19 4	pBR322 1	Bpu1102 p UC18/19 0	21 (ESPI). pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 0		e scriptIISK O		pACYC177 5		CYC184 3
CG Sensitivity to CpG Methylation	Therm Inactiv	· · · · · · · · · · · · · · · · · · ·	High Concentra	ition	Genome Qualified	Bulk quantities		Blue	mulation e/White tified	ns availal	ble on FastDi Enzym	gest™



R	nu1	1021	(Fshl
	yu i	1021	(LSPI

Tango 37º 👬 🎘 🖉 🗡 🥯

В

G

0

R

Tango 2X Tango

5′ G C↓T N A 3′ C G A N T ↑			Concentr 10u/µl	ration			50-100 50-1 <i>Ligation a</i> After 50-fold	nd Recle	avage	100 20-50 pu11021, more
#ER0091 Supplied with: 10X Buffer Tango™	200u 1ml		1X Buffer T 33mM Tris	Fango™: -acetate	e (pH 7.9 at	2	a reaction m	nixture con of fragme	taining 20-4 ints and 10	an be ligated in 40u of T4 DNA % PEG. More
#ER0092 Supplied with: 10X Buffer Tango™	1000u 1ml		0.1mg/ml Incubate at	BSA.			<i>Methylatic</i> Dam: never			
-		—		is suppl -HCI (pl	H 7.4 at 25	°C), 100mM KCl, /ml BSA and	Dcm: never CpG: may of EcoKI: never EcoBI: may of	overlap – r • overlaps •	no effect (p. – no effect.	
		λ DNA 0.7% agarose	50% (v/v) g			,		units of th gestion of 7	e enzyme a	Ided DNA re required for ose-embedded
Lambda Φ X174 6 0	M13mp18/19 0	pBR322 pU	C18/19 p	UC57	pTZ19R/U 0	pBluescriptIIKS(-/+)	pBluescript 0	IISK(-/+)	pACYC177 0	pACYC184 0

BpuAl	Fermentas enzyme Bpil, p.38
Bsal	Fermentas enzyme Eco31I, p.62
BsaAl	Fermentas enzyme Ppu21I, p.97
BsaBl	Fermentas enzyme BseJI, p.41
BsaHI	Fermentas enzyme Hin11, p.74
BsaJI	Fermentas enzyme BseDI, p.40
BsaMI	Fermentas enzyme Mva1269I, p.88
BseAl	Fermentas enzyme <mark>Kpn21</mark> , p.80

BseDI (Se	ecl)		Tango 5	5° 95% 🖄	80°		В	Activity G	/ in Five 0	Buffer Syst		2V Tongo
							В 50-100	20-50	0-20	к 0-20	Tango 100	2X Tango 50-100
5′ C↓C N N G 3′ G G N N C ↑			Сопсе 10u/µl	entration				o n and l O-fold o		avage estion with	n Bse	DI, more
#ER1081 Supplied with:	300u			tions for fer Tango™	100% Activ	/ity	than 95 and rec		e DNA i	fragments	can t	e ligated
10X Buffer Tango™	1ml				e (pH 7.9 at		-	lation E				
#ER1082 Supplied with:	1500u		0.1mg/	/ml BSA.	e, 66mM K-a	acetate and	Dcm: r	nay over	lap – n	- no effect o effect (p	.134).	
10X Buffer Tango™	1ml			te at 55°C.						o effect (p - no effect		
				ge Buffer s supplied						- no effect		
			10mM 1mM D	Tris-HCI (p)TT, 0.1mN	H 7.5 at 25° 1 EDTA, 0.2m	C), 50mM KCI, ng/mI BSA and	Note			ults in 109		rity.
		λ DNA 1.4% agarose		/v) glycerol	Ι.							
Lambda ФX174			pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	scriptIISK	(-/+)	pACYC177	p P	CYC184
105 6	9	8	5	4	5	6		6		12		18
www.fermenta	is.com www	v.fermentas.	com/dou	bledigest	www.ferr	mentas.com/resea	rch					
Tango Recommended Buffer	d DTT Requir	es SAM	Requires SAM	37	Incubation Temperature	95% Ligation Efficiency	y	Star Acti		meiliam	ensitivi am Me	ty to hylation

ISO ISO 9001 14001

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Recombinant Enzyme

1. RESTRICTION ENDONUCLEASES

Bulk quantities & custom formulations available on request Blue/White Certified

FastDigest[™] Enzyme

ISO ISO 9001 14001	1. RI	ESTRICTION ENDONUCLEASES Product Description	0
BseGI (FokI*)	Tango 55º 957 1580	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 50-100 20-50 20-50 100 20-50	5
* Unlike Fokl, BseGl cleaves closer to the recognition sequence and produces DI fragments with a 2-base 3'-extension 5'G G A T G N N↓3' 3'C C T A C N N5' #ER0871 500u Supplied with: 10X Buffer Tango™ 10X Buffer Tango™ 1ml	CULCETITATION	Ligation and Recleavage After 50-fold overdigestion with BseGI, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – no effect (p.136). EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined. Mote Incubation at 37°C results in 25% activity.	1
Lambda ΦΧ174 Μ13mp18/19 150 8 4	50% (v/v) glycerol. λ DNA 1.0% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 12 5 5 4 4	pBluescriptIISK(-/+) pACYC177 pACYC184 4 10 7	
•	λ DNA 1.0% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	4 10 7 Activity in Five Buffer System, % 7 B G 0 R Tango 2X Tango NR 100* 100 NR NR 100* * Star activity appears at a greater than 5-fold overdigestion (5u x 1h). Ligation and Recleavage After 10-fold overdigestion with BseJI, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: may overlap – blocked (p.133). Dcm: never overlaps – no effect.	



BseLI (Bsi	YI)		Tango 55° 📆 Dam C.G. 🖉	Activity in Five Buffer System, % B G O R Tango 2X Ta
5′ ССNNN 3′ GGNN[^]NI			<i>Concentration</i> 10u/µl	20-50 100 50-100 20-50 100 50-7 <i>Ligation and Recleavage</i> After 50-fold overdigestion with BseLI, m
#ER1201 <i>Supplied with:</i> 10X Buffer Tango™	500u 1ml		Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C),	than 95% of the DNA fragments can be liga and recut. <i>Methylation Effects</i>
#ER1202 Supplied with: 10X Buffer Tango™	2500u 1ml		10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 55°C. Storage Buffer	Dam: never overlaps – no effect. Dcm: may overlap – cleavage impaired (p.1 CpG: may overlap – cleavage impaired (p.1 EcoKI: never overlaps – no effect.
			BseLI is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	EcoBI: never overlaps – no effect. <i>Note</i> Incubation at 37°C results in 40% activity.
Lambda ΦΧ174 176 19	M13mp18/19 17	pBR322 20	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 6 6 7 8	8 8 21
	17			
176 19 BseMI (Bs	זז דDI) גרDI)		6 6 7 8	8 8 21 Activity in Five Buffer System, % B G O R Tango 2X Ta 0-20 20-50 0-20 100 50-100 50-70 Ligation and Recleavage After 50-fold overdigestion with BseMI, m BseMI, m BseMI, m
176 19	זז דDI) גרDI)		6 6 7 8 R 55° (ه.) 55° (ه.) 8 Concentration 5u/µl 5u/µl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ ,	8 8 21 Activity in Five Buffer System, % B G 0 R Tango 2X Tage 0-20 20-50 0-20 100 50-100 50-70 Ligation and Recleavage After 50-fold overdigestion with BseMI, m than 95% of the DNA fragments can be ligation and approximately 80% of these can be recut Methylation Effects
176 19 BSEMI (BS 5'G C A A T G 3'C G T T A C 3'C G T T A C 4'ER1261 Supplied with: 10X Buffer R 10X Buffer Tango ^{**} #ER1262 Supplied with: 10X Buffer R	17 (TDI) (TNNJ3' (TNN5' 100u 1ml 1ml 500u 1ml		6 6 7 8 Concentration 5u/μl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCl and 0.1mg/ml BSA. Incubate at 55°C. Storage Buffer BseMI is supplied in:	8 8 21 Activity in Five Buffer System, % B G 0 R Tango 2X Tage 0-20 20-50 0-20 100 50-100 50-700 Ligation and Recleavage After 50-fold overdigestion with BseMI, m than 95% of the DNA fragments can be ligated and approximately 80% of these can be recuted and approximately 80% of these can be recuted to the text overlaps – no effect. Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.136). EcoKI: no effect.
176 19 BSEMI (BS 5'G C A A T G 3'C G T T A C #ER1261 Supplied with: 10X Buffer R 10X Buffer Tango [™] #ER1262 Supplied with:	17 TTDI) TNN3' TNN5' 100u 1ml 1ml 500u		6 6 7 8 R 55° 55° 55° Concentration 5u/μl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/ml BSA. Incubate at 55°C. Storage Buffer	Activity in Five Buffer System, % B G O R Tango 2X Tagge 0-20 20-50 0-20 100 50-100 50-700 Ligation and Recleavage After 50-fold overdigestion with BseMI, m than 95% of the DNA fragments can be ligation and approximately 80% of these can be recuted and approximately 80% of these can be recuted to the theory overlaps – no effect. Dcm: never overlaps – no effect. Dcm: never overlaps – no effect. Dcm: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.136).

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com www.fe	ermentas.co	pm/doubledigest www.fermentas.com/research

37^o Incubation Temperature

95% Ligation Efficiency

Star Activity

Dam Sensitivity to Dam Methylation

DTT Requires DTT

Tango Recommended Buffer

SAM Requires SAM

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

ISO ISO 9001 14001	1. RE	STRICTION ENDONUCLEASES Product Description
BseMII	Tango SAM 55° 📆 🎉	Activity in Five Buffer System, % B_ssm G_ssm 0_ssm R_ssm Tango_ssm 2X Tango_ssm 50-100 50-100 50-100 50-100 50-100 50-100
5'C T C A G (N) 103' 3'G A G T C (N) 85' #ER1401 100u Supplied with: 10X Buffer Tango TM 1ml 50X SAM 0.1ml #ER1402 500u Supplied with: 10X Buffer Tango TM 1ml 50X SAM 2x0.1ml	Concentration 1-3u/μlConditions for 100% Activity [1X Buffer Tango [™]] + SAM: [33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA] + 0.01mM S-adenosylmethionine. Incubate at 55°C.Storage Buffer BseMII is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 10-fold overdigestion with BseMII, more than 90% of the DNA fragments can be ligated, but none of these can be recut due to the methylation of the recognition sequence by this enzyme (see Note). Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoBI: never overlaps – no effect. Mote • Incubation at 37°C results in 30% activity. • Requires SAM for activity. Sinefungin can replace SAM in the restriction reaction. In this case DNA is not methylated and more than 95% of the ligated BseMII fragments can be recut by this enzyme.
	R322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 7 5 5 4 4	pBluescriptIISK(-/+) pACYC177 pACYC184 4 13 8
BseNI (BsrI)	B 65° 95% ×80	Activity in Five Buffer System, % B G O R Tango 2X Tango 100 20-50 0-20 0-20 50-100 20-50
5' A C T G G N ↓3' 3' T G A C[↑]C N 5' #ER0881 1000u Supplied with:	Concentration 10u/µl Conditions for 100% Activity 1X Buffer B:	Ligation and Recleavage After 50-fold overdigestion with BseNI, more than 95% of the DNA fragments can be ligated and recut.

5′ A C T G G I 3′ T G A C[↑]C I			Concen 10u/µl	tration			<i>Ligation and Recle</i> After 50-fold overdig	gestion with	
#ER0881 Supplied with: 10X Buffer B 10X Buffer Tango™	1000u 1ml 1ml		1X Buffer	B: s-HCI (p		vity IC), 10mM MgCl ₂	than 95% of the DNA and recut. Methylation Effect Dam: never overlaps	's	an be ligated
#ER0882 Supplied with: 10X Buffer B 10X Buffer Tango [™]	5000u 2x1ml 1ml			higher age reac	efficiency of tion under pa	digestion, perform araffin oil.	Dcm: never overlaps CpG: never overlaps EcoKI: may overlap – EcoBI: may overlap –	 no effect. effect not det 	
		λDNA	BseNI is s 10mM Tri	supplied s-HCI (p 7, 1mM E	in: H 7.4 at 25° EDTA, 0.2mg	C), 100mM KCI, /ml BSA and	<i>Note</i> Incubation at 37°C activity.	results in les	ss than 10%
Lambda ΦX174				pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
110 9	18	19	11	11	12	12	12	14	15

Genome Qualified

HC High Concentration

BsePl Fermentas enzyme Paul, p.93

Thermal Inactivation

CG Sensitivity to CpG Methylation

Bulk quantities & custom formulations available on request

FastDigest" Enzyme

Blue/White

Certified

Recombinant Enzyme



λ DNA 0.7% agarose B/19 pBR322 p 3 BseSI has only one site at	Linque 65° 95% 100 Concentration 1-3u/μl Conditions for 100% Activity 1X Buffer BseXI:	20-50 100 0-20 20-50 50-100 0-2 Methylation Effects Dam: never overlaps – no effect (p. 134). CpG: may overlap – no effect (p. 134). CpG: may overlap – no effect (p. 136). EcoKI: may overlap – cleavage impaired (p. 13 EcoBI: no effect. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1µg of agarose-embedded λ DNA in 16 hours. Note • Incubation at 37°C results in 20% activity. • Low salt, high glycerol (>5%) concentratic pH >8.0 or a large excess of enzyme n result in star activity. • pBluescriptIISK(-/+) pACYC177 pACYC18 3 2 1 • Activity in Five Buffer System, % B G 0 R Tango 2X Ta NR NR NR NR NR NR NR NR NR NR NR NR NR
3	DUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+, 3 3 4 2 3 at position 2088. Unique 65° 95% ★ 100 Concentration 1-3u/µl Conditions for 100% Activity 1X Buffer BseXI:	3 2 1 Activity in Five Buffer System, % B G 0 R Tango 2X Tall NR NR NR NR NR NR NR Ligation and Recleavage After 10-fold overdigestion with BseXI, m than 95% of the DNA fragments can be ligation
	Linque 65° 95% 100 Concentration 1-3u/μl Conditions for 100% Activity 1X Buffer BseXI:	B G O R Tango 2X Tango X Tango
=	Concentration 1-3u/µl Conditions for 100% Activity 1X Buffer BseXI:	B G O R Tango 2X Ta NR NR NR NR NR NR Ligation and Recleavage After 10-fold overdigestion with BseXI, m than 95% of the DNA fragments can be ligation DNA DNA DNA DNA DNA
	1-3u/µl Conditions for 100% Activity 1X Buffer BseXI:	After 10-fold overdigestion with BseXI, n than 95% of the DNA fragments can be liga
		recut.
λ DNA 1.4% agarose	50mM Tris-HCI (pH 7.5 at 37°C), 2mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 65°C. To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil. Storage Buffer BseXI is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 200mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	 Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.136). EcoKl: never overlaps – no effect. EcoBl: may overlap – effect not determined. Note Assayed using pBR322 DNA (#SD0041). Incubation at 37°C results in 10% activity Greater than 40-fold overdigestion with B may result in the star activity. BseXI may remain associated with the clear DNA. This may cause DNA band shift during electrophoresis. To avoid an atyp DNA band pattern, use the 6X Loading Dy SDS Solution (#R1151) for sample prepara or heat the digested DNA in the presence SDS prior to electrophoresis.
8/19 pBR322 p 21	12 12 12 13) pBluescriptIISK(-/+) pACYC177 pACYC18 13 7 15
p.121 p.369 tion p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R universal Tango[™] buffer, specially formulated On-line technical support: 	for double digests
	21 p.121 p.369	21 12 12 12 13 p.121 p.369 p.336 PureExtreme™ Quality & Performance on p.336 Supplied with 10X concentrated buffers: - color-coded optimal buffer (one of B, G, O, R - universal Tango™ buffer, specially formulated

Ligation 95% Efficiency Star Activity Dam Sensitivity to Dam Methylation

37^o Incubation Temperature

1

Tango Recommended Buffer DTT Requires DTT

SAM Requires SAM

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Recombinant Enzyme

1. RESTRICTION ENDONUCLEASES

50 IS0 001 14001		STRICTION ENDONUCLEASES Product Description
Bsh1236I (FnuDII)	R 37º 55% CG 555	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 0-20 50-100 100 20-50 50-100
C G↓C G3' G C↑G C5' ER0921 500u Supplied with: 10X Buffer R 1ml 10X Buffer Tango [™] 1ml ER0922 2500u Supplied with: 10X Buffer R 1ml 10X Buffer Tango [™] 1ml	Concentration 10u/μl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Bsh1236l is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with Bsh1236I, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.
	λ DNA 1.4% agarose	
Lambda ФX174 M13mp18/19 157 14 18 Bsh1285I (McrI) с с ри ру↓с с3'	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 23 10 11 11 15 G 37° 95% CG 500 X	Activity in Five Buffer System, % B G R Tango 2X Tango 20-50 100 20-50 0-20 0-20 20-50
157 14 18 Bsh1285I (McrI)	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 23 10 11 11 15 G 37° 95% CG ∑ee ∑ee G 37° 95% CG ∑ee ∑ee Concentration 10u/µl Conditions for 100% Activity 1X Buffer G: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl₂, 50mM NaCI and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Bsh12851 is supplied in: Storage Buffer Bsh12851 is supplied in:	151018Activity in Five Buffer System, %BGORTango2X Tango20-5010020-500-200-2020-50Ligation and RecleavageAfter 50-fold overdigestion with Bsh1285I, morethan 95% of the DNA fragments can be ligatedand recut.Methylation EffectsDam: may overlap – no effect (p. 133).Dcm: never overlaps – no effect.CpG: completely overlaps – no effect.
157 14 18 Bsh1285I (McrI) C G Pu Py↓C G3' G C [↑] Py Pu G C5' ER0891 600u Supplied with: 10X Buffer G 1ml	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 23 10 11 11 15 G 37° 95% CG See X Concentration 10u/µl Conditions for 100% Activity 1X Buffer G: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 50mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Storage Buffer X	151018Activity in Five Buffer System, %BGORTango2X Tango20-5010020-500-200-2020-50Ligation and RecleavageAfter 50-fold overdigestion with Bsh1285I, morethan 95% of the DNA fragments can be ligatedand recut.Methylation EffectsDam: may overlap – no effect (p. 133).Dcm: never overlaps – no effect.CpG: completely overlaps – blocked (p. 135).



Bulk quantities & custom formulations available on request Blue/White Certified

FastDigest[™] Enzyme



BshNI (Hg	iCI)	0 37º 95% Den CG 🏷 🗡	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 20-50 100 50-100 0-20 100
5′G↓G Py Pu C 3′C C Pu Py G #ER1001 Supplied with: 10X Buffer O 10X Buffer Tango™		Concentration 10u/μ1 Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer BshNI is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 200mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with BshNI, more than 95% of the DNA fragments can be ligate and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – cleavage impaired (p.134 CpG: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.
Lambda ΦΧ174 25 3	M13mp18/19 pBR322 j 7 9	bUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 4 4 4 4	pBluescriptIISK(-/+) pACYC177 pACYC184 4 1 10
BshTI (Age	el)	0 37° 📆 CG 🎘 🖌 🕯	Activity in Five Buffer System, % B G Q R Tango 2X Tan 0-20 20-50 100 50-100 20-50 20-50
5' A ↓ C C G G T 3' T G G C C ↑ A #ER1461 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER1462 Supplied with: 10X Buffer O 10X Buffer Tango™ 10X Buffer Tango™	200u 1ml 1ml 1000u 1ml 1ml λ DNA 0.7% agarose	Concentration 10u/µl Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer BshTl is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 50-fold overdigestion with BshTl, more than 95% of the DNA fragments can be ligated and recut. bUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined. Digestion of Agarose-embedded DNA Minimum 20 units of the enzyme are required complete digestion of 1µg of agarose-embedded λ DNA in 16 hours. Note Low salt, high glycerol (>5%) concentration pH >8.0 or a large excess of enzyme may ress in star activity.
13 0 Dcil	0 0	0 0 0 0	0 2 4
Bsil BsiEl	Fermentas enzyme B		
BSIHKAI	Fermentas enzyme B		
	Fermentas enzyme A	· · · · ·	
BsiWI BsiVI	Fermentas enzyme P	· · · · · · · · · · · · · · · · · · ·	
BsiYI Bsll	Fermentas enzyme B	· · · · · · · · · · · · · · · · · · ·	
Bsll	Fermentas enzyme B	· ·	
Bsml BsmAl	Fermentas enzyme M	·	
BsmAl	Fermentas enzyme A		
BsmBl	Fermentas enzyme E	sp3I , p.71	

www.fermentas.com/doubledigest www.fermentas.com/research

37^o Incubation Temperature

95% Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

SAM Requires SAM

1

46

www.fermentas.com

DTT Requires DTT

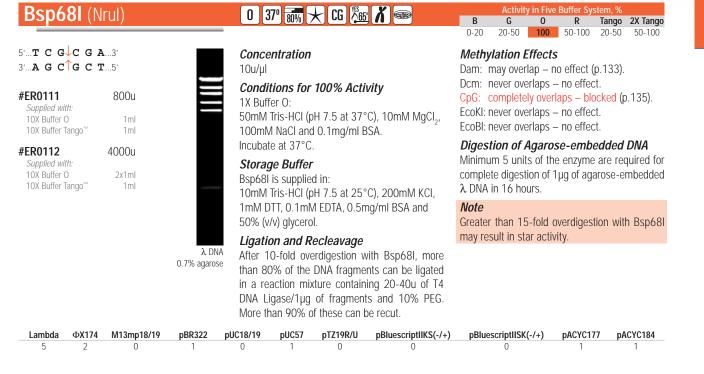
Tango Recommended Buffer

0000 1. RESTRICTION ENDONUCLEASE

Product Description







Bsp106 Fermentas enzyme Bsu15I, p.53

Bsp119I (Asull)		Tango 3	7º 95% (.G <u>(580</u> /		В	G	0		ingo 2X Tango
5' T T↓C G A # 3' A A G C ↑ T 1 #ER0121			10u/µl Condi	ntration tions for er Tango™	100% Acti	vity	After 5	on and Ro 0-fold ove 5% of the	rdigest	v age tion with B	i 00 100 sp119I, more can be ligated
<i>Supplied with:</i> 10X Buffer Tango™	1ml		33mM 10mM 0.1mg/ Incubat Storag	Tris-aceta	te (pH 7.9 at te, 66mM K-;		Dam: r Dcm: r CpG: c EcoKI: r		aps – I aps – I <mark>overla</mark> p – eff	no effect. <mark>ps – block</mark> ect not det	ed (p.135). ermined.
		λ DN 0.7% agaros	10mM 1mM D 50% (v.	Tris-HCI (p	oH 7.4 at 25° EDTA, 0.2mg	°C), 100mM KCI, /ml BSA and	Minimu comple	m 5 units	, of the n of 1µ		ded DNA e required for se-embedded
Lambda ΦX174 7 0	M13mp18/19 0	pBR322 0	pUC18/19 0	pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 0	pBlue	scriptIISK(-/ 0	'+)	pACYC177 1	pACYC184
CG Sensitivity to CpG Methylatic	n (15) Therm		HC High Concentr		K Genome Qualified	Bulk quantities	ant (om formu Blue/M	/hite	F F	e on request astDigest [™] .nzyme



Bsp1201 (Apal*)

B 37º 📆 Dan CG 🎘 HC 🗡 🥯

	Activit	y in Five	Buffer Sy	stem, %	
В	G	0	R	Tango	2X Tango
100	20-50	0-20	20-50	50-100	0-20

Dam Sensitivity to Dam Methylation

* Unlike Apal, Bsp1 fragments with a 5′G↓G G C C 3′C C C G G	4-base 5'-extension		10u/μl 50u/μl		n r 100% Acti	ivitv	Ligation and Recter After 50-fold overdig than 95% of the DNA and recut.	estion with E	
#ER0131 Supplied with: 10X Buffer B 10X Buffer Tango™	1500u 1mi 1mi		1X Buf 10mM and 0.	fer B: Tris-HCI (1mg/ml B	pH 7.5 at 37 SA.	°C), 10mM MgCl ₂	<i>Methylation Effect</i> Dam: never overlaps Dcm: may overlap –	 no effect. blocked (p.13) 	
#ER0132 Supplied with: 10X Buffer B	5x1500u 2x1ml		Stora	te at 37°C ge Buffe Ol is suppl	r		CpG: may overlap – EcoKI: never overlaps EcoBI: never overlaps	– no effect.	30).
10X Buffer Tango [™] #ER0133 Supplied with:	^{1ml} HC, 7500u		10mM 1mM E	Tris-HCI ()TT, 1mM	pH 7.4 at 25 EDTA, 0.2mg	°C), 100mM KCI, g/mI BSA and	Digestion of Agare Minimum 5 units of the complete digestion of	ne enzyme ar	re required for
10X Buffer B 10X Buffer Tango™	2x1ml 1ml	λ DN/ 0.7% agarose	4	v/v) glycer	UI.		λ DNA in 16 hours.	rµy or ayaro	se-empedded
Lambda Φ X1	74 M13mp18/19	pBR322	pUC18/19 0	pUC57	pTZ19R/U 0	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184

Bsp143I ((Nhol*)		Unique 37° 95% CG <u>65</u>		Activit	iy in Five I	Buffer Sy	ystem, %	
DSP1431				B	G	0	R	Tango	J
* Unlike Mbol, Bsp14: by Dam methylation			<i>Concentration</i> 10u/µl		20-50 on and O-fold o		•	50-100 th Bsn1/	20-50 431 more
5' ↓GATC 3 3' СТА G ↑5		_	Conditions for 100% Activity 1X Buffer Bsp143I:		5% of th	0			be ligated
#ER0781 Supplied with:	300u		33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate,	Dam: 0	r lation l completenever over	ely overla	aps – no	o effect ((p.133).
10X Buffer Bsp143I 10X Buffer Tango™	1ml 1ml	=	0.02% Triton X-100 and 0.1mg/ml BSA. Incubate at 37°C.	CpG: I	may over never over	rlap – bl	ocked (p.136).	
#ER0782 Supplied with: 10X Buffer Bsp143I 10X Buffer Tango™	1500u 1ml 1ml		Storage Buffer Bsp143I is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.15% Triton X-100,					(p.138).	
		λ DNA 1.4% agarose	0.2mg/ml BSA and 50% (v/v) glycerol.						

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
116	0	7	22	15	15	15	15	15	22	15

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

www.fermentas.com www.fermentas.com/doubledigest www.fermentas.com/research

Tango Recommended DTT Requires SAM Requires SAM SAM 370 Incubation Efficiency Star Activity	
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1. RESTRICTION ENDONUCLEASES Product Description

ISO ISO 9001 14001

#ER0791

#ER0792

Supplied with:

Supplied with:

10X Buffer Tango™

2x1ml

10X Buffer Tango™

Bsp143II (Haell) Tango 37º 37 🛧 CG 嫣 🗡 🔿 В G 0 R Tango 2X Tango 5′...**Pu G C G C↓Py**...3′ Concentration Ligation and Recleavage 3'...**Py** C G C G Pu...5' 10u/µl After 5-fold overdigestion with Bsp143II more than 95% of the DNA fragments can be ligated Conditions for 100% Activity 1000u and recut. 1X Buffer Tango[™]: Methylation Effects 33mM Tris-acetate (pH 7.9 at 37°C), 1ml 10mM Mg-acetate, 66mM K-acetate and Dam: never overlaps - no effect. 5000u 0.1mg/ml BSA. Dcm: may overlap - no effect (p.134).

Incubate at 37°C. Storage Buffer Bsp143II is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 250mM KCI, 1mM DTT, 0.1mM EDTA, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

λDNA 0.7% agarose EcoBI: may overlap - effect not determined. Digestion of Agarose-embedded DNA

CpG: completely overlaps – blocked (p.135).

EcoKI: never overlaps - no effect.

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Greater than 5-fold overdigestion with Bsp143II may result in star activity.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
48	8	6	11	3	3	4	4	4	2	11



Fermentas enzyme Sdul, p.105

Bsp1407I	Tango 37º 95%	1	•	B 0-20	Activity in Fiv G 0 20-50 0-20		m, % Tango 2X Tango 100 50-100
5′ T↓G T A C A 3′ 3′ A C A T G↑T 5′ #ER0931 300u		for 100% Acti	ivity	<i>Ligation</i> After 50- than 95%	fold overdige of the DNA	eavage estion with E	sp1407l more can be ligated
Supplied with: 10X Buffer Tango™ 1ml #ER0932 1500u Supplied with:		etate (pH 7.9 at etate, 66mM K- N.		Dam: ne Dcm: ne	ation Effect ever overlaps ever overlaps	no effect.no effect.	
10X Buffer Tango™ 1ml	Storage Buf Bsp1407l is si	fer		EcoKI: ne	ever overlaps ever overlaps ever overlaps	- no effect.	
0.7		M EDTA, 0.2mg	°C), 100mM KCI, //mI BSA and	Minimum complete		he enzyme a	Ided DNA are required for ose-embedded
			pBluescriptIIKS(-/+)	pBluesc	riptIISK(-/+)	pACYC177	pACYC184
		5	pBR322 pUC18/19 pUC57 pTZ19R/U	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluesc	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+)	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177

BspCl	Fermentas enzyme Pvul, p.100
BspCNI	Fermentas enzyme BseMII, p.43
BspDI	Fermentas enzyme Bsu151, p.53
BspEl	Fermentas enzyme Kpn2I, p.80
BspHI	Fermentas enzyme PagI, p.92

Genome

Qualified

High Concentration

HC

0.000

Bulk quantities & custom formulations available on request FastDigest Enzyme Blue/White Certified

Recombinant

Enzyme

 \bigcirc



BspL	(Nla	V)		Tango 3	17° <u>90%</u> (iam CG 765		В	Activit G	y in Five O	Buffer Sys R		2X Tang
				_				50-100	50-100	0-20	20-50	100	20-50
5′ G G N 3′ C C N				Сопсе 10и/µl	entration			After 5		erdiges	stion with		
#ER1151 Supplied wit	h:	200u		1X Buf	fer Tango [™]		2				fragmen these car		
10X Buffer T		1ml				te (pH 7.9 at			lation E				
#ER1152		1000u			ivig-aceta /ml BSA.	te, 66mM K-	acetate and				- no effec leavage ir		d (n 134
Supplied wit 10X Buffer T		1ml		0	te at 37°C						leavage ir		
			λ DI 1.4% agaro	BspLI is 10mM 1mM D 50% (v		in: oH 7.4 at 25° VI EDTA, 0.2r	°C), 200mM NaCl, ng/ml BSA and				- no effec - no effec		
Lambda 82	Φ Χ174	M13mp18/19 18	pBR322	pUC18/19	pUC57 12	pTZ19R/U 13	pBluescriptIIKS(-/+)	pBlue	scriptIISK 15	(-/+)	pACYC17 10	7 p/	23
BspL BspN	11	Fermen	tas enzyme	: Psci , p.97 : Bvei , p.54 : Kpn2i , p.8(
BspN													

)		Tango 55º 70%	Dam <u>(580°</u>					Buffer Syst	
BspPI (Bin	'/					B 20-50	G 20-50	0 0-20		Tango 2X Tango 100 0-20
5'G G A T C(N) 3'C C T A G(N)) ₄↓3′) ₅↑5′		<i>Concentratio</i> 1-3u/µl	n		After 1		erdiges	tion with I	3spPI, approxi
#ER1321 Supplied with: 10X Buffer Tango™ #ER1322 Supplied with: 10X Buffer Tango™	100u 1ml 500u 1ml		Conditions for 1X Buffer Tango 33mM Tris-acet 10mM Mg-acet 0.1mg/ml BSA. Incubate at 55°	™: ate (pH 7.9 at ate, 66mM K-a	37°C),	in a re DNA Li More th <i>Methy</i>	action mi gase/1µg nan 50% r lation E	ixture o g of fra of thes ffects	containing agments a e can be r	s can be ligate 20-40u of T and 10% PEC ecut. ked (p.133).
lov build lange			Storage Buffe BspPI is supplie	e r d in: (pH 7.4 at 25°	C), 100mM KCl, /ml BSA and	Dcm: r CpG: r EcoKI:r	nay overl nay overl never ove	ap — no ap — no rlaps —	o effect (p. o effect (p. no effect.	.134). .136).
		λ DNA (<i>dam</i> ⁻) 1.4% agarose	50% (v/v) glycer							0% activity. #SD0021).
Lambda ΦX174	M13mp18/19	pBR322 pl	JC18/19 pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK(-/+)	pACYC177	pACYC184
58 0	4	12	10 10	10	10	pbruo	10	,	13	4
58 0	4	12				parao		,	13	4
	4 ts	12 p.121 p.369 p.336	 Fermentas Re PureExtreme Supplied with – color-coded 	estriction En [™] Quality & Pe 10X concentr d optimal buffe	donucleases:	Fango™)	10		13	4
580Supporting Product• Dilution Buffer• 0.5M EDTA, pH 8.0	4 ts	p.121 p.369	 Fermentas Re PureExtreme Supplied with – color-coded 	estriction En [™] Quality & Pe 10X concentr d optimal buffe ango [™] buffer, s	donucleases: rformance rated buffers: er (one of B, G, O, R, T specially formulated f	Fango™)	10		13	4
58 0 Supporting Product • Dilution Buffer • 0.5M EDTA, pH 8.0	4 ts	p.121 p.369	Fermentas Re • PureExtreme ¹ • Supplied with - color-codec - universal Ta On-line technology - DoubleDige	estriction En [™] Quality & Pe 10X concentr d optimal buffe ango [™] buffer, s hical support est [™] at www.fe	donucleases: rformance rated buffers: er (one of B, G, O, R, T specially formulated f	Fango™) or double digest fo	10 e digests or optimal	double	e digest bu	4 Iffer
58 0 Supporting Product • Dilution Buffer • 0.5M EDTA, pH 8.0	4 ts SDS Solution	p.121 p.369 p.336	Fermentas Re • PureExtreme ¹ • Supplied with - color-codec - universal Ta On-line technology - DoubleDige	estriction En M Quality & Pe a 10X concentr d optimal buffer ango [™] buffer, s bical support est [™] at <u>www.ferme</u>	donucleases: rformance rated buffers: er (one of B, G, O, R, T specially formulated f ermentas.com/double	Fango™) or double digest fo or compl	10 e digests or optimal	double	e digest bu	4 Iffer

1. RESTRICTI	on Endoi	VUCLEASES
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ISO ISO 9001 14001

										00	C 20
ISO ISO 9001 14001			1. RESTRICTION ENDONUCLEASES Product Description								
BspTI (Afli)		0 3	70 95% 2	165 X 🕯		B 0-20	Activity in Five G O 0-20 100		m, % ango 2X Tango D-20 50-100	
5′ C↓T T A A (3′ G A A T T ↑C			10u/µl	ntration			After 50		gestion with	BspTI, more ments can be	
#ER0831 Supplied with: 10X Buffer O 10X Buffer Tango™	1000u 1ml		Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ ,				ligated ir of T4 DN PEG. Mor	1			
#ER0832 Supplied with: 10X Buffer O 10X Buffer Tango™	10X Buffer Tango [™] 1ml #ER0832 5000u Supplied with: 10X Buffer O 10X Buffer O 2x1ml		100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer BspTI is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 200mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.				Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.				
		λ DNA 0.7% agarose		, 9.9.00			Minimum complete		ne enzyme al	Ided DNA re required for ose-embedded	
Lambda ΦX174 3 2	M13mp18/19 0	pBR322	0 0	pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 0	pBlueso	criptIISK(-/+)	pACYC177 0	pACYC184	
BspT104I	Fermen	tas enzyme <mark>B</mark>	sp119I , p.4	17							
BspT107I	Fermen	tas enzyme <mark>B</mark>	shNI , p.46								
Bsrl	Fermen	tas enzyme <mark>B</mark>	<mark>seNI</mark> , p.43								
BsrBl	Fermen	tas enzyme <mark>N</mark>	bil , p.82								

BsrBl	Fermentas enzyme Mbil, p.82
BsrDI	Fermentas enzyme BseMI, p.42
BsrFI	Fermentas enzyme Cfr10I, p.56
BsrGl	Fermentas enzyme Bsp1407I, p.49
BsrSI	Fermentas enzyme BseNI, p.43
BssHII	Fermentas enzyme Paul, p.93
BssKI	Fermentas enzyme Bme1309I (different cleavage position), p.37
BssSI	Fermentas enzyme Baul, p.32
Bst98I	Fermentas enzyme BspTI, p.51

FastDigest[™] Enzyme

Blue/White Certified

HC High Concentration

Recombinant Enzyme



	(Snal)				<u>EG (565</u> X		B 20-50	G O 50-100 100	R 100	Tango 20-50	2X Tang 100
5′G T A↓T A (3′C A T [↑] A T (10u/µl				<i>Ligation and Recleavage</i> After 50-fold overdigestion with Bst1107I, mor than 90% of the pBR322 DNA fragments can b				
#ER0701	500u		1X Buf		100% Act	ivity	ligated and recut.				
Supplied with: 10X Buffer O	1ml					°C), 10mM MgCl ₂ ,	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect.				
10X Buffer Tango™	1ml			/I NaCI and te at 37°C	d 0.1mg/ml I	BSA.					
#ER0702 Supplied with:	2500u			qe Buffei	CpG: may overlap – cleavage impaired (p.136						
10X Buffer O 10X Buffer Tango™	1ml 1ml	Bst1107I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI,						EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.			
				1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.				Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedde λ DNA in 16 hours.			
		λ DN 0.7% agaros					λDNA	in 16 hours.			
Lambda Φ X174 3 0	M13mp18/19 0			pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 0		in 16 hours. scriptIISK(-/+)0	pACYC17 1	17 pA	ACYC184
		0.7% agaros pBR322	se pUC18/19					scriptIISK(-/+)		77 pA	ACYC184 1
	0	0.7% agaros pBR322 1	se pUC18/19	0				scriptIISK(-/+)		77 p#	ACYC184 1
3 0	o Ferment	0.7% agaros pBR322 1 tas enzyme	pUC18/19 0	.47				scriptIISK(-/+)		77 pA	1 1
³ 0 BstBl	o Ferment Ferment	0.7% agaros pBR322 1 tas enzyme tas enzyme	p UC18/19 0 Bsp119I, p	0 .47 .57				scriptIISK(-/+)		77 pA	ACYC184 1
³ 0 BstBl BstEll	o Ferment Ferment Ferment	0.7% agaros pBR322 1 tas enzyme tas enzyme tas enzyme	pUC18/19 0 Bsp119I, p Eco91I, p.6 BseGI, p.4	0 .47 .67 1	0		pBlue	scriptIISK(-/+)	1		1
³ 0 BstBl BstEll BstF5l	o Ferment Ferment Ferment Ferment	0.7% agaros pBR322 1 tas enzyme tas enzyme tas enzyme tas enzyme	puC18/19 0 Bsp119I, p Eco91I, p.¢ BseGI, p.4 s EcoRII (di	0 .47 .0 .7 .1 	0 avage positic	0	pBlue	scriptIISK(-/+) 0 nethylation), p.	.70 and Mv	val, p.8	1
³ 0 BstBl BstEll BstF5l BstNl	ò Ferment Ferment Ferment Ferment	0.7% agaros pBR322 1 tas enzyme tas enzyme tas enzyme tas enzymes	puC18/19 0 Bsp119I, p Eco91I, p.¢ BseGI, p.4 s EcoRII (di	0 .47 .7 1 ferent clea	0 avage positic	n and different sensi	pBlue	scriptIISK(-/+) 0 nethylation), p.	.70 and Mv	val, p.8	1

BstX				0 5	5° 95% >	Dem X565	X 📾 🕐			n, % Ingo 2X Tango -100 100	
5' C C A N N N N N √N T G G 3' 3' G G T N[↑]N N N N N A C C 5' #ER1021 500u						100% Acti	vity	<i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: may overlap – cleavage impaired (p.134).			
Supplied wit 10X Buffer C 10X Buffer T)	1ml 1ml			Tris-HCI (p	H 7.5 at 37° I 0.1mg/ml E	C), 10mM MgCl ₂ , SSA.	CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.			
#ER1022 Supplied wit 10X Buffer C)	2500u 1ml 1ml		Stora BstXI is	te at 55°C. ge Buffer s supplied i Tris-HCI (n	n:	C), 50mM KCl,	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of $1\mu g$ of agarose-embedded λ DNA in 16 hours.			
FastDigest #ER1024 Supplied wit 10X FastDig	50 r€	eactions	λDN	1mM [50% (\ <i>Ligati</i>	DTT, 0.1mN //v) glycero fon and R	1 EDTA, 0.2n I. e cleavage	ng/ml BSA and	 Note Incubation at 37°C results in 50% activity. Greater than 15-fold overdigestion with BstXI may result in star activity. 			
	* 1/474		0.7% agaros	e mately and ree	95% of the cut.	e DNA fragme	ents can be ligated		401/01/27		
Lambda 13	ΦΧ174 3	M13mp18/1 0	0	pUC18/19 0	pUC57	pTZ19R/U 0	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	0 PACYC177	0 0	
WWW.feri	nmended		ww.fermentas quires r		bledigest		mentas.com/resea	Star	i liam i i	sitivity to n Methylation	

ISO ISO 9001 14001

BstYI

BstZl

1. RESTRICTION ENDONUCLEASES

Product Description

-0 0000



Bsu15I (Clal)	Tango 37º 📆 Davn CG 🎘 🖌 🥯 🕐	Activity in Five Buffer System, % B G O R Tango 2X Tango				
5' A T↓C G A T 3' 3' T A G C[↑]T A 5'	<i>Concentration</i> 10u/µl	20-50 20-50 20-50 20-50 100 20-50 <i>Ligation and Recleavage</i> After 50-fold overdigestion with Bsu15I, mor				
#ER0141 600u Supplied with:	Conditions for 100% Activity 1X Buffer Tango™:	than 95% of the DNA fragments can be ligated and recut.				
10X Buffer Tango™ 1ml	33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and	<i>Methylation Effects</i> Dam: may overlap – blocked (p.133).				
#ER0142 3000u Supplied with: 10X Buffer Tango [™] 2x1ml	0.1mg/ml BSA. Incubate at 37°C.	Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135).				
FastDigest [™] Bsu15I (see p.2)	Storage Buffer Bsu15I is supplied in:	EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.				
#ER0144 50 reactions Supplied with: 10X FastDigest [™] Buffer 1ml	10mM Tris-HCl (pH 8.0 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol. λ DNA 0.7% agarose					
Lambda ΦX174 M13mp18/19 15 0 2) pBluescriptIISK(-/+) pACYC177 pACYC184 1 1 1				

Bsu36l Fermentas enzyme Eco81I, p.66

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

								20-50 20-50 50-10		-100 100		
5′G G↓C 8′C C ¹ G				Сопсе 10u/µl	entration			Ligation and Recleavage After 50-fold overdigestion with BsuRI, mo				
ER0151 Supplied with		3000u	Du Conditions for 100% Activity 1X Buffer R:					than 95% of the DNA fragments can be ligate and recut.				
10X Buffer R 10X Buffer Ta	ango™	2x1ml 1ml		100mN		0.1mg/ml BS		<i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134).				
		x3000u	_		ge Buffer			CpG: may overlap –				
#ER0152 5x300Ou Supplied with: 10X Buffer R 2x1ml 10X Buffer Tango [™] 1ml			BsuRI i	s supplied	in:	C), 50mM KCl,	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.					
			λ DNA 1.4% agarose	1mM D 50% (v		/I EDTA, 0.2n	ng/ml BSA and					
Lambda	ФХ174	M13mp18/19	1.4% agarose	1mM D 50% (v	TT, 0.1mÑ /v) glycero	Л EDTA, 0.2n l.	ng/ml BSA and			DACYC18		
Lambda 149	ΦΧ174 11	_ M13mp18/19 15		1mM D 50% (v	TT, 0.1mN	/I EDTA, 0.2n		pBluescriptIISK(-/+) 14	pACYC177 13	pACYC18 24		

Genome Qualified

Recombinant Enzyme

Blue/White Certified

FastDigest[™] Enzyme



Bvel (BspMI)

0 Oligo 37º 📆 🔀 🎽

Concentration

5u/µl

	Activity	y in Five	Buffer Sy	stem, %	
В	G	0	R	Tango	2X Tango
0-20	20-50	100	20-50	50-100	100

At least two copies of Bvel recognition site are

Dam Sensitivity to Dam Methylation

Note

5'....**ACCTGC (N)** 3′...**T G G A C G (N)**₈[↑]...5′

	8	50/µi	
10X Buffer Tango [™] 1	Du ml ml 5μl λ DNA 0.7% agarose	Conditions for 100% Activity[1X Buffer O] + 1μM of oligonucleotide:[50mM Tris-HCl (pH 7.5 at 37°C),10mM MgCl ₂ , 100mM NaCl, 0.1mg/ml BSA] +1μM of oligonucleotide (see Note).Incubate at 37°C.Storage BufferBvel is supplied in:10mM Tris-HCl (pH 7.5 at 25°C), 150mM KCl,1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and50% (v/v) glycerol.Ligation and RecleavageAfter 50-fold overdigestion with Bvel, more than90% of the DNA fragments can be ligated andrecut.Methylation EffectsDam: never overlaps – no effect.Dcm: never overlaps – no effect.CpG: may overlap – cleavage impaired (p.136).EcoKl: may overlap – effect not determined.EcoBl: may overlap – effect not determined.	 required for efficient cleavage. Inclusion of 1µM oligonucleotide with the Bvel recognition sequence in the reaction mixture significantly improves cleavage of plasmid DNAs, especially of those with a single Bvel site. Still, a complete cleavage of some substrates with Bvel is difficult to achieve. Bvel concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase. Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity. Bvel may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.
Lambda ΦX174 M13 41 3	mp18/19 pBR322 pl 3 1	JC18/19 pUC57 pTZ19R/U pBluescriptlIKS(-/+) 1 0 1 0	pBluescriptIISK(-/+) pACYC177 pACYC184 0 0 1
Cail (AlwNI)		Tango 37º 📆 Deur 🏷 🗡	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 20-50 20-50 50-100 100 50-100
#ER1392 2500 Supplied with:	Du	Concentration 10u/µI Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/mI BSA. Incubate at 37°C. Storage Buffer Cail is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/mI BSA and	Ligation and Recleavage After 50-fold overdigestion with Cail, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – blocked (p.134). CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for

M13mp18/19 ФХ174 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 Lambda 41 3 1 **Supporting Products** Fermentas Restriction Endonucleases: p.121 • Dilution Buffer • PureExtreme[™] Quality & Performance • 0.5M EDTA, pH 8.0 p.369 • Supplied with 10X concentrated buffers: 6X Loading Dye & SDS Solution p.336 color-coded optimal buffer (one of B, G, O, R, Tango[™]) - universal Tango[™] buffer, specially formulated for double digests **On-line technical support:** DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes www.fermentas.com www.fermentas.com/doubledigest www.fermentas.com/research 37^o Incubation

Temperature

95% Ligation Efficiency

Activity Star

0.7% agarose

SAM Requires

Requires

DTT DTT

Tango Buffer

Recommended

1. RESTRICTION ENDONUCLEASE

Product Description

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Caull	Fermentas enzyme BcnI, p.33	
Celll	Fermentas enzyme Bpu1102I, p.40	
Cfol	Fermentas enzymes Hin6I (different cleavage position), p.75 and HhaI, p.73	

Cfrl			Tango 37º 📆 Davy TCG 🏷 🔏 🔿					Activit G	ty in Five O	Buffer Sys R		2X Tango
5′ Py√GGCC 3′ PuCCG G			<i>Concentration</i> 10u/µl					50-100* 50-100 0-20 0-20 100 0-20 * Star activity appears at a greater than 5-fold overdigestion (5u x 1h). <i>Ligation and Recleavage</i> After 50-fold overdigestion with CfrI, more than				0-20 n (5u x 1h).
#ER0161 Supplied with: 10X Buffer Tango™ #ER0162 Supplied with: 10X Buffer Tango™		1X Buf 33mM 10mM 0.1mg Incuba Stora Cfrl is 10mM	fer Tango" Tris-aceta Mg-aceta /ml BSA. te at 37°C ge Buffe supplied ir Tris-HCl (r	te (pH 7.9 a te, 66mM K :. r n: pH 8.5 at 25	5	recut. Methy Dam: 1 Dcm: 1 CpG: 1 EcoKI: 1 EcoBI: 1 Diges	viation in never over may over may over never over never over never over	Effects erlaps – rlap – b rlap – b erlaps – erlaps – Agaros	nents car - no effec: locked (p. locked (p. locked (p. - no effec: - no effec: se-embe e enzyme	t. 134). 136). t. t.	DNA	
		λ DNA (<i>dcm</i> ⁻) 1.0% agarose	(dcm^{-}) 50% (v/v) glycerol. complete digestion of 1µg of agarose-embedde λ DNA in 16 hours.									
Lambda Φ X174 39 2	M13mp18/19 3	pBR322 p 6	UC18/19 3	pUC57 3	pTZ19R/U 3	pBluescriptIIKS(-/+) 4	5	escriptIISK 4		pACYC17 4		CYC184 8
Cfr9I (Sma	al*)		Unique 3	70 95% (CG (<u>×65</u>		B 0-20	Activit G 0-20	t <mark>y in Five</mark> 0 0-20	Buffer Sys R 0-20		2X Tango 0-20

* Unlike Smal, Cfr9l	produces DNA
fragments with a 4	4-base 5'-extension

5'...C C C G G G...3' 3'...**G G G C C**C...5'

#ER0171 Supplied with: 10X Buffer Cfr9I 10X Buffer Tango™	300u 1ml 1ml	
#ER0172 Supplied with: 10X Buffer Cfr9I 10X Buffer Tango™	1500u 1ml 1ml	

Concentration 10u/µl

Conditions for 100% Activity

1X Buffer Cfr9I: 10mM Tris-HCI (pH 7.2 at 37°C), 5mM MgCl₂, 200mM sodium glutamate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Cfr9I is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 250mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 50-fold overdigestion with Cfr9I, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

Dam: never overlaps - no effect.

Dcm: never overlaps - no effect.

CpG: completely overlaps - cleavage impaired (p.135).

EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

To achieve a complete digestion of substrate with Cfr9I, the concentration of DNA should be no less than 50µg/ml in the reaction buffer.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	1	0	1	1	1	1	1	1	0

Genome

Qualified

High Concentration 1herman 1nactivation Thermal HC

 λ DNA

0.7% agarose

Bulk quantities & custom formulations available on request Recombinant Blue/White

Certified

FastDigest Enzyme



200u

1ml

1ml

1ml

1ml

1000u

Cfr10I

#ER0181

#ER0182

Supplied with:

Supplied with:

10X Buffer Cfr10I

10X Buffer Tango™

10X Buffer Cfr10I

10X Buffer Tango™

5′...**Pu↓C C G G Py**...3′

3'....**Py G G C C¹Pu**....5'

luique 37° 📆 😾 CG 🗏 🗡 🔘

10mM Tris-HCI (pH 8.0 at 37°C), 5mM MgCl₂,

10mM potassium phosphate (pH 7.4 at 25°C),

100mM KCI, 2mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Conditions for 100% Activity

100mM NaCl, 0.02% Triton X-100

Concentration

1X Buffer Cfr10I:

and 0.1mg/ml BSA.

Incubate at 37°C.

Storage Buffer

λ DNA 1.0% agarose Cfr10I is supplied in:

10u/µl

Activity in Fiv

 B
 G
 O
 R
 Tango
 2X Tango

 0-20
 20-50
 20-50
 50-100*
 20-50
 50-100

 * Star activity appears at a greater than 5-fold overdigestion (5u x 1h).
 50-100
 50-100
 50-100

Ligation and Recutting

After 5-fold overdigestion with Cfr10I, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Greater than 5-fold overdigestion with Cfr10I may result in star activity.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
61	0	1	7	1	1	2	2	2	5	12

Cfr13I (As	sul)		Tango 3	7º 95% D	KU CG AGE	Activity in Five Buffer System, % B G Q R Tango 2X Tago 50-100 50-100 20-50 20-50 100 20-							
5′GJG N С С 3′С С N G [^] G #ER0191	10u/µl <i>Condi</i>	ntration tions for er Tango™	100% Acti	ivity	After 50	% of the	verdige	ivage istion with fragments					
Supplied with: 10X Buffer Tango™ #ER0192	1ml 5000u		33mM 10mM	Tris-aceta	te (pH 7.9 at te, 66mM K-		<i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: may overlap – blocked (p.134).						
<i>Supplied with:</i> 10X Buffer Tango [™]	2x1ml	λ DN 1.0% agaros	Incubat <i>Storag</i> Cfr13I i 10mM 100mM 7mM 2 A 50% (v.	Incubate at 37°C. Storage Buffer Cfr13I is supplied in: 10mM potassium phosphate (pH 7.5 at 25°C), 100mM KCI, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.				nay overla ever over	a <mark>p – bl</mark> Taps –	locked (p. no effect no effect	136).		
Lambda ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	criptIISK(·/+)	pACYC177	7 pA	CYC184	
74 2	4	15	6	8	6	8		8		9		11	

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 	p.121 p.369	PureExtreme [™] Quality & Performance
6X Loading Dye & SDS Solution	p.336	 Supplied with 10X concentrated buffers: – color-coded optimal buffer (one of B, G, O, R, Tango[™]) – universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
C 1		

Tango Recommended Buffer	DTT Requires DTT	SAM Requires SAM	37 ^o Incubation Temperature	95% Ligation Efficiency	Star Activity	Dam Sensitivity to Dam Methylation

1. RESTRICTION ENDONUCLEASES

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

ISO ISO 9001 14001	1. RI	ESTRICTION ENDONUCLEASES Product Description			
Cfr42I (SacII)	B 37º 📆 CG 🎘 🔿 🥯	Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100 0-20 0-20 50-100 0-20			
5′ C C G C√G G 3′ 3′ G G[↑]C G C C 5′	Concentration	Methylation Effects			
#ER0201 1200u <i>Supplied with:</i> 10X Buffer B 1ml 10X Buffer Tango [™] 1ml	10u/μl Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA.	Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.			
#ER0202 5x1200u Supplied with: 10X Buffer B 2x1ml 10X Buffer Tango™ 1ml	Incubate at 37°C. Storage Buffer Cfr42I is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C),	Digestion of Agarose-embedded DNA Minimum 20 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.			
	^λ DNA Ligation and Recleavage	 Note Certain sites in λ and ΦX174 DNAs are difficult to cleave with Cfr42l, the same as with its prototype SacII. 			
	0.7% agarose After 50-fold overdigestion with Cfr42I, more than 95% of the DNA fragments can be ligated and recut.	 Cfr42I activity is affected by high salt con- centration. Trace amounts of sodium chloride remaining in the substrate DNA after com- pletion of upstream applications may inhibit enzyme activity and result in impaired DNA cleavage. 			
Lambda ΦX174 M13mp18/1 4 1 0	19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 0 0 0 0 1	Ũ			
Clal Ferm	nentas enzyme <mark>Bsu15I</mark> , p.53				

Cpol (RsrII)	Tango 37º 55% CG 🏷 🕯	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 50-100 50-100 20-50 100 50-100						
T 5'C G↓G A C C G3' 3'G C C T G↑G C5' A	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] :	<i>Ligation and Recleavage</i> After 50-fold overdigestion with Cpol, more than 95% of the DNA fragments can be ligated and recut.						
#ER0741 200u <i>Supplied with:</i> 10X Buffer Tango™ 1ml	 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. 	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135).						
#ER0742 1000u Supplied with: 10X Buffer Tango [™] 1ml	Storage Buffer Cpol is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 0.7% agarose	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.						
Lambda ΦX174 M13mp18/19 5 0 0	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 0 0 0 0 0	pBluescriptIISK(-/+) pACYC177 pACYC184 0 0 0						

Genome Qualified

Recombinant Enzyme

Bulk quantities & custom formulations available on request Blue/White Certified

FastDigest[™] Enzyme





R 37° 55% CG 🔊 🔿

B

G

0

R

Tango 2X Tango

10X Buffer Tango™	1ml	pBR322 DNA 1.4% agarose	10mM 1mM [50% (v		H 7.5 at 25 1 EDTA, 0.2r	°C), 100mM KCI, ng/mI BSA and	EcoKI: never overlaps EcoBI: may overlap – Note • Assayed using pBR • Low salt, high glyc pH >8.0 or a larg result in star activit • Csel may remain a DNA. This may co during electrophor DNA band pattern,	effect not det 322 DNA (#S erol (>5%) cc e excess of y. ssociated with cause DNA to esis. To avoid use the 6X L	D0041). oncentrations enzyme ma h the cleaved band shifting d an atypica oading Dye 8
	4 M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	SDS Solution (#R11 or heat the digeste SDS prior to electro pBluescriptIISK(-/+)	ed DNA in the	

Csp6I (Rsal*) B 37° 95% 2565 В G 0 R Tango 2X Tango **100** 50-100 0-20 0-20 50-100 0-20 * Unlike Rsal, Csp6l produces DNA Concentration Ligation and Recleavage fragments with a 2-base 5'-extension After 50-fold overdigestion with Csp6I, more 10u/µl than 95% of the DNA fragments can be ligated 5'....G T A C....3' Conditions for 100% Activity and recut. 3'...C A T G...5' 1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl, Methylation Effects #ER0211 1500u and 0.1mg/ml BSA. Dam: never overlaps - no effect. Supplied with: Incubate at 37°C. Dcm: never overlaps - no effect. 10X Buffer B CpG: may overlap – no effect (p.136). 10X Buffer Tango™ 1ml Storage Buffer EcoKI: never overlaps - no effect. Csp6l is supplied in: EcoBI: never overlaps - no effect. 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 1.4% agarose pUC57 pBR322 pUC18/19 pTZ19R/U pBluescriptIISK(-/+) pACYC177 pACYC184 M13mp18/19 pBluescriptIIKS(-/+) 113 11 19 3 3 3 2 4 3 2 Csp45I Fermentas enzyme Bsp119I, p.47 **CviAll** Fermentas enzyme Hin1II (different cleavage position), p.74 Ddel Fermentas enzyme HpyF3I, p.79

Incubation

37°

Tango Recommended Buffer

DTT

www.fermentas.com/doubledigest www.fermentas.com www.fermentas.com/research DTT Requires SAM Requires

SAM







SES iption **1. RESTRICTION ENDONUCLEAS**

Pr	oduct	Desc	crip	oti

0000

ISO ISO 9001 14001					1. RE	RESTRICTION ENDONUCLEASES Product Description						
Dpnl			Tango 3	7º 70%	80 🔘		Acti B G 100 100	-	R Tar	ngo 2X Tango	Ve	
CH ₃ 5'G A↓T C3' 3'C T↑A G5' CH ₃ #ER1701 Supplied with: 10X Buffer Tango [™] #ER1702 Supplied with: 10X Buffer Tango [™]	500u 1ml 2500u 1ml		10u/µl Condit 1X Buffe 33mM 1 10mM I 0.1mg/i Incubate Storag Dpnl is 1 10mM 1	ditions for 100% Activity uffer Tango [™] : M Tris-acetate (pH 7.9 at 37°C), M Mg-acetate, 66mM K-acetate and ng/mI BSA. bate at 37°C. rage Buffer			Ligation and Recleavage After 50-fold overdigestion with DpnI, more than 70% of the pBR322 DNA fragments can be ligated and more than 95% of these can be recut. Methylation Effects Dam: does not cut dam ⁻ DNA. Dcm: never overlaps – no effect. CpG: may overlap – no effect. CpGI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoBI: may overlap – effect not determined.					
		pBR322 DNA 1.4% agarose	50% (v/	/v) glycerol	Ι.	ng/ml BSA and	adenine w cleave DN/ • Assayed u	vithin the red A. Ising pBR322	cognition 2 DNA (#S			
Lambda ΦΧ174 116 0	M13mp18/19 7	pBR322 p 22	DUC18/19 15	pUC57 15	pTZ19R/U 15	pBluescriptIIKS(-/+) 15	pBluescriptII 15	ISK(-/+) p/	22	pACYC184 15		
Dpnll	Ferment	tas enzymes [nethylation), p.48,						

Fermentas enzymes Bsp143I (different sensitivity to methylation), p.48, DpnI (different cleavage position and different sensitivity to methylation), above and MboI, p.83

Dral (Ahalli)	Tango 37º 95% 🏷 HC 🗡	Activity in Five Buffer System, % B G R Tango 2X Tango 50-100 50-100 20-50 20-50 100 50-100
5' T T T↓A A A 3' 3' A A A[↑]T T T 5'	<i>Concentration</i> 10u/µl, 50u/µl, HC	<i>Ligation and Recleavage</i> After 50-fold overdigestion with Dral, more than 95% of the DNA fragments can be ligated and
#ER0221 1500u Supplied with: 10X Buffer Tango [™] 1ml	Conditions for 100% Active 1X Buffer Tango™:	Methylation Effects
#ER02225x1500uSupplied with:2x1ml	33mM Tris-acetate (pH 7.9 at 3 10mM Mg-acetate, 66mM K-ac 0.1mg/mI BSA. Incubate at 37°C.	
#ER0223 HC, 7500u Supplied with: 10X Buffer Tango [™] 2x1ml	Storage Buffer Dral is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C	
	1mM DTT, 0.1mM EDTA, 0.15% λ DNA 0.2mg/ml BSA and 50% (v/v) g 0.7% agarose	plycerol. λ DNA in 16 hours.
Lambda ΦX174 M13mp18/19 13 2 5	pBR322 pUC18/19 pUC57 pTZ19R/U 3 3 3 3	pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 3 3 3 2

Drall Fermentas enzyme Eco0109I, p.69 Dralll Fermentas enzyme Adel, p.26 Drdl Fermentas enzyme Aasl, p.24 Eael Fermentas enzyme Cfrl, p.55 Eagl Fermentas enzyme Eco52I, p.64

Genome Qualified

HC High Concentration

CG Sensitivity to	Thermal
CpG Methylation	Inactivation



Eam1104I (Ksp632I)

Tango 37º 📆 🌾 🔿

Tango 2X Tango В G 0 R 50-100 50-100 0-20 0-20 100 0-20

5'...C T C T T C(N) 1 3'...GAGAAG(#ER0231 Supplied with: 10X Buffer Tango™ #ER0232

Supplied with:

10X Buffer Tango™

(N) ₄ [↑] 5′		10u/µl
300u 1mi 1500u 1mi		Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37° 10mM Mg-acetate, 66mM K-acet 0.1mg/mI BSA. Incubate at 37°C.
		Storage Buffer Eam1104I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 1mM DTT, 1mM EDTA, 0.2mg/ml 50% (v/v) glycerol.
	λ DNA 1.0% agarose	<i>Ligation and Recleavage</i> After 50-fold overdigestion with Ea than 95% of the DNA fragments

Concentration

1

°C), etate and

100mM KCI, BSA and

am1104I, more can be ligated and recut.

Methylation Effects Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap – no effect (p.136). EcoKI: never overlaps - no effect. EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Certain sites in λ DNA are difficult to cleave with Eam1104I, the same as with its prototype Ksp632I.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
34	2	2	2	3	3	3	3	3	2	1

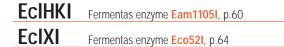
		l Ini		65"		_		·		stem, %	
Eam1105			que 37º 80% 🖔			B 20-50	G 50-100	0 0-20	R 0-20	Tango 50-100	2X Tang 20-50
	√NNGTC3′ INNCAG5′		o ncentration Du/µl			Dam:	/lation L never ove	erlaps –	no effe		
ER0241 Supplied with: 10X Buffer Eam1105I 10X Buffer Tango™	1000u 1ml 1ml	1)	Conditions for Conditions for Comm Tris-HCI (p DomM NaCI and	051: oH 7.5 at 37°	C), 5mM MgCl ₂ ,	CpG: EcoKI:	never ove may over never ove never ove	iap – n erlaps –	o effect • no effe	(p.136) ct.	
ER0242 Supplied with: 10X Buffer Eam1105I 10X Buffer Tango™	5000u 2x1ml 1ml		cubate at 37°C t orage Buffer Im1105I is supp ImM Tris-HCI (p	- plied in:	C), 100mM KCl,	Minimu comple	<i>tion of J</i> um 5 unit ete digest in 16 ho	ts of the ion of 1	e enzyme	e are re	equired f
		1r 50 <i>Li</i>	nM DTT, 1mM [°] I)% (v/v) glycero gation and R	EDTA, 0.2mg/ ol. P ecleavage			alt, high 0 or a la activity.				
Lambda ΦX174	M13mp18/19 pl	a i Liq tha BR322 pUC18	reaction mixture gase/1µg of fra an 90% of thes /19 pUC57	DNA fragment e containing 2 agments and	s can be ligated in 20-40u of T4 DNA 10% PEG. More	pBlue	escriptIISK	(-/+)	pACYC1	77 p.	ACYC184
Lambda Φ Χ174 9 1		a i Liq tha	reaction mixture gase/1µg of fra an 90% of thes	DNA fragment e containing 2 agments and e can be recu	s can be ligated in 20-40u of T4 DNA 10% PEG. More ut.	pBlue	escriptIISK 1	(-/+)	_ pACYC1 1	77 p.	ACYC184 1
9 1	<u>M13mp18/19</u> pl 0 : <u>ts</u>	BR322 pUC18 1 1 p.121 p.369 p.336	reaction mixture gase/1µg of fra an 90% of thes /19 pUC57 1 ermentas Res PureExtreme™ Supplied with 7 – color-coded	DNĂ fragment e containing 2 agments and e can be recu pTZ19R/U 1 striction En Quality & Per 10X concentra optimal buffe	s can be ligated in 20-40u of T4 DNA 10% PEG. More ut. pBluescriptIIKS(-/+) 1 donucleases: formance	Tango™)	1	· ·	pACYC1 1	77 p.	ACYC18 4 1
9 1 Cupporting Product Dilution Buffer 0.5M EDTA, pH 8.0	<u>M13mp18/19</u> pl 0 : <u>ts</u>	BR322 pUC18 1 1 p.121 p.369 p.336	reaction mixture gase/1µg of fra an 90% of thes /19 pUC57 1 ermentas Res PureExtreme™ Supplied with – color-coded – universal Tar n-line technic – DoubleDiges	DNĂ fragment e containing 2 agments and e can be recu pTZ19R/U 1 striction En Quality & Per 10X concentra optimal buffer, s cal support. t™ at <u>www.fe</u>	s can be ligated in 20-40u of T4 DNA 10% PEG. More ut. pBluescriptIIKS(-/+) 1 donucleases: formance ated buffers: r (one of B, G, O, R, pecially formulated f	Tango™) for doubl	1 e digests or optima	s Il double	e digest	buffer	1
9 1 Supporting Product Dilution Buffer 0.5M EDTA, pH 8.0	M13mp18/19 pl 0 ts SDS Solution	BR322 pUC18 1 1 p.121 p.369 p.336	reaction mixture gase/1µg of fra an 90% of thes /19 pUC57 1 ermentas Res PureExtreme™ Supplied with 7 – color-coded – universal Tar n-line technic – DoubleDiges – REsearch™ a	DNĂ fragment e containing 2 agments and e can be recu pTZ19R/U 1 striction Enu Quality & Per 10X concentra optimal buffe ngo™ buffer, s cal support. t™ at www.ferme	s can be ligated in 20-40u of T4 DNA 10% PEG. More ut. pBluescriptIIKS(-/+) 1 donucleases: formance ated buffers: r (one of B, G, O, R, ¹ pecially formulated f : rmentas.com/double	Tango™) for doubl edigest fo or compl	1 e digests or optima	s Il double	e digest	buffer	1

1. RESTRICTION ENDONUCLEASES Product Description

Earl

Fermentas enzyme Eam1104I, p.60

Ecl136II (Sacl*)		Unique 3	7º <u>90%</u> (K 565 X		B 50-100	Activit G 20-50	y in Five 0 0-20	Buffer Sy: R 0-20	stem, % Tango 50-100	2X Tango 0-20
* Unlike SacI, EcI1361 fragments with blur 5'G A G \downarrow C T (3'C T C \uparrow G A (nt ends □3'	-	<i>Concentration</i> 10u/µl <i>Conditions for 100% Activity</i> 1X Buffer Ecl136II: 10mM Bis-Tris Propane-HCI (pH 6.5 at 37°C),			After 5)% of th	verdige	stion with		6II, more be ligated	
#ER0251 Supplied with: 10X Buffer Ecl136II 10X Buffer Tango™	1500u 1ml 1ml	_	10mM Incubat Storag Ecl136	MgCl ₂ and le at 37°C ge Buffer Il is suppli	I O.1mg/ml B ed in:		Dam: r Dcm: r CpG: r EcoKI: r	never over may over never over	erlaps – erlaps – <mark>lap – c</mark> erlaps –	no effect no effect	ot. I <mark>mpaireo</mark> ot.	d (p.136). ned.
		λ DN 0.7% agaros	1mM E 50% (v		EDTA, 0.2mg	/ml BSA and	Digest Minimu comple	t ion of J m 5 unit	Agaros is of the ion of 1	e enzyme	<i>edded</i> e are ree	
Lambda ФX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK	(-/+)	pACYC17	77 pA	CYC184
2 0	I	0	I	I	1	I		I		0		0



CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Eco24I (HgiJII)	Tango 37º 📆 🎉 🗡 🥯	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 50-100 0-20 20-50 100 0-20
5′G Pu G C Py↓C3′ 3′C [↑] Py C G Pu G5′ #ER0281 1500u	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango™:	<i>Ligation and Recleavage</i> After 50-fold overdigestion with Eco24I, more than 95% of the DNA fragments can be ligated and recut.
<i>Supplied with:</i> 10X Buffer Tango [™] 1ml	33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Eco24I is supplied in:	Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – no effect (p.136). EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.
λ 0.7% aga	10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. DNA	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours.
Lambda ΦX174 M13mp18/19 pBR322 7 0 2 2	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 1 2 2 3	pBluescriptIISK(-/+) pACYC177 pACYC184 3 1 2

Genome Qualified

Bulk quantities & custom formulations available on request

Blue/White Certified

Recombinant Enzyme FastDigest[®] Enzyme



1000u

5000u

2x1ml

1ml

λ DNA (dcm-)

0.7% agarose

1ml

1ml

Eco31

#ER0291

#ER0292

Supplied with:

10X Buffer Tango™

10X Buffer G

Supplied with.

10X Buffer G

10X Buffer Tango™

5'....G G T C T C(N) 1

3'...C C A G A G(N) 1...5

G 37º 95% Den CG 565 X 🔿

10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl₂,

10mM Tris-HCI (pH 7.5 at 25°C), 200mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and

After 50-fold overdigestion with Eco31I, more

than 95% of DNA fragments can be ligated

Conditions for 100% Activity

50mM NaCl and 0.1mg/ml BSA.

Ligation and Recleavage

Concentration

Incubate at 37°C.

Storage Buffer

50% (v/v) glycerol.

and recut.

Eco311 is supplied in:

10u/µl

1X Buffer G:

Activity in Five Buffer System, %								
В	G	0	R	Tango	2X Tango			
50-100	100	0-20	0-20	50-100	20-50			

Methylation Effects

Dam: never overlaps - no effect.

Dcm: may overlap - cleavage impaired (p.134). CpG: may overlap – cleavage impaired (p.136). EcoKI: never overlaps - no effect. EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

- Assayed using λ DNA (*dcm*⁻) (#SD0021), as one of the two Eco31I recognition sites in λ DNA is difficult to cleave.
- Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.
- Eco31I cleaves downstream of its recognition site and can generate any desired 4 base 5'-overhangs. This feature is useful for direct PCR product cloning (p.140).

La	mbda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
	2	0	0	1	1	1	1	1	1	1	0



5'....G A T A T C....3'

3'...C T A T A G...5

#ER0301 Supplied with.

#ER0302

#ER0303

TOX Buffer Tango™

#ER0304

Supplied with: 10X FastDigest[™] Buffer

10X Buffer R

Supplied with. 10X Buffer R

Supplied with:

10X Buffer R

10X Buffer Tango™

10X Buffer Tango™

FastDigest[™] Eco32I (see p.2)

2000u

5x2000u

HC, 10000u

100 reactions

1ml

1ml

2x1ml

2x1ml

1ml

R 37° 📆 🌾 K 🖌 🕯 🕐

Conditions for 100% Activity

100mM KCI and 0.1mg/ml BSA.

10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl_a,

25mM Tris-HCI (pH 7.5 at 25°C), 200mM NaCl,

1mM DTT, 1mM EDTA, 0.2mg/ml BSA and

Concentration

10u/µl

50u/µl, HC

1X Buffer R:

Incubate at 37°C.

Storage Buffer

50% (v/v) glycerol.

Eco32I is supplied in:



After 50-fold overdigestion with Eco32I, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

Star

Activity

 \star

Sensitivity to

Dam Methylation

Dam

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap – no effect (p.136). EcoKI: never overlaps - no effect. EcoBI: may overlap – effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
21	0	0	1	0	1	0	1	1	0	1
Supporting • Dilution • 0.5M ED • 6X Loadi	Buffer TA, pH 8.0		p.121 p.369 p.336	 Pure Supp – co – un On-lin – Do 	Extreme [™] blied with lor-coded iversal Tar te techni publeDiges	' Quality & Pe 10X concentr optimal buffe ngo™ buffer, ical support	rated buffers: er (one of B, G, O, R, T specially formulated fo t: ermentas.com/doubled			

www.fermentas.com www.fermentas.com/doubledigest

www.fermentas.com/research

95% Ligation Efficiency

Recommended Requires Requires Incubation Tango Buffer DTT SAM 370 DTT SAM Temperature

 λ DNA

1.0% agarose

1. RESTRICTION ENDONUCLEASES

1. RESTRICTION ENDONUCLEASES

Bulk quantities & custom formulations available on request

Blue/White Certified

FastDigest[™] Enzyme

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Recombinant Enzyme

SO SO 9001 14001	I. KE	ESTRICTION ENDONUCLEASES Product Description	
E co47I (Avall)	R 37º 55% Den CG 🖉 🔿	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 50-100 50-100 100 50-100 50-100	
T G↓G A C C3' C C T G↑G5' A ER0311 800u Supplied with: 10X Buffer R 1ml ER0312 4000u Supplied with: 10X Buffer Tango™ 1ml ER0312 4000u Supplied with: 10X Buffer R 10X Buffer R 2x1ml 10X Buffer Tango™ 1ml	Concentration 10u/μl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Eco47l is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with Eco47I, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – blocked (p.134). CpG: may overlap – blocked (p.136). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.	
	λ DNA		
Lambda ΦX174 M13mp18/19 35 1 1 Eco47III	λ DNA 1.0% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 8 2 2 2 2 2 2 0 37° m CG Σε Σ σ) pBluescriptIISK(-/+) pACYC177 pACYC184 2 4 5 Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 20-50 100 100 50-100 100	
Lambda Φ X174 M13mp18/19 35 1 1	1.0% agarose 	2 4 5 Activity in Five Buffer System, % B G O R Tango 2X Tango	



;′C↓G G C C G. ⊱G C C G G↑C. #ER0331 Supplied with:			<i>Concentration</i> 10u/μl	0-20 0-20 0-20 20-50 0-20 20-5 <i>Ligation and Recleavage</i> After 50-fold overdigestion with Eco52I, m
10X Buffer Eco52I 10X Buffer Tango™ #ER0332 <i>Supplied with:</i> 10X Buffer Eco52I 10X Buffer Tango™	1ml 1ml 2500u 1ml 1ml	λ DNA	<i>Conditions for 100% Activity</i> 1X Buffer Eco52I: 10mM Tris-HCl (pH 8.5 at 37°C), 3mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. <i>Storage Buffer</i> Eco52I is supplied in: 10mM Tris-HCl (pH 8.2 at 25°C), 500mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	than 95% of the DNA fragments can be liga and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135) EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1μg of agarose-embedded λ DNA in 16 hours.
Lambda ΦΧ174 2 0 Ecco57I	M13mp18/19 0	0.7% agarose pBR322 pL 1	JC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 0 0 0 1 G SAM 37° Time Sec	pBluescriptIISK(-/+) pACYC177 pACYC18 1 0 1 Activity in Five Buffer System, % B _{*SAM} G _{*SAM} O _{*SAM} R _{*SAM} Tango _{*SAM} 2X Tango
SC T G A A G SG A C T T C ER0341 Supplied with: 10X Buffer G 50X SAM 10X Buffer Tango™ ER0342 Supplied with: 10X Buffer G 50X SAM 10X Buffer Tango™	(N) 163' (N) 145' 200u 1ml 0.1ml 1ml 1000u 1ml 2x0.1ml 1ml	λ DNA 1.0% agarose	Concentration 5u/µl Conditions for 100% Activity [1X Buffer G] + SAM: [10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 50mM NaCl and 0.1mg/ml BSA] + 0.01mM S-adenosylmethionine. Incubate at 37°C. Storage Buffer Eco57l is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol and 50% (v/v) glycerol. Ligation and Recleavage After 10-fold overdigestion with Eco57l, approximately 70% of the DNA fragments can be ligated, but none of these can be recut due to the methylation of the recognition sequence by this enzyme. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect.	 100 100 20-50 20-50 50-100 50-100 Note Eco57I requires only Mg²⁺ for its activibut is stimulated by S-adenosylmethion 0.01mM S-adenosylmethionine gives 100-fold increase in Eco57I activity. Stil complete cleavage of some substrates w Eco57I is difficult to achieve. Eco57I concentration is determined by maximal cleavage level achieved when change in the fragmentation patterns observed with the further enzyme increass Low salt, high glycerol (>5%) concentration pH >8.0 or a large excess of enzyme result in star activity. Eco57I may remain associated with cleaved DNA. This may cause DNA band string during electrophoresis. To avoid an aty cal DNA band pattern, use the 6X Load Dye & SDS Solution (#R1151) for sam preparation or heat the digested DNA in presence of SDS prior to electrophoresis.



1. RESTRICTION ENDONUCLEASES

Activity in Five Bu

 B_+SAM
 G_+SAM
 0_+SAM
 R_+SAM
 Tango_+SAM
 2X Tango_+SAM

 100
 50-100
 0-20
 20-50
 50-100
 0-20

Product Description

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Eco57MI[®]

B SAM 37º 📆 Dem 🌾

5' C T G Pu A G (N) 163'	Concentration	Methylation Effects
3' G A C Py T C (N) 145'	1-3u/μl	Dam: never overlaps – no effect.
#ER1671 50u	Conditions for 100% Activity	Dcm: may overlap – blocked (p.134).
Supplied with:	[1X Buffer B] + SAM:	CpG: never overlaps – no effect.
10X Buffer B 1ml	[10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂	EcoKI: never overlaps – no effect.
50X SAM 0.1ml	and 0.1mg/ml BSA] +	EcoBI: may overlap – effect not determined.
10X Buffer Tango™ 1ml	PBP222 pHC12/19 pHC52 pT210PL/19 pHC52 pT210PL/19 pHC52 pT210PL/19 pPL025 pT210PL/19 pT210PL/19	 Note Eco57MI requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine. 1µM S-adenosylmethionine gives more than a 100-fold increase in Eco57MI activity. Still, a complete cleavage of some substrates with Eco57MI is difficult to achieve. Eco57MI concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase. Eco57MI may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
65	3	2	6	3	3	3	3	4	2	6

* This product and process is covered by US patent No 6893854 and corresponding counterparts.

Eco72I (PmaCI)

Tango	37º	80%	\star	CG	YES ▲65 °	X	\bigcirc	

Concentration

		C↓G		
3′ G	т	G↑C	A	C 5′

3'G T G ¹ C A #ER0361 Supplied with: 10X Buffer Tango [™] #ER0362	2000u 1ml 5x2000u		1X Bufi 33mM 10mM				Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined. Digestion of Agarose-embedded DNA				
<i>Supplied with:</i> 10X Buffer Tango™	2x1ml		Incubat Storag	e at 37°C ge Buffe i	-		Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours.				
			Eco72I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.			<i>Note</i> Greater than 10-fold overdigestion with Eco72I may result in star activity.					
		λ. DNA 0.7% agarose	After 1 than 80 in a re DNA L	0-fold ov 0% of the action miz igase/1µg	DNA fragme xture contain	with Eco72I, more nts can be ligated ing 20-40u of T4 is and 10% PEG. be recut.					
Lambda Φ X1 3 0	74 M13mp18/19 0	pBR322	0 UC18/19	pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+) 0	pACYC177 0	pACYC184 0		
CG Sensitivity to CpG Methyla		nal H	High Concent	ration	Genome Qualified	Bulk quantitie	s & custom formulatio	F F	e on request astDigest [™] inzyme		

Activity in Five Buffer S

R

0-20

0

0-20

В

NR

G

NR

Methylation Effects

Tango 2X Tango

100 20-50



500u

2500u

1ml

Eco81I (Saul)

5'...C C T N A G G...3'

3'...G G A N T C C...5'

#ER0371

#ER0372

Supplied with:

Supplied with:

10X Buffer Tango

10X Buffer Tango™

Tango 37º 📷 🏷 🕼 🗡 🥯

Conditions for 100% Activity

33mM Tris-acetate (pH 7.9 at 37°C),

10mM Mg-acetate and 66mM K-acetate and

10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI,

1mM DTT, 1mM EDTA, 0.2mg/ml BSA and

Concentration

1X Buffer Tango[™]:

0.1mg/ml BSA.

Incubate at 37°C.

Storage Buffer

50% (v/v) glycerol.

Eco81I is supplied in:

10u/µl

Activity in Five Buffer System, %						
В	G	0	R	Tango	2X Tango	
50-100	100	0-20	0-20	100	0-20	

 λ DNA 0.7% agarose

Ligation and Recleavage

After 10-fold overdigestion with Eco81I, more than 80% of the M13mp18 DNA fragments can be ligated in a reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments and 10% PEG. More than 95% of these can be recut.

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	0	0	0	0	0	0	1

Eco88I (A	val)		Tango 3	Tango 37º 😥 CG 🏷 🖉 💭 🥯					0	Buffer Sys R	Tango	v	
5′C↓Py C G Pu 3′G Pu G C Py			10u/µl	<i>Concentration</i> 10u/µl					10050-1000-200-2010020-50Ligation and RecleavageAfter 50-fold overdigestion with Eco881, more than 95% of the DNA fragments can be ligated				
#ER0381 Supplied with:	1000u		1X Buf	fer Tango [™]		and re	cut.		0		ingatou		
10X Buffer Tango™	1ml		10mM 0.1mg	33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate and 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.					Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – cleavage impaired				
			Storage Buffer(p. 135).Eco88I is supplied in:EcoKI: never overlaps – no effect.10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI,EcoBI: never overlaps – no effect.										
		λ DN 0.7% agaros	1mM [50% (v		EDTA, 0.2m	g/ml BSA and	Minim comple		ts of the tion of 1	e enzyme	are red	DNA quired for mbedded	
Lambda ФX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlu	escriptIISK	(-/+)	pACYC17	7 pA	CYC184	
8 1	2	1	1	1	1	2		2		2		1	

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™])
		 – universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.formontoc.com	rmontos co	m /daubladigaet

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www.fermentas.com/doubledigest www.fermentas.com

SAM

www.fermentas.com/research 95% Ligation Efficiency Requires Requires Incubation Star SAM

Recommended

Tango Buffer

DTT DTT 37° Temperature

Activity



1. RESTRICTION ENDONUCLEASE



Product Description

ISO ISO 9001 14001	1. RESTRICTION ENDONUCLEASES Product Description
Eco91I (BstEll)	0 37º 55% № 1 ∞ Activity in Five Buffer System, % B G 0 R Tango 2X Tango 20-50 20-50 100 50-100 100
5'G↓G T N A C C3' 3'C C A N T G G5' #ER0391 1000u Supplied with: 10X Buffer O 1ml 10X Buffer Tango™ 1ml #ER0392 5000u Supplied with: 10X Buffer O 10X Buffer O 2x1ml 10X Buffer Tango™ 1ml	Concentration 10u/µl Ligation and Recleavage Conditions for 100% Activity 1X Buffer 0: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Ligation and Recleavage Methylation Effects After 50-fold overdigestion with Eco91I, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – no effect (p.136). Eco911 is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 150mM KCl, EcoBI: may overlap – effect not determined.
<u>Lambda ΦX174 M13mp18/19</u> 13 0 0	Imm DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1μg of agarose-embedded λ DNA in 16 hours.pBR322pUC18/19pUC57pTZ19R/UpBluescriptIIKS(-/+)pBluescriptIISK(-/+)pACYC177pACYC1840000010
Eco105I (SnaBI)	Tango 37° Tango 27° CG Tango X O Activity in Five Buffer System, % B G O R Tango 2X Tango

		1001	101	iu D
	_			

5' T A C↓G T A 3' 3' A T G↑C A T 5'							
#ER0401	600u						
<i>Supplied with:</i> 10X Buffer Tango™	1ml						
#ER0402	3000u						
<i>Supplied with:</i> 10X Buffer Tango™	2x1ml						
		λ DNA					
		0.7% agarose					

Tango 37º 📷 🛧		X	\bigcirc
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Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango™: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate and 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Eco105I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 1mM phenylmethylsulfonylfluoride,

0.2mg/ml BSA and 50% (v/v) glycerol.

100* 50-100 0-20 0-20 * Star activity appears at a greater than 5-fold or Ligation and Recleavage

After 2-fold overdigestion with Eco105I, more than 80% of the phage M13mp18 DNA fragments can be ligated and more than 90% of these can be recut.

100 0-20 verdigestion (5u x 1h).

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Greater than 15-fold overdigestion with Eco105I may result in star activity.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	1	0	0	0	0	0	0	0	0

HC

Bulk quantities & custom formulations available on request

FastDigest Enzyme



Eco130I (July						R	Tango	
				0-20	20-50	100	50-100	50-100	100
A A 5′C↓C T T G G 3′G G A A C↑C T T			Concentration 10u/µl Conditions for 100% Activity 1X Buffer O:	After 50	o n and F D-fold ov 5% of the ut.	verdiges	tion with		
#ER0411 2500u Supplied with: 10X Buffer O 1ml 10X Buffer Tango [™] 1ml			50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCI and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Eco130I is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI,	Dam: n Dcm: n CpG: n EcoKI: n	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.				
		λ DNA 1.0% agarose	1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Minimu complet	t ion of A m 5 units te digesti in 16 hou	s of the ion of 1	enzyme	e are rec	quired
Lambda ΦΧ174 10 0	M13mp18/19 0	pBR322 pL 1	JC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 0 0 0 0 0 0 0) pBlues	o O	(-/+)	pACYC1 0	77 pA	2 2
	Ō	I		В	0 Activity G	/ in Five O	0 Buffer Sy: R	stem, % Tango	2 2X Tar
10 0 ECO147I (3	° Stul) ェ ₃ [,]		0 0 0 0 0 Β 37° 📷 🔤 🎢 Ο 📾 Concentration 10u/μl Conditions for 100% Activity	B 100 <i>Ligatic</i> After 50	0 Activity G 50-100 Don and H D-fold ov 0% of the	<mark>y in Five I</mark> 0 20-50 Reclea verdiges	0 Buffer Sy R 20-50 Wage	stem, % Tango 50-100 h Eco14	2 2X Tar 0-20
10 0	0 Stul) I3' A5'		0 0 0 0 0 Β 37° m Den Ear Λ Ο Φ Concentration 10u/μl	B 100 <i>Ligatic</i> After 50 than 90 and rec <i>Methy</i> Dam: n	0 Activity G 50-100 Don and H D-fold ov 0% of the	y in Five 0 20-50 Reclea verdiges e DNA f Fffects rlaps –	0 Buffer Syr 20-50 Vage tion with ragment	stem, % Tango 50-100 h Eco14 ts can b ct.	2 2X Tar 0-20

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
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1. RESTRICTION ENDONUCLEASES —

www.fermentas.com www.fermentas.com/doubledigest www.fermentas.com/research



1. RESTRICTION ENDONUCLEAS

Activity in Five Buffer

Product Description

Tango 2X Tango

EcolCRI	Fermentas enzymes Ecl136II, p.61 and SacI (different cleavage position), p.102
EcoNI	Fermentas enzyme XagI, p.115
EcoO65I	Fermentas enzyme Eco91I, p.67

Eco0109 (Drall)

Tango 37º 95% Dem 1565 🗡

		50-100 20-50 20-50 20-50 100 100				
5′ Pu G↓G N C C Py 3′	Concentration	Ligation and Recleavage				
3' Py C C N G¹G Pu 5'	10u/µl	After 50-fold overdigestion with EcoO109I, more				
#ER0261 2000u	<i>Conditions for 100% Activity</i> 1X Buffer Tango™:	than 95% of the DNA fragments can be ligated and recut.				
Supplied with: 10X Buffer Tango™ 1ml	33mM Tris-acetate (pH 7.9 at 37°C),	Methylation Effects				
Tox Buildi lango	10mM Mg-acetate, 66mM K-acetate and	Dam: never overlaps – no effect.				
	0.1mg/ml BSA.	Dcm: may overlap – blocked (p.134).				
	Incubate at 37°C.	CpG: may overlap – no effect.				
	Storage Buffer Eco0109I is supplied in:	EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.				
	10mM potassium phosphate (pH 7.5 at 25°C) 100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme is required for complete digestion of $1\mu g$ of agarose-embedded λ DNA in 16 hours.				
	0.7% agarose					
Lambda	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184				

EcoRI

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3

0

0

4

 λ DNA 0.7% agarose

Thermal

1 Inactivation

1

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Concentration

10u/µl

5′G↓AATI 3′CTTAA #FD0071	↑G 5′
#ER0271 Supplied with: 10X Buffer EcoRI 10X Buffer Tango™	2ml 1ml
#ER0272 Supplied with: 10X Buffer EcoRI 10X Buffer Tango [™]	5x5000u ^{5x1ml} 1ml
#ER0273 H Supplied with: 10X Buffer EcoRI 10X Buffer Tango™	IC, 25000u 5x1ml 1ml
FastDigest ^{***} EC #ER0274 20 Supplied with:	

CG Sensitivity to CpG Methylation

Unique 37º 📆 🎉 HC 🗡 🔿 🥯 🕐

0

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50u/µl, HC Conditions for 100% Activity 1X Buffer EcoRI: 50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl₂, 100mM NaCl, 0.02% Triton X-100 and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer EcoRI is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 300mM NaCl, 1mM EDTA, 1mM DTT, 0.2mg/ml BSA, 0.15% Triton X-100 and 50% (v/v) glycerol.

Activity in Five Buffer В G 0 R Tango 2X Tango

1

4

0-20 NR 100 100* NR 100 * Star activity appears at a greater than 5-fold overdigestion (5u x 1h).

Ligation and Recleavage

After 50-fold overdigestion with EcoRI, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

1

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap - no effect (p.136). EcoKI: never overlaps - no effect. EcoBI: may overlap - no effect (p.138).

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Recombinant

Enzyme

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- Low salt concentration, large excess of the enzyme, pH >8.0, or the replacement of Mg2+ by Mn²⁺ may result in star activity.
- One hour DNA digestion with the FastDigest[™] EcoRI in the FastDigest[™] buffer may result in a relaxation of enzyme specificity. Cleavage of DNA in the EcoRI buffer is recommended in such a case.

Enzyme

		pUC18/19	p0007	pTZ19R/U	pBluescriptlikS(-/+)	pBluescriptIISK(-/+)	рАСҮС1//	pACYC184
5 0 1	1	1	1	1	1	1	0	1
							-	

Genome

Qualified

High Concentration

HC

Bulk quantities & custom formulations available on request FastDigest

Blue/White

Certified





0 37º 📆 Dcm 🖉 🔿

Activity in Five Buffer System В G 0 R Tango 2X Tango 20-50 50-100 **100** 50-100 20-50 50-100

T 5'↓C C A G (3' G G T C (A	
#ER1921 Supplied with:	200
10X Buffer O 10X Buffer Tango™	1m 1m
#ER1922	1000

′↓C C A G (′ G G T C (A		
ER1921 Supplied with: 10X Buffer O 10X Buffer Tango™	200u 1ml 1ml	
ER1922 Supplied with: 10X Buffer O 10X Buffer Tango™	1000u 1ml 1ml	
		λ DNA (<i>dcm</i> ⁻) 1.4% agarose

Concentration

10u/µl

www.fermentas.com/doubledigest

SAM

Requires

SAM

Requires

DTT

DTT

Conditions for 100% Activity

1X Buffer O: 50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl_a, 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

EcoRII is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 50-fold overdigestion with EcoRII, more than 90% of the DNA fragments can be ligated and recut.

Methylation Effects

Dam: never overlaps - no effect. Dcm: completely overlaps - blocked (p.134). CpG: never overlaps - no effect.

EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect

Note

- At least two copies of EcoRII recognition site are required for efficient cleavage. For cleavage of DNA substrates with only one copy of recognition site Mval, neoschizomer (#ER0551) of EcoRII, is recommended.
- Mval, the Dcm methylation insensitive neoschizomer of EcoRII, is recommended for the cleavage of Dcm methylated DNA.
- EcoRII may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis. Assayed using pBR322 DNA (dcm⁻).

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
70	2	7	6	5	5	5	5	5	8	12
EcoR	ECORV Fermentas enzyme Eco32I, p.62									
EcoT	14I	Fermentas enzyme Eco130I, p.68								
EcoT	221	Fermentas enzyme Mph1103I, p.85								

Tango 37º 📆 CG 🏷 🗡 Ehel (Narl*) ngo Unlike Narl, Ehel produces DNA Concentration Methylation Effects fragments with blunt ends 10u/µl Dam: never overlaps - no effect. Dcm: may overlap - no effect (p.134). Conditions for 100% Activity 5'...**G G C G C C**...3' CpG: completely overlaps - blocked (p.135). 1X Buffer Tango[™]: EcoKI: never overlaps - no effect. 33mM Tris-acetate (pH 7.9 at 37°C), EcoBI: never overlaps - no effect. 10mM Mg-acetate, 66mM K-acetate and #ER0441 500u 0.1mg/ml BSA. Digestion of Agarose-embedded DNA Supplied with: 10X Buffer Tango" Incubate at 37°C. Minimum 20 units of the enzyme are required for complete digestion of 1µg of agarose-embedded Storage Buffer λ DNA in 16 hours. Ehel is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 100mM NaCl, Note 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and • Unlike Narl, Ehel completely digests λ and 50% (v/v) glycerol. pBR322 DNAs. Low salt, high glycerol (>5%) concentrations, λ DNA Ligation and Recleavage 0.7% agarose pH >8.0 or a large excess of enzyme may After 50-fold overdigestion with Ehel, more result in star activity. than 80% of the pBR322 DNA fragments can be ligated in a reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments and 10% PEG. More than 90% of these can be recut. pUC18/19 pACYC177 pACYC184 ΦX174 M13mp18/19 pBR322 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) I ambda 0 0 4 2 4 1 0 0 Espl Fermentas enzyme Bpu1102I, p.40

Incubation

Temperature

370

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95% Ligation Efficiency

Star

Activity

 \star

3′...**C C G[↑]C G G**...5′

www.fermentas.com Recommended Tango Buffer

Activity in Five Buffer System, %									
В	G	0	R	Tango	2X Tan				
20-50	50-100	0-20	0-20	100	20-50				

Bulk quantities & custom formulations available on request

Blue/White Certified

FastDigest[™] Enzyme

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Recombinant Enzyme

		000
SO ISO 3001 14001	1. RE	ESTRICTION ENDONUCLEASES Product Description
Esp3I	Tango DTT 37º 📆 CG 🏂 🗡 🔘	Activity in Five Buffer System, % B_+DIT G_+DIT 0_+DIT R_+DIT Tango_+DIT 100 20-50 0-20 100 0-20
YC G T C T C (N) $1 \\ \dots 3'$ YG C A G A G A G (N) $5 \\ \dots 5'$ ER0451200uSupplied with:1ml10X Buffer Tango TM 1mlER04521000uSupplied with:1ml10X Buffer Tango TM 1ml	Concentration 10u/μlConditions for 100% Activity [1X Buffer Tango [™]] + DTT: [33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA] + 1.0mM DTT. Incubate at 37°C.Storage Buffer 	 Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours. Note The enzyme requires DTT (#R0861/2). Freshly made DTT should be added to the reaction buffer. Esp31 cleaves downstream of its recognition site and can generate any desired 4 base 5'-overhangs. This feature is useful for PCR product cloning (p.140).
Lambda ΦX174 M13mp18/19 14 0 1	and recut. pBR322 pUC18/19 pUC57 pTZ19R/U pBluescript1IKS(-/+) 1 2 2 0 0) pBluescriptIISK(-/+) pACYC177 pACYC184 0 1 2
Faql (Finl)	Tango SAM 37º 📆 🔀 🎘 📾	Activity in Five Buffer System, % B_sam G_sam O_sam R_sam Tango_sam 2X Tango_sam 20-50 20-50 0-20 0-20 100 20-50
Faql (Finl)	A. DNA37°ImageImageImageImageA. DNA1-3u/µlA. DNAA. DNA1.0% agaroseA. DNA1.0% agaroseA. DNAA. DNA1.0% agaroseA. DNAA. DNA<	B G SAM Tango SAM ZX Tango 20-50 20-50 0-20 0-20 100 20-50 Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – blocked (p.136). EcoKl: never overlaps – no effect. EcoBl: never overlaps – no effect. EcoBl: never overlaps – no effect. Schwart (p. 136). EcoKl: never overlaps – no effect. Vote • Faql requires only Mg ²⁺ for its activity, but is stimulated by S-adenosylmethionine. 0.05mM S-adenosylmethionine gives more than a 2-fold increase in Faql activity. Still, a complete cleavage of some substrates is difficult to achieve. • Faql concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase. • Faql may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation
$a = G G G A C(N)_{10} \downarrow3'$ $a = C C C T G(N)_{14} \uparrow5'$ ER1811 100u Supplied with: 10X Buffer Tango \checkmark 1ml	Concentration1-3u/μlConditions for 100% Activity[1X Buffer Tango [™]] + SAM:[33mM Tris-acetate (pH 7.9 at 37°C),10mM Mg-acetate, 66mM K-acetate and0.1mg/ml BSA] +0.05mM S-adenosylmethionine.Incubate at 37°C.Storage BufferFaql is supplied in:10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl,1mm DTT, 0.1mM EDTA, 0.2mg/ml BSA and50% (v/v) glycerol.Ligation and RecleavageAfter 10-fold overdigestion with Faql, more than90% of the DNA fragments can be ligated, butnone of these can be recut due to the methyla-	 B sam G sam O sam R sam Tango sam 2X Tango sam 220-50 20-50 0-20 0-20 100 20-50 Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – blocked (p.136). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoBI: never overlaps – no effect. Faql requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine. 0.05mM S-adenosylmethionine gives more than a 2-fold increase in Faql activity. Still, a complete cleavage of some substrates is difficult to achieve. Faql concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase. Faql may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.
Lambda $\Phi X174$ M13mp18/19 38 2 2	Concentration $1-3u/\mul$ Conditions for 100% Activity $1-3u/\mul$ Conditions for 100% Activity $[1X Buffer Tangom] + SAM:[33mM Tris-acetate (pH 7.9 at 37°C),10mM Mg-acetate, 66mM K-acetate and0.1mg/ml BSA] +0.05mM S-adenosylmethionine.Incubate at 37°C.Storage BufferFaql is supplied in:10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl,1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and50\% (v/v) glycerol.Ligation and RecleavageAfter 10-fold overdigestion with Faql, more than90\% of the DNA fragments can be ligated, butnone of these can be recut due to the methyla-tion of the recognition sequence by this enzyme.pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)4$	B G SAM D CAM R Tango SAM 2X Tango SAM ZX Tango Z
Lambda ФХ174 M13mp18/19 38 38 2 2 Fatl Fermen	A DNA A. DNA A. DNA B. 200% of the DNA fragments can be ligated, but nore of these can be recut due to the methyla- tion of the recognition sequence by this enzyme. PBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	B G SAM D CAM R Tango SAM 2X Tango SAM ZX Tango Z



Finl	Fermentas enzyme Faq, p.71
FnuDII	Fermentas enzyme Bsh1236, p.45
Fnu4HI	Fermentas enzyme SatI, p.103
Fokl	Fermentas enzyme BseGI (different cleavage position), p.41
Fspl	Fermentas enzyme Nsbl, p.91

FspAl			0	87° 80%	CG <u>(65</u>) 🥯	9	B 0-20	G		em, % Tango 2X Tango 0-20 50-100		
5' Pu T G C↓G C A Py 3' 3' Py A C G↑C G T Pu 5' #ER1661 100u			Concentration 5u/µl Conditions for 100% Activity 1X Buffer O:				<i>Ligation and Recleavage</i> After 50-fold overdigestion with FspAI, more than 80% of the DNA fragments can be ligated and more than 90% of these can be recut.					
Supplied with: 10X Buffer O 10X Buffer Tango™ #ER1662	1ml 1ml 500u	-	50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCI ₂ , 100mM NaCI and 0.1mg/ml BSA. Incubate at 37°C.		<i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect.							
#EK 1002 SUDU Supplied with: 10X Buffer O 1ml 10X Buffer Tango™ 1ml							CpG: completely overlaps – blocked (p.135). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined.					
		λ DNA					<i>Note</i> Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.					
Lambda Φ X174	M13mp18/19 0	0.5% agarose pBR322 2	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK(-/-	•) pACYC177	pACYC184		

FspBI (Mael)			Tango 3	7º 80% 2	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 20-50 0-20 -20 100 0-20							
5′C↓T A G3′ 3′G A T [↑] C5′ #FR1761	500u		Concentration 10u/µl Conditions for 100% Activity		<i>Ligation and Recleavage</i> After 50-fold overdigestion with FspBI, more than 80% of the DNA fragments can be ligated in a reaction mixture containing 20-40u of T4							
<i>Supplied with:</i> 10X Buffer Tango™	1ml	_	33mM		: te (pH 7.9 at te, 66mM K-		DNA Li	gase/1µg an 95% of	of fragi	ments	and 1	
#ER1762 Supplied with: 10X Buffer Tango™	2500u 1ml		0.1mg/ Incubat	'ml BSA. e at 37°C			Dam: n	<i>lation Eff</i> never overla never overla	aps – no			
		-	Storage Buffer FspBI is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and					CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.				
		λ DN 0.7% agaros	50% (v	/v) glycero			Minimu complet	tion of Ag m 10 units te digestior in 4 hours.	of the e	enzyme	are rec	quired for
Lambda ΦX174	M13mp18/19	pBR322 5	pUC18/19	pUC57	pTZ19R/U 5	pBluescriptIIKS(-/+)	pBlues	scriptIISK(-/	⊦) p <i>l</i>	ACYC177	7 pA	CYC184
14 J	5	J	7	4	J	U		U		4		Ŧ

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95% Ligation Efficiency



1. RESTRICTION ENDONUCLEASES Product Description

ISO ISO 9001 14001

Gsul			B 30° 📆 Dem 🎽 🔿		Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100 20-50 20-50 100 50-100
5' C T G G A C 3' G A C C T C #ERO461 <i>Supplied with:</i> 10X Buffer B 10X Buffer Tango [™]			Concentration 5u/µl Conditions for 100% Action 1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37° and 0.1mg/ml BSA.	-	Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – blocked (p.134). CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.
#ER0462 Supplied with: 10X Buffer B 10X Buffer Tango™	500u 1ml 1ml	λ DNA 1.0% agarose	Incubate at 30°C. Storage Buffer Gsul is supplied in: 10mM potassium phosphate (1mM EDTA, 7mM 2-mercapto 0.2mg/ml BSA and 50% (v/v) Ligation and Recleavage After 10-fold overdigestion w mately 90% of the DNA fr ligated and recut.	ethanol, glycerol. ith Gsul, approxi-	 Note Incubation at 37°C results in 70% activity. Gsul requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine. 0.01mM S-adenosylmethionine gives a 2-fold increase in activity. Gsul may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.
Lambda ΦX174 25 3	M13mp18/19 2	4	UC18/19 pUC57 pTZ19R/U 1 1 1 1	pBluescriptIIKS(-/+) 1	pBluescriptIISK(-/+) pACYC177 pACYC184 2 1 4
Haell Haelll		itas enzyme Bs itas enzyme Bs	· ·		

Haelli	Fermentas enzyme BsuRI, p.53
Hapll	Fermentas enzymes Hpall, p.77 and Mspl (different sensitivity to methylation), p.86
Hgal	Fermentas enzyme Csel, p.58
HgiAl	Fermentas enzyme Alw211, p.29
HgiCl	Fermentas enzyme BshNI, p.46
HgiJII	Fermentas enzyme Eco24I, p.61

Hhal			Tanno 3	70 95% (CG (<u>580</u>			<u> </u>	Buffer Sys		
i inai				95%			B G 50-100 50-100	0 20-50	R 20-50	Tango 2X Tang 100 20-50	
;′ G C G↓C 3′ ;′ C¹G C G 5′			Сопсе 10и/µl	entration	1		Ligation and Recleavage After 50-fold overdigestion with Hhal, more that				
ER1851 Supplied with:	2000u			tions for fer Tango™	95% of the DN recut.						
10X Buffer Tango™	1ml	λ.Di 1.4% agarc	33mM 10mM 0.1mg/ Incubat Storag Hhai is 10mM 1mM E 50% (v	Tris-aceta Mg-aceta (ml BSA. te at 37°C ge Buffei supplied i Tris-HCI (p	te (pH 7.9 at te, 66mM K-a r n: oH 7.4 at 25° 1 DTT, 0.2mg	acetate and C), 100mM KCI,					
Lambda Φ X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIIS	K(-/+)	pACYC17	7 pACYC184	
215 18	26	31	17	17	20	24	24		16	26	
CG Sensitivity to CpG Methylati	on (15) Thern (15) Inacti	nal vation	HC High Concent		K Genome Oualified	Bulk quantities		mulatio e/White tified	ns availa	ble on reque FastDigest™ Enzvme	



Hin1l (Acy)	G 37º 5 % Den CG 🎘 🖌	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 100 20-50 20-50 20-50 20-50 20-50
5′G Pu√C G Py 3′C Py G C [^] Pu		<i>Concentration</i> 10u/µl	Ligation and Recleavage After 50-fold overdigestion with Hin1I, more that
#ERO471 <i>Supplied with:</i> 10X Buffer G 10X Buffer Tango [™]	300u	Conditions for 100% Activity 1X Buffer G: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCI ₂ , 50mM NaCI and 0.1mg/mI BSA.	95% of the DNA fragments can be ligated ar recut. <i>Methylation Effects</i> Dam: never overlaps – no effect.
#ER0472 Supplied with: 10X Buffer G 10X Buffer Tango™	1500u 1ml 1ml	Incubate at 37°C. <i>Storage Buffer</i> Hin1I is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C),	Dcm: may overlap – cleavage impaired (p.13 ² CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.
	λDNA	100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required f complete digestion of 1μg of agarose-embedde λ DNA in 16 hours.
Lambda ΦX174	1.0% agarose M13mp18/19 pBR322	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	
<u>Lambda</u> Φ X174 40 7 Hin1II (NIa	1.0% agarose <u>M13mp18/19 pBR322 j</u> 1 6	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 3 3 1 1 G 370 ⊕5% ^{¥8} 65	1 2 4 Activity in Five Buffer System, % B G 0 R Tango 2X Tango
40 7	1.0% agarose <u>M13mp18/19 pBR322 1</u> 1 6 ())	3 3 1 1 G 37º ∰	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 100 20-50 50-100 50-100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hin1II, mo
40 7 Hin1II (NIa 5' CATG ↓3 3' 1 G TAC 5 #ER1831	1.0% agarose <u>M13mp18/19 pBR322 1</u> 1 6 ())	3 3 1 1 G 37 ⁰ 05% (55) Concentration	Activity in Five Buffer System, % B G O R Tango 2X Tan 50-100 100 20-50 50-100 50-100 50-100 Ligation and Recleavage Eleavage Eleavage Eleavage Eleavage Eleavage
40 7 Hin111 (Nia 5' c a t g↓ 3' 1 g t a c	1.0% agarose M13mp18/19 pBR322 1 1 6 	3 3 1 1 G 37 ⁰ 37 ⁰ 565 <i>Concentration</i> 5u/μl <i>Conditions for 100% Activity</i>	Activity in Five Buffer System, % B G O R Tango 2X Tan 50-100 100 20-50 50-100 50-100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hin1II, model than 95% of the DNA fragments can be ligation 95% 100

 λ DNA 1.4% agarose

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
181	22	15	26	11	11	9	8	8	16	23

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		 On-line technical support: DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

www.fermentas.com www.fermentas.com/doubledigest www.fermentas.com/research - -

		Tango Recommended Buffer	DTT Requires DTT	SAM Requires	
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37^o Incubation Temperature **95%** Ligation Efficiency





1. RESTRICTION ENDONUCLEASES

Product Description

Hin4l

Tango SAM 37º 📆 Davy CG 🌾

5'... \downarrow_8 (N)GAPy(N)₅(A/C/G)TC(N)₁₃₋₁₄ \downarrow ...3' 3'... (**N**) CTPu (**N**) (**T**/G/C) AG (**N**) #ER1601 50u Supplied with: 10X Buffer Tango™ 1ml 50X SAM 0.1ml #ER1602 250u Supplied with: 10X Buffer Tango™ 50X SAM 2x0.1ml λ DNA (dam⁻) 1.0% agarose

Concentration

1-3u/µl

Conditions for 100% Activity

[1X Buffer Tango[™]] + SAM: [33mM Tris-acetate (pH 7.9 at 37°C). 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA] + 0.05mM S-adenosylmethionine. Incubate at 37°C.

Storage Buffer

Hin4I is supplied in: 10mM potassium phosphate (pH 7.4 at 25°C), 100mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 10-fold overdigestion with Hin4I, more than 95% of the DNA fragments can be ligated and only 50% of these can be recut due to the methylation of the recognition sequence by restriction enzyme.

G_{+SAM} 20-50 0_{+SAM} R_{+SAM} Tango_{+SAM} 2X Tango_{+SAM} 0-20 0-20 100 0-20 B_{+SAM} 20-50

Methylation Effects

Dam: may overlap - blocked (p.133). Dcm: never overlaps - no effect. CpG: may overlap - cleavage impaired (p.136). EcoKI: never overlaps - no effect.

EcoBI: may overlap - effect not determined.

Note

- Hin4I requires only Mg2+ for its activity, but is stimulated by S-adenosylmethionine. 0.05mM S-adenosylmethionine gives more than a 10-fold increase in Hin4I activity. Still, a complete cleavage of some substrates with Hin4I is difficult to achieve.
- Hin4I concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.
- Hin4I produces double-strand cuts on both sides from the interrupted recognition site. Its unique feature is a degenerate cleavage point on the 3' side of the recognition sequence (13 or 14 nt away).
- Assayed using λ DNA (dam⁻) (#SD0021).

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
58	9	5	5	2	3	2	2	2	8	6

Hin6I (Hh	ial*)		Tango 3	7º <u>95%</u> (CG (<u>565</u>		B G		m, % ango 2X Tango 100 50-100
* Unlike Hhal, Hin6l fragments with a .	produces DNA 2-base 5'-extension		Сопсе 10и/µl	ntration			Ligation and Red After 50-fold over	leavage	
5′ G↓C G C 3′ 3′ C G C ↑ G 5′				tions for Ter Tango™	100% Acti	vity	than 95% of the D and recut.	0	
#ER0481 Supplied with:	2000u		10mM	Mg-acetat	te (pH 7.9 at te, 66mM K-		<i>Methylation Effe</i> Dam: never overla	os – no effect.	
10X Buffer Tango™ #ER0482	1ml 5x2000u		Incubat	ml BSA. e at 37°C			Dcm: never overlap CpG: completely o	verlaps – block	ed (p.135).
<i>Supplied with:</i> 10X Buffer Tango™	2x1ml		Hin6l is	je Buffer supplied	in:		EcoKI: never overla EcoBI: never overla		
			100mN	1 NaCl, 1m	nM EDTA,	pH 7.4 at 25°C), mg/ml BSA and			
		λ DNA 1.4% agarose		/v) glycero		-			
Lambda ΦX17	4 M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U 20	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+	pACYC177	pACYC184

HinP1I

CG Sensitivity to CpG Methylation

Thermal

1 Inerman

High

Concentration

HC

Fermentas enzymes Hhal (different cleavage position), p.73 and Hin6I, see above

Genome

Qualified

Certified

FastDigest Enzyme



Hincll (Hi	ndll)		Tango (3	7º 95%	CG (55)		Activity in Five Buffer System, % B G O R Tango 2X Tan 50-100 50-100 20-50 50-100 100 50-100					
5′ G T Py↓Pu 3′ C A Pu↑Py			Сопсе 10и/µl	entration			<i>Ligation and Recleavage</i> After 50-fold overdigestion with Hincll, mo					
#ER0491 500u Supplied with: 10X Buffer Tango [™] 1ml				tions for fer Tango™	100% Acti	ivity	than 95% of the DNA fragments can be ligate and recut.					
		=			te (pH 7.9 at	37°C),	Methylation Effects					
#ER0492 Supplied with:	2500u		10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.					Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.136				
10X Buffer Tango™ 1ml		λ DNA 1.0% agarose	Hincll i 10mM 1mM E		in: oH 7.5 at 25° ⁄I EDTA, 0.5r	°C), 200mM KCI, ng/mI BSA and	EcoKI: may ove EcoBI: may ove					
				pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIIS		pACYC177	pACYC184		

3' T T C G	'TT AÎA			10u/µl	entration	1		Ligation and Recleavage After 50-fold overdigestion with HindIII, mor than 95% of the DNA fragments can be ligate			
#ER0501 Supplied with:		5000u		50u/µl		[.] 100% Acti	i vitu	and recu		tragments of	can be liga
10X Buffer R 10X Buffer Tan	10 [™]	1ml 1ml		1X Buf		100 % ACI	Vity	Methyl	lation Effect	'S	
#ER0502	, ,	x5000u					°C), 10mM MgCl ₂ ,		ever overlaps		
Supplied with: 10X Buffer R		2x1ml			te at 37°C	0.1mg/ml BS	SA.		ever overlaps ever overlaps		
10X Buffer Tanç	J0 [™]	1ml		Stora	ge Buffel	r		EcoKI: n	ever overlaps	– no effect.	
#ER0503 Supplied with:	HC,2	25000u			is supplied				nay overlap –	U 1	
10X Buffer R]0 [™]	2x1ml 1ml		1mM [)TT, 0.1ml	M EDTA, 0.2r	°C), 250mM KCI, ng/ml BSA and	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required f complete digestion of 1µg of agarose-embedde			
		(<i>see</i> p.2) eactions	λ DNA 0.7% agarose	Α	v/v) glycero	ol.			e digestion of n 16 hours.	1µg of agaro	ise-embed
<i>Supplied with:</i> 10X FastDigest	™ Buffer	1ml	0								
	X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	criptIISK(-/+)	pACYC177	pACYC18
7	0	1	1	1	1	1	1		1	1	1
Supporting P		ts			entas Re.	striction Er	donucleases:				
			p.121 p.369	iuit		Quality & Pe					
Dilution Bu	рп о.	SDS Solution	p.336	• Sub			rated buffers: er (one of B, G, O, R, [*]	Tanqo™)			
Dilution Bu0.5M EDTA,	Dye &						specially formulated		diaests		
Dilution Bu0.5M EDTA,	Dye &			– ur	IIVEI SAI TAI	ngo bunon,	specially ronnalated		algosts		
Dilution Bu0.5M EDTA,	Dye &			<u>On-lir</u>	ne techni	ical suppor					

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

1

Tango Recommended Buffer DTT Requires DTT

SAM Requires SAM

1. RESTRICTION ENDONUCLEASES

ISO ISO 9001 14001	1. RESTRICTION ENDONUCLEASES Product Description	
Hinfl	B G R Tango 2X Tango 0-20 20-50 50-100 100 50-100 50-100	10
5'G↓ANTC3' 3'CTNA [↑] G5' #ER0801 2000u Supplied with: 10X Buffer Tango [™] 1ml #ER0802 5x2000u Supplied with: 10X Buffer Tango [™] 1ml #ER0803 HC, 10000u Supplied with: 10X Buffer R 2x1ml 10X Buffer R 2x1ml 10X Buffer R 2x1ml 10X Buffer Tango [™] 1ml	ConcentrationLigation and Recleavage10u/µl50u/µl, HC50u/µl, HCAfter 50-fold overdigestion with Hinfl, more than 95% of the DNA fragments can be ligated and recut.1X Buffer R:10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl2, 100mM KCl and 0.1mg/ml BSA. Incubate at 37°C.Methylation EffectsStorage Buffer Hinfl is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.Dam: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.137). EcoKI: never overlaps – no effect. EcoBI: may overlap – blocked (p.138).	1
Lambda ΦX174 M13mp18/19 148 21 27	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 10 6 5 9 8 8 13 9	
Hpal Fermenta	tas enzyme <mark>KspAI</mark> , p.81	

Hpall				Tango 3	7° 95%	CG (<u>65</u>		B 50-100	Activit G 50-100	y in Five 0 0-20	Buffer Sy R 20-50	stem, % Tango 100	2X Tango 20-50
5′ C↓C G G 3′ G G C↑C #ER0511 Supplied with: 10X Buffer Tang	5′	1000u 1ml		10u/μl Condi 1X Bufi	fer Tango	r 100% Act		Ligation After 50 95% of recut.	on and D-fold ov	Reclea erdigesi A fragn	tion with nents ca	Hpall, r	nore than lated and
#ER0512 Supplied with: 10X Buffer Tang	jo [™]	5000u 2x1ml		0.1mg/ Incubat	/ml BSA. te at 37°(acetate and	Dcm: r CpG: c	never over never over complete never over	erlaps – ely overla	no effec aps – blo	ot. <mark>ocked (</mark> p	o.135).
			λ DN 1.4% agaros	Hpall is 10mM 1mM E 50% (v		in: (pH 7.4 at 25 M EDTA, 0.21	°C), 100mM NaCl, mg/ml BSA and		never ove	•			
Lambda Ф 328	X174	M13mp18/19 18	pBR322 26	pUC18/19 13	pUC57 13	pTZ19R/U 12	pBluescriptIIKS(-/+) 13	pBlue	scriptIISK 13	(-/+)	pACYC1 16	77 pA	CYC184 34

Genome Qualified



Hphl			B 37º 7777 Dan 🏝 🔘	Activity in Five Buffer System, % B G O R Tango 2X Tang 100 0-20 0-20 0-20 20-50 0-20
5'GGTGA(N) 3'CCACT(N)	, ↑ 5′		<i>Concentration</i> 10u/µl <i>Conditions for 100% Activity</i>	<i>Ligation and Recleavage</i> After 50-fold overdigestion with HphI, more tha 70% of the DNA fragments can be ligated ar
#ER1101 Supplied with: 10X Buffer B 10X Buffer Tango™	300u 1ml 1ml		1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCI ₂ and 0.1mg/ml BSA. Incubate at 37°C.	more than 90% of these can be recut. <i>Methylation Effects</i> Dam: may overlap – blocked (p.133). Dcm: may overlap – no effect.
#ER1102 Supplied with: 10X Buffer B 10X Buffer Tango™	1500u 1ml 1ml		<i>Storage Buffer</i> Hphl is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI,	CpG: may overlap – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – blocked (p.138).
		λ DNA (<i>dam</i> -) 1.4% agarose	1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	 Note High glycerol (>5%) concentrations, pH >8 or a large excess of enzyme may result in st activity. Assayed using λ DNA (<i>dam⁻</i>) (#SD0021).
Lambda ΦΧ174 168 9	M13mp18/19 18	pBR322 p	DUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+)	pBluescriptIISK(-/+) pACYC177 pACYC184 6 17 16
Hnv81 (Mia	1\/)		Tanna 370 🛲 ILC Kar	Activity in Five Buffer System, %
Hpy8I (Mja			Tango 37º 📆 CG 🎦 🔿	B G O R Tango 2X Tan
5′G T N↓N A C 3′C A N [↑] N T G	!3' !5'		<i>Concentration</i> 10u/μl <i>Conditions for 100% Activity</i>	B G O R Tango 2X Tar 50-100 50-100 0-20 20-50 100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hpy8l, mothematical than 95% of the DNA fragments can be ligation 100 50-100 100
5′ G T N↓N A C	!3 [,]		<i>Concentration</i> 10u/µl <i>Conditions for 100% Activity</i> 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C),	B G O R Tango 2X Tan 50-100 50-100 0-20 20-50 100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hpy8I, mo than 95% of the DNA fragments can be ligat and recut. Methylation Effects
5'G T N↓N A C 3'C A N↑N T G #ER1571 Supplied with:	3' 5' 200u		Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.	B G O R Tango 2X Tar 50-100 50-100 0-20 20-50 100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hpy8l, mothan 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.13)
5'G T N↓N A C 3'C A N [↑] N T G #ER1571 Supplied with: 10X Buffer Tango [™] #ER1572 Supplied with:	3' 5' 200u 1ml 1000u	λ DNA 1.4% agarose	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA.	B G O R Tango 2X Tan 50-100 50-100 0-20 20-50 100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hpy8I, mo than 95% of the DNA fragments can be ligat and recut. Methylation Effects Dam: never overlaps – no effect.
5'G T N↓N A C 3'C A N [↑] N T G #ER1571 Supplied with: 10X Buffer Tango [™] #ER1572 Supplied with:	3' 5' 200u 1ml 1000u	1.4% agarose	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Hpy8I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and	B G O R Tango 2X Tar 50-100 50-100 0-20 20-50 100 50-100 Ligation and Recleavage After 50-fold overdigestion with Hpy8l, mothan 95% of the DNA fragments can be ligat and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.13) EcoKI: may overlap – effect not determined.
5'G T N↓N A C 3'C A N N T G #ER1571 Supplied with: 10X Buffer Tango™ #ER1572 Supplied with: 10X Buffer Tango™	3' 200u 1ml 1000u 1ml 1ml <u>M13mp18/19</u> 11	1.4% agarose pBR322 p	Concentration $10u/\mu$ lConditions for 100% Activity1X Buffer Tango [™] :33mM Tris-acetate (pH 7.9 at 37°C),10mM Mg-acetate, 66mM K-acetate and0.1mg/ml BSA.Incubate at 37°C.Storage BufferHpy8I is supplied in:10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl,1mM DTT, 1mM EDTA, 0.2mg/ml BSA and50% (v/v) glycerol.	B G O R Tango 2X Tar 50-100 50-100 0-20 20-50 100 50-10 Ligation and Recleavage After 50-fold overdigestion with Hpy8I, modeling 100 50-10 than 95% of the DNA fragments can be ligated and recut. Methylation Effects 100 100 Dam: never overlaps – no effect. 100 100 100 100 Dcm: never overlaps – no effect. 100 100 100 100 Dcm: never overlaps – no effect. 100 100 100 100 Dcm: never overlaps – cleavage impaired (p.13) 100 100 100 100 EcoBI: may overlap – effect not determined. 100 100 100 100 100 pBluescriptillSK(-/+) pACYC177 pACYC184

Supporting Products		Fermentas Restriction Endonucleases:
• 0.5M EDTA, pH 8.0	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com www.ferme	entas.co	m/doubledigest www.fermentas.com/research

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

1

78

Tango Recommended Buffer DTT Requires DTT

SAM Requires SAM

			ESTRICTION ENDONUCLEASES Product Description
HpyF3I (Ddel)		Tango 37º 📆 🎘 🔘	Activity in Five Buffer System, % B G O R Tango 2X Tango 20-50 20-50 20-50 100 50-100
5' C↓T N A G 3' 3' G A N T[↑]C 5' #ER1881 5000 <i>Supplied with:</i> 10X Buffer Tango [™] 1n #ER1882 25000 <i>Supplied with:</i> 10X Buffer Tango [™] 1n		Concentration 10u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer HpyF3I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with HpyF3I, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.
	29 8	IC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 6 6 4 4 Tango 37° 05% ℃G ∑80 ◯	pBluescriptIISK(-/+) pACYC177 pACYC184 4 14 9 Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 0-20 0-20 100 50-100
5'G C N N N N N N ↓ N 3'C G N N N N N N N #ER1731 3000 Supplied with: 10X Buffer Tango™ 1n #ER1732 15000	N C G 5'	Concentration 10u/µI Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/mI BSA. Incubate at 37°C. Storage Buffer HpyF10VI is supplied in:	Ligation and Recleavage After 50-fold overdigestion with HpyF10VI, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.137). EcoBI: never overlaps – no effect. EcoBI: never overlaps – no effect.
Supplied with: 10X Buffer Tango™ 1n	λDNA	10mM Tris-HCI (pH 7.4 at 25°C), 100mM NaCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	
10X Buffer Tango™ 1n LambdaΦX174M13n	λ DNA 1.4% agarose np18/19 pBR322 pU0	ImM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. IC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 13 13 14 16	e) pBluescriptIISK(-/+) pACYC177 pACYC184 16 11 36

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Certified

Recombinant Enzyme

FastDigest" Enzyme



1

1. RESTRICTION ENDONUCLEASES —

Kpnl	Unique 37º 📆 🎉 HC 🔏 🥯 🕐	Activity in Five Buffer System, % B G O R Tango 2X Tang 20-50 0-20 0-20 0-20 20-50 0-20
5' G G T A C↓C 3' 3' C[↑]C A T G G 5' #ER0521 4000u <i>Supplied with:</i> 10X Buffer Kpnl 2x1ml 10X Buffer Tango [™] 1ml	Concentration 10u/µl 50u/µl, HC Conditions for 100% Activity 1X Buffer Kpnl:	Methylation Effects Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134). CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.
#ER0522 5x4000u Supplied with: 10X Buffer Kpnl 10X Buffer Tango™ 1ml	10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 0.02% Triton X-100 and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedde λ DNA in 16 hours.
#ER0523 HC, 20000u Supplied with: 10X Buffer Kpnl 4x1ml 10X Buffer Tango™ 1ml FastDigest™ Kpnl (see p.2)	KpnI is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/mI BSA and 50% (v/v) glycerol.	<i>Note</i> High glycerol (>5%) concentrations, pH >8. or a large excess of enzyme may result in sta
#ER0524 100 reactions Supplied with: 10X FastDigest ^{**} Buffer 1ml	 λ DNA 0.7% agarose Ligation and Recleavage After 50-fold overdigestion with Kpnl, more than 95% of the DNA fragments can be ligated and recut. 	activity.
Lambda ΦX174 M13mp18/19 2 0 1	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 0 1 1 1 1	pBluescriptIISK(-/+) pACYC177 pACYC184 1 0 0

	Tango 55º	95%	G (<u>580</u> /		Activity in Five Buffer System, % B G Q R Tango 2X Tango 50-100 50-100 0-20 20-50 100 50-100									
ï…T↓CCGGA ï…AGGCC [↑] T	Concent i 10u/µl				<i>Ligatio</i> After 50	Ligation and Recleavage After 50-fold overdigestion with Kpn2I, more than 0.5% of the DNA fragments can be ligated								
#ER0531 Supplied with:	500u		Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and					than 95% of the DNA fragments can be ligated and recut.						
10X Buffer Tango [™]	1ml							Methylation Effects						
FR0532 Supplied with:	2500u		0.1mg/ml			Dam: may overlap – no effect (p.133). Dcm: never overlaps – no effect.								
10X Buffer Tango [™]	1ml	Incubate at 55°C. CpG: completely overlaps – blocke Storage Buffer EcoKI: never overlaps – no effect.								<mark>d</mark> (p.135).				
		_	<i>Storage Buffer</i> Kpn2I is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.					ever overla ever overla						
		λ DNA					Digesti Minimun complete	ion of Ag m 5 units d	arose-ei of the enzy α of 1μg of	nbedd yme are	led DNA e required for e-embeddec			
		0.7% agarose					<i>Note</i> Incubatio	on at 37°C	c results ir	n 50% a	activity.			
		pBR322 pl	UC18/19 g	UC57	pTZ19R/U	pBluescriptIIKS(-/+)		criptIISK(-/-		(C177	pACYC184			

Supporting Products	Fermentas Restriction Endonucleases:
Dilution Bufferp.10.5M EDTA, pH 8.0p.36X Loading Dye & SDS Solutionp.3	 Supplied with 10X concentrated buffers;
	On-line technical support:
	 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com www.ferment	as.com/doubledigest www.fermentas.com/research

37^o Incubation Temperature

95% Ligation Efficiency

Star Activity

Dam Sensitivity to Dam Methylation

Tango Recommended Buffer

DTT Requires DTT

SAM Requires SAM

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

1. RESTRICTION ENDONUCLEASES Product Description

Bulk quantities & custom formulations available on request Blue/White

Certified

FastDigest[®] Enzyme

Recombinant Enzyme



KspAI (Hpal)	B 37º 📆 🔀 🎘 🗡	Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100* 20-50 20-50 100* 50-100				
5' G T T↓A A C 3' 3' C A A↑T T G 5' #ER1031 500u <i>Supplied with:</i> 10X Buffer B 1ml 10X Buffer Tango [™] 1ml	Concentration 10u/μl Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA.	 Star activity appears at a greater than 5-fold overdigestion (5u x 1h). Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.137) EcoKI: may overlap – blocked (p.138). EcoBI: never overlaps – no effect. 				
#ER1032 2500u Supplied with: 10X Buffer B 1ml 10X Buffer Tango [™] 1ml	Incubate at 37°C. Storage Buffer KspAI is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 50-fold overdigestion with KspAI, more	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours. Note High glycerol (>5%) concentration, pH >8. or a large excess of enzyme may result in state activity.				
LambdaΦX174M13mp18/19 140	0.7% agarose initial control of the DNA fragments can be ligated and recut. pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) 0 0 0 0 0	pBluescriptIISK(-/+) pACYC177 pACYC184 0 0 0				

5' G C T C T 3' C G A G A			5u/µl	entration			Ligation and Recle	estion with Lg		
#ER1931 Supplied with: 10X Buffer Tango™	100u 1ml		1X Buf	fer Tango™	100% Acti : e (pH 7.9 at	2	90% of the DNA frag recut. <i>Methylation Effect</i>		e ligated ar	
#ER1932 Supplied with: 10X Buffer Tango™		0.1mg	Mg-acetate /ml BSA. te at 37°C.	e, 66mM K-	acetate and	Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect.				
TOX Duner Tango	1ml		Stora	<i>ge Buffer</i> supplied in		EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.				
	λ DNA			Tris-HCI (p	H 7.4 at 25° DTA, 0.2mg	C), 100mM KCI, /ml BSA and	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours.			
		0.7% agarose					<i>Note</i> Low salt, high glyce pH >8.0 or a large ex in star activity.			
Lambda ФX174	M13mp18/19	pBR322 pl	JC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
10 1	0	1	1	1	1	1	1	0	0	

Genome Qualified

O CO



Lwel (SfaN	ll)		Tango 3	87° 95% 1	KG 🕌 📿		B 0-20	Activi G 0-20	ty in Five 0 0-20	Buffer Sys R 20-50		2X Tang 20-50		
5'G C A T C(N 3'C G T A G(N	() ₅↓3′ () ₅↑5′		Сопсе 10u/µl	entration			After 5		verdiges	tion with				
#ER1621 Supplied with:	100u			tions for fer Tango™	100% Act	vity	95% o recut.	f the DN	IA fragn	nents ca	n be lig	ated ar		
10X Buffer Tango™	1ml			33mM Tris-acetate (pH 7.9 at 37°C),					Methylation Effects					
#ER1622	500u	=		10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.				Dam: never overlaps – no effect. Dcm: never overlaps – no effect.						
<i>Supplied with:</i> 10X Buffer Tango™	1ml							CpG: may overlap – cleavage impaired (p.137)						
-				<i>Storage Buffer</i> Lwel is supplied in:						no effect		ined.		
			10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.			Note								
						ng/mi BSA and	Lwel may remain associated with the cleaved DNA. This may cause DNA band shifting during							
		λDNA	λ.	, i, gijoore			electro	phoresis	. To avo	id an at	ypical E	NA bar		
		1.4% agarose	9							iding Dye				
										preparat presence				
								phoresis						
Lambda ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIIS	((-/+)	pACYC17	77 pA	CYC184		
169 12	/	22	8	9	4	4		4		17		16		
Mael	Ferme	ntas enzyme I	EspBL p 72)										
Maell		ntas enzyme 1			e position), p	.110								
Maml	Ferme	ntas enzyme	BseJI , p.41											

Mbil (BsrBI)

5′...**G A G C G G**...3′ 3'...C T C¹G C C...5'

#ER1271 1000u Supplied with: 10X Buffer Tango™ 1ml

Tango 37º 80% CG <u>४</u>६५ 🗡

Concentration 10u/µl

Conditions for 100% Activity

1X Buffer Tango[™]: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Mbil is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 200mM NaCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

 λ DNA Ligation and Recleavage 1.4% agarose

After 50-fold overdigestion with Mbil, approximately 80% of the DNA fragments can be ligated. No more than 50% of these can be recut due to the asymmetric recognition sequence of Mbil. The remaining uncleaved ligation products may be cut by Cfr42I (SacII) and Ecl136II (SacI)

Activity in Five Buffer System, %									
В	G	0	R	Tango	2X Tango				
20-50	100	20-50	20-50	100	20-50				

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: completely overlaps - cleavage impaired (p.135). EcoKI: never overlaps - no effect.

EcoBI: may overlap - effect not determined.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
17	1	4	2	3	3	4	5	5	3	2	
 www.feri	mentas	.com www	.fermenta	is.com/doul	oledigest	www.ferm	nentas.com/researc	h			
Tango Recon Buffer	nmended	DTT Require	es S/	Requires SAM	370	Incubation Temperature	95% Ligation Efficiency	Star Activity	Dam Sen Dan	sitivity to n Methylation	

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

60 0000

			Product Description
Mbol		R 37º 95% Dam 🔀	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 50-100 50-100 50-100 100 50-100 100
Supplied with: 10X Buffer R 10X Buffer Tango [™] ER0812 Supplied with: 10X Buffer R	20u 1ml 1ml 20u 1ml 1ml 1ml	Concentration 10u/μl Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Mbol is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Ligation and Recleavage After 50-fold overdigestion with Mbol, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: completely overlaps – blocked (p.133). Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect. EcoBI: may overlap – blocked (p.138). Note Assayed using λ DNA (dam ⁻) (#SD0021).
	1.4% agarose		
116 0 Mboll	3mp18/19 pBR322 p 7 22	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(- 15 15 15 15 15 B 370 mm ★ Dam 🎉 ◯	pBluescriptIISK(-/+) pACYC177 pACYC184 15 22 15 Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100 20-50 0-20 50-100 20-50
116 0 Mboll	3mp18/19 pBR322 r 7 22	15 15 15 15 B 37º ₩ ★ ₩ ₩ ₩ ♥ ♥ Concentration	Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100 20-50 0-20 50-100 20-50 Methylation Effects Effects Effects Effects Effects Effects
116 0 Mboll G A A G A (N) , ↓ C T T C T (N) , ↑ ER0821 30 <i>Supplied with:</i> 10X Buffer B 10X Buffer Tango [™] ER0822 150	3 <u>mp18/19 pBR322 r</u> 7 22 .3' .5' DOu 1ml 1ml	15 15 15 15 15 15 15 15 B 37° 37° 37° 37° Concentration 5u/µl 566 566 Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA. Incubate at 37°C. 15	152215Activity in Five Buffer System, %BG0RTango2X Tango10050-10020-500-2050-10020-50Methylation EffectsDam:may overlapblocked (p.133).Dcm:never overlapsno effect.CpG:may overlapno effect (p.137).EcoKI:never overlapsno effect (p.138).Note
116 0 Mboll	3 <u>mp18/19 pBR322 r</u> 7 22 .3' .5' DOu 1ml 1ml	15 15 15 15 15 15 15 15 B 37° 15 15 15 Concentration 5u/µl Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl₂ and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Mboll is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	15Activity in Five Buffer System, %BGORTango2X Tango10050-10020-500-2050-10020-50Methylation EffectsDam:may overlap– blocked (p. 133).Dcm:never overlaps– no effect.CpG:may overlap– no effect (p. 137).EcoKI:never overlaps– no effect (p. 138).
116 0 Mboll $a = a = a = a = a = a = a = a = a = a =$	3mp18/19 pBR322 r 7 22 22 .3' .5' .5' 00u 1ml 1ml 1ml 1ml 1ml 1ml 1ml 1ml	15 15 15 15 15 15 15 15 B 37° 37° 15 15 Concentration 5u/µl Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl₂ and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Mboll is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and	Activity in Five Buffer System, % B G O R Tango 2X Tango 100 50-100 20-50 0-20 50-100 20-50 Methylation Effects Dam: may overlap – blocked (p.133). Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect (p.138). Mote • Greater than 15-fold overdigestion with Mboll may result in star activity. • Mboll may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis. • Assaved using λ DNA (dam-) (#SD0021)

Mcrl	Fermentas enzyme Bsh12851, p.45
Mfel	Fermentas enzyme MunI, p.87
Mfll	Fermentas enzyme Psul, p.99
MjalV	Fermentas enzyme Hpy8I, p.78

Genome Qualified

HC High Concentration



5. T G G \downarrow C C A.3: 3. A C C \uparrow G G T.5: Concentration 5. \downarrow μ Concentration 5.	5T G G C C A3' 3A C C G G T5' #ER1211 200u Supplied with: 10X Buffer R 1ml 10X Buffe	50 50-11 I, more the ligated a st. .). ed DNA required e-embedo				
3: A C C C G G T5' #ER1211 200u Supplied with: 10X Buffer R 1ml 10X Buffer R 1ml 10X Buffer R 1000u Supplied with: 10X Buffer R 1000u Supplied with: 10X Buffer R 1000u Supplied with: 10X Buffer R 1ml 10X Buffer	3' A C C C G G T5' 5u/μ Supplied with: Supplied with: Supplied with: Oconditions for 100% Activity After 50-fold overdigestion with Mist 10X Buffer R 1ml Conditions for 100% Activity After 50-fold overdigestion with Mist 10X Buffer R 1ml Conditions for 100% Activity Methylation Effects 10X Buffer Rango [™] 1ml 1000u Storage Buffer Dom KCl and 0.1mg/ml BSA. Dam: never overlaps – no effect. 10X Buffer R 1ml 1ml Storage Buffer Mist is supplied in: Dom M Tris-HCl (pH 7.4 at 25°C), 100mM KCl, Digestion of Agarose-embedded 10X Buffer Tango [™] 1ml Note So% (v/v) glycerol. Digestion of 1µg of agarose A DNA (dcm [™]) 0.7% agarose pUC18/19 pUC57 pTZ19R/U pBluescriptilKS(-/+) pBluescriptilKS(-/+) pBluescriptilKS(-/+)	ligated a it.). ed DNA required e-embedo				
#ER1211200uTX Buffer R: 10X Buffer R 10X Buffer Tango"more than 95% of these can be recut. Methylation Effects Dam: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.JOX Buffer Tango"1mlImlImlImlA DNA (dorn) 0.7% agaroseNota 0.0% (v/v) glycerol.Storage Buffer MISI is supplied in: 100M Tris-HCI (pH 7.4 at 25°C), 100M KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.Methylation Effects Dorm: may overlap - blocked (p.134). CpG: never overlaps - no effect. EcoBI: never overlaps - no effect. EcoBI: never overlaps - no effect. Digestion of Agarose-embedded DA Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DA Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DA Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DA Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DA Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DA Note Assayed using λ DNA (dorm) 0.7% agaroseLambda $\Phi X174$ M13mp18/19pBR322pUC18/19pUC57pT219R/UpBluescriptilKS(-/+)pBluescriptilKS(-/+)pACtivity in Five Buffer System, % Tango	#ER1211 200u Supplied with: 10X Buffer R 1ml 10X Buffer Tango [™] 1ml #ER1212 1000u Supplied with: 1ml 10X Buffer R 1ml 10X Buffer Tango [™] 1ml 10X Buffer R 1ml 10X Buffer Tango [™] 1ml 10X Buffer Tango [™] 1ml 10X Buffer R 1ml 10X Buffer Tango [™] 1ml 10X Buffer Tango [™] 1ml 10X Buffer Tango [™] 1ml 10X Buffer R 1ml 10X Buffer R ango [™] 1ml 10X Buffer R 1ml 10X Buffer R ango [™]	ıt.). e d DNA required e-embedd				
10X Buffer Tango"1ml $JOOMM KCI and 0.1mg/ml BSA.Incubate at 37°C.Dam: never overlaps – no effect.Dcm: may overlaps – no effect.Supplied with:10X Buffer Tango"1ml10X Buffer Tango"1mlJORA Edfer Tango1mlJORA Edf$	10X Buffer Tango [™] 1ml #ER1212 1000u Supplied with: 1000u 10X Buffer R 1ml 10X Buffer Tango [™] 1ml 10X Buffer R 1ml 10X Buffer Tango [™] 1ml <	ed DNA required e-embedo				
Supplied vith: 100000 10X Buffer R 1ml 10X Buffer R 1ml 10X Buffer Tango	Supplied with: 10X Buffer R 1ml 10X Buffer Tango [™] <	e d DNA required e-embedd				
Imm DTT, 1m/ EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Digestion of Agarose-embedded DM Minimum 20 units of the enzyme are requir complete digestion of 1µg of agarose-embed by DNA in 16 hours. λ DNA (dcm ⁻) 0.7% agarose Note Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pACYC177 pACYC 18 0 1 0 0 0 0 0 2 MIUI R 37° TG C G C G T3' Concentration Second Contraction B G 0 R Tango 2X Str	1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Digestion of Agarose-embedded λ DNA (dcm ⁻) 0.7% agarose A DNA (dcm ⁻) 0.7% agarose 0.7% agarose A DNA (dcm ⁻) Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIIKS(-/+) pACYC177	required e-embed				
Mile Assayed using λ DNA (dcm ⁻) (#SD0021). Lambda $\Phi X174$ M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC 18 0 1 1 0 0 0 0 0 2 Milui R 370 \overline{m} \overline{c} </td <td>Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177</td> <td>001)</td>	Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177	001)				
Lambda $\Phi X174$ M13mp18/19pBR322pUC18/19pUC57pTZ19R/UpBluescriptIIKS(-/+)pBluescriptIISK(-/+)pACYC177pACY	Lambda ФX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIIKS(-/+) pACYC177	0211.				
3' T G C G C A5' Conditions for 100% Activity Light of and Recicavage Conditions for 100% Activity 95% of the DNA fragments can be ligate	0-20 20-50 50-100 100 20-5					
	$3'\mathbf{T} \mathbf{G} \mathbf{C} \mathbf{G} \mathbf{C}^{\uparrow} \mathbf{A}_{5'}$ 10u/µl After 50-fold overdigestion with Mlul					
Supplied with: 10000 1X Buffer R: recut.	#ER0561 1000u IV Birlifor D. recut.	ligated				
10X Buffer R1ml10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl₂,Methylation Effects10X Buffer Tango™1ml100mM KCl and 0.1mg/ml BSA.Dam: never overlaps – no effect.	10X Buffer R1ml10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl2,Methylation Effects10X Buffer Tango [™] 1ml100mM KCl and 0.1mg/ml BSA.Dam: never overlaps – no effect.	Dam: never overlaps – no effect.				
#ER0562 5000u Incubate at 37°C. Dcm: never overlaps – no effect.	Supplied with: Storage Buffer CpG: completely overlaps – blocked					
	10X Buffer Tango [™] 1ml 10MM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, EcoBI: may overlap – no effect (p.13	8).				
10X Buffer R 10X Buffer Tango [™] 2x1mlEcoKI: may overlap – no effect (p.138).10X Buffer Tango [™] 1mlImlEcoKI: may overlap – no effect (p.138).10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI,EcoBI: may overlap – no effect (p.138).	50% (v/v) glycerol. Minimum 5 units of the enzyme are complete digestion of 1 μ g of agarose λ DNA λ DNA in 16 hours.	required				
10X Buffer R 2x1ml 10X Buffer Tango [™] 1ml Mlul is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. EcoKl: may overlap – no effect (p.138). Digestion of Agarose-embedded DM Minimum 5 units of the enzyme are requir complete digestion of 1µg of agarose-embedded DM	U. 1/0 0U0U05					

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 (X Logging Due & SDS Solution 	p.121 p.369	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers:
6X Loading Dye & SDS Solution	p.336	 color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

www.fermentas.com www.fermentas.com/doubledigest www.fermentas.com/research

	Tango Re Bu	commended DTT	Requires DTT SAM	Requires 37°	Incubation Temperature	Ligation Efficiency
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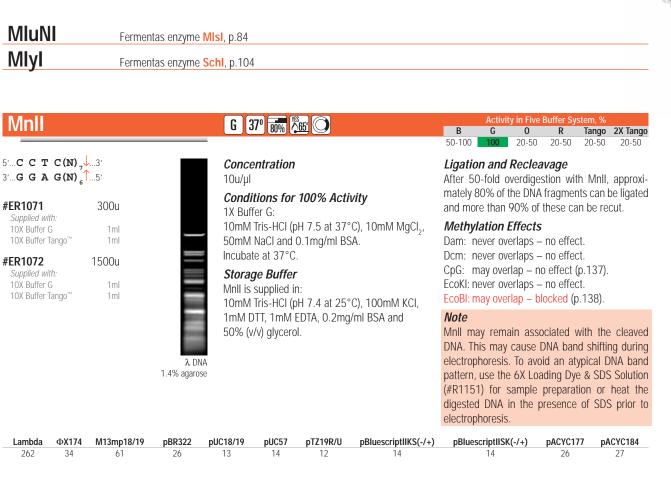
CG Sensitivity to CpG Methylation

1herman 1nactivation Thermal

1. RESTRICTION ENDONUCLEASE

Product Description

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wpni	1031	(AvallI)		R 3	7° 95% 2	<u></u>		B G O 0-20 50-100 20-5		ango 2X Tang 0-100 50-100	
5′ A T G (3′ T[↑]A C (5′		10u/µl	entration tions for	- 100% Acti	vity	<i>Ligation and Recl</i> After 50-fold overdig than 95% of the DN	estion with Mp		
#ER0731 Supplied with 10X Buffer R 10X Buffer Ta		1000u 1ml 1ml	_	100mN	Tris-HCI (p /I KCI and	0.1mg/ml BS	°C), 10mM MgCl ₂ , SA.	and recut. <i>Methylation Effect</i> Dam: never overlaps	s – no effect.		
#ER0732 5000u Supplied with: 10X Buffer R 2x1ml 10X Buffer Tango [™] 1ml			λDNA	Storag Mph11 10mM 1mM E 50% (v		r plied in: pH 7.4 at 25° EDTA, 0.2mg	°C), 200mM KCI, /mI BSA and				
Lambda	ΦΧ174	M13mp18/19	0.7% agarose pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
	0	0	0	0	1	0	0		2	0	

Mrol	Fermentas enzyme Kpn2I, p.80
Mscl	Fermentas enzyme MISI, p.84
Msel	Fermentas enzyme Tru1I, p.112

Genome

Qualified

High Concentration

HC

Bulk quantities & custom formulations available on request

Blue/White

Certified

Recombinant

Enzyme

 \bigcirc

FastDigest Enzyme



Mspl (Hpa	II)		Tango 3	70 95% 2	<u>)65'</u>		Activity in Fi B G O 50-100 50-100 0-20		m, % ango 2X Tang 100 50-100
5'C↓C G G3' 3'G G C↑C5' #ER0541 10X Buffer Tango™ #ER0542 5 Supplied with: 10X Buffer Tango™	3000u 1ml 5x3000u 2x1ml		10u/µl Condii 1X Buff 33mM 10mM 0.1mg/ Incubat Storag Mspl is 10mM 200mM 0.2mg/	Tris-aceta Mg-aceta Mg-aceta (ml BSA. te at 37°C ge Buffer supplied potassium / NaCl, 1n	te (pH 7.9 at te, 66mM K-;	37°C), acetate and pH 7.5 at 25°C), 1 EDTA,	Ligation and Recleavage After 50-fold overdigestion with Mspl, more the 95% of the DNA fragments can be ligated a recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – no effect (p.135) EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.		
Lambda Φ X174 328 5	M13mp18/19 18	1.4% agarose pBR322 26	pUC18/19 13	pUC57	pTZ19R/U 12	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177 16	pACYC184
Mssl (Pme	i)		B 3	70 📆 🏌	<u>)</u> 65 X 📾			ve Buffer Syste	
	, , , , , , , , , , , , , , , , , , ,						B G O 100 0-20 0-20		ango 2X Tan 0-50 0-20
5' G T T T↓A A 3' C A A A↑T 1 #ER1341 <i>Supplied with:</i> 10X Buffer B 10X Buffer Tango™		5u/µl Condi 1X Buff 10mM	fer B:	° 100% Acti oH 7.5 at 37°	vity C), 10mM MgCl ₂	Ligation and Recleavage After 10-fold overdigestion with MssI, more th 90% of the DNA fragments can be ligated i reaction mixture containing 20-40u of T4 D Ligase/1µg of fragments and 10% PEG. M than 90% of these can be recut.			
FER1342 Supplied with: 10X Buffer B 10X Buffer Tango™	1250u 1ml 1ml		Incubat Storag Mssl is 10mM 1mM D	te at 37°C ge Buffer supplied i Tris-HCI (p	• • • T.5 at 25° • M EDTA, 0.2n	C), 50mM KCI, ng/ml BSA and	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: may overlap – blocked (p.138). EcoBI: never overlaps – no effect.		
		λ DNA 0.7% agarose		, , , , , , , , , , , , , , , , , , , ,			Digestion of Agar Minimum 5 units of t complete digestion o λ DNA in 16 hours.	the enzyme a	re required f
Lambda Φ X174 2 0	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U 0	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184 0
Mstl	Fermen	tas enzyme <mark>N</mark>	isbi , p.91						
Supporting Produc	cts		Ferme	entas Reg	striction Fr	donucleases:			
 Dilution Buffer 		p.121			Quality & Pe				
0.5M EDTA, pH 8.	0	p.369				ated buffers:			

- 6X Loading Dye & SDS Solution p.336
- Supplied with 10X concentrated buffers: - color-coded optimal buffer (one of B, G, O, R, Tango[™])
 - universal Tango[™] buffer, specially formulated for double digests

On-line technical support:

- $\mathsf{DoubleDigest}^{\mathsf{\tiny M}} \text{ at } \underline{\mathsf{www.fermentas.com/doubledigest}} \text{ for optimal double digest buffer}$
- REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

Star Activity

www.fermentas.com/doubledigest www.fermentas.com Tango Recommended Buffer DTT Requires

www.fermentas.com/research 37^o Incubation Temperature SAM Requires SAM Ligation 95% Efficiency

Temperature

1

Dam Sensitivity to Dam Methylation

1. RESTRICTION ENDONUCLEASES

10X Buffer R

Supplied with:

10X Buffer R

#ER0552

10X Buffer Tango™

10X Buffer Tango™

1ml

1ml

2x1ml

1ml

5x2000u

000°

ISO ISO 9001 14001						1. RE	STRICT	ION EN		CLEASES of Description	
Munl (Mfe)		G 3	7° 95% (č	165 X O		В	Activity in Fiv G O 100 0-20		<mark>m, % ango 2X Tango</mark> 100 0-20	
5′ C↓A A T T (3′ G T T A A ↑			10u/µl	entration			After 50-fo		estion with M	unl, more than	6
#ER0751 Supplied with: 10X Buffer G 10X Buffer Tango""	1X Buff 10mM ⁻ 50mM I	fer G: Tris-HCI (p	0.1mg/ml BS	°C), 10mM MgCl ₂ ,	 95% of the DNA fragments can be ligated and recut. <i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect. 				1		
#ER0752 1500u Supplied with:		Storag Munl is 10mM	ge Buffer s supplied i Tris-HCI (p	. in: oH 7.4 at 25°	°C), 100mM KCI,	CpG: neve EcoKI: neve EcoBI: may					
		λ DNA 0.7% agarose	50% (v/)TT, 1mM E //v) glycero	EDTA, 0.2mg/)l.	/ml BSA and	Minimum 5	5 units of th ligestion of		Ided DNA re required for ose-embedded	
Lambda ФX174	M13mp18/19		pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)		ptIISK(-/+)	pACYC177	pACYC184	
8 1	0	0	0	0	0	0	(0	0	0	

R 37° 📆 🕺 🔿 Mval (EcoRII*) * Unlike EcoRII, Mval produces DNA **Concentration** fragments with a 1-base 5'-extension 10u/µl and is not blocked by Dcm methylation Conditions for 100% Activity т 1X Buffer R: 5'...C C A G G...3' 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl₂, 3'...G G T[^]C C...5' 100mM KCl and 0.1mg/ml BSA. Α Incubate at 37°C. #ER0551 2000u Supplied with:

> λDNA 1.4% agarose

Storage Buffer

Mval is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 400mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Activity in Five Buffer Syst B G 0 R Tango 2X Tango 20-50 20-50 50-100 100 20-50* 100 Star activity appears at a greater than 5-fold overdigestion (5u x 1h).

Ligation and Recleavage

After 10-fold overdigestion with Mval, more than 90% of the DNA fragments can be ligated in a reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments and 10% PEG. More than 90% of these can be recut.

Methylation Effects

Dam: never overlaps - no effect. Dcm: completely overlaps - no effect (p.134). CpG: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Note

- Low salt, high glycerol (>5%) concentrations or a large excess of enzyme may result in star activity.
- Unlike its neoschizomer EcoRII, Mval does not require multiple copies of recognition site for efficient cleavage.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
70	2	7	6	5	5	5	5	5	8	12

CG Sensitivity to CpG Methylation 1herman 1nactivation Thermal

High Concentration

HC

Bulk quantities & custom formulations available on request



Mva12691	(Bsml)		R	370 95% 2	<u>55</u> X 📾	•	В	Activity G	y in Five 0	Buffer Syste R		2X Tang		
							0-20		50-100		0-20	50-100		
5' G A A T G C 3' C T T A C^ G				entration	1			ion and l						
3'C T T A C G	N 5′		10u/µl				After 50-fold overdigestion with Mva1269I, mo than 95% of the DNA fragments can be ligat							
#ER0961	200u		1X Buf		- 100% Acti	vity	and recut.							
Supplied with: 10X Buffer R	1ml	_	10mM	Tris-HCI (°C), 10mM MgCl ₂ ,	Methylation Effects							
10X Buffer Tango™	1ml			VIKCI and te at 37°(0.1mg/ml BS	SA.	Dam: never overlaps – no effect. Dcm: never overlaps – no effect.							
#ER0962 Supplied with:	1000u	_					CpG: may overlap – no effect (p.137).							
10X Buffer R 10X Buffer Tango™	1ml 1ml		Storage Buffer Mva1269I is supplied in:					EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.						
				10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/mI BSA and					•					
				/v) glycer					e -embed e enzyme a					
				, , , , , , , ,			comple	ete digesti	ion of 1	µg of agar				
		λ DNA 1.0% agarose					λDNA	in 16 ho	urs.					
Lambda ΦX174	M13mp18/19	0	UC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlu	escriptIISK	(-/+)	pACYC177	pА	CYC184		
46 4	1	1	0	1	0	0		0		2		3		
Mvnl	Fermen	tas enzyme Bs	sh1236l,	p.45										
Mwol	Fermen	tas enzyme Hr	oyF10VI,	p.79										
Nael	Fermen	tas enzyme Pc	ii , p.94											
			. /	- 70										
Narl	Fermen	tas enzyme Eh	iel (differ	ent cleava	ge position),	0.70								

Ncol		Tango 37º 95% 🖔	<u>65</u> 👗 🥯		B G	0		ingo 2X Tango
5′ C↓C A T G G 3′ 3′ G G T A C[^]C 5′		<i>Concentration</i> 10u/µl			20-50 20-50 <i>Methylation</i> Dam: never o	Effects		100 100
#ER0571 500u Supplied with: 10X Buffer Tango [™] 1ml		<i>Conditions for</i> 1X Buffer Tango [™] 33mM Tris-acetat 10mM Mg-acetat	: e (pH 7.9 at	37°C),	Dcm: never o CpG: never o EcoKI: never o EcoBI: never o	verlaps – verlaps –	no effect. no effect.	
#ER0572 2500u Supplied with: 10X Buffer Tango™ 10X Buffer Tango™ 1ml FastDigest™ Ncol (see p.2) 1ml		0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Ncol is supplied ir			Digestion of Minimum 5 ur complete dige λ DNA in 16 h	nits of the stion of 1	e enzyme ar	e required fo
#ER0574 50 reactions Supplied with: 10X FastDigest [™] Buffer 1ml		10mM Tris-HCI (p 1mM DTT, 0.1mM 50% (v/v) glycero	H 7.5 at 25° 1 EDTA, 0.2n		<i>Note</i> Low salt, high pH >8.0 or a	arge exce		
	λ DNA 0.7% agarose		digestion with	th Ncol, more than can be ligated and	in star activity			
Lambda Φ X174 M13mp18/		DUC18/19 pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIIS	GK(-/+)	pACYC177	pACYC184
4 0 0	0	0 0	0	0	0		0	1

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37^o Incubation Temperature

SAM Requires SAM

Ligation 95% Efficiency Star Activity Dam Sensitivity to Dam Methylation

1

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DTT Requires DTT

Tango Recommended Buffer



CG Sensitivity to CpG Methylation

Thermal Inactivation

5′ C A↓T 3′ G T A				10u/µl	entration	100% Acti		<i>Ligation and Recle</i> After 50-fold overdige 95% of the DNA frag	stion with Nd		(
#ER0581 Supplied with 10X Buffer O		500u 1ml		1X Buff	er O:	100% Acti H 7.5 at 37°	C), 10mM MgCl _a ,	recut. Methylation Effect			
10X Buffer Ta	ingo™	1ml 2500u		100mN		0.1mg/ml B		Dam: never overlaps Dcm: never overlaps			
Supplied with 10X Buffer O 10X Buffer Ta		1ml 1ml		Ndel is	ge Buffer supplied in Tric, HCL (p	ו:	C), 100mM KCl,	CpG: never overlaps EcoKI: never overlaps EcoBI: may overlap –			
FastDigest [*] #ER0584 Supplied with 10X FastDige	50 re	(see p.2) actions 1ml	λ DNA 0.7% agarose	1mM D 50% (v		EDTA, 0.2mg		Digestion of Agaro Minimum 5 units of the complete digestion of λ DNA in 16 hours.	se-embeda ne enzyme are	<i>ded DNA</i> e required for	
Lambda	ΦΧ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	

Nhel			Tango 3	70 95%		B 100	Activity G 20-50	/ in Five 0 0-20	Buffer Sys R 0-20	stem, % Tango 100		
5′ G↓C T A G (3′ C G A T C ↑(Concentration Methylation Effects 10u/µl Dam: never overlaps – Dom: never overlaps – Dom: never overlaps –										0-20
#ER0971 Supplied with: 10X Buffer Tango™	500u 1ml		1X Buff 33mM	^f er Tango™ Tris-aceta	100% Acti ': te (pH 7.9 at te, 66mM K-	37°C),	<mark>CpG:</mark> EcoKI:		<mark>ap – cl</mark> rlaps –	leavage i · no effec	<mark>mpair</mark> e :t.	d (p.137).
#ER0972 Supplied with: 10X Buffer Tango™	2500u 1ml		0.1mg/ Incubat <i>Storag</i>	′mľ BSA. te at 37°C ge Buffer	-		Minimi	ation of A um 5 unite te digesti	s of th∈ on of 1	e enzyme	e are re	quired for
		λΟΝ	10mM 1mM D 50% (v)TT, 0.1ml /v) glycerc	oH 8.0 at 25° / EDTA, 0.2r I.	C), 50mM KCI, ng/ml BSA and	pH >8					entrations, nay result
		0.7% agaros	se After 5	50-fold ov 5% of D		with Nhel, more is can be ligated						
Lambda Φ X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	escriptIISK((-/+)	pACYC17	77 p/	ACYC184

NIaIII Fermentas enzyme Hin1II, p.74 NIalV Fermentas enzyme BspLI, p.50

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HC High Concentration

Recombinant Enzyme

Bulk quantities & custom formulations available on request



NmuCl (T	sp45I)		R 37° 55% CG 555	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 20-50 50-100 100 20-50 50-100
С 5′↓G Т G А С 3′ С А С Т G [´] G			Concentration 10u/µl Conditions for 100% Activity	<i>Ligation and Recleavage</i> After 50-fold overdigestion with NmuCl, m than 95% of the DNA fragments can be liga and recut.
#ER1511 Supplied with: 10X Buffer R 10X Buffer Tango [™] #ER1512 Supplied with:	200u 1ml 1ml		1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgC 100mM KCI and 0.1mg/ml BSA. Incubate at 37°C. <i>Storage Buffer</i> NmuCI is supplied in:	I ₂ , <i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p. 13 EcoKI: never overlaps – no effect.
10X Buffer R 10X Buffer Tango™	1ml 1ml	λ DNA 1.4% agarose	10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	, ,
	1140 40/40	55444	pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS	(-/+) pBluescriptIISK(-/+) pACYC177 pACYC18
Lambda ΦX174 81 8	M13mp18/19 8	рВR322 р 9	4 4 4 4	4 5 7
				4 5 7 Activity in Five Buffer System, %
⁸¹ 8	8			Activity in Five Buffer System, % B G R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-51
81 8	8 E_G_C 3'		4 4 4 4 4 0 37° 55% CG 580 HC 7 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	Activity in Five Buffer System, % B G R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-5 Ligation and Recleavage Classical Classical
81 8 Notl 5′g c↓g g c d	8 E_G_C 3'			Activity in Five Buffer System, % B G O R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-4 Ligation and Recleavage After 50-fold overdigestion with Notl, m than 95% of DNA fragments can be ligation 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100 20-50 100
81 8 Notl 5'G C↓G G C C 3'C G C C G C	8 C G C3′ ≰↑C G5′		4 4 4 4 4 0 37 ⁰ σ CG δ HC γ σ () Concentration 10u/μl 50u/μl, HC Conditions for 100% Activity	Activity in Five Buffer System, % B G O R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-10 Ligation and Recleavage After 50-fold overdigestion with NotI, m than 95% of DNA fragments can be ligated and recut.
81 8 Not! 5'G C↓G G C C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™	8 C G C3′ ≸↑C G5′ 300u 1ml 1ml		4 4 4 4 4 0 37⁰ ³⁷⁰ ¹ ¹⁰⁵ ¹⁰⁵	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-50 Ligation and Recleavage After 50-fold overdigestion with Notl, m than 95% of DNA fragments can be ligated and recut. Methylation Effects
81 8 Not! 5'G C↓G G C C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0592 Supplied with:	8 C G C 3' 300u 1ml 1ml 1500u		4 4 4 4 4 0 37° ³⁷⁰ CG ³⁷⁰ HC X ³⁷⁰ ³⁷⁰ CG ³⁷⁰ CG ³⁷⁰ CG ³⁷⁰ CG ³⁷⁰ C	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-4 Ligation and Recleavage After 50-fold overdigestion with Not1, m than 95% of DNA fragments can be ligated and recut. Methylation Effects I Dam: no effect. Dcm: no effect. 12,1 Dam: never overlaps – no effect. Dcm: no effect.
81 8 Not! 5'G C↓G G C C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0592	8 C G C3′ ≸↑C G5′ 300u 1ml 1ml		4 4 4 4 4 0 37º 570 CG ∑80 HC X € € € Concentration 10u/µl 50u/µl, HC Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgC 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C.	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-5 Ligation and Recleavage After 50-fold overdigestion with Not1, m than 95% of DNA fragments can be liga and recut. Methylation Effects I I I I I2, Dam: noverlaps – no effect. I
81 8 Notl 5'G C↓G G C C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango [™] #ER0592 Supplied with: 10X Buffer O 10X Buffer O 10X Buffer Tango [™] #ER0593 HC	8 C G C 3' 300u 1ml 1ml 1500u 1ml		4 4 4 4 4 0 37 ⁰ 55 [∞] CG ∑10 HC X ∞ € Concentration 10u/µl 50u/µl, HC Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgC 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Notl is supplied in:	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-7 Ligation and Recleavage After 50-fold overdigestion with Not1, m than 95% of DNA fragments can be ligated and recut. Methylation Effects I2, Dam: never overlaps – no effect. Dcm: never overlaps – no effect. DCF: completely overlaps – no effect. CpG: completely overlaps – no effect. EcoBI: never overlaps – no effect. EcoBI: never overlaps – no effect.
81 8 5'G C↓G G C G 3'C G C C G C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0592 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0593 #ER0593 HO Supplied with: 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer Tango™ HO	8 c g c 3' f c g 5' 300u 1ml 1ml 1500u 1ml 1ml 2, 1500u 1ml 1ml		4 4 4 4 0 37° 50% CG Concentration 10u/µl 50u/µl, HC Image: Conditions for 100% Activity 1mu Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgC 100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Notl is supplied in: 20mM Tris-HCl (pH 7.8 at 25°C), 100mM NaC 0.1mM EDTA, 10mM 2-mercaptoethanol, 0.1mM EDTA, 10mM 2-mercaptoethanol,	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-4 Ligation and Recleavage After 50-fold overdigestion with NotI, m than 95% of DNA fragments can be liga and recut. Methylation Effects I2, Dam: never overlaps – no effect. DCm: never overlaps – no effect. CpG: completely overlaps – no effect. CpG: completely overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoBI: never overlaps – no effect. C1, Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required
81 8 5'G C↓G G C G 3'C G C C G C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0592 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0593 #ER0593 HC Supplied with: 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer Tango™ #ER0593 #ER0594 50 m	8 c g c 3' f c g 5' 300u 1ml 1ml 1500u 1ml 1ml 2, 1500u 1ml 1ml	9 Ad2 DNA	4 4 4 4 4 0 37 ⁰ 5 ⁵ CG 5 ⁶ HC X 6 € 6 6 € € € 6 € 6 € € 6 € € 6 € € € € € 6 € € € € € € € € € €	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-10 Ligation and Recleavage After 50-fold overdigestion with NotI, m than 95% of DNA fragments can be ligated and recut. Methylation Effects J2, Dam: never overlaps – no effect. Dcm: never overlaps – no effect. DCG: completely overlaps – blocked (p. 135) EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. CoBI: never overlaps – no effect. C1, Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1µg of agarose-embedded DNA Adenovirus-2 DNA in 16 hours.
81 8 5'G C↓G G C G 3'C G C C G C 3'C G C C G C #ER0591 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0592 Supplied with: 10X Buffer O 10X Buffer Tango™ #ER0593 #ER0593 HC Supplied with: 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer O 10X Buffer Moth	8 C G C 3 [,] T C G 5 [,] 3 00u 1ml 1ml 1500u 1ml 1ml 2, 1500u 1ml 1ml (see p.2) eactions	9	4 4 4 4 0 37° 5% CG	4 5 7 Activity in Five Buffer System, % B G 0 R Tango 2X Ta 0-20 0-20 100 20-50 0-20 20-4 Ligation and Recleavage After 50-fold overdigestion with NotI, m than 95% of DNA fragments can be liga and recut. Methylation Effects I2, Dam: never overlaps – no effect. DCm: never overlaps – no effect. CpG: completely overlaps – no effect. CpG: completely overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. EcoKI: never overlaps – no effect. C1, Digestion of Agarose-embedded DIVA Minimum 5 units of the enzyme are required complete digestion of 1µg of agarose-embedded

• 0.5M EDTA, pH 8.0 p.369 • Si	PureExtreme [™] Quality & Performance Supplied with 10X concentrated buffers:
	– color-coded optimal buffer (one of B, G, O, R, Tango™) – universal Tango™ buffer, specially formulated for double digests
<u>On-</u>	- Universial range — burler, specially formulated for double digests - Inne technical support: - DoubleDigest [™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer - REsearch [™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

1

1. RESTRICTION ENDONUCLEASES —

Tango Recommended Buffer

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1. RESTRICTION ENDONUCLEASES Product Description

Nrul

CG Sensitivity to CpG Methylation

Thermal Inactivation

Fermentas enzyme Bsp68I, p.47

Nsbl (Mst)		Tango 3	8 7 ° 80% (CG 🔀 🗡		В	G	0	Buffer Sy R	stem, % Tango	2X Tango			
5′ T G C↓G C . 3′ A C G ↑C G			Сопсе 10и/µl	entration			After 5		erdiges	stion with		20-50 nore than			
#ER1221 Supplied with:	400u	_		itions for fer Tango™	100% Acti 1:	vity			0	ments ca e can be		jated and			
10X Buffer Tango™	1ml				te (pH 7.9 at		-	lation l							
#ER1222 Supplied with:	2000u		0.1mg	/ml BSA.	te, 66mM K-	acetate and	Dcm: I	never ove	erlaps -	- no effe - no effe	ct.				
10X Buffer Tango™	1ml										y overlaps – blocked (p.135). ap – no effect (p.138). laps – no effect.				
		λDN 0.7% agaro	10mM 1mM E 50% (v	Tris-HCI (p	oH 7.4 at 25° EDTA, 0.2mg	°C), 100mM KCI, /ml BSA and	Minimu comple	um 5 unit	is of the	2	e are re	DNA quired for mbedded			
Lambda ФX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK	(-/+)	pACYC1	77 p/	ACYC184			
15 1	1	4	2	2	2	2		2		1		3			

Nsil	Fermentas enzyme Mph1103I, p.85
Nspl	Fermentas enzyme Xcel, p.116
NspV	Fermentas enzyme Bsp119I , p.47

Olil			R	70 80%	XI (<u>)65</u>		B 0-20	Activit G 0-20	y in Five 0 0-20		Tango 2	2X Tango 50-100
5′C A C N N↓ 3′G T G N N↑ #ER1631			10u/µl Condi		- 100% Acti	ivity	After 50 80% of	the DN	/erdige A fragr	avage stion with (ments can se can be r	be ligat	
Supplied with: 10X Buffer R 10X Buffer Tango™ #ER1632	1ml 1ml 1000u		100m	Tris-HCI (p	0.1mg/ml B	°C), 10mM MgCl ₂ , SA.	<i>Methy</i> Dam: n Dcm: n	lation E never ove never ove	E ffects erlaps - erlaps -	s - no effect. - no effect.		
Supplied with: 10X Buffer R 10X Buffer Tango™	1ml 1ml	Ξ	Olil is s	ge Buffel supplied in Tris-HCI (p	:	°C), 100mM KCI,	EcoKI: n	nay over	iap – b	leavage im blocked (p.1 blocked (p.1	38).	(p.137).
		λ DNA 0.7% agarose	50% (\)TT, 1mM /v) glycero		g/ml BSA and	Minimu complet	m 5 unit	is of the	se-embed e enzyme a 1µg of agan	are requ	lired for
Lambda Φ X174 20 1 * According to our expe	0*	0	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlues	scriptIISK 1	(-/+)	pACYC177 0		YC184 0

Genome Qualified

HC High Concentration Recombinant Enzyme 1

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Blue/White Certified FastDigest" Enzyme



Pael (Sphl)	В	37° 95% 燆 🖌		Activity in Five Buffer System, B G O R Tan 100 50-100 0-20 0-20 50-1						
5' G C A T G↓C 3' 3' C[↑]G T A C G 5'	Conc 10u/μ	e ntration		Ligation and Recleav After 50-fold overdigestid	on with Pael, more t					
#ER0601 500u		litions for 100% ffer B:	Activity	95% of the DNA fragme recut.	inis can be ligated					
Supplied with: 10X Buffer B 1ml 10X Buffer Tango™ 1ml	10mM and 0	1 Tris-HCI (pH 7.5 a .1mg/ml BSA.	at 37°C), 10mM MgCl ₂	<i>Methylation Effects</i> Dam: never overlaps – r						
#ER0602 2500u <i>Supplied with:</i> 10X Buffer B 1ml 10X Buffer Tango [™] 1ml	Stora	ate at 37°C. age Buffer s supplied in:		Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect.						
FastDigest [™] Pael (see p.2) #ER0604 50 reactions	50mN	1 potassium-phosp 1 KCI, 1mM DTT, 0 6 Triton X-100, 0.5		EcoBI: may overlap – blo Digestion of Agarose Minimum 5 units of the e	-embedded DNA					
Supplied with: 10X FastDigest [™] Buffer 1ml	λ DNA 0.7% agarose	0% (v/v) glycerol.		complete digestion of 1 μ λ DNA in 16 hours.	g of agarose-embed					
Lambda	pBR322 pUC18/19	pUC57 pTZ19	PR/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) p	DACYC177 pACYC18					
6 0 1	1 1	1 1	0	0	0 1					

PaeR7I

Fermentas enzyme Xhol, p.117

Pagl (BspHI)			0 37º	95% Dax)		B 0-20	Activity G 50-100	y in Five O 100	Buffer Sy R NR		2X Tange NR
5′ T↓C A T G A 3′ 3′ A G T A C↑T 5′			Concentr 10u/µl	ation				Methy	<i>Iation E</i>	ffects			
Supplied with: 10X Buffer O	20u 1ml 20u 1ml		Condition 1X Buffer C 50mM Tris 100mM Na Incubate at Storage E Pagl is sup 10mM Tris 1mM DTT, 50% (v/v) g Ligation a): -HCI (pH aCI and (: 37°C. Buffer plied in: -HCI (pH 0.1mM I glycerol. and Red	7.5 at 37°).1mg/ml B 7.4 at 25° EDTA, 0.2m c leavage	C), 10mM Mg	laCl, d	Dcm: I CpG: I EcoKI: I EcoBI: I Diges Minimu comple λ DNA Note Low sa	never over never over may over tion of J im 5 unit in 16 hor alt, high 0 or a lar	erlaps – erlaps – erlaps – lap – no Agaros s of the ion of 1 urs. glycero	no effec no effec no effect (effect (enzyme µg of ag	st. ct. (p.138). edded e are rec arose-ei	DNA quired for mbedde
_Lambda ΦX174 Μ1 8 3		'% agarose BR322 pU 4	recut.		pTZ19R/U	n be ligated a pBluescriptII 2		pBlue	scriptIISK	(-/+)	pACYC1	77 рА	1 1
Supporting Products Dilution Buffer O.5M EDTA, pH 8.0 GX Loading Dye & SD	S Solution	p.121 p.369 p.336	PureExtrSupplied	reme™ Q I with 10	r <mark>iction En</mark> uality & Pel X concentr	donuclease		anqo™)					
			– univer <u>On-line te</u> – Double	sal Tang echnica eDigest™	o [™] buffer, s a l support ′at <u>www.fe</u>	pecially form	ulated fo	or doubl	or optima	l double			zvmes

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

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Tango Recommer Buffer	nded DTT Requires DTT	SAM Requires SAM
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0000 **1. RESTRICTION ENDONUCLEASES**

Pasl	1. R	ESTRICTION ENDONUCLEASES Product Description Activity in Five Buffer System, % B G 0 R Tango 2X Tango
A 5'C C↓C T G G G3' 3'G G G A C↑C C5' T #ER1861 200u Supplied with: 10X Buffer Pasl 1ml	Concentration 10u/µl Conditions for 100% Activity 1X Buffer Pasl: 10mM Bis-Tris Propane-HCI (pH 6.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/ml BSA. Incubate at 55°C.	NR NR NR NR NR Methylation Effects Dam: never overlaps – no effect. Dcm: completely overlaps – no effect. CpG: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. T EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 10 units of the enzyme are required for
	Storage Buffer Pasl is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 0.7% agarose After 10-fold overdigestion with PasI, more than 90% of the DNA fragments can be ligated and recut.	
Lambda ΦX174 M13mp18/19 2 0 0	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 0 0 0 0 0 0	pBluescriptIISK(-/+) pACYC177 pACYC184 0 0 2



Fermentas enzyme BsuRI, p.53

Paul (Bsel)		R 3	R 37º 📆 CG 🎘 🕷 🐼					in Five 0 100	Buffer Sys R 100		2X Tango 100
5′G↓C G C G C 3′C G C G C10	10u/µl	<i>Concentration</i> 10u/µl					<i>Methylation Effects</i> Dam: never overlaps – no effect.					
#ER1091 Supplied with: 10X Buffer R 10X Buffer Tango™	200u 1ml 1ml		Conditions for 100% Activity Dcm: never overlaps – no effect. 1X Buffer R: Dcm: never overlaps – blocked (p 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ , EcoKl: never overlaps – no effect. 100mM KCl and 0.1mg/ml BSA. EcoBl: never overlaps – no effect.								<mark>cked (</mark> p t.	o.135).
#ER1092 Supplied with: 10X Buffer R 10X Buffer Tango™	2 1000u Incubate at 37°C. <i>Storage Buffer</i> r R 1ml Paul is supplied in:					Digestion of Agarose-embedded DNA Minimum 10 units of the enzyme are required for complete digestion of $1\mu g$ of agarose-embedded λ DNA in 16 hours.						
					ng/ml BSA and		rge exces				entrations Ilt in star	
		0.7% agaros		f the DNA								
Lambda ΦX174 6 1	M13mp18/19 0	pBR322 0	pUC18/19 0	pUC57 0	pTZ19R/U 0	pBluescriptIIKS(-/+) 2	pBlue	escriptIISK(2	-/+)	pACYC17 0	7 рА	0 0

Genome Qualified

Pcil

CG Sensitivity to CpG Methylation

Fermentas enzyme Pscl, p.97

HC High Concentration

Thermal Inactivation



Pdil (Nael)

Tango 37º 30% CG 🏷 🗡 🔘 🕯

]		Activit	y in Five I	Buffer Sys	stem, %	
j	В	G	0	R	Tango	2X Tango
	50-100	20-50	0-20	0-20	100	50-100

Dam: never overlaps - no effect. Dcm: never overlaps - no effect.

EcoKI: never overlaps - no effect.

Methylation Effects

5′...G C C G G C...3′ 3'...C G G C C G...5'

#ER1521 Supplied with:	200u	
10X Buffer Tango™	1ml	_
#ER1522 Supplied with:	1000u	
10X Buffer Tango™	1ml	
		pBR322 DNA
		0.7% agarose

Concentration

10u/µl

Conditions for 100% Activity 1X Buffer Tango[™]: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Pdil is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 500mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA, 0.15% Triton X-100 and 50% (v/v) glycerol.

Ligation and Recleavage

After 50-fold overdigestion with Pdil, more than 90% of the DNA fragments can be ligated and recut.

EcoBI: never overlaps - no effect. Digestion of Agarose-embedded DNA Minimum 20 units of the enzyme are required for

CpG: completely overlaps - blocked (p.135).

complete digestion of 1µg of agarose-embedded pBR322 DNA in 16 hours.

Note

• Certain sites in pBR322 are difficult to cleave with Pdil, the same as with its prototype Nael.

Assayed using pBR322 DNA (#SD0041).

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	1	4	0	0	1	1	1	0	5

Pdml (Xm	nl)		Tango 37º 95% CG (565 🗡					Activity in Five Buffer System, % B G Q R Tango 2X Tango 20-50 50-100 0-20 0-20 100 0-20						
5′G A A N N↓ 3′C T T N N↑1 #ER1531		10u/µl <i>Condi</i>		r 100% Act	Ligation and Recleavage After 50-fold overdigestion with Pdml, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect.									
<i>Supplied with:</i> 10X Buffer Tango [™]	500u 1ml		33mM	ër Tango" Tris-aceta Mg-aceta										
			Incubat Storag	ml BSA. e at 37°C je Buffe i	r	Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.137) EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined.								
		λ DNA 0.7% agarose	Pdml is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM KCl, 1mM DTT, 5mM MgCl ₂ , 0.2mg/ml BSA and 50% (v/v) glycerol.					Digestion of Agarose-embedded DNA Minimum 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.						
Lambda Φ X174 24 3	M13mp18/19 2	pBR322 p	UC18/19	pUC57 1	pTZ19R/U 1	pBluescriptIIKS(-/+) 1	pBlue	escriptIISK 1	(-/+)	pACYC1 2	77 p/	ACYC184 1		

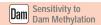
Supporting Products		Fermentas Restriction Endonucleases:
Dilution Buffer	p.121	 PureExtreme[™] Quality & Performance
0.5M EDTA, pH 8.06X Loading Dye & SDS Solution	p.369 p.336	 Supplied with 10X concentrated buffers: – color-coded optimal buffer (one of B, G, O, R, Tango[™])
		 – universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

Recommended

DTT Requires

Tango Recons

www.fermentas.com/doubledigest www.fermentas.com www.fermentas.com/research



ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

Ptifi	Fermentas enzyme Psyl, p.100
PfIMI	Fermentas enzyme Van911, p.114

Genome Qualified



Bulk quantities & custom formulations available on request Recombinant Enzyme FastDigest[™] Enzyme Blue/White Certified



Pfol				4 9 9%	Davn Devn CQ		B 0-20	G 20-50 5	0 60-100	R 0-20	Tango 100	2X Tang 50-100
5' T \downarrow C C N G G A 3' 3' A G G N C C \uparrow T 5'				ntration tions for	- 100% Acti	ivity	After 5 95% o	on and R O-fold over f the DNA	rdigest	ion with		
#ER1751 Supplied with: 10X Buffer Tango™	200u 1ml	=	33mM 10mM 0.1mg/		te (pH 7.9 at te, 66mM K-		recut. <i>Methylation Effects</i> Dam: may overlap – cleavage impaired (p.13 Dcm: may overlap – blocked (p.134). CpG: may overlap – blocked (p.137).					d (p.133
	λ.DN 0.7% agaros	Pfol is s 10mM 1mM D 50% (v		n: pH 7.4 at 25 EDTA, 0.2mg	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 10 units of the enzyme are required for complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours.							
Lambda ΦΧ17 15 Ο	M13mp18/19 0	pBR322	pUC18/19 1	pUC57 1	pTZ19R/U 0	pBluescriptIIKS(-/+) 0	pBlue	escriptIISK(-/ 0	/+)	pACYC17 1	7 p/	1 1
Phol	Formor		BsuRI , p.53									

Phoi	Fermentas enzyme BsuRI, p.53
PinAl	Fermentas enzyme BshTI, p.46
Plel	Fermentas enzyme Schl (different cleavage position), p.104
PmaCl	Fermentas enzyme Eco72I, p.65
Pmel	Fermentas enzyme MssI, p.86
Pmll	Fermentas enzyme Eco72I, p.65

Ppil			R 3	0° <u>90%</u> -			В	G	0	Buffer Sy R	Tango	2X Tango		
$5 = \frac{1}{2} (\mathbf{N}) \mathbf{G} \mathbf{A} \mathbf{A} \mathbf{C}$ $3 = \frac{1}{2} (\mathbf{N}) \mathbf{C} \mathbf{T} \mathbf{T} \mathbf{G}$	(N) ₅ C T C(1 (N) ₅ G A G(N	1) ⁸ ↓3, 1) ¹³ ↓3,	Сопсе 1-3u/µ	entration	,		Dam:	0-20 / lation / never ov	erlaps -	- no effe		50-100		
#ER1541 Supplied with: 10X Buffer R 10X Buffer Tango™	50u 1ml 1ml		<i>Conditions for 100% Activity</i> 1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCI ₂ , 100mM KCI and 0.1mg/mI BSA.					Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired (p.137). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.						
#ER1542 Supplied with: 10X Buffer R 10X Buffer Tango™	250u 1ml 1ml	λ DNA 0.7% agarose	Storag Ppil is 10mM 1mM E 50% (v Ligati After 1	Incubate at 30°C. Storage Buffer Ppil is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 10-fold overdigestion with Ppil, more than 90% of the DNA fragments can be ligated and recut.				frecogniti	s certa r 8 nt a lon sequ A A C (N) T T G (N) ce of Sa completa n 10-fol	ain DNA way on Jence: ∫₅ C T C (N ₅ G A G (N AM in a e cleavag Id overdi	A seque the top $I_{13} \downarrow3'$ $I_{3} \uparrow5'$ reaction ge with	ences at strand of n mixture		
Lambda Φ X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIIS	<(-/+)	pACYC1	77 p.	ACYC184		

37^o Incubation Temperature

96

www.fermentas.com/doubledigest www.fermentas.com/research

95% Ligation Efficiency



SAM Requires SAM

1. RESTRICTION ENDONUCLEAS Product Description



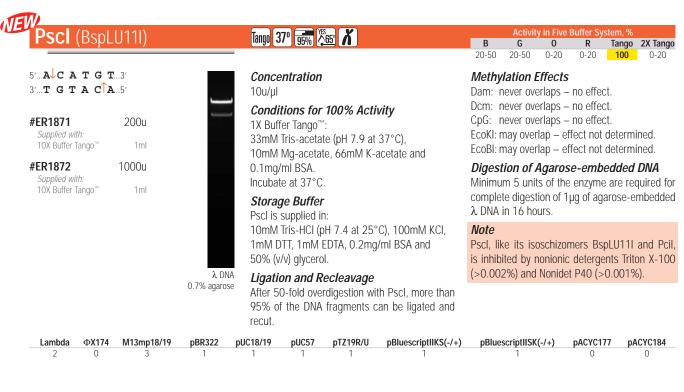
Ppu21I (E	saAl)		Unique (30° 90% (CG <u>(565</u> /		В	G	0	Buffer Syst R	Tango	2X Tango
5' Py A C↓G T 3' Pu T G ↑C A			Conc 10u/μ	entration	,		Methy	100* ity appears at lation E never ove	ffects			NR on (5u x 1h).
#ER1971 Supplied with: 10X Buffer Ppu21I	500u 1ml		1X Bu 10mN 150m	ffer Ppu21 I Tris-HCI (J	oH 7.2 at 37 d 0.1mg/ml	°C), 3mM MgCl ₂ ,	CpG: 0 EcoKI: r EcoBI: r	never ove completel may overl may overl tion of A	<mark>ly overla</mark> lap – eff lap – eff	ps – bloo fect not c fect not c	c <mark>ked</mark> (p leterm leterm	ined. ined.
			Ppu21 10mN 100m	M NaCl, 1r	ed in: n phosphate nM EDTA,	(pH 7.4 at 25°C), 2mg/ml BSA and	Minimu comple λ DNA <i>Note</i>	im 5 unit: te digesti in 16 hou bation at	s of the ion of 1 ₁ urs.	enzyme ıg of aga	are rei rose-e	quired fo mbedde
		λ DNA 0.7% agarose	<i>Ligat</i> After	50-fold ov	Recleavage erdigestion	with Ppu21I, more ents can be ligated	pH >	ity. salt, high >8.0 or a It in star a	a large			
Lambda ФX174	M13mp18/19	pBR322 p	and re UC18/19	cut. pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	nPluo	scriptIISK(pACYC177	7	ACYC184



CG Sensitivity to CpG Methylation

Thermal

1 Inactivation





Genome

Qualified

High Concentration

HC

800-0

Bulk quantities & custom formulations available on request Blue/White

Certified

Recombinant

Enzyme

 \bigcirc

FastDigest Enzyme



Psp5II (Pp	ы (МЛ) <u> </u>		G 37° 95% Dem 🖉					ly III Five	Buffer Sy	stem, %	
			a 37 95% DMU (<u>78</u>			B 0-20	G 100	0 20-50	R 20-50	Tango 50-100	2X Tang 100
T ′…Pu G↓G A C			<i>Concentration</i> 10u/µl			Ligatio	on and	<i>Reclea</i> /erdigest	vage		
3' Py C C T G A	G Pu 5′	-	<i>Conditions for 100%</i> 1X Buffer G:	% Activity			5% of th	e DNA fr			
#ER0761 Supplied with: 10X Buffer G 10X Buffer Tango™	500u 1ml 1ml		10mM Tris-HCI (pH 7.5 50mM NaCI and 0.1mg. Incubate at 37°C.		0mM MgCl ₂ ,	Dam: r	never ov	Effects erlaps – rlap – bl	no effe ocked (r	ct. p.134).	
TOX BUILEL LANGO	1111		Storage Buffer Psp5II is supplied in: 10mM Tris-HCI (pH 7.4	at 25°C). 10	00mM KCL	CpG: r EcoKI: r	never ov never ov	erlaps – erlaps – rlap – ef	no effe no effe	ct. ct.	ined.
		λ DNA 0.7% agarose	1mM DTT, 1mM EDTA, (0.2mg/ml BSA and 50%	0.1% Triton X	X-100,	Minimu comple	ım 5 uni	Agaros ts of the tion of 1 ours.	enzyme	e are red	quired f
		0.770 agarose									
Lambda Φ X174 3 0	M13mp18/19 0		· · ·	19R/U pBlu O	o 0	pBlue	scriptIISK O	((-/+)	pACYC1 0	77 pA	2 2
	0	pBR322 p		0		В	0 Activit G	ty in Five O	0 Buffer Sy R	rstem, %	2 2X Tan
³ 0 Psp1406l	(AcII)	pBR322 p	0 0 0	0		B 100	0 Activit G 50-100	t <mark>y in Five 0</mark> 0-20	0 Buffer Sy R 20-50	rstem, %	2 2X Tan
з 0 Рѕр14061 5аа↓сата	o (AcII)	pBR322 p	0 0 0 0 Tango 370 35% CG Σ6 Concentration 10u/μl			в 100 <i>Ligatic</i> After 50	0 Activit G 50-100 Don and D-fold ov	ty in Five I 0 0-20 Reclea verdigest	0 Buffer Sy R 20-50 Vage ion with	rstem, % Tango 100	2 2X Tan 0-20 06l, mc
3 0 Psp14061 5 ^{аа↓с с т т 3^тт с с1а а}	o (AcII)	pBR322 p	0 0 0 0 Tango 37 ⁰ 95% CG μ Concentration 10u/μl Conditions for 100%			в 100 <i>Ligatic</i> After 50	0 Activit G 50-100 On and D-fold ov 5% of th	ty in Five 0 0-20 <i>Reclea</i>	0 Buffer Sy R 20-50 Vage ion with	rstem, % Tango 100	2 2X Tan 0-20 06l, mc
3 0 Psp14061 5аа↓с д т т 3т т д с↑а а	0 (AcII) 3' 5'	pBR322 p	0 0 0 0 Tango 37º 95% CG № Concentration 10u/µl Conditions for 100% 1X Buffer Tango™: 33mM Tris-acetate (pH	0 65 X & Activity 7.9 at 37°C)	0	B 100 Ligatia After 50 than 95 and rec Methy	0 Activit G 50-100 on and D-fold ov 5% of th cut.	<mark>ty in Five 0</mark> 0-20 Reclea verdigest ne DNA f Effects	0 Buffer Sy 20-50 Vage ion with ragmen	<mark>rstem, %</mark> Tango 100 n Psp14(ts can b	2 2X Tan 0-20 06l, mc
3 0 Psp1406 5' A ↓C G T 1 3' T T G C ↑ A 2 #ER0941 Supplied with:	0 (AcII) 3' 5' 300u	pBR322 p	0 0 0 0 Tango 37 ⁰ 95% CG ^{γS6} Concentration 10u/μl Conditions for 100% 1X Buffer Tango™: 33mM Tris-acetate (pH 10mM Mg-acetate, 66m	0 65 X & Activity 7.9 at 37°C)	0	B 100 Ligatii After 50 than 95 and rec Methy Dam: r	0 Activit G 50-100 On and D-fold ov 5% of th cut. vlation in hever ov	t <mark>y in Five 0</mark> 0-20 Reclea verdigest ne DNA f Effects erlaps –	0 Buffer Sy 20-50 Vage tion with tragmen	rstem, % Tango 100 n Psp14(ts can b ct.	2 2X Tan 0-20 D6l, mo
3 0 Psp1406 5' A ↓C G T T 3' T T G C ↑ A Z #ER0941 <i>Supplied with:</i> 10X Buffer Tango [™]	o (AcII) 3' 5' 300u 1ml	pBR322 p	0 0 0 0 Tango 37º 95% CG № Concentration 10u/µl Conditions for 100% 1X Buffer Tango™: 33mM Tris-acetate (pH	0 65 X & Activity 7.9 at 37°C)	0	B 100 Ligatii After 50 than 95 and rec Methy Dam: r Dcm: r CpG: c	0 Activit G 50-100 On and D-fold ov 5% of the cut. Action of hever ov hever ov complete	ty in Five 0 0-20 <i>Reclea</i> verdigest erdigest erdigest erlaps – erlaps – erlaps – erlaps –	0 Buffer Sy R 20-50 Vage ion with ragmen no effer no effer no effer aps – bl	rstem, % Tango 100 n Psp14(ts can b ct. ct. ct. ocked (p	2 2X Tan 0-20 06I, mo be ligate
3 0 Psp14061 5'a a↓C g T T 3'T T g C↑a z #ER0941 Supplied with: 10X Buffer Tango [™] #ER0942 Supplied with:	0 (AcII) 3' 5' 300u 1ml 1500u	pBR322 p	0 0 0 0 Tango 37º 95% CG № Concentration 10u/µl Conditions for 100% 1X Buffer Tango [™] : 33mM Tris-acetate (pH 10mM Mg-acetate, 66n 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer	o 65° X 7.9 at 37°C) mM K-acetate	0	B 100 Ligatii After 50 than 95 and rec Methy Dam: r Dcm: r CpG: c EcoKI: r	0 Activit G 50-100 On and D-fold ov 5% of th cut. Action of hever ov hever ov complete may ove	ty in Five 0-20 <i>Reclea</i> verdigest he DNA f <i>Effects</i> erlaps – erlaps – erlaps – ely overla rlap – ef	0 Buffer Sy R 20-50 vage tion with tragmen no effer no effer no effer aps – bl fect not	rstem, % Tango 100 n Psp14(ts can b ct. ct. ct. ocked (p determi	2 2X Tan 0-20 06I, mo oe ligate 0.135). ined.
3 0 Psp14061 5'a a↓C g T T 3'T T g C↑a z #ER0941 Supplied with: 10X Buffer Tango [™] #ER0942 Supplied with:	0 (AcII) 3' 5' 300u 1ml 1500u	pBR322 p	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 65 X 7.9 at 37°C) mM K-acetate n: at 25°C), 10), e and)0mM KCI,	B 100 Ligatia After 56 than 95 and rec Methy Dam: r Dcm: r CpG: c EcoKI: r EcoBI: r Diges Minimu comple	Activit G 50-100 on and D-fold ov 5% of th cut. Alation in hever ov complete may ove may ove may ove may ove may ove	ty in Five 0 0-20 Reclea verdigest the DNA f erlaps – erlaps – erlaps – erlap – ef rlap – ef Agaros hits of the tion of 1	0 Buffer Sy 20-50 Vage ion with ragmen no effer aps – bl fect not fect not e-emb	rstem, % Tango 100 n Psp140 ts can b ct. ct. ocked (p determi determi determi eedded e are rea	2X Tan 0-20 06I, mo be ligate b.135). ined. ined. DNA quired f

				5							
Lan	nbda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
	7	3	2	4	2	2	2	2	2	2	2

Supporting Products		Fermentas Restriction Endonucleases:
 Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution 	p.121 p.369 p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

1

1. RESTRICTION ENDONUCLEASES —

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASE

Product Description

PspAl	Fermentas enzymes Cfr91, p.55 and Sma1 (different cleavage position), p.106
PspGI	Fermentas enzymes EcoRII, p.70 and Mval (different cleavage position and different sensitivity to methylation), p.87
PspOMI	Fermentas enzymes Apal (different cleavage position), p.30 and Bsp120I, p.48

Pstl		0 37º 📆 🔊 HC 🗡 📟 🕐	Activity in Five Buffer System, %						
1 Su		U 37 95% 🏊 nu 📶 📟 🔛		В	G	0	R	Tango	2X Tango
				50-100	50-100	100	100	50-100	50-100
5' C T G C A		Concentration		Ligatio	on and	Reclea	vage		
3'G `A C G T	C 5′	 10u/µl		After 50	0-fold ov	verdigest	tion wit	h Pstl, n	nore than
#ER0611	3000u	50u/µl, HC		95% of	f the DN	A fragm	ents ca	an be lig	gated and
Supplied with:		Conditions for 100% Activity		recut.					
10X Buffer O	2x1ml	1X Buffer O:		Methv	lation l	Effects			

50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl_a,

10mM Tris-HCI (pH 7.4 at 25°C), 200mM NaCl,

1mM DTT, 0.1mM EDTA, 0.15% Triton X-100,

0.2mg/ml BSA and 50% (v/v) glycerol.

100mM NaCl and 0.1mg/ml BSA.

Incubate at 37°C.

Storage Buffer

Pstl is supplied in:

High Concentration

HC

 λ DNA

0.7% agarose

Methylation Effects Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
28	1	1	1	1	1	1	1	1	1	0

Psul (Xhol	1)		B	87° 95% X	ES 80°				ty in Five					
	')			90%	.00		B 100	G 20-50	0 0-20	R 0-20	Tango 50-100	2X Tango 0-20		
fPu↓GATCPy3' fPyCTAG ^P Pu5' ER1551 500u Supplied with: 10X Buffer B 1ml 10X Buffer Tango [™] 1ml			5u/µl Condi 1X Buf 10mM and 0. Incuba	Conditions for 100% Activity 1X Buffer B: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer					Ligation and Recleavage After 50-fold overdigestion with Psul, more th 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: completely overlaps – no effect (p.133 Dcm: may overlap – no effect. CpG: may overlap – no effect (p.137).					
			10mM 1mM [pH 7.4 at 25 EDTA, 0.2mg	°C), 200mM NaCl, g/ml BSA and	EcoBI: <i>Note</i>	never ov may over lycerol (rlap – el	fect not	determ	ined. pH >8.C		
		λ DN 0.7% agaros	A e				or a la activity	rge exce	ess of e	nzyme r	may resi	ult in star		
Lambda Φ X174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+) pBlue	scriptIISK	K(-/+)	pACYC1	// p/	CYC184		

Genome Qualified Genome

#ER0611 30 Supplied with: 10X Buffer O 10X Buffer Tango™ 1ml #ER0612 5x3000u Supplied with: 10X Buffer O 2x1ml 10X Buffer Tango™ 1ml #ER0613 HC, 15000u Supplied with: 10X Buffer O 2x1ml NEW 10X Buffer Tango™ 1ml FastDigest[™] Pstl (see p.2) #ER0614 200 reactions Supplied with:

10X FastDigest[™] Buffer

Bulk quantities & custom formulations available on request Recombinant FastDigest Enzyme Blue/White

Certified



5′G A C N↓N 3′C T G N N↑			Сопс а 10и/µl	entration				on and R 0-fold ove			Psvl n	nore thar
3' C T G N N[↑]N C A G 5' #ER1331 1000u Supplied with: 10X Buffer B 1ml 10X Buffer Tango [™] 1ml			Condu 1X Buf 10mM and 0. Incuba Stora Psyl is 10mM 1mM [itions for fer B: Tris-HCI (p 1mg/ml BS te at 37°C ge Buffer supplied ir Tris-HCI (p	SA. H 7.4 at 25 1 EDTA, 0.2	ivity °C), 10mM MgCl ₂ °C), 50mM NaCl, mg/ml BSA and	60% of reaction Ligase/ than 95 <i>Methy</i> Dam: r Dcm: r CpG: r EcoKI: r	f the DNA n mixture /1µg of fra 5% of thes /lation Ef never over never over may overla never over may overla	fragm conta agmer se can f fects laps – laps – n laps –	nents car ining 20- nts and 7 be recut - no effect - no effect - no effect - no effect	n be lig 40u of 10% Pl t. t. t. 5.137). t.	ated in a T4 DNA EG. More
		λ DNA 0.7% agarose	·	, , , ,			Minimu comple	tion of A um 5 units ete digestic in 16 hou	of the	e enzyme	are red	quired fo
$\begin{array}{c c} Lambda & \Phi X174 \\ \hline 2 & 0 \end{array}$	M13mp18/19	pBR322 pl	JC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	escriptIISK(-	/+)	pACYC17	7 pA	CYC184

3' G C[↑]T A			Сопсе 10u/µl	entration	CG 💒 🗡		B G O 0-20 20-50 50-1 Ligation and Rec After 50-fold overdig	100 100 50 <i>leavage</i>	ango 2X Tango)-100 100
	G C 5'		10u/µl				•	•	ul more than
Supplied with:	300u		Condi						
		-	1X Buff		100% Acti	ivity	90% of the DNA fra more than 95% of the		
10X Buffer Tango	1ml 1ml	-	100mN	/ KCI and	0.1mg/ml BS	°C), 10mM MgCl ₂ , SA.	Methylation Effect Dam: completely ov	verlaps – no ef	fect (p.133).
#ER0622 Supplied with:	1500u			te at 37°C ge Buffer			Dcm: never overlap CpG: completely ov		<mark>ed</mark> (p.135).
10X Buffer R 10X Buffer Tango	1ml ™ 1ml		Pvul is	supplied in	n:	°C), 300mM KCl,	EcoKI: never overlap EcoBI: never overlap	s – no effect.	
		λ Dľ 0.7% agaro	0.1mM 50% (v		nM DTT, 0.2r	ng/ml BSA and	Digestion of Aga Minimum 5 units of complete digestion α λ DNA in 16 hours.	the enzyme ar	e required for
Lambda ФX	(174 M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0 1	1	2	2	2	2	2	2	0

Supporting Products• Dilution Bufferp.121• 0.5M EDTA, pH 8.0p.369• 6X Loading Dye & SDS Solutionp.336	- color-coded optimal burler (one of B, G, O, K, Tango)
	 universal Tango[™] buffer, specially formulated for double digests On-line technical support: DoubleDigest[™] at www.fermentas.com/doubledigest for optimal double digest buffer REsearch[™] at www.fermentas.com/research for complete information on restriction enzymes
www.fermentas.com www.fermentas	com/doubledigest www.fermentas.com/research

37^o Incubation Temperature

Ligation 95% Efficiency Star Activity Dam Sensitivity to Dam Methylation

1

100

Tango Recommended Buffer DTT Requires DTT

SAM Requires SAM

1. RESTRICTION ENDONUCLEASES



#ER1122

Supplied with:

10X Buffer Tango™

CG Sensitivity to CpG Methylation

Product	Description
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ISO ISO 9001 14001	1. R	ESTRICTION ENDONUCLEASES Product Description
Prull5c $A \subseteq J \subseteq T \subseteq3^{\circ}$ $a \subseteq T \subseteq J \subseteq A \subseteq3^{\circ}$ $a \subseteq T \subseteq J \subseteq A \subseteq3^{\circ}$ *ERO631 2500u $Bupfied with:$ $DX Buffer G = 1ml$ $DX Buffer TangoTM = 1ml$ *ERO632 5x2500u $Supplied with:$ $DX Buffer G = 2x1ml$ $DX Buffer TangoTM = 1ml$	G 37º 0 € En HC K Concentration 10u/µl 50u/µl, HC Conditions for 100% Activity 1X Buffer G: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 50mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Pvull is supplied in: 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 10-fold overdigestion with Pvull, more than 90% of the DNA fragments can be ligated and more than 95% of these can be recut.	
Lambda ΦX174 M13mp18/19 15 0 3 Rcal Ferment \$\mathbf{S}_{\mathbf{C}}\$ \$\mathbf{F}_{\mathbf{C}}\$ \$\mathbf{C}_{\mathbf{C}}\$ \$\mathbf{C}_{\mathbf{C}}\$ \$\mathbf{M}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$ \$\mathbf{L}_{\mathbf{C}}\$	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/- 1 2 2 2 2 2 tas enzyme PagI, p.92 Tango 37° Tigo Tigo <thtigo< th=""> Tigo <thtigo< th=""> <thtigo< th=""> Tigo<th> PBluescriptIISK(-/+) pACYC177 pACYC184 2 0 2 Activity in Five Buffer System, % B G 0 R Tango 2X Tango 50-100 20-50 0-20 0-20 100 0-20 Ligation and Recleavage After 50-fold overdigestion with Rsal, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects </th></thtigo<></thtigo<></thtigo<>	 PBluescriptIISK(-/+) pACYC177 pACYC184 2 0 2 Activity in Five Buffer System, % B G 0 R Tango 2X Tango 50-100 20-50 0-20 0-20 100 0-20 Ligation and Recleavage After 50-fold overdigestion with Rsal, more than 95% of the DNA fragments can be ligated and recut. Methylation Effects

Methylation Effects Dam: never overlaps - no effect.

Dcm: never overlaps - no effect. CpG: may overlap – cleavage impaired (p.137). EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA 1.4% agarose pTZ19R/U M13mp18/19 pBR322 pUC18/19 pUC57 pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 113 11 19 3 3 3 2 2 4 3

Genome Qualified

High Concentration

HC

10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI,

10mM Mg-acetate, 66mM K-acetate and

0.1mg/ml BSA.

Incubate at 37°C.

Storage Buffer

Rsal is supplied in:



Thermal Inactivation

5000u

2x1ml

Blue/White

Certified

Enzyme

FastDigest[®] Enzyme



Sacl	Linne 37º 誡 🏍 HC 🗡 🥯 🕐	Activity in Five Buffer System, %
		B G O R Tango 2X Tan 50-100 20-50 0-20 0-20 50-100 20-50
5′G A G C T↓C3′ 3′C [↑] T C G A G5′	Concentration 10u/ul	Ligation and Recleavage After 50-fold overdigestion with Sacl, more th
#ER1131 1200u	50u/μl, HC	95% of the DNA fragments can be ligated a recut.
Supplied with: 10X Buffer Sacl 1ml 10X Buffer Tango™ 1ml	Conditions for 100% Activity 1X Buffer Sacl:	Methylation Effects
#ER1132 5x1200u Supplied with:	10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA. Incubate at 37°C.	Dam: never overlaps – no effect. Dcm: never overlaps – no effect.
10X Buffer Sacl2x1ml10X Buffer Tango™1ml	Storage Buffer	CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect. EcoBI: may overlap – no effect (p.138).
#ER1133 HC, 6000u Supplied with: 10X Buffer Sacl 2x1ml	SacI is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI,	Digestion of Agarose-embedded DNA
10X Buffer Tango [™] 1ml FastDigest [™] SacI (<i>see</i> p.2)	1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.	Minimum 5 units of the enzyme are required complete digestion of 1µg of agarose-embedd
#ER1134 50 reactions <i>Supplied with:</i>	λ DNA 0.7% agarose	λ DNA in 16 hours.
10X FastDigest [™] Buffer 1ml Lambda ΦX174 M13mp18/19	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescript1IKS(-/+)	pBluescriptIISK(-/+) pACYC177 pACYC184
Coll		Activity in Five Buffer System, %
Sall	0 37° 📆 CG 🏂 HC 🗡 🕯	B G O R Tango 2X Tar 0-20 0-20 100 20-50 0-20 50-10
5′G↓T C G A C3′ 3′C A G C T [^] G5′	Concentration 10u/ul	<i>Methylation Effects</i> Dam: never overlaps – no effect.
#ER0641 1500u	50u/µl, HC <i>Conditions for 100% Activity</i>	Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135).
Supplied with: 10X Buffer O 1ml 10X Buffer Tango™ 1ml	1X Buffer O: 50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCI ₂ ,	opol. completely overlaps blocked (p. 199).
#ER0642 5x1500u		EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.
Supplied with:	100mM NaCl and 0.1mg/ml BSA. Incubate at 37°C.	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA
10X Buffer O 2x1ml 10X Buffer Tango™ 1ml	Incubate at 37°C. Storage Buffer	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.
10X Buffer O 2x1ml	Incubate at 37°C. Storage Buffer Sall is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C),100mM KCI,	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1 μ g of agarose-embedded λ DNA in 16 hours. Note
10X Buffer O 2x1ml 10X Buffer Tango [™] 1ml #ER0643 HC, 7500u Supplied with:	Incubate at 37°C. Storage Buffer Sall is supplied in:	EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1 μ g of agarose-embedd λ DNA in 16 hours.

recut. Lambda ФХ174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 0 2 0 1 1 1 1 1 1 1 1

Temperature

95% of the DNA fragments can be ligated and

Sapl

Tango Recommended Buffer

Fermentas enzyme Lgul, p.81

1

1. RESTRICTION ENDONUCLEASES —

DTT Requires DTT

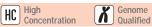
95% Efficiency



SAM Requires SAM

											00	100
ISOISO 9001 14001						1. RE	STRICT	ION EN			ASES scription	
Satl (Fnu4	HI)		G 3	70 60%	CG 565		В	<mark>ctivity in Five</mark> G O 00 20-50	R		2X Tango 20-50	
5'G C↓N G C 3'C G N [↑] C G!	-		10u/µl	ntration			<i>Ligation a</i> After 50-fo 60% of the	ld overdige	stion with			0
#ER1641 Supplied with: 10X Buffer G	200u 1ml 1ml		1X Buff 10mM	er G: Tris-HCI (p		°C), 10mM MgCl ₂ ,	reaction m Ligase/1µg than 90% (1				
10X Buffer Tango [™] #ER1642 <i>Supplied with:</i> 10X Buffer G 10X Buffer Tango [™]	Incubat Storag SatI is s 10mM 1mM D	e at 37°C Je Buffei supplied ir Tris-HCI (p	r n: oH 7.4 at 25° EDTA, 0.2mç	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – blocked (p.137). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.								
Lambda ФХ174 380 31	M13mp18/19 17	λ DN. 1.4% agaros pBR322 42		pUC57 19	pTZ19R/U 20	pBluescriptIIKS(-/+)	pBluescrip	• •	pACYC1 19	77 p#	ACYC184 37	
Saul	Fermen	tas enzyme	Eco81I , p.6	6								
Sau96I	Ferment	tas enzyme	Cfr13I , p.56)								
Sau3AI		tas enzymes different sens				leavage position and	different sen	sitivity to m	ethylatior	ו), p.59	and	
Sbfl	Ferment	tas enzyme	<mark>Sdal</mark> , p.104									

Scal			Unique 3	70 80%	N N			Activity in Five Buffer System, % B G O R Tango 2X Tango							
								0-20	0-20	0-20	0-20	0-20	0-20		
5′ A G T↓A C 3′ T C A[↑]T G			Сопсе 10и/µl	entration	1			<i>Ligation and Recleavage</i> After 50-fold overdigestion with Scal, more than							
#ER0431 Supplied with:	1000u			tions for ^f er Scal:	[.] 100% Act	tivity		80% of the DNA fragments can be ligated and more than 90% of these can be recut.							
10X Buffer Scal	1ml					(pH 6.5 at 37°C		<i>Methylation Effects</i> Dam: never overlaps – no effect.							
#ER0432	5000u			MgCI ₂ , TC te at 37°C		nd 0.1mg/ml BS	SA.		never ov never ov						
<i>Supplied with:</i> 10X Buffer Scal	2x1ml		Scal is	ge Buffei supplied i Tris-HCI (r		CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – blocked (p.138).									
	1mM D	10mM Tris-HCI (pH 7.5 at 25°C), 50mM KCI, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.					Digestion of Agarose-embedded DNA Minimum 20 units of the enzyme are required for complete digestion of $1\mu g$ of agarose-embedded λ DNA in 16 hours.								
Lambda ΦX17	74 M13mp18/19	0.7% agarose pBR322 pl	UC18/19	pUC57	pTZ19R/U	pBluescriptIIK	(S(-/+)	pBlue	scriptIISk	((-/+)	pACYC1	77 p/	ACYC184		
5 0	0	1	1	1	1	1		P-140	1		1	p.	1		





Bulk quantities & custom formulations available on request

FastDigest[™] Enzyme



1000u

Schl (Plel*)

* Unlike Plel, Schl produces

5'...G A G T C(N) 5

3'...C T C A G(N) (1...5'

#ER1371

Supplied with: 10X Buffer Tango™

ScrFI

DNA fragments with blunt ends

Tango 37º 📆 🛧 🏷 🔿

Conditions for 100% Activity

33mM Tris-acetate (pH 7.9 at 37°C),

10mM Mg-acetate, 66mM K-acetate and

10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI,

After 10-fold overdigestion with Schl, approxi-

mately 90% of DNA fragments can be ligated and more than 90% of these can be recut.

1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and

Concentration

1X Buffer Tango[™]:

0.1mg/ml BSA.

Incubate at 37°C.

Storage Buffer

Schl is supplied in:

50% (v/v) glycerol.

Ligation and Recleavage

10u/µl

Activity in Five Buffer System, %											
В	G	0	R	Tango	2X Tango						
20-50	50-100	0-20	0-20	100	0-20						

Methylation Effects

Dam: never overlaps - no effect. Dcm: never overlaps - no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps - no effect. EcoBI: may overlap - effect not determined.

Note

- · Greater than 10-fold overdigestion with Schl may result in star activity.
- Schl may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
61	10	8	4	4	3	7	6	6	6	4

Fermentas enzyme Bme1390I, p.37

 λ DNA

1.4% agarose

Sdal (SSP8	3871)		Inique 3	70 .						Buffer Sys		
	0300	13071)		Unique	/ 95% /			B NR	G	0 0-20	R 0-20	Tango NR	2X Tango 20-50
5'C C T C 3'G G A G #ER1191 Supplied with: 10X Buffer Sd 10X Buffer Tar #ER1192 Supplied with: 10X Buffer Sd 10X Buffer Tar	C G T al ıgo™			10u/µl Condi 1X Buff 37mM 15mM 0.1mg/ Incubat Storag Sdal is 10mM 1mM D	Tris-aceta Mg-aceta Mg-aceta MI BSA. e at 37°C ge Buffer supplied in Tris-HCI (p	te (pH 7.0 at te, 150mM k	5	Dam: Dcm: CpG: EcoKI: EcoBI: Diges Minim comple λ DNA Note Greate	never ov never ov never ov never ov stion of um 5 un ete diges v in 16 h	verlaps - verlaps - verlaps - verlaps - verlaps - verlaps - Agaro its of th stion of ours.	s - no effec - no effec - no effec - no effec - no effec se-embo e enzyme 1µg of aga	t. t. t. e dded are re arose-e	quired for mbedded
Lambda	Φ Χ174	M13mp18/19	λ DNA 0.7% agarose pBR322 0	After 5	-fold over		h Sdal, more than can be ligated and pBluescriptIIKS(-/+)	pBlu	escriptIIS	K(-/+)	pACYC17 0	'7 p/	ACYC184
5	U	I	0	I	0	I	0		0		0		0
Supporting Products p.121 • Dilution Buffer p.369 • 6X Loading Dye & SDS Solution p.369 • 6X Loading Dye & SDS Solution p.369 • DureExtreme™ Quality & Performance • Supplied with 10X concentrated buffers: • color-coded optimal buffer (one of B, G, O, R, Tango™) • universal Tango™ buffer, specially formulated for double digests On-line technical support: • DoubleDigest™ at www.fermentas.com/doubledigest for optimal double digest buffer											Izymes		
www.ferm	entas	.com www	<i>i.</i> fermentas	.com/doul	oledigest	www.fer	mentas.com/resea	rch					
Tango Recomm	nended	DTT Requir	_		37		e 95% Efficience	y	St Ac	ar ctivity		Sensitivi Dam Me	ity to thylation

1. RESTRICTION ENDONUCLEASE

ISO ISO 9001 14001

Product Description

Sdul		Unique 37º 📆	65'		Activity in Five Buffer System, % B G O R Tango 2X Tango					
T T A A 5'G G G C C↓C3 3'C ¹ C C G G G5		Concentration 10u/μl Conditions for	100% Activ	ity	NR 50-100* 50-100 0-20 NR NR * Star activity appears at a greater than 5-fold overdigestion (5u x 1h). <i>Ligation and Recleavage</i> After 50-fold overdigestion with Sdul, more than 95% of the DNA fragments can be ligated and					
Т Т А А	500u	1X Buffer Sdul: 10mM Tris-HCl (p 150mM NaCl and Incubate at 37°C.	0.1mg/ml BS		recut. <i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: may overlap – no effect (p.134).					
Supplied with: 10X Buffer Sdul	1ml	Storage Buffer Sdul is supplied in 10mM Tris-HCI (p	n: H 7.4 at 25°0		CpG: may overlap – no effect (p.137). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined.					
	λ 1.0% ag	1mM EDTA, 1mM 50% (v/v) glycerol . DNA arose		ml BSA and	<i>Note</i> Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.					
Lambda ΦX174 M 38 3	M13mp18/19 pBR322 5 10	2 pUC18/19 pUC57 5 6	pTZ19R/U 5	pBluescriptIIKS(-/+) 6	pBluescriptIISK(-/+) 6	pACYC177 4	pACYC184 8			
Secl	Fermentas enzyr	ne <mark>BseDI</mark> , p.40								
SfaNI	Fermentas enzyr	me Lwel , p.82								
Sfcl	Fermentas enzyr	me Bfml , p.35								
Sfel	Fermentas enzyr	me Bfml , p.35								

Sfil

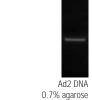
G 50° 35% Den CG 🎘 🖝 🔏 🥯

Activity in Five Buffer System, G 100 В 0 R Tango 2X Tango 50-100 20-50 0-20 100 0-20

5'...G G C C N N N N V N G G C C...3' 3'...CCGGNÎNNNNCCGG...5'

#ER1821	1000u	
Supplied with:		
10X Buffer G	1ml	
10X Buffer Tango™	1ml	
5		

CG Sensitivity to CpG Methylation



Thermal

1 Inerman

Concentration 10u/µl Conditions for 100% Activity

1X Buffer G: 10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl₂, 50mM NaCl and 0.1mg/ml BSA. Incubate at 50°C.

Storage Buffer

High Concentration

HC

Sfil is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 300mM NaCl, 10mM MgCl₂, 1mM DTT, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage

After 50-fold overdigestion with Sfil, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects Dam: never overlaps - no effect. Dcm: may overlap - cleavage impaired (p.134).

CpG: may overlap - cleavage impaired (p.137). EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded Adenovirus-2 DNA in 16 hours.

Note

• At least two copies of Sfil recognition site are required for efficient cleavage.

FastDigest Enzyme

- Incubation at 37°C results in 10% activity.
- Assayed using Adenovirus-2 DNA.

Blue/White

Certified

Enzyme

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	Ad2
0	0	0	0	0	0	0	0	0	0	0	3

Sfol Fermentas enzyme Ehel, p.70 Sful Fermentas enzyme Bsp119I, p.47 Bulk quantities & custom formulations available on request Recombinant

Genome

Qualified



Sgsl (Ascl	Tango 37º 📆 CG 🏷 🖝 🕯					Activity in Five Buffer System, % B G O R Tango 2X Tango							
							0-20	0-20	0-20	50-100	100	50-100	
5′ G G C G C		Conce	entration	Ligation and Recleavage									
3' C C G C G	C G G 5'		5u/µl						After 50-fold overdigestion with Sgsl, more than 95% of the DNA fragments can be ligated and				
#ER1891 Supplied with:	_		<i>tions for</i> fer Tango™	100% Acti	vity	95% of recut.	f the DNA	, fragn	nents can	be lig	ated and		
10X Buffer Tango™ 1ml			33mM Tris-acetate (pH 7.9 at 37°C),						Methylation Effects				
#ER1892 1500u Supplied with: 10X Buffer Tango [™]			10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA.					Dam: never overlaps – no effect. Dcm: may overlap – no effect.					
			Incubate at 37°C. Storage Buffer						CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect.				
				supplied i			EcoBI: r	never over	laps –	- no effect			
					°C), 300mM KCI,	Diges	tion of A	garos	se-embe	dded	DNA		
)TT, 0.1mľ /v) glycero		ng/ml BSA and				e enzyme a µg of agar				
	λ DN/ 0.7% agaros					λ DNA	in 16 hou	Irs.					
Lambda ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK(-	-/+)	pACYC177	р рА	CYC184	
2 0	0	0	0	0	0	0		0		0		0	

Sinl

Fermentas enzyme Eco47I, p.63

Smal	Tango 30º 📆 CG 🎘 HC 🗡 🕯 🕐	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 0-20 0-20 0-20 100 0-20				
5'C C C G G G G3' 3'G G G C C C C5' #ER0661 1200u Supplied with: 10X Buffer Tango™ 1ml #ER0662 5x1200u Supplied with: 10X Buffer Tango™ 2x1ml #ER0663 HC, 6000u Supplied with: 10X Buffer Tango™ 2x1ml FastDigest™ Smal (see p.2) #ER0664 50 reactions Supplied with: 10X FastDigest™ Buffer 1ml	Concentration 10u/µl 50u/µl, HC Conditions for 100% Activity 1X Buffer Tango™: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 30°C. Storage Buffer Smal is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.					
0.7% agai use		 Note Incubation at 37°C results in 50% activity. Incubation at 25°C results in 100% activity. 				
		 Incubation at 25°C results in 100% activity. 				
Lambda ΦX174 M13mp18/19 pBR322 p 3 0 1 0	DUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/- 1 1 1 1 1	 Incubation at 25°C results in 100% activity. 				
		 Incubation at 25°C results in 100% activity. pBluescriptIISK(-/+) pACYC177 pACYC184 1 0 1 0 R, Tango[™]) 				
3 0 1 0 Supporting Products • • • • Dilution Buffer p.121 • • • 0.5M EDTA, pH 8.0 p.369 • • • 6X Loading Dye & SDS Solution p.336	1 1 1 Fermentas Restriction Endonucleases: • PureExtreme™ Quality & Performance • Supplied with 10X concentrated buffers: - color-coded optimal buffer (one of B, G, O, F - universal Tango™ buffer, specially formulater On-line technical support: - DoubleDigest™ at www.fermentas.com/doul	 Incubation at 25°C results in 100% activity. pBluescriptIISK(-/+) pACYC177 pACYC184 1 1 0 R, Tango[™]) d for double digests pledigest for optimal double digest buffer for complete information on restriction enzymes 				

1. RESTRICTION ENDONUCLEASES

Bulk quantities & custom formulations available on request

Blue/White Certified

FastDigest[™] Enzyme

Recombinant Enzyme

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Smil (Swal)		0 30" 📷 🎘 🐼 🥯	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 0-20 100 20-50 0-20 20-50
Supplied with: 10X Buffer O 10X Buffer Tango™ #ER1242 50 Supplied with:		Concentration 10u/µl Conditions for 100% Activity 1X Buffer O: 50mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA. Incubate at 30°C. Storage Buffer Smil is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 200mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Ligation and Recleavage After 50-fold overdigestion with Smil, more than 80% of the DNA fragments can be ligated in a reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments and 10% PEG. More	 Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKl: never overlaps – no effect. EcoBl: may overlap – effect not determined. Digestion of Agarose-embedded DNA Minimum 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded Adenovirus-2 DNA in 16 hours. Mote Incubation at 37°C results in 70% activity. Assayed using Adenovirus-2 DNA.
Smll		o, see below	
Smol (Smll)		Tango 55° 📆 Dem Ker X	Activity in Five Buffer System, %BGORTango2X Tango50-10020-500-2020-5010020-50Ligation and Recleavage
5C↓T Py Pu A G 3G A Pu Py T↑C	5 3'	Tango 55° 55% Dem ∑ Concentration 10u/μl Conditions for 100% Activity 1X Buffer Tango™:	BGORTango2X Tango50-10020-500-2020-5010020-50Ligation and RecleavageAfter 50-fold overdigestion with Smol, more than95% of the DNA fragments can be ligated and recut.
5"C↓T Py Pu A G 3"G A Pu Py T↑C #ER1981 2	2 3' 25'	Tango 55° 55° Tango X Concentration X X X X 10u/μl Conditions for 100% Activity	B G O R Tango 2X Tango 50-100 20-50 0-20 20-50 100 20-50 Ligation and Recleavage After 50-fold overdigestion with Smol, more than 95% of the DNA fragments can be ligated and



Smul (Faul)	Tango 3	3 7 ° 95%		1	Activity in Five Buffer System, % B G O R Tango 2X Tango						
5'CCCGC(N) 3'GGGCG(N)	Сопсе 1-3u/µ	entration	1		50-100 50-100 0-20 20-50 100 20-50 Ligation and Recleavage After 10-fold overdigestion with Smul, more than						
#ER1691 Supplied with: 10X Buffer Tango [™] #ER1692 Supplied with: 10X Buffer Tango [™]	50u 1ml 250u 1ml	1X Buf 33mM 10mM 0.1mg Incuba Stora	fer Tango" Tris-aceta	ate (pH 7.9 at ate, 66mM K- C. r	37°C),	 95% of the DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect. 					
		λ DM	1mM E 50% (v		M EDTA, 0.2r	°C), 100mM KCI, ng/ml BSA and	<i>Note</i> Greater than 40-fold overdigestion with Sm may result in star activity.				
		1.0% agaro	5e								
Lambda Φ X174 90 0	M13mp18/19 10	pBR322 10	pUC18/19 5	pUC57	pTZ19R/U 5	pBluescriptIIKS(-/+) 5	pBluescriptIISK(-/ 5	+) pACYC177 1	pACYC184 10		
⁹⁰ 0 Snal	io Fermen	pBR322 10 tas enzyme	pUC18/19 5 Bst11071, p	5 0.52			pBluescriptIISK(-/ 5	+) pACYC177 1			
90 0 Snal SnaBl	io Fermeni Fermeni	pBR322 10 tas enzyme tas enzyme	pUC18/19 5 Bst1107I, p	5 0.52			pBluescriptIISK(-/ 5	+) pACYC177 1			
90 0 Snal SnaBl Spel	io Fermeni Fermeni	pBR322 10 tas enzyme tas enzyme	pUC18/19 5 Bst11071, p	5 0.52			pBluescriptIISK(-/ 5	+) pACYC177 1			
90 0 Snal SnaBl	io Fermeni Fermeni Fermeni	pBR322 10 tas enzyme tas enzyme tas enzyme	pUC18/19 5 Bst1107I, p	5 0.52			pBluescriptIISK(-/ 5	+) pACYC177 1			
90 0 Snal SnaBl Spel	io Fermeni Fermeni Fermeni Fermeni	pBR322 10 tas enzyme tas enzyme tas enzyme tas enzyme	pUC18/19 5 Bst1107I, ; Eco105I, p Bcul, p.33	5 0.52 .67			pBluescriptIISK(-/ 5	+) pACYC177 1			

Ssil (Ad	cil)			03	7º 95% (CG (<u>*8</u> 65)		B	Activity G 20-50	in Five Buffer S 0 R 100 50-100	Tang	6 o 2X Tango 100	
5'C↓C G C3' 3'G G C↑G5' #ER1791 200u Supplied with: 10X Buffer O 1ml 10X Buffer Tango™ 1ml		1ml		<i>Concentration</i> 10u/μl <i>Conditions for 100% Activity</i> 1X Buffer O: 50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCl and 0.1mg/ml BSA.					Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.				
			Incuba Storag Ssil is 10mM 1mM E			Ibate at 37°C. rage Buffer is supplied in: nM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, M DTT, 1mM EDTA, 0.2mg/mI BSA and 6 (v/v) glycerol.				glycerol (>59 ge excess of é			
			λ DNA 2.0% agarose	Ligation After 5 mately ligated. due to a The rer	on and R O-fold over 95% of No more asymmetr	ecleavage erdigestion w the DNA fi than 50% of ic recognitior ncleaved liga	with Ssil, approxi- ragments can be these can be recut n sequence of Ssil. tion products may						
	₽ X174 36	M13mp18/19 42	pBR322 67	pUC18/19 34	pUC57 34	pTZ19R/U 32	pBluescriptIIKS(-/+) 36	pBlu	escriptIISK(-	-/+) pACYC	177	pACYC184 56	
www.ferme			v.fermentas				30 mentas.com/resea	ırch	30	32		50	

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

SAM Requires SAM

DTT Requires DTT

1

Tango Recommended Buffer

Dr	oduct	Doc	crir
PI	UUUULI	Des	CHI

3'T T A T A A5' 10u/µl Dam: never overlaps - no effect. Dam: never overlaps - no effect. 10u/µl Supplied with: 10x Buffer G 1ml Dom M Tris-HCI (pH 7.5 at 37°), 10mM MgCl, 50mM NaCl and 0.1mg/ml BSA. Dam: never overlaps - no effect. 10mM Tris-HCI (pH 7.5 at 37°), 10mM MgCl, 50mM NaCl and 0.1mg/ml BSA. EcoRI: never overlaps - no effect. 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCl, 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCl, 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCl, 11mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Digestion of Agarose-embedded DNA 10X Buffer Tango [™] 1ml Note Greater than 10-fold overdigestion with Sspl may result in star activity.	Spin B C O R Tango 2X Tango 5: A A T A T T3' S: T T A T A A5' #ER0771 500u South and the construction Sou	Schl		
B:T T A T A A5' 10u/µl Dam: never overlaps – no effect. Dam: never overlaps – no effect. 10u/µl Supplied with: 10x Buffer G 1ml Dom M Tris-HCI (pH 7.5 at 37°), 10mM MgCI ₂ , 50mM NaCI and 0.1mg/ml BSA. Dom Storage Buffer Cookitions for 100% Activity EcoKI: never overlaps – no effect. Tokitions for 100% Activity YER0772 2500u Storage Buffer Storage Buffer Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded DNA NOX Buffer Tango** 1ml Dom M Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 100M MrscI, 100M MrscI, 100M Mrs-HCI (pH 7.4 at 25°C), 100mM KCI, 100M MrscI, 100M MrscI, 100M Mrs-HCI (pH 7.4 at 25°C), 100mM KCI, 100M MrscI, 100M MrscI, 100M Mrs-HCI (pH 7.4 at 25°C), 100mM KCI, 100M MrscI, 100M MrsC	Sum T T A T A A5' 10υ/μl Doulut Doulut Dam: never overlaps – no effect. Do: Not overlap – no effect. Do: No: Not overlap – no effect.			B G O R Tango 2X Tango 20-50 100 0-20 50-100 100 20-50
10X Buffer Tango [™] 1ml Spir is supplied inf. 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 10mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Note Ligation and Recleavage After 10-fold overdigestion with SspI, more than Mote	10X Buffer Tango [™] 1ml Spirits supplied inf. 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, λ DNA in 16 hours. 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mm DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. Note Ligation and Recleavage After 10-fold overdigestion with Sspl, more than 90% of the DNA fragments can be ligated and recut. may result in star activity. Note Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184	FT T A ↑T A A5' FER0771 500u Supplied with: 10X Buffer G 1ml 10X Buffer Tango [™] 1ml FER0772 2500u Supplied with:	10u/µl Conditions for 100% Activity 1X Buffer G: 10mM Tris-HCl (pH 7.5 at 37°), 10mM MgCl ₂ , 50mM NaCl and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer	Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – no effect (p.138). Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for
	90% of the DNA fragments can be ligated and recut. Lambda ΦX174 M13mp18/19 pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184		10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. <i>Ligation and Recleavage</i> After 10-fold overdigestion with Sspl. more that	 λ DNA in 16 hours. Note Greater than 10-fold overdigestion with Sspl may result in star activity.

<u></u>	rementas enzyme corisor, p.00
StyD4I	Fermentas enzyme Bme1390I (different cleavage position), p.37
Swal	Fermentas enzyme Smil , p.107

Taal (Tsp4	CI)		Tango 6	5° 90% (K 🏷	Activity in Five Buffer System, % B G O R Tango						
5′ A C N↓G T 3′ T G[↑]N C A			- Conce 10u/μl	entration			After 5		verdige	estion with		
#ER1361	200u			<i>tions for</i> fer Tango™	100% Acti	vity				fragments these can		
<i>Supplied with:</i> 10X Buffer Tango™	1ml		33mM	Tris-aceta	te (pH 7.9 at			lation E				
#ER1362	1000u	_		Mg-aceta ml BSA.	te, 66mM K-a	acetate and				 no effect. no effect. 		
<i>Supplied with:</i> 10X Buffer Tango™	1ml		Incubat To ensu	te at 65°C ure higher		digestion, perform araffin oil.	CpG: I EcoKI: I	<mark>may over</mark> may over	l <mark>ap – c</mark> lap – e	leavage im ffect not de ffect not de	i <mark>paired</mark> etermi	nëd.
		λ DN/ 1.4% agarose	Taal is 10mM A 1mM D		n: oH 7.5 at 25° ⁄I EDTA, 0.2n	°C), 100mM KCI, ng/ml BSA and	Note Incubat	tion at 37	7°C res	ults in 10%	6 activ	ity.
Lambda ΦX174	M13mp18/19	pBR322	pUC18/19 8	pUC57 8	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK 8	(-/+)	pACYC177 14	рA	CYC184 20
	(YK) Thorr	nal		1	V) Genome	Bulk quantitie:				ns availat		request
CG Sensitivity to CpG Methylation	on VES Therr Inact	nal ivation	HC High Concent	ration	X Genome Qualified	Recombin Enzyme	ant	Blue Certi	/White ified		FastDig Enzym	



Tail (Maell*)	R 65° 95% CG 🖉	Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 50-100 20-50 100 100 50-100
* Unlike Maell, Tail produces DNA fragments with a 4-base 3'-extension 5' A C G T↓3' 3'↑T G C A5'	Concentration 10u/µl Conditions for 100% Activity 1X Buffer R:	<i>Ligation and Recleavage</i> After 50-fold overdigestion with Tail, more that 95% of DNA fragments can be ligated and recut.
#ER1141 400u Supplied with: 10X Buffer R 10X Buffer Tango™ 1ml #ER1142 2000u Supplied with: 10X Buffer R 10X Buffer R 1ml	 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl₂, 100mM KCI and 0.1mg/ml BSA. Incubate at 65°C. To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil. <i>Storage Buffer</i> Tail is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. 	Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: completely overlaps – blocked (p.135). EcoKI: may overlap – effect not determined. EcoBI: may overlap – effect not determined. Mote Incubation at 37°C results in less than 10% activity.
Lambda ΦΧ174 M13mp18/19 143 19 22	1.4% agarose pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 10 5 5 8 8) pBluescriptIISK(-/+) pACYC177 pACYC184 8 7 9
	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+	8 7 9 Activity in Five Buffer System, % B G O R Tango 2X Tang
143 19 22	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 10 5 5 8 8	8 7 9 Activity in Five Buffer System, %

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
121	10	12	7	4	4	5	7	7	10	10

Supporting Products Dilution Buffer 0.5M EDTA, pH 8.0 6X Loading Dye & SDS Solution	p.121 p.369 p.336	 Fermentas Restriction Endonucleases: PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests
		On-line technical support:
		 DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> for optimal double digest buffer REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes
www.fermentas.com www.fe	ermentas.co	om/doubledigest www.fermentas.com/research

37^o Incubation Temperature **95%** Ligation Efficiency Star Activity Dam Sensitivity to Dam Methylation

SAM Requires SAM

DTT Requires DTT

Tango Recommended Buffer

CG Sensitivity to CpG Methylation

Thermal Inactivation

ISO ISO 9001 14001	1. R	ESTRICTION ENDONUCLEASES Product Description
Tasl (TspEl)	B 65° 📆	B G O R Tango 2X Tango 100 50-100 20-50 0-20 20-50 0-20
5' ↓ A A T T 3' 3' T T A A ↑5' #ER1351 1000u <i>Supplied with:</i> 10X Buffer B 1ml 10X Buffer Tango™ 1ml #ER1352 5000u <i>Supplied with:</i> 10X Buffer B 2x1ml 10X Buffer Tango™ 1ml	 A DNA A DNA A DNA Concentration 10u/µl Conditions for 100% Activity TX Buffer B: 10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl₂ and 0.1mg/ml BSA. Incubate at 65°C. To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil. Storage Buffer Tasl is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. 	Ligation and Recleavage After 50-fold overdigestion with Tasl, more than 95% of DNA fragments can be ligated and recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoBI: nay overlap – blocked (p. 138). Note Incubation at 37°C results in less than 10% activity.
Lambda 02X174 M13mp18/19 189 25 64 Tatl	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+ 8 7 7 10 12	 pBluescriptIISK(-/+) pACYC177 pACYC184 12 14 13 Activity in Five Buffer System, % B G O R Tango 2X Tango NR 50-100* 20-50 20-50 100 0-20
' (A/T) ↓ G T A C (A/T)3' ' (A/T) C A T G (A/T)5' ER1291 100u Supplied with: 10X Buffer Tango™ 1ml ER1292 500u Supplied with: 10X Buffer Tango™	Concentration 5u/µl Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 65°C. To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.	 * Star activity appears at a greater than 5-fold overdigestion (5u x 1h). <i>Ligation and Recleavage</i> After 5-fold overdigestion with Tatl, more than 95% of the DNA fragments can be ligated and recut. <i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect.
	Storage BufferTatl is supplied in:10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI,λ DNA1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and1.0% agarose50% (v/v) glycerol.	 Note Incubation at 37°C results in 20% activity. Greater than 5-fold overdigestion with Tatl may result in star activity.

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	0	5	2	2	2	1	1	1	1	1





G 5′G C C G↓C3′ 3′C ¹ G G C G5′ C #ER1651		=	1-3u/ <i>Cona</i> 1X Bu 10mN	litions foi ffer B:	r 100% Acti pH 7.5 at 37°	r vity °C), 10mM MgCl ₂	Dam: Dcm: CpG: EcoKI:	ylation Eff never overla never overla completely never overla never overla	aps – no efi aps – no efi overlaps – aps – no efi	^f ect. <mark>blockec</mark> ^f ect.	d (p.135).
Supplied with: 10X Buffer B 10X Buffer Tango [™] #ER1652 Supplied with: 10X Buffer B 10X Buffer Tango [™]	λ DNA 1.4% agarose	Incuba Stora Taul is 10mM 1mM 50% (Ligat After	ate at 55°C nge Buffe s supplied i 1 Tris-HCI (DTT, 1mM v/v) glycer ion and F 10-fold c	C. r pH 7.4 at 25° EDTA, 0.2mg ol. Recleavage overdigestion	² C), 100mM KCI, /ml BSA and with Taul, more ts can be ligated						
Lambda ΦX174 181 17	M13mp18/19 7	pBR322 p 21	and re 0 UC18/19 7	ecut. pUC57 7	pTZ19R/U 8	pBluescriptIIKS(-/+) 10	pBlue	e scriptIISK(-/ 10	+) pACY(12		pACYC184 22

Tru11 (Msel)		R 6	50 90%	₩ HC		B 50-100	G	Five Buffer Sy O R 0-50 100	/stem, % Tango 2X Tango 50-100 100		
5′ T↓T A A 3′ 3′ A A T[↑]T 5′			Сопсе 10u/µl 50u/µl,	ntration			After 5	0-fold ove		with Tru1I, more s can be ligated		
#ER0981 Supplied with: 10X Buffer R 10X Buffer Tango™	300u 1ml 1ml	Conditions for 100% Activity and recut. 1X Buffer R: Methylation Effects 10mM Tris-HCl (pH 8.5 at 37°C), 10mM MgCl ₂ , Dam: never overlaps – n							ects	Ŭ		
#ER0982 Supplied with: 10X Buffer R 10X Buffer Tango™	1500u 1ml 1ml		Incubat To ensu	e at 65°C Ire higher		digestion, perform	Dcm: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: may overlap – blocked (p.138). EcoBI: never overlaps – no effect.					
#ER0983 HC, Supplied with: 10X Buffer R 10X Buffer Tango™	1500u 1ml 1ml	λ DN 1.4% agaros	Storag Tru1l is 10mM A 1mM D	re Buffei supplied Tris-HCI (r TT, 1mM	- in: bH 7.4 at 25° EDTA, 0.2mg	°C), 100mM KCl, //ml BSA and	<i>Note</i> Incubati	on at 37°(C results in	10% activity. We ER0983) at 37°C.		
Lambda ΦΧ174 195 35	M13mp18/19 63	pBR322 15	pUC18/19 13	/v) glycerc pUC57 13	л. pTZ19R/U 18	pBluescriptIIKS(-/+) 19	pBlues	s criptIISK(-/- 19	•) pACYC1 16	77 pACYC184 12		

Tru9l

Fermentas enzyme Tru1I, p.112

112

1

1. RESTRICTION ENDONUCLEASES —

www.fermentas.com/doubledigest www.fermentas.com/research 37^o Incubation Temperatur

Temperature

95% Ligation Efficiency



24

0

Sensitivity to

CG CpG Methylation

1

Thermal

1 Inerman

0

High HC

Concentration

0

0

Genome

A Qualified

0

Recombinant

Enzyme

0

Bulk quantities & custom formulations available on request Blue/White

Certified

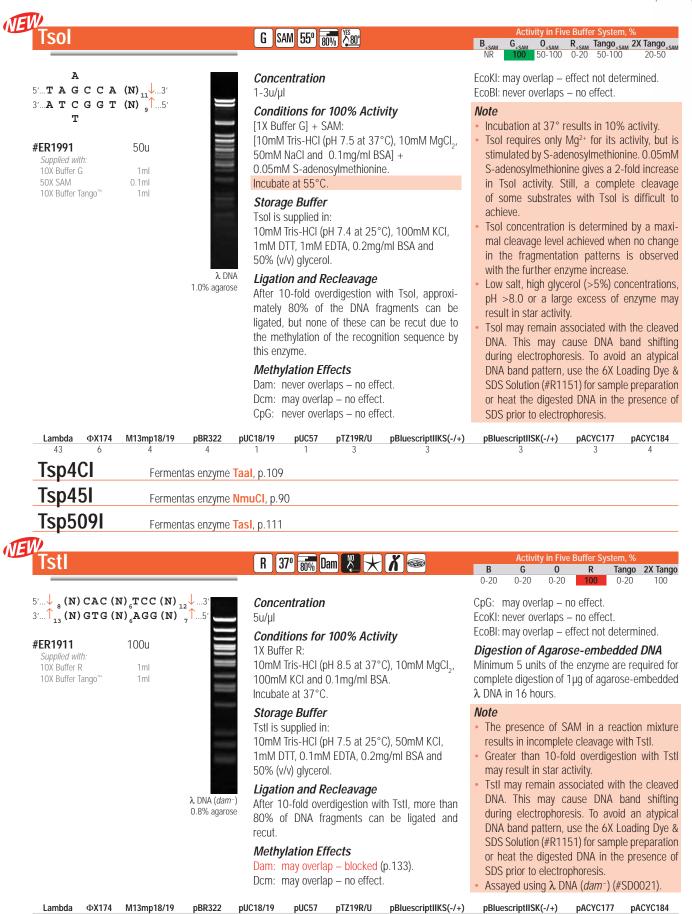
2

2

FastDigest Enzyme

1. RESTRICTION ENDONUCLEASE

Product Description







Fermentas enzyme Psyl, p.100

Van91I (P				87° 90% D			B 0-20	G 0 50-100 50-1	R 00 100	Tango 2X Tango 20-50 50-100
5'C C A N N I 3'G G T NÎN I #ER0711			10u/µl		100% Act	ivity	After 5	i on and Recl 50-fold overdi 20% of DNA cut.	gestion with	
<i>Supplied with:</i> 10X Buffer R 10X Buffer Tango™	1ml 1ml		10mM 100mM	Tris-HCI (p /I KCI and	0.1mg/ml B	°C), 10mM MgCl ₂ , SA.	Dam:	ylation Effect never overlaps	s – no effect	
#ER0712 2000u Supplied with: 10X Buffer R 10X Buffer Tango™ 1ml			Stora Van91	te at 37°C ge Buffei is supplie Tris-HCI (r	d in:	°C), 100mM KCI,	CpG: EcoKI:	may overlap – never overlaps never overlaps never overlaps	s – no effect s – no effect	· · ·
		λ DN 0.7% agaros	1mM E 50% (v		EDTA, 0.2mg	g/ml BSA and	Minimu comple	tion of Agar um 5 units of ete digestion o in 16 hours.	the enzyme	are required t
Lambda ФX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	escriptIISK(-/+)	pACYC17	7 pACYC184

Vspl			0 37	95%	65 X		B 0-20	G	n Five Buffer 0 R 100 20-5	Tang	go 2X Tango
5' A T↓T A A 3' T A A T↑T # ER0911 Supplied with:			1X Buffe	ons for r 0:	100% Acti	2	After than of and re	50-fold ov 95% of Dl cut.	IA fragmen	with	Vspl, more be ligated
10X Buffer O 10X Buffer Tango™	1ml 1ml		100mM	NaCI and	l 0.1mg/ml	°C), 10mM MgCl ₂ , BSA.	Dam:		aps – no ef		
#ER0912 Supplied with: 10X Buffer O 10X Buffer Tango™	5000u 2x1ml 1ml		Incubate Storage Vspl is su 10mM Tr	e Buffer upplied ir):	°C), 100mM KCI,	CpG: EcoKI:	never over never over	aps – no efi aps – no efi aps – no efi p – effect n	fect. fect.	rmined.
		λ DNA 1.0% agarose		T, 1mM E	EDTA, 0.2mg	/ml BSA and	Diges Minim compl	stion of A um 5 units	garose-em of the enzyr n of 1μg of a	bedde ne are	
Lambda ΦX174	M13mp18/19	pBR322 pU	IC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlu	escriptIISK(-	/ +) pACY(pACYC184
 Supporting Produ Dilution Buffer 0.5M EDTA, pH 8 6X Loading Dye 	.0	p.121 p.369 p.336	Fermen • PureE: • Suppli – colo – univ On-line – Dou	ttas Ress xtreme™ ed with 1 r-coded o ersal Tan technic bleDigesi	triction Er Quality & Pe IOX concent optimal buffer, cal suppor	erformance rated buffers: er (one of B, G, O, R, specially formulated	for doub	le digests or optimal	Jouble dige:	st buffer	

Temperature

www.fermentas.com/doubledigest www.fermentas.com/research www.fermentas.com 37^o Incubation Temperatur SAM Requires SAM **95%** Ligation Efficiency

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Star Activity Dam Sensitivity to Dam Methylation

Bulk quantities & custom formulations available on request

Blue/White Certified

FastDigest[™] Enzyme

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

Genome Qualified

Recombinant Enzyme

ISO ISO 9001 14001	1. RESTRICTION ENDONUCLEASES Product Description
Xagl (EcoNI)	B G R Tango ZX Tango 0-20 20-50 50-100 100 20-50 50-100
5′C C T N N↓N N N A G G. 3′G G A N N N^N N T C C.	Concentration Ligation and Recleavage
#ER1301 1000u <i>Supplied with:</i> 10X Buffer R 1ml 10X Buffer Tango™ 1ml	1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/mI BSA. 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/mI BSA.
#ER1302 5000u Supplied with: 10X Buffer R 2x1ml 10X Buffer Tango [™] 1ml	Incubate at 37°C.Methylation EffectsStorage Buffer Xagl is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.Dam: never overlaps – no effect. CpG: never overlaps – no effect. CpG: never overlaps – no effect. EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.
	λ DNA Digestion of Agarose-embedded DNA λ DNA Minimum 5 units of the enzyme are required for 0.7% agarose complete digestion of 1µg of agarose-embedded λ DNA in 16 hours. λ DNA in 16 hours.
Lambda ΦX174 M13mp18/19 9 0 0	pBR322 pUC18/19 pUC57 pTZ19R/U pBluescriptIIKS(-/+) pBluescriptIISK(-/+) pACYC177 pACYC184 1 0 0 0 0 1 1

Xapl (Apol)		Tango 37º	95%	★ X		Activity in Five Buffer System, % B G O R Tango 2X Tango 50-100 100 0-20 0-20 100 20-50						
5′ Pu↓AATT 3′ PyTTAA			Concen 10u/µl	tration	1		<i>Methylation Effects</i> Dam: never overlaps – no effect.						
Supplied with:			1X Buffer 33mM Tri	[™] Tango" is-aceta	* 100% Act [™] : ite (pH 7.9 a ite, 66mM K-	Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect. EcoBI: may overlap – effect not determined.							
			0.1mg/m Incubate Storage Xapl is su	Ĭ BSA. at 37°C Buffe l). r	Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required for complete digestion of 1μg of agarose-embedded λ DNA in 16 hours.							
			10mM Tri	is-HCI (j F, 1mM	pH 7.4 at 25 EDTA, 0.2mg	<i>Note</i> Greater than 15-fold overdigestion with Xap may result in star activity.							
		λ DNA 0.7% agarose	After 10-1	fold ove		ith XapI, more than can be ligated and							
Lambda ΦX174 58 7	M13mp18/19 11	pBR322 pU 1	C18/19 1	pUC57 1	pTZ19R/U 3	pBluescriptIIKS(-/+) 3	pBlues	scriptIISK 3	((-/+)	pACYC1	77 p	ACYC184 3	



1ml

2x1ml

2x1ml

 λ DNA (dam⁻)

0.7% agarose

5x1500u

HC, 7500u

200 reactions

Xbal

#ER0681

#ER0682

#ER0683

NEW^{10X} Buffer Tango"

#ER0684

Lamb

Supplied with:

10X FastDigest[™] Buffer

1. RESTRICTION ENDONUCLEASES

Supplied with:

Supplied with:

Supplied with:

10X Buffer Tango™

FastDigest[™] Xbal (see p.2)

10X Buffer Tango™

5'...**T \C T A G A**...3'

3'...**A G A T C^T...**5'

		Tango 37º 95% Daxy 🏷65° HC 🔏 🕯 🕐	
			B
			50-100 50
3′		Concentration	Ligation
5′		10u/µl	After 50-
	TOIL STOL	50u/µl, HC	than 95%
1500u		Conditions for 100% Activity	and recut.

1X Buffer Tango™: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Xbal is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 100mM KCI, 10mM 2-mercaptoethanol, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

		y 111 1 1 V C 1	Junci Jy		
В	G	0	R	Tango	2X Tango
50-100	50-100	20-50	0-20	100	50-100

and Recleavage

-fold overdigestion with Xbal, more % of DNA fragments can be ligated

Methylation Effects

Dam: may overlap – blocked (p.133). Dcm: never overlaps - no effect. CpG: never overlaps - no effect. EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Note

								Assayed using λ DNA	(<i>dam</i> ⁻) (#SD	0021).	
nbda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	
1	0	1	0	1	1	1	1	1	0	1	

Xcel (Nspl)		Tango (3	70 95%			В	Activi G	ty in Five O	Buffer Sy R		2X Tango		
5′ Pu C A T G 3′ Py[↑]G T A C			<i>Сопсе</i> 10и/µl	Concentration 10u/µl					50-100 0-20 0-20 0-20 100 0-20 <i>Ligation and Recleavage</i> After 50-fold overdigestion with Xcel, more tha					
#ER1471 Supplied with: 10X Buffer Tango [™] #ER1472 Supplied with: 10X Buffer Tango [™]	200u 1ml 1000u 1ml	λ DNA 0.7% agarose	1X Bufi 33mM 10mM 0.1mg, Incubat Storag Xcel is 10mM 1mM E 50% (v	Conditions for 100% Activity 1X Buffer Tango [™] : 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C. Storage Buffer Xcel is supplied in: 10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.				95% of the DNA fragments can be ligated a recut. Methylation Effects Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – no effect (p.137). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.						
Δambda ΦΧ174 32 0	M13mp18/19 6	pBR322 4	pUC18/19 3	pUC57 3	pTZ19R/U 2	pBluescriptIIKS(-/+) 1	pBlue	scriptIISI 1	<u> </u>	pACYC1 1	77 p.	2 2		
Supporting Produ Dilution Buffer 0.5M EDTA, pH 8		p.121 p.369			striction Er Quality & Pe	idonucleases: iformance								

- 0.5M EDTA, pH 8.0
- Supplied with 10X concentrated buffers:
- 6X Loading Dye & SDS Solution p.336
- color-coded optimal buffer (one of B, G, O, R, Tango[™])
- universal Tango[™] buffer, specially formulated for double digests

On-line technical support:

DoubleDigest[™] at www.fermentas.com/doubledigest for optimal double digest buffer

95% Ligation Efficiency

REsearch[™] at <u>www.fermentas.com/research</u> for complete information on restriction enzymes

Star

Activity

 \star

Sensitivity to

Dam Dam Methylation

www.fermentas.com www.fermentas.com/doubledigest

SAM

Requires

DTT

DTT

www.fermentas.com/research SAM Requires 37^o Incubation

Temperature

Tango Buffer

Recommended

1. RESTRICTION ENDONUCLEASES Product Description

CG Sensitivity to CpG Methylation

Thermal Inactivation

HC High Concentration

ISO ISO 9001 14001	1. R	ESTRICTION ENDONUCLEASES Product Description
Xhol	R 37º 📆 CG 🎘 HC 🗡 🕯 🕐	Activity in Five Buffer System, % B G O R Tango 2X Tango 0-20 50-100 50-100 100 20-50 100
5'C↓T C G A G3' 3'G A G C T [↑] C5'	<i>Concentration</i> 10u/µl 50u/µl, HC	Ligation and Recleavage After 50-fold overdigestion with Xhol, more than 95% of the DNA fragments can be ligated and
#ER0691 2000u Supplied with: 10X Buffer R 10X Buffer Tango™ 1ml	Conditions for 100% Activity 1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ ,	recut. <i>Methylation Effects</i> Dam: never overlaps – no effect.
#ER0692 5x2000u Supplied with: 10X Buffer R 2x1ml	100mM KCl and 0.1mg/ml BSA. Incubate at 37°C.	Dcm: never overlaps – no effect. CpG: completely overlaps – cleavage impaired
10X Buffer Tango [™] 1ml #ER0693 HC. 10000u	Storage Buffer	(p.135). EcoKI: never overlaps – no effect.

#ER0693 Supplied v	ith:	10000u			supplied in Tris-HCI (p		°C), 100mM KCl,	ECORI: never overlaps EcoBI: never overlaps		
10X Buffer		2x1ml 1ml					/ml BSA and	Digestion of Agaro	ose-embed	ded DNA
FastDige #ER0694 Supplied v	st [™] Xhol 200 r€	(<i>see</i> p.2) eactions	λ DN 0.7% agaro	50% (v	/v) glycero	0		Minimum 5 units of the complete digestion of λ DNA in 16 hours.	ne enzyme ar	e required for
Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	1	0	0	0	0	0	1	1	1	0

Xholl	Fermentas enzyme Psul, p.99
Xmal	Fermentas enzymes Cfr9I, p.55 and SmaI (different cleavage position), p.106
XmallI	Fermentas enzyme Eco52, p.64
XmaCl	Fermentas enzymes Cfr9I, p.55 and Smal (different cleavage position), p.106

XmaJI (A	vrll)		Tango 3	7º 95% 2	<u>80</u> X @		B 20-50	G	n Five Buffer 1 0 R 0-100 50-10	Tar	igo 2X Tango	
5′C↓C T A G 3′G G A T C [↑]			Сопсе 10и/µl	ntration	1		<i>Ligatic</i> After 5	on and R O-fold ove	ecleavage erdigestion	with X	(maJI, more	
#ER1561 Supplied with:	200u			<i>tions for</i> er Tango™	• 100% Acti ":	ivity	than 95% of the DNA fragments can be ligated and recut.					
10X Buffer Tango™	1ml				ite (pH 7.9 at		-	lation Ef				
#ER1562 Supplied with: 10X Buffer Tango™	1000u 1ml		0.1mg/	Mg-aceta ml BSA. e at 37°C	te, 66mM K- ;.	acetate and	Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: never overlaps – no effect.					
0				je Buffei s supplied					aps – no eff aps – no eff			
		λ DN 1.0% agaro	10mM 1mM D 50% (v	Tris-HCI (p	oH 7.4 at 25° EDTA, 0.2mg	Minimu comple	m 10 units	of the enzy n of 1µg of a	embedded DNA zyme are required for of agarose-embedded			
Lambda ΦX174		pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBlue	scriptIISK(-/		177	pACYC184	
2 0	0	0	0	0	0	0		0	0		0	

Genome Qualified

Recombinant Enzyme

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Bulk quantities & custom formulations available on request Blue/White Certified

FastDigest[™] Enzyme



Xmil (Accl)		B 3	/* 95%	<u>565</u> X		B 100	G 0-20	0 0-20	Buffer Sys R 0-20		2X Tang 20-50		
A T 5'G T↓C G A (3'C A G C↑T (T A			10u/µl		100% Acti	ivity	After 50		/erdiges	avage stion with nents car				
#ER1481 Supplied with: 10X Buffer B 10X Buffer Tango™	400u 1ml 1ml		and 0.1	10mM Tris-HCl (pH 7.5 at 37°C), 10mM MgCl ₂ and 0.1mg/ml BSA. Incubate at 37°C.					<i>Methylation Effects</i> Dam: never overlaps – no effect. Dcm: never overlaps – no effect.					
#ER1482 Supplied with:	2000u		Xmil is	ge Buffer supplied in Tris-HCI (r	n:	CpG: may overlap – blocked (p.137). EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.								
10X Buffer Tango™	10X Buffer B 1ml 10X Buffer Tango™ 1ml			10mM Tris-HCl (pH 7.5 at 25°C), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol. λ DNA % agarose					Digestion of Agarose-embedded DNA Minimum 5 units of the enzyme are required complete digestion of 1 μ g of agarose-embed λ DNA in 16 hours.					
Lambda Φ X174 9 2	M13mp18/19 1	pBR322	pUC18/19 1	pUC57 1	pTZ19R/U 1	pBluescriptIIKS(-/+) 1	pBlue	scriptIISK 1	(-/+)	pACYC17 1	'7 p/	ACYC184 2		
Xmnl	Fermen	tas enzyme I	Pdml , p.94											
Xspl	Fermen	tas enzyme	spBI , p.72											
Zral	Fermen	tas enzyme	Aatll (differ	ent cleava	n 25									

Supporting Products	Fermentas Restriction Endonucleases:					
• Dilution Buffer p.121 • 0.5M EDTA, pH 8.0 p.369 • 6X Loading Dye & SDS Solution p.336	 PureExtreme[™] Quality & Performance Supplied with 10X concentrated buffers: color-coded optimal buffer (one of B, G, O, R, Tango[™]) universal Tango[™] buffer, specially formulated for double digests 					
	On-line technical support: – DoubleDigest [™] at www.fermentas.com/doubledigest for optimal double digest buffer – REsearch [™] at www.fermentas.com/research for complete information on restriction enzymes					
	com/doubledigest www.fermentas.com/research Requires 37 ⁰ Incubation Ligation SAM 37 ⁰ Temperature Incubation Efficiency Activity Dam Dam Methylation					



1. RESTRICTION ENDONUCLEASE Product Description

00-00

Nicking Enzyme

Nb.Bpu10I is a site and strand specific endonuclease artificially engineered from restriction endonuclease Bpu10I. It cleaves only one strand of the DNA within its recognition sequence on a double-stranded DNA substrate.

Nb.Bpu101

5'...G C T N A G G...3' 3'...C G A N T C C...5'

#ER1681	100u
Supplied with:	
10X Buffer R	1ml
10X Buffer Tango™	1ml

R 37º <u>580</u> 🔘 🥯

Concentration 5u/µl

Conditions for 100% Activity

1X Buffer R: 10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl_a, 100mM KCl and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

Nb.Bpu10I is supplied in: 10mM Tris-HCI (pH 7.5 at 25°C), 200mM KCI, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% glycerol.

Nicking and Cleavage

- Incubation of 10 units of enzyme with 1µg pUC19 DNA (lacking the recognition sequence of Bpu10I) for 1 hour at 37°C in 50µl reaction buffer results in <10% conversion to circular form.
- Incubation of 1 unit of enzyme with 1µg pBR322 DNA for 1 hour at 37°C in 50µl reaction buffer results in <5% conversion to linear form.

	Activit	y in Five I	Buffer Sy	stem, %	
3	G	0	R	Tango	2X Tango
20	20-50	20-50	100	20-50	50-100

Applications

E 0.

- · Production of single-stranded circular DNA from supercoiled double-stranded plasmids in vitro with subsequent use in DNA sequencing, site-specific mutagenesis, etc.
- · Creation of nested deletions.
- Vector preparation for ligation independent cloning method.
- Preparations of covalently closed, doublestranded linear DNA molecules.

Note

Nb.Bpu10I may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

For protocols visit www.fermentas.com

Lambda	ФХ174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
19	7	4	1	0	0	0	0	0	5	3

Genome

Qualified

* This product and process are covered by US patent No 6867028 and corresponding counterparts.

CG Sensitivity to CpG Methylation Thermal 1 Inerman

Enzyme

Recombinant

Enzyme

 \bigcirc



Homing Enzyme

I-Scel is a site-specific homing endonuclease encoded by a mitochondrial intron of Saccharomyces cerevisiae (1, 2). Intron-encoded endonucleases are proteins that promote the first step in mobility of the intron at the DNA level. They recognize and cleave an intronless allele of their cognate gene to insert a copy of the intron by a double-strand-break repair mechanism that results in the recipient allele also becoming intronplus (3-5). Homing endonucleases recognize long,

14-40 base pairs sequences and are, therefore, extremely rare-cutting enzymes. They allow the introduction of a single or several double-strand breaks into complex genomes. This capability makes these enzymes powerful tools in highresolution physical mapping, genome organization analysis, gene cloning and site-directed inducedrecombination and for studying double-strandbreak repair in diverse biological systems (4, 6).

Scel

Tango 37º 📆 🄀 街 🔿 🕯

5'...TAGGG ATAA CAGGGTAAT...3'

3'...ATCCC TATT GTCCCATTA..5'

#ER1771 Supplied with:	250u
10X Buffer Tango [™] 10X Buffer Tango [™]	1ml
(without Mg-acetate) 100mM Mg-acetate	1ml 1ml
roomini nig-acetate	1111

10u/µl

Conditions for 100% Activity

1X Buffer Tango™: 33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA. Incubate at 37°C.

Storage Buffer

I-Scel is supplied in: 10mM Tris-HCI (pH 7.4 at 25°C), 500mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% glycerol.

pUC-I-Scel DNA

Ligation and Recleavage

After 50-fold overdigestion with I-Scel, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects

Dam: never overlaps – no effect. Dcm: never overlaps - no effect. CpG: never overlaps – no effect. EcoKI: never overlaps - no effect. EcoBI: never overlaps - no effect.

Activity in Five Buffer System, %									
В	G	0	R	Tango	2X Tango				
50-100	50-100	50-100	50-100	100	50-100				

Digestion of Agarose-embedded DNA

Minimum 20 units of the enzyme are required for complete digestion of 1µg of agarose-embedded pUC-I-Scel DNA in 1 hour (see the protocol below).

Note

- Homing endonucleases do not have stringently defined recognition sequences. They can tolerate minor sequence changes, which only partially affect the cleavage reaction.
- I-Scel may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid an atypical DNA band pattern, use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.
- Diffusion of the enzyme in the absence of Mg-acetate prior to digestion is necessary, because I-Scel is unstable in the presence of Mg²⁺ ions.
- Assayed using pUC-I-Scel DNA.

References

- 1. Colleaux, L., et al., Recognition and cleavage site of the intron-encoded omega transposase, Proc. Natl. Acad.Sci. U.S.A.,85, 6022-6026, 1988.
- 2. Monteihet, C., et al., Purification and characterization of the in vitro activity of I-Scel, a novel and highly specific endonuclease encoded by a group I intron, Nucleic Acids Res., 18, 1407-1413, 1990.
- 3. Dujon, B., Group I introns as mobile genetic elements: facts and mechanistic speculations - review, Gene, 82, 91-114, 1989
- 4. Belfort, M., Roberts R.J., Homing endonucleases: keeping the house in order, Nucleic Acids Res., 25, 3379-3388, 1997
- 5. Chevalier, B.S., Stoddard, B.L., Homing endonucleases: structural and functional insight into the catalysis of intron/ intein mobility, Nucleic Acids Res., 29, 3757-3774, 2001.
- 6. Jasin, M., Genetic manipulation of genomes with rare-cutting endonucleases, Trends in Genetics, 12, 224-228, 1996.

DTT

Requires

DTT

Recommended

Tango Buffer

Protocol for Digestion of the Agarose-embedded DNA with I-Scel

- Immerse an agarose plug in 50-100µl of the 1X Tango[™] buffer without Mg-acetate (supplied with the enzyme). The volume of the buffer should be sufficient to completely cover the plug.
- Add 20u of the enzyme.
- Incubate 2 hours on ice.
- 4 Add 1/10 volume of the 100mM Mg-acetate solution (supplied with the enzyme).

95% Ligation Efficiency

Incubate at 37°C for 1 hour.

Incubation

Temperature

370

Requires

SAM



1.4% agarose

Reaction Conditions for Restriction Endonucleases

General Protocol for DNA Digestion

We recommend digesting DNA with a 2-fold to 10-fold excess of enzyme in the total volume of 20µl using 0.2-1.5µg of DNA. A typical restriction endonuclease digestion protocol is presented on the right.

)	Add	compone	ents in	the	following	order:
-	nuu	compone	/11.5 111	uic	ronowing	orucr.

Water, nuclease-free (#R0581)	16µI
10X recommended REase buffer	2µI
Substrate DNA	1µl (~1µg)
Restriction Endonuclease	0.5-1µl (5-10u)

O Mix gently, spin down briefly.

Incubate at the optimum temperature for 1-16 hours.

The digestion reaction may be scaled either up or down.

Note

Some enzymes require additional components to obtain the stated activity. In these cases, add the required additive and adjust the volume of water appropriately.

Five Buffer System

Our unique Five Buffer System features the optimum reaction conditions for each restriction enzyme. The system consists of B (blue), G (green), O (orange), R (red) and Tango[™] (yellow) buffers (for buffer compositions, please see Table 1.6). All restriction endonucleases are packed in color-coded tubes to indicate the recommended reaction buffer. The 10X recommended buffer and/or the universal 10X Tango[™] buffer are supplied with each enzyme. Tango[™] buffer has been specifically designed for double digestions. For more information on double digestion, please refer to the section "How do I perform Double Digestion?" (p.127) or visit the Fermentas DoubleDigest[™] engine online at www.fermentas.com/doubledigest.

The Five Buffer System ensures optimal enzyme performance, simplicity and convenience. To ensure dependable and reproducible restriction endonuclease performance, Fermentas buffers contain BSA, which enhances the stability of many restriction endonucleases and binds contaminants that may be present in DNA preparations. Due to the stringent requirements involved in our BSA preparations, Fermentas buffers containing BSA can be freeze-thawed multiple times without BSA precipitation.

Fermentas restriction endonucleases exhibit 100% of their certified activity in the recommended buffer. Some restriction endonucleases require additives to achieve 100% activity. For example, Ajul, Alfl, Bdal, Bpll, BseMII, Faql, Eco57I, Eco57MI, Hin4I, Tsol require S-adenosylmethionine, which is supplied with the enzyme, while Aarl and Bvel require oligonucleotide (also supplied with the enzyme), and Esp3I requires DTT*.

The following enzymes require unique buffers for optimal digestions: Aarl, Ajil, BamHI, Bful, Bpu10I, BseXI, Bsp143I, Cfr9I, Cfr10I, Eam1105I, Ecl136II, Eco52I, EcoRI, KpnI, PasI, Ppu21I, SacI, Scal, Sdal, Sdul and TaqI. For the compositions of unique buffers, *see* the Table 1.7 (p.122), or *see* the descriptions of restriction endonucleases in the catalog, or the Certificate of Analysis provided with each enzyme.

Recommended storage conditions.

All Fermentas buffers with BSA should be stored at -20°C.

* DTT is not stable in solution. A freshly prepared DTT solution should be added directly to the reaction mixture before digestion (to order DTT (#R08671), see p.369).

Table	1.6.	Fermentas	Five	Buffer	System
Tubic	1.0.	i cimentu s	1100	Dunoi	System.

			, ,
Fermentas buffer	Cat. #	Quantity	1X buffer composition
Buffer B	BB5	5x1ml 10X buffer	10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCI ₂ and 0.1mg/ml BSA.
Buffer G	BG5	5x1ml 10X buffer	10mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ , 50mM NaCI and 0.1mg/ml BSA.
Buffer 0	B05	5x1ml 10X buffer	50mM Tris-HCI (pH 7.5 at 37°C), 10mM MgCl ₂ , 100mM NaCI and 0.1mg/ml BSA.
Buffer R	BR5	5x1ml 10X buffer	10mM Tris-HCI (pH 8.5 at 37°C), 10mM MgCl ₂ , 100mM KCI and 0.1mg/ml BSA.
Buffer Tango [™]	BY5	5x1ml 10X buffer	33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA.
Buffer Set for Restriction Endonucleases	B30	1ml 10X each of B, G, O, R, Tango [™] buffers	
Dilution Buffer for Restriction Endonuclease	B19	5x1ml 1X buffer	10mM Tris-HCI (pH 7.4 at 25°C), 100mM KCI, 1mM EDTA, 1mM DTT, 50% glycerol, 0.2mg/ml BSA

For the compositions of unique buffers see p.122.



Reaction Buffers for Restriction Endonucleases

										Table 1.7	. Reaction Bu	Iffers for F	Restricti	on Endon	ucleases.
Buffer	Cat.	Quantity					1X bu	ffer co	mposition						
Duilei	Саг. #	10X buffer, ml	Tris-HCI, mM	Tris-acetate, mM	Bis-Tris Propane-HCI, mM	MgCl _{2'} mM	NaCI, mM	KCI, mM	Mg-acetate, mM	K-acetate, mM	Sodium glutamate, mM	Triton X-100, %	BME, mM	BSA, mg/ml	pH at 37°C
FIVE BUFFE	R SYS	ГЕМ													
В	BB5	5x1	10			10								0.1	7.5
G	BG5	5x1	10			10	50							0.1	7.5
0	B05	5x1	50			10	100							0.1	7.5
R	BR5	5x1	10			10		100						0.1	8.5
Tango™	BY5	5x1		33					10	66				0.1	7.9
UNIQUE BU	FFERS														
Aarl, Ajil, Bpu10l, Scal, Pasl	B27	1			10	10		100						0.1	6.5
BamHI	B57	5x1	10			5		100				0.02	1	0.1	8.0
Bful	B59	1		50					15	100				0.1	7.9
BseXI	B31	1	50			2	100							0.1	7.5
Bsp143I	B13	1		33					10	66		0.02		0.1	7.9
Cfr9l	B02	1	10			5					200			0.1	7.2
Cfr10I	B04	1	10			5	100					0.02		0.1	8.0
Eam1105I	B25	1	10			5	100							0.1	7.5
Ecl136II, Sacl	B26	1			10	10								0.1	6.5
Eco52I	B22	1	10			3	100							0.1	8.5
EcoRI	B12	5x1	50			10	100					0.02		0.1	7.5
Kpnl	B29	1	10			10						0.02		0.1	7.5
Sdal	B24	1		37					15	150				0.1	7.0
Sdul, Ppu21I	B23	1	10			3	150							0.1	7.2
Taql	B28	1	10			5	100							0.1	8.0

Note

• The buffers listed above are available from Fermentas and may be ordered separately.

• For activity of DNA/RNA Modifying Enzymes in Fermentas restriction endonuclease buffers see p.162.

General Properties of Restriction Endonucleases

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Dilution of Restriction Endonucleases

Dilution Buffer (#B19) is available from Fermentas for applications that require diluted enzymes.

The diluted enzymes retain 50-100% activity after storage for one month at -20°C.

Stability During Prolonged Incubation

The stability of restriction endonucleases in a reaction mixture depends on the nature of the enzyme, the buffer composition and the incubation temperature.

If a restriction endonuclease retains its activity in the reaction mixture for more than one hour, DNA can be digested with less enzyme, by using a prolonged incubation period. For exact quantities of enzymes sufficient for overnight digestion, refer to the table "Reaction Conditions for Restriction Endonucleases" on pp.124-126.

Inactivation

Before subsequent manipulation of the digested DNA, restriction endonucleases present in the reaction mixture should be inactivated or removed.

Thermal inactivation of restriction enzymes is the most convenient method for terminating the digestion reaction. Most restriction enzymes can be heat-inactivated at 65°C or 80°C in 20min. Information on the susceptibility of Fermentas restriction enzymes to thermal inactivation is presented in the table "Reaction Conditions for Restriction Endonucleases" (*see* pp.124-126), in the product descriptions and in the Certificate of Analysis supplied with each enzyme.

An alternative method to stop the reaction is by the addition of EDTA, which chelates Mg²⁺, thereby preventing DNA digestion. The recommended final concentration of EDTA is 20mM.

However, high EDTA concentration is not compatible with most of downstream applications. Therefore, we recommend purification of the digested DNA with our DNA Extraction Kit (#K0513) or phenol/chloroform extraction using the protocol shown in the box.

Note. Bfil is the only known restriction endonuclease that does not require Mg²⁺ for DNA cleavage. Therefore a digestion reaction catalyzed by this enzyme can not be terminated by the addition of EDTA. The enzyme can be inactivated by heating at 65°C for 20min.

Considerations for Partial Digestion of DNA

In certain cloning experiments, incomplete cleavage of the DNA is desirable. Such partial digestion of the DNA requires the following conditions:

- low amounts of restriction endonuclease in the reaction mixture;
- short incubation time;
- · incubation at a suboptimal temperature.

Protocol for DNA Purification after Enzymatic Reaction by Phenol/Chloroform Extraction and Alcohol Precipitation

- Mix your sample with 0.5 volume of TE-saturated phenol and 0.5 volume of chloroform. Then, centrifuge (10,000rpm, 5min, room temperature).
- Transfer the upper phase to a fresh tube. Add an equal volume of chloroform and mix. Then, centrifuge (10,000rpm, 5min, room temperature).
- Transfer the upper phase to a fresh tube. Add 1/10 volume of 3M sodium acetate or 2M sodium chloride.
- 4 Add an equal volume of isopropanol or 2.5 volumes of ethanol to precipitate DNA.
- S Incubate the mixture for 30-60min at -20°C.
- O Centrifuge for 10min at 10,000rpm. Then discard the supernatant and rinse the pellet twice with 70% cold ethanol.
- Ø Air-dry the pellet. Dissolve in Water, nuclease-free (#R0581) or TE buffer for further use.

Note

Use Glycogen (#R0561) to maximize the yield of DNA during precipitation. For a detailed protocol *see* p.370.

For certain targets, partial cleavage of the desired DNA site is inefficient due to site preferences of restriction enzymes (*see* Site Preferences by Restriction Endonucleases on p.130).



Chart: Reaction Conditions for Restriction Endonucleases

	Recommended buffer	Units for overnight	Thermal inactivation			Table 1.8. Reaction Conditions for Restriction Endonucleases Enzyme activity, %				
	bullel	incubation, u/µg DNA		B (blue) 1X	G (green) 1X	0 (orange) 1X	R (red) 1X	Tango™ (1X		
Aarl	Aarl +oligo	1.0	65°C	NR (+oligo)	NR (+oligo)	0-20 (+oligo)	0-20 (+oligo)	NR (+oligo)	50-100 (+oligo)	
	В	0.1	3°08	100	20-50	0-20	0-20	50-100*	0-20	
Aatll	Tango™	0.3	65°C	50-100	20-50	0-20	0-20	100	20-50	
	0	0.3	65°C	0-20	20-50	100	20-50	20-50	50-100	
	G	0.5	No	0-20	100	20-50	100	100*	20-50	
	Ajil	0.5	65°C	NR	NR	20-50*	NR	NR	20-50*	
J ·	R +SAM	0.5	65°C	0-20 (+SAM)	50-100 (+SAM)	20-50 (+SAM)	100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	
	R +SAM	1.0	65°C	0-20 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)	
. ,	R	0.1	65°C	0-20	0-20	0-20	100	20-50	100	
	Tango™	0.1	65°C	50-100	0-20	0-20	0-20	100	20-50	
	0	0.1	65°C	0-20	20-50	100	50-100	20-50	50-100	
	Tango™	0.2	65°C	50-100	100	0-20	0-20	100	100	
	Tango™	0.1	65°C	50-100	100	0-20	50-100	100	50-100	
1.1	B	0.2	65°C	100	20-50	0-20	0-20	20-50	0-20	
	BamHI Tommo™	0.5	80°C (10u)	20-50*	100	20-50	50-100*	100*	50-100	
	Tango [™]	0.2	65°C	0-20	50-100	0-20	50-100 20-50	100 100*	50-100	
	G Tango™	0.1	80°C (10u) 65°C	20-50 20-50	100 50-100	20-50 50-100	20-50	100 ^m	100 50-100	
	Tango Tango™	0.2	No	50-100	50-100	0-20	20-50	100	0-20	
	G +SAM	1.0	65°C	NR (+SAM)	100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)	50-100* (+SAM)	50-100 (+SAM)	
	Tango [™]	1.0	65°C	20-50	20-50	0-20 (+3Aivi) 0-20	0-20	100 (+3AN)	0-20	
	Tango [™]	1.0	65°C	0-20	50-100	0-20	0-20	100	20-50	
	Bful	1.0	80°C	NR	NR	0-20	0-20	NR	50-100*	
	0	0.1	65°C	0-20	50-100	100	100	0-20	100	
	0	0.1	No	0-20	20-50	100	50-100	0-20	100	
<u> </u>	0	0.2	80°C	20-50	50-100	100	50-100	50-100	50-100	
	Tango [™]	0.5	80°C	0-20	0-20	0-20	20-50	100	20-50	
	G	0.3	65°C	20-50	100	50-100	50-100	50-100	50-100	
	Tango [™] +SAM	0.3	65°C	0-20 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)	
	Bpu10I	0.2	80°C	0-20	20-50*	50-100*	100*	50-100*	100*	
	Tango™	0.1	80°C	50-100	50-100	20-50	20-50	100	20-50	
	Tango™	0.2	80°C	50-100	20-50	0-20	0-20	100	50-100	
	Tango™	0.2	80°C	20-50	50-100	20-50	20-50	100	20-50	
	0	0.1	No	NR	100*	100	NR	NR	100*	
BseLI (55°C)	Tango™	0.1	No	20-50	100	50-100	20-50	100	50-100	
BseMI (55°C)	R	0.3	80°C	0-20	20-50	0-20	100	50-100	50-100	
BseMII (55°C)	Tango [™] +SAM	0.5	30°C	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	100 (+SAM)	50-100 (+SAM)	
BseNI (65°C)	В	0.1	3°08	100	20-50	0-20	0-20	50-100	20-50	
BseSI (55°C)	G	0.1	80°C (10u)	20-50	100	0-20	20-50	50-100	0-20	
BseXI (65°C)	BseXI	0.3	80°C	NR	NR	NR	NR	NR	NR	
	R	0.1	65°C	0-20	0-20	50-100	100	20-50	50-100	
	G	0.2	3°08	20-50	100	20-50	0-20	0-20	20-50	
	0	0.3	65°C	0-20	20-50	100	50-100	0-20	100	
	0	0.1	65°C	0-20	20-50	100	50-100	20-50	20-50	
	0	0.2	65°C	0-20	20-50	100	50-100	20-50	50-100	
	Tango™	0.1	0°C	20-50	0-20	0-20	0-20	100	100	
	B	0.1	80°C	100	20-50	0-20	20-50	50-100	0-20	
	Bsp143I	0.1	65°C	20-50	20-50	0-20	0-20	50-100	20-50	
	Tango [™]	0.5	65°C	50-100*	20-50	0-20	20-50	100*	20-50	
Bsp1407I	Tango™	0.1	65°C	0-20	20-50	0-20	20-50	100	50-100	
	Tango™	0.3	65°C	50-100	50-100	0-20	20-50	100	20-50	
	Tango™	0.5	80°C	20-50	20-50	0-20	0-20	100	0-20	
	0	0.1	65°C	0-20	0-20	100	20-50	0-20	50-100	
	0	0.1	65°C	20-50	50-100	100	100	20-50	100	
	0 Tanga™	0.1	65°C	20-50	100	100	50-100 20 E0	50-100	100	
Bsu151	Tango [™]	0.1	65°C	20-50	20-50	20-50	20-50	100	20-50	
	R O volizo	0.1	80°C	20-50	20-50	50-100	100	50-100	100 (Lealize)	
	0 +oligo Tanga™	0.2	65°C	0-20 (+oligo)	20-50 (+oligo)	100 (+oligo)	20-50 (+oligo)	50-100 (+oligo)	100 (+oligo)	
	Tango [™] Tango [™]	0.2	65°C	20-50	20-50	20-50	50-100	100 100	50-100	
		1.0	65°C	50-100*	50-100	0-20	0-20	20-50	0-20	
	Cfr9I Cfr10I	0.2	65°C No	0-20	0-20 20-50	0-20 20-50	0-20 50-100*	20-50	0-20 50-100	
	and the second sec	111	TWO I	11-711	211-211	/11-111	111-1111	(11-11)		

General Properties of Restriction Endonucleases

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Enzyme	Recommended buffer	Units for overnight	Thermal inactivation							
	build		in 20min	B (blue) 1X	G (green) 1X	0 (orange) 1X	R (red) 1X	Tango [™] 1X		
fr13I	Tango™	0.3	65°C	50-100	50-100	20-50	20-50	100	20-50	
fr42l	В	0.1	65°C	100	50-100	0-20	0-20	50-100	0-20	
pol	Tango™	0.5	65°C	20-50	50-100	50-100	20-50	100	50-100	
sel	R	0.5	80°C (10u)	NR	50-100*	50-100	100	100*	50-100	
sp6l	В	0.1	65°C	100	50-100	0-20	0-20	50-100	0-20	
pnl	Tango™	0.1	80°C	100	100	50-100	50-100	100	50-100	
ral	Tango™	0.1	65°C	50-100	50-100	20-50	20-50	100	50-100	
am11041	Tango™	0.5	65°C	50-100	50-100	0-20	0-20	100	0-20	
am11051	Eam1105I	0.1	65°C	20-50	50-100	0-20	0-20	50-100	20-50	
cl136ll	Ecl136II	0.2	65°C	50-100	20-50	0-20	0-20	50-100	0-20	
co24l	Tango™	0.2	65°C	50-100	50-100	0-20	20-50	100	0-20	
co31l	G	0.3	65°C	50-100	100	0-20	0-20	50-100	20-50	
co32l	R	0.1	65°C 65°C	0-20	50-100	50-100	100	20-50 50-100	100	
co47I co47III	R O	0.3	65°C	0-20 0-20	50-100 20-50	50-100 100	100 100	50-100	50-100 100	
co4711 co52l	Eco52I	0.1	65°C	0-20	0-20	0-20	20-50	0-20	20-50	
c0521	G +SAM	1.0	65°C	100 (+SAM)	100 (+SAM)	20-50 (+SAM)	20-50 20-50 (+SAM)	0-20 50-100 (+SAM)	20-50 50-100 (+SAN	
co57MI	B +SAM	1.0	65°C	100 (+SAM) 100 (+SAM)	50-100 (+SAM)	20-50 (+SAIVI) 0-20 (+SAM)	20-50 (+SAM)	50-100 (+SAIVI) 50-100 (+SAM)	0-20 (+SAM)	
co72l	Tango [™]	0.5	65°C	NR	NR	0-20 (+ SAIVI) 0-20	0-20	100 (+SAIVI)	20-50	
co81l	Tango [™]	0.5	80°C	50-100	100	0-20	0-20	100	0-20	
co88l	Tango [™]	0.1	65°C	100	50-100	0-20	0-20	100	20-50	
co91l	0	0.2	65°C	20-50	20-50	100	50-100	50-100	100	
co105I	Tango™	0.5	65°C	100*	50-100	0-20	0-20	100	0-20	
co130I	0	0.2	65°C	0-20	20-50	100	50-100	50-100	100	
co147I	B	0.1	80°C	100	50-100	20-50	20-50	50-100	0-20	
co01091	Tango™	0.2	65°C	50-100	20-50	20-50	20-50	100	100	
coRI	EcoRI	0.2	65°C	0-20	NR	100	100*	NR	100	
coRII	0	0.1	80°C	20-50	50-100	100	50-100	20-50	50-100	
hel	Tango™	1.0	65°C	20-50	50-100	0-20	0-20	100	20-50	
sp3l	Tango [™] +DTT	0.2	65°C	100 (+DTT)	20-50 (+DTT)	0-20 (+DTT)	0-20 (+DTT)	100 (+DTT)	0-20 (+DTT)	
aql	Tango [™] +SAM	1.0	80°C	20-50 (+SAM)	20-50 (+SAM)	0-20(+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)	
spAl	0	0.2	65°C	0-20	0-20	100	50-100	0-20	50-100	
spBl	Tango™	0.3	65°C	50-100	20-50	0-20	0-20	100	0-20	
sul (30°C)	В	1.0	65°C	100	50-100	20-50	20-50	100	50-100	
hal	Tango™	0.1	80°C	50-100	50-100	20-50	20-50	100	20-50	
lin1l	G	0.1	65°C	20-50	100	20-50	20-50	20-50	20-50	
lin1ll	G	0.3	65°C	50-100	100	20-50	50-100	50-100	50-100	
lin4l	Tango [™] +SAM	0.2	65°C	20-50 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	0-20 (+SAM)	
lin6l	Tango [™]	0.1	65°C	50-100	50-100	20-50	20-50	100	50-100	
lincll	Tango™	0.1	65°C	50-100	50-100	20-50	50-100	100	50-100	
lindIII	R	0.1	65°C	0-20	20-50	0-20	100	50-100	50-100	
linfl	R	0.1	65°C	0-20	20-50	50-100	100	50-100	50-100	
Ipall	Tango™	0.1	65°C	50-100	50-100	0-20	20-50	100	20-50	
lphl	В	0.1	65°C	100	0-20	0-20	0-20	20-50	0-20	
lpy8l	Tango™	0.1	0°C	50-100	50-100	0-20	20-50	100	50-100	
lpyF3I	Tango™	0.2	65°C	20-50	20-50	20-50	20-50	100	50-100	
pyF10VI	Tango™	0.1	0°08	0-20	0-20	0-20	0-20	100	50-100	
(pnl	Kpnl Tanana™	0.2	0°08	20-50	0-20	0-20	0-20	20-50	0-20	
(pn2l (55°C)	Tango™	0.1	80°C	50-100	50-100	0-20	20-50	100	50-100	
spAl	B	0.5	65°C	100	50-100*	20-50	20-50	100*	50-100	
gul	Tango™	0.5	65°C	20-50	50-100	20-50	20-50	100	20-50	
wel	Tango™	0.2	65°C	0-20	0-20	0-20	20-50	100	20-50	
lbil Ibol	Tango™	0.2	65°C	20-50	100	20-50	20-50	100	20-50	
lbol	R	0.1	65°C	50-100	50-100	50-100	100	50-100	100	
	B	1.0	65°C	100	50-100	20-50	0-20	50-100	20-50	
llsl	R	0.5	65°C	0-20	20-50	0-20	100	20-50	50-100	
llul Inll	R	0.1	80°C	0-20	20-50	50-100	100	20-50	50-100	
	G	0.5	65°C	50-100	100	20-50	20-50	20-50	20-50	
Iph1103I	R Tanga™	0.3	65°C	0-20	50-100	20-50	100	50-100	50-100	
lspl loci	Tango [™]	0.3	65°C	50-100	50-100	0-20	0-20	100	50-100	
lssl	B	0.5	65°C	100	0-20	0-20	0-20	20-50	0-20	
luni Mual	G	0.1	65°C	100	100	0-20	0-20	100	0-20	
Aval Ava12691	R	0.1	80°C (10u)	20-50	20-50	50-100 50-100	100	20-50*	100	
m/a 17691	R	0.1	65°C	0-20	20-50	50-100	100	0-20	50-100	

* Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour).
 NR: buffer is not recommended, because of high star activity.
 80°C(10u) indicates that only small amounts of the restriction enzyme (up to 10 units) can be inactivated at 80°C in 20min.

Bulk quantities & custom formulations available on request



Enzyme	Recommended buffer	Units for overnight	Thermal inactivation		1.8. Reaction Cor activity, %	n Conditions for Restriction Endonucleases			
		incubation, u/µg DNA		B (blue) 1X	G (green) 1X	0 (orange) 1X	R (red) 1X	Tango [™] (1X	
Ncol	Tango™	0.1	65°C	20-50	20-50	20-50	50-100	100	100
Ndel	0	0.2	65°C	0-20	0-20	100	50-100	0-20	50-100
Nhel	Tango™	0.2	65°C	100	20-50	0-20	0-20	100	0-20
NmuCl	R	0.1	65°C	0-20	20-50	50-100	100	20-50	50-100
Notl	0	0.1	0°C	0-20	0-20	100	20-50	0-20	20-50
Nsbl	Tango™	0.1	65°C	20-50	50-100	0-20	20-50	100	20-50
Olil	R B	0.2	65°C	0-20	0-20	0-20	100	0-20	50-100
Pael	0	0.1	65°C 80°C	100 0-20	50-100 50-100	0-20	0-20 NR	50-100 NR	0-20 NR
Pagl Pasl (55°C)	Pasl	0.2	80°C	NR	NR	NR	NR	NR	NR
Paul	R	0.2	0°C	0-20	0-20	100	100	0-20	100
Pdil	Tango™	0.5	65°C	50-100	20-50	0-20	0-20	100	50-100
Pdml	Tango™	0.5	65°C	20-50	50-100	0-20	0-20	100	0-20
Pfel	0	0.1	65°C	0-20	20-50	100	50-100	20-50	50-100
Pfl23II	Tango™	0.3	65°C	20-50	50-100	20-50	20-50	100	0-20
Pfol	Tango™	0.1	65°C	0-20	20-50	50-100	0-20	100	50-100
Ppil (30°C)	R	0.1	65°C	0-20	0-20	0-20	100	50-100	50-100
Ppu21I (30°C)		0.5	65°C	50-100*	100*	20-50	NR	NR	NR
Pscl	Tango™	0.2	65°C	20-50	20-50	0-20	0-20	100	0-20
Psp5II	G	0.2	0°C	0-20	100	20-50	20-50	50-100	100
Psp1406l	Tango™	0.5	65°C	100	50-100	0-20	20-50 100	100	0-20 50-100
Pstl Psul	0 B	0.2	80°C (10u) 80°C	50-100 100	50-100 20-50	100 0-20	0-20	50-100 50-100	0-20
Psyl	В	0.5	30°C 80°C	100	50-100	0-20	0-20	50-100	0-20
Pvul	R	0.2	80°C (10u)	0-20	20-50	50-100	100	50-100	100
Pvull	G	0.2	80°C	50-100*	100	20-50	50-100	20-50*	20-50*
Rsal	Tango™	0.2	65°C	50-100	20-50	0-20	0-20	100	0-20
Sacl	Sacl	0.2	65°C	50-100	20-50	0-20	0-20	50-100	20-50
Sall	0	0.1	65°C	0-20	0-20	100	20-50	0-20	50-100
Satl	G	0.1	65°C	20-50	100	20-50	20-50	50-100	20-50
Scal	Scal	0.5	80°C (10u)	0-20	0-20	0-20	0-20	0-20	0-20
Schl	Tango™	0.2	65°C	20-50	50-100	0-20	0-20	100	0-20
Sdal	Sdal	0.3	65°C	NR	NR	0-20	0-20	NR	20-50
Sdul	Sdul	0.3	65°C	NR	50-100*	50-100	0-20	NR	NR
Sfil (50°C)	G	0.2	80°C	50-100	100	20-50	0-20	100	0-20
Sgsl	Tango [™] Tango [™]	0.1	65°C 65°C	0-20 50-100	0-20	0-20 0-20	50-100 0-20	100 100	50-100 0-20
Smal (30°C) Smil (30°C)	0	0.2	65°C	0-20	0-20	100	20-50	0-20	20-50
Smol (55°C)	Tango [™]	0.1	80°C	50-100	20-50	0-20	20-50	100	20-50
Smul	Tango™	0.2	65°C	50-100	50-100	0-20	20-50	100	20-50
Ssil	0	0.5	65°C	NR	20-50	100	50-100	NR	100
Sspl	G	0.1	65°C	20-50	100	0-20	50-100	100	20-50
Taal (65°C)	Tango™	0.2	No	0-20	0-20	0-20	50-100	100	100
Tail (65°C)	R	0.3	No	50-100	50-100	20-50	100	100	50-100
Taql (65°C)	Taql	0.3	No	0-20	20-50	20-50	20-50	20-50	20-50
Tasl (65°C)	В	0.3	No	100	50-100	20-50	0-20	20-50	0-20
Taul (55°C)	B	1.0	No	100	50-100	0-20	0-20	20-50	0-20
Tatl (65°C)	Tango [™]	0.2	No	NR	50-100*	20-50	20-50	100 *	0-20
Tru1l (65°C)	R G +SAM	0.2	No	50-100	50-100	20-50	100	50-100	100 20 E0 (L SAM)
Tsol (55°C) Tstl	G +SAM R	1.0 0.1	80°C No	NR (+SAM) 0-20	0-20	50-100 (+SAM) 0-20	0-20 (+SAM) 100	50-100 (+SAM) 0-20	20-50 (+SAM) 100
Van91I	R	0.1	65°C	0-20	50-100	50-100	100	20-50	50-100
Vspl	0	0.1	65°C	0-20	50-100	100	20-50	100	100
Xagl	R	0.1	65°C	0-20	20-50	50-100	100	20-50	50-100
Xapl	Tango™	0.1	80°C	50-100	100	0-20	0-20	100	20-50
Xbal	Tango™	0.1	65°C	50-100	50-100	20-50	0-20	100	50-100
Xcel	Tango™	0.2	65°C	50-100	0-20	0-20	0-20	100	0-20
Xhol	R	0.1	80°C	0-20	50-100	50-100	100	20-50	100
XmaJI	Tango™	0.2	80°C	20-50	50-100	50-100	50-100	100	50-100
Xmil	В	0.1	65°C	100	0-20	0-20	0-20	50-100	20-50
I-Scel	Tango™	0.5	65°C	50-100	50-100	50-100	50-100	100	50-100
Nb.Bpu10I	R	0.3	80°C	0-20	20-50	20-50	100	20-50	50-100

* Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour). NR: buffer is not recommended, because of high star activity. 80°C(10u) indicates that only small amounts of the restriction enzyme (up to 10 units) can be inactivated at 80°C in 20min.

General Properties of Restriction Endonucleases

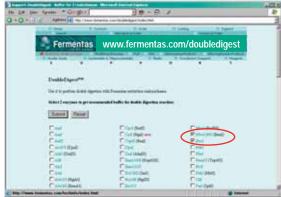
How do I Perform a Double Digest?

Here are three simple methods to achieve a successful Double Digest:

1. The Fermentas DoubleDigest[™] Engine

Use <u>www.fermentas.com/doubledigest</u> for the automated on-line set up of your double digests. Simply select two restriction enzymes, submit the query and read the recommendations.

1. Select two restriction endonucleases



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2. "Double Digestion using Universal Tango[™] Buffer" Chart

Use this table (pp.128-129) to set up optimal conditions for your double digest in the universal TangoTM buffer.

- Determine the concentration of universal Tango[™] buffer recommended for each restriction enzyme.
- 2 If the same Tango[™] buffer concentration is recommended for both enzymes, use it.
- If the two restriction enzymes require different Tango[™] buffer concentrations, perform the first digestion with the enzyme recommended for the 1X Tango[™] buffer. After digestion, add an additional aliquot of the 10X Tango[™] buffer (1/8 of initial reaction volume) to get 2X Tango[™] buffer. Then, digest DNA with the second enzyme.

Note

If both the 1X and the 2X concentrations of Tango[™] buffer are suitable for double digestion, use the 2X concentrated buffer to avoid star activity.

3. "Reaction Conditions for Restriction Endonucleases" Chart

This table (pp.124-126) presents the relative activity (% of the activity in the optimal buffer) of the Fermentas restriction enzymes in the Five Buffer System.

- Determine which color-coded buffers are recommended for each enzyme.
- 2 If the recommended color-coded buffer for both enzymes is the same, use that buffer.
- If such a buffer is not indicated, choose the buffer in which both enzymes maintain at least 20% of their activity. Increase the amount of the enzymes in your digest according to their activity in that buffer.

Note

For enzymes that are prone to relaxation of specificities, use a buffer in which they do not exhibit star activity.

Note

To achieve effective digestion with both mesophilic and thermophilic enzymes (e.g., Smal, Taal), we recommend DNA digestion at the lower temperature first, and then increase the digestion temperature. The optimal reaction temperature for each restriction enzyme is indicated both in the product description and in the Certificate of Analysis supplied with each enzyme. Information about activity of thermophilic restriction enzymes at 37°C is presented in Table 1.10 on p.130.

Sequential Digestion

If neither buffer is compatible with both restriction endonucleases due to low enzyme activity (lower than 20%) or to the star activity, then perform sequential digestions in buffers optimal for each enzyme.

- Digest the DNA with the first restriction enzyme in its optimal buffer.
- 2 Purify the digested DNA by phenol/chloroform extraction and ethanol precipitation (see p.123).
- **③** Digest the DNA with the second restriction enzyme in its optimal buffer.



Chart: Double Digestions using Universal Tango[™] Buffer

	Buffers	for double digestion	ns
Fermentas	Recommended		ngo™
enzyme	buffer	1X	2X
Aarl	Aarl +oligo	NR	+oligo
Aasl	В	*	NR
Aatll	Tango™		
Acc65I	0		
Adel	G	*	
Ajil	Ajil	NR	*
Ajul	R +SAM	+SAM	+SAM
Alol (30°C)	R		
Alfi	R +SAM	NR	+SAM
Alul Alw21I	Tango [™] 0		
Alw26l	Tango [™]		
Alw201	Tango [™]		
Apal	B		NR
BamHI	BamHI	*	
Baul	Tango™		
Bcll (55°C)	G	*	
Bcnl	Tango™		
Bcul	Tango™		NR
Bdal (30°C)	G +SAM	+SAM *	+SAM
Bfil	Tango™		NR
Bfml	Tango™	1/2	
Bful	Bful	NR	*
Bgll	0	NR	
BgIII	0	NR	
Bme1390I Boxl	0 Tango [™]		
Boil	G		
Bpll	Tango [™] +SAM	+SAM	NR
Bpu10I	Bpu10I	*	*
Bpu1102I	Tango™		
BseDI (55°C)	Tango™		
BseGI (55°C)	Tango™		
BseJI (65°C)	0	NR	*
BseLI (55°C)	Tango™		
BseMI (55°C)	R		
BseMII (55°C)	Tango [™] +SAM	+SAM	+SAM
BseNI (65°C)	В		
BseSI (55°C)	G	ND	NR
BseXI (65°C)	BseXI R	NR	NR
Bsh1236l Bsh1285l	G	NR	
BshNI	0	NR	
BshTl	0	DUX	
Bsp68l	0		
Bsp119I	Tango™		
Bsp120I	B		NR
Bsp143I	Bsp143I		
Bsp143II	Tango™	*	
Bsp1407I	Tango™		
BspLI	Tango™		
BspPI (55°C)	Tango™	1/2	NR
BspTI	0	NR	
Bst1107I	0		
BstXI (55°C)	0 Tango [™]		
Bsu15I BsuRI	R R		
Bvel	K O +oligo	+oligo	+oligo
Cail	Tango™	- Ongo	rongo
Cfrl	Tango™		NR
Cfr9I	Cfr9I		NR
Cfr10I	Cfr10I		
Cfr13I	Tango™		
	-		

	Buffers fo	r double digestio	ins
ermentas nzyme	Recommended buffer	Ta 1X	ngo™ 2X
fr42l	B		NR
pol	Tango [™]		INIX
sel	R	*	
sp6l	В		NR
)pnl	Tango™		
)ral	Tango™		
am11041	Tango™		NR
am11051	Eam1105I		
cl136ll	Ecl136II		NR
co24l	Tango [™]		NR
co31l co32l	G G		
co321	R		
co47III	0		
co52l	Eco52I	NR	
co571	G +SAM	+SAM	+SAM
co57MI	B +SAM	+SAM	NR
co72l	Tango™		
co81I	Tango™		NR
co88l	Tango™		
co91l	0		
co105l	Tango™		NR
co130l	0		
co147l	B		NR
co0109I	Tango [™]	ND	
coRI	EcoRI	NR	
coRII	0 Tanga™		
hel	Tango [™] . DTT	. DTT	ND
sp3l aql	Tango [™] +DTT Tango [™] +SAM	+DTT +SAM	NR +SAM
spAl	0	+ SAM NR	+SAIVI
spAl	Tango [™]	IVIX	NR
Spol Ssul (30°C)	B		INIX
lhal	 Tango™		
lin1l	G		
lin1ll	G		
lin4l	Tango [™] +SAM	+SAM	NR
lin6l	Tango™		
lincll	Tango™		
lindIII	R		
linfl	R		
Ipall	Tango™		
lphl	В		NR
lpy8l	Tango™		
IpyF3I	Tango [™]		
lpyF10VI	Tango [™]		ND
	Kpnl Tango [™]		NR
(pn2l (55°C)	B	*	
(spAl .gul	B Tango™	· · · · · · · · · · · · · · · · · · ·	
.yui .wel	Tango [™]		
/bil	Tango [™]		
/bol	R		
/boll	В		
Alsi	R		
Alul	R		
/inii	G		
/iph1103l	R		
/Ispl	Tango™		
	J		NR
Assl	В		NK
	B G		NR

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(continued on next page)

General Properties of Restriction Endonucleases

Table 1.9. Double Digestions using Universal Tango[™] Buffer.

	Table 1.9. Double Dig		
		r double digestic	
Fermentas	Recommended		ngo™
enzyme	buffer	1X	2X
Ncol	Tango™		
Ndel	0	NR	
Nhel	Tango™		NR
NmuCl	R		
Notl	0	NR	
Nsbl	Tango™		
Olil	R	NR	
Pael	В		NR
Pagl	0	NR	NR
Pasl (55°C)	Pasl	NR	NR
Paul	R	NR	
Pdil	Tango™		
Pdml	Tango™		NR
Pfel	0		
Pfl23II	Tango™		NR
Pfol	Tango™		
Ppil (30°C)	R		
Ppu21I (30°C)	Ppu21I	NR	NR
Pscl	Tango™		NR
Psp5II	G		
Psp1406l	Tango™		NR
Pstl	0		
Psul	В		NR
Psyl	В		NR
Pvul	R		
Pvull	G	*	*
Rsal	Tango™		NR
Sacl	Saci		
Sall	0	NR	
Satl	G		
Scal	Scal	NR	NR
Schl	Tango™		NR
Sdal	Sdal	NR	
Sdul	Sdul	NR	NR
Sfil (50°C)	G		NR
Sgsl	Tango™		
Smal (30°C)	Tango™		NR
Smil (30°C)	0	NR	
Smol (55°C)	Tango™		
Smul	Tango™		
Ssil	0	NR	
Sspl	G		
Taal (65°C)	Tango™		
Tail (65°C)	R		
Taql (65°C)	Taql		
Tasl (65°C)	В		NR
Taul (55°C)	В		NR
Tatl (65°C)	Tango™	*	NR
Tru11 (65°C)	R		
Tsol (55°C)	G +SAM	+SAM	+SAM
		NR	
Tstl	R	INIX	
Tstl Van91I		INIX	
	R	NIX	
Van91I Vspl	R R O R	NK	
Van91l Vspl Xagl	R R O R Tango [™]	IVIX	
Van91I Vspl	R R O R Tango [™]	NK.	
Van91l Vspl Xagl Xapl	R R O R Tango [™] Tango [™]	IVIX	NR
Van91l Vspl Xagl Xapl Xbal Xcel	R R O R Tango [™]		NR
Van91l Vspl Xagl Xapl Xbal Xcel Xhol	R R O R Tango [™] Tango [™] R	NK.	NR
Van91l Vspl Xagl Xapl Xbal Xcel	R R O R Tango [™] Tango [™]	ΝK.	NR
Van91l Vspl Xagl Xapl Xbal Xcel Xhol XmaJl	R R O R Tango™ Tango™ R Tango™		NR

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Note

Cleavage efficiency:

yellow shaded buffers50-100%grey shaded buffers20-50%

 $\ensuremath{\text{NR:}}$ buffer is not recommended since enzyme activity is less than 20% or star activity is too high.

* Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour).

Optimal temperature for the enzymes listed is $\rm 37^\circ C$ unless otherwise indicated in parenthesis.



Activity of Mesophilic and Thermophilic Enzymes at 37°C

		Table 1.10. /	Activity of Mesop	philic and Thermop	hilic Enzymes at 37°C
Enzyme	Optimal temperature	Activity at 37°C, %	Enzyme	Optimal temperature	Activity at 37°C, %
Alol	30	20	Pasl	55	30
Bcll	55	50	Ppil	30	60
Bdal	30	30	Ppu21I	30	<30
BseDI	55	10	Sfil	50	10
BseGI	55	25	Smal (HC)*	30	50
BseJI	65	<10	Smil	30	70
BseLl	55	40	Smol	55	10
BseMI	55	20	Taal	65	10
BseMII	55	30	Tail	65	<10
BseNI	65	<10	Tasl	65	<10
BseSI	55	20	Taul	55	30
BseXI	65	10	Taql (HC)*	65	10
BspPI	55	30	Tatl	65	20
BstXI	55	50	Tru1I (HC)*	65	10
Gsul	30	70	Tsol	55	10
Kpn2I	55	50			

* - high concentration enzyme preparations are available for incubation at non-optimal temperature.

Site Preferences by Restriction Endonucleases

In 1975, Thomas and Davis discovered that EcoRI cleaves the five recognition sites on λ DNA at rates that differ by an order of magnitude (1). Similar examples have been documented for other restriction enzymes. Factors such as flanking sequences and the number of cleavage sites appear to influence cleavage efficiency (2). There are numerous restriction endonucleases (EcoRII, Nael, Narl, Ksp632l, BspMl, Eco57l, etc.), which are known to never achieve complete cleavage of certain unmethylated target DNAs, even when using an excess of enzyme or a prolonged incubation (3-6). Most of these enzymes are members of the expanding group of type II restriction endonucleases which require simultaneous interaction with two copies of the target site for effective cleavage (7). These enzymes cleave DNA molecules with one recognition site very slowly. In the case of type IIE enzymes (EcoRII, Nael), one of the target sequences serves as an allosteric effector for the effective cleavage of the other recognition site (3, 8-10). Type IIF endonucleases (Sfil, Cfr10l, NgoMIV, BspMl) cleave both recognition sequences in a concerted reaction (11-14). Type IIS enzymes, such as Fokl, Bpml, Bsgl, Mboll, also interact with two copies of their recognition sequence before cleaving DNA by different mechanisms (15).

Cleavage of resistant sites was found to be significantly enhanced by the addition of cleavable DNA, recognition site containing oligodeoxyribonucleotide, or spermidine (4, 6, 14, 16, 17).

Different restriction enzymes recognizing the same nucleotide sequence (isoschizomers) often do not cleave the same resistant recognition site (e. g.,

Fermentas' enzymes Bvel, Cfr42l, Eam1104l and Pdil and their prototypes BspMI, SacII, Ksp632I and Nael). However, some isoschizomers cleave "resistant" sites at the same rate as other normal sites. For example, Ehel cleaves the target DNA more efficiently than its prototype Narl. Thus, one recognition site of Narl on λ DNA and two sites on pBR322 are not cleaved to completion. even after incubation with 50 units of Narl for 16 hours. Unlike Narl, Fermentas' neoschizomer Ehel cleaves λ DNA and pBR322 DNA completely under standard conditions. Site preferences are a characteristic feature of the following Fermentas prototype enzymes: Aarl, Ajul, Alfl, Alol, Bdal, Bpll, BseMII, Eco57I, Eco57MI, Ppil, Tsol and Tstl. The properties of these enzymes differ significantly from other type II enzymes.

References

- Thomas M., Davis R.W., Studies on the cleavage of bacteriophage lambda DNA with EcoRI restriction endonuclease, J. Mol. Biol., 91, 315-328, 1975.
- Sapienza P.J., et al., Thermodynamic and Kinetic Basis for the relaxed DNA Sequence Specificity of "Promiscuous" Mutant EcoRI endonuclease, J. Mol. Biol., 348, 307-324, 2005.
- Kruger, D.H., et al., EcoRII can be activated to cleave refractory DNA recognition sites, Nucleic Acids Res., 16, 3997-4008, 1988.

- Oller, A. R., et al., Ability of DNA and spermidine to affect the activity of restriction endonucleases from several bacterial species, Biochemistry, 30, 2543-2549, 1991.
- Bolton, B.J., et al., Ksp632I: a novel class IIS restriction endonuclease from *Kluyvera species* 632 with the asymmetric hexanucleotide recognition sequence: 5'-CTCT-TCN^-3' 3'-GAGAAGNNNN^-5', Gene, 66, 31-43, 1988.
- Reuter, M., et al., Use of specific oligonucleotide duplexes to stimulate cleavage of refractory DNA sites by restriction endonucleases, Anal. Biochem., 209, 232-237, 1993
- Halford, S.E., Hopping, jumping and looping by restriction endonucleases, Biochem. Soc. Trans, 29, 363-373, 2001.
- Gabbara, S., Bhagwat, A.S., Interaction of EcoRII endonuclease with DNA substrates containing single recognition sites, J.Biol.Chem., 267, 18623-18630, 1992.
- Yang, C.C., Topal, M.D., Nonidentical DNA-binding sites of endonuclease Nael recognize different families of sequences flanking the recognition site, Biochemistry, 31, 9657-9664, 1992.
- Huai, Q., et al., Crystal structure of Nael an evolutionary bridge between DNA endonuclease and topoisomerase, EMBO J., 19, 3110-3118, 2000.
- Wentzell, L.M., et al., The Sfil restriction endonuclease makes a four-strand DNA break at two copies of its recognition sequence, J. Mol. Biol., 248, 581-595, 1995.
- Siksnys, V., et al., The Cfr10I restriction enzyme is functional as a tetramer, J. Mol. Biol., 291, 1105-1118, 1999.
 Deibert, M., et al., Structure of the tetrameric restriction
- endonuclease NgoMIV in complex with cleaved DNA, Nat. Struct. Biol., 7, 792-799, 2000.
- 14.Gormley, N.A., et al., The type IIs restriction endonuclease BspMI is a tetramer that acts concertedly at two copies of an asymmetric DNA sequence, J. Biol. Chem., 277, 4034-4041, 2002.
- Bath, A.J., et al., Many type IIs restriction endonucleases interact with two recognition sites before cleaving DNA, J. Biol. Chem., 277, 4024-4033, 2002.
- Conrad, M., Topal, M.D., DNA and spermidine provide a switch mechanism to regulate the activity of restriction enzyme Nael, Proc. Natl.Acad Sci. USA, 86, 9707-9711, 1989.
- Grigaite, R.J., et al., Aarl, a restriction endonuclease from Arthrobacter aurescens SS2-322, which recognizes the novel non-palindromic sequence 5'-CACCTGC(N)_{4/8}-3', Nucleic Acids Res., 30, e123, 2002.

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Star Activity (Relaxation of Specificity)

Restriction endonucleases recognize specific nucleotide sequences within DNA molecules. However, the recognition specificity of restriction endonucleases can be reduced *in vitro* (1). Under certain conditions, enzymes are able to recognize and cleave nucleotide sequences which differ from the canonical site. At low ionic strength, for example, BamHI (with the recognition sequence GGATCC) is able to cleave the following sequences: NGATCC, GPuATCC and GGNTCC (2, 3). This phenomenon is called "relaxed" or "star" activity (4, 5).

In most practical applications of restriction endonucleases, star activity is not desirable. Analysis of several reports (4, 6-10) on the star activity suggests the following causes for this phenomenon:

- · prolonged incubation;
- high enzyme concentration in the reaction mixture;
- high glycerol concentration in the reaction mixture;
- presence of organic solvents, such as ethanol or dimethyl sulfoxide, in the reaction mixture;
- low ionic strength of the reaction buffer;
- suboptimal pH values of the reaction buffer;
- substitution of Mg²⁺ for other divalent cations, such as Mn²⁺ or Co²⁺.

In some cases, the termini generated by DNA cleavage with a restriction enzyme at the canonical site have been shown to stimulate the enzyme's star activity (11).

Star activity and incomplete DNA digestion result in atypical electrophoresis patterns which can be identified by careful examination of gel images (see Fig.1.2). Here, incomplete DNA digestion results in additional low-intensity bands above the expected DNA bands on the gel. No additional bands below the smallest expected fragment are observed. These additional bands disappear when the incubation time or amount of enzyme is increased. On the contrary, star activity results in additional DNA bands below the expected bands and no additional bands above the largest expected fragment. These additional bands become more intense with the increase of either the incubation time or the amount of enzyme, while the intensity of the expected bands decreases.

Some restriction endonucleases may remain associated with the substrate DNA after cleavage and thus change the mobility of digestion products during electrophoresis. The resulting atypical pattern is not related to star activity. To avoid confusing electrophoresis patterns, use a loading dye with SDS (e.g., the Fermentas 6X Loading Dye & SDS Solution, #R1151). Then, heat the sample for 10min at 65°C and place it on ice prior to loading it on the gel.

Any tendency of a restriction endonuclease to exhibit star activity is indicated both in the product description (*see* pp.24-120) and in the Certificate of Analysis supplied with each enzyme.

References

- Nasri, M., Thomas, D., Relaxation of recognition sequence of specific endonuclease HindIII, Nucleic Acids Res., 14, 811-821, 1986.
- George, J., Chirikjian, J.G., Sequence-specific endonuclease BamHI: Relaxation of sequence recognition, Proc. Natl. Acad. Sci. USA, 79, 2432-2436, 1982.
- Kolesnikov, V.A., et al., Relaxed specificity of endonuclease BamHI as determined by identification of recognition sites in SV40 and pBR322 DNAs, FEBS Letters, 132, 101-103, 1981.
- Polisky, B., et al., Specificity of substrate recognition by the EcoRI restriction endonuclease, Proc. Natl. Acad. Sci. USA, 72, 3310-3314, 1975.
- Woodbury, C.P., et al., DNA site recognition and reduced specificity of the EcoRI endonuclease, J. Biol. Chem., 255, 11534-11546, 1980.
- George, J., et al., Sequence-specific endonuclease BamHI, J. Biol. Chem., 255, 6521-6524, 1980.
- Malyguine, E., et al., Alteration of the specificity of restriction endonucleases in the presence of organic solvents, Gene, 8, 163-177, 1980.
- Hsu, M., Berg, P., Altering the specificity of restriction endonuclease: effect of replacing Mg²⁺ with Mn²⁺, Biochemistry, 17, 131-138, 1978.
- Mayer, H., Optimization of the EcoRI* activity of EcoRI endonuclease, FEBS Letters, 90, 341-344, 1978.
- Nasri, M., Thomas, D., Alteration of the specificity of Pvull restriction endonuclease, Nucleic Acids Res., 15, 7677-7687, 1987.
- Bitinaite, J., Schildkraut, I., Self generated DNA termini relax the specificity of SgrAl restriction endonuclease, Proc. Natl. Acad. Sci. USA, 99, 1164-1169, 2002.

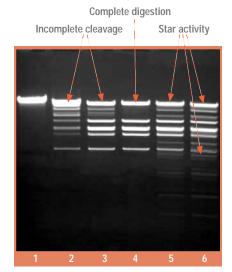


Figure 1.2. Enzyme star activity.

- 1 Lambda DNA
- 2 Lambda DNA incubated 1 hour with 0.15u of EcoRI (incomplete cleavage)
- 3 Lambda DNA incubated 1 hour with 0.4u of EcoRI (incomplete cleavage)
- 4 Lambda DNA incubated 1 hour with 1u of EcoRI (complete digestion)
- 5 Lambda DNA incubated 16 hours with 40u of EcoRI (star activity)
- 6 Lambda DNA incubated 16 hours with 70u of EcoRI (star activity)

O CO D



Digestion of Methylated DNA

DNA methylation is the process of transfering a methyl group from a donor molecule to either a cytosine or to an adenine by DNA methyltransferases. Such methylation is the most common and abundant DNA modification process in living organisms. Three types of methylated bases are predominantly found in DNA:

5-methylcytosine (m5C),

N4-methylcytosine (m4C),

N6-methyladenine (m6A).

Other modified bases, such as 5-hydroxymethylcytosine (hm5C) and 5-hydroxymethyluracil (hm5U), have also been described. The organism-specific pattern of methylation depends on the methyltransferases' specificity.

In prokaryotes, DNA cleavage by a cognate restriction endonuclease is prevented by the methylation of DNA by a sequence-specific methyltransferase which is an integral component of every restriction-modification system (1, 2).

The majority of *E.coli* strains used for propagation of plasmid DNA contain two site-specific DNA methyltransferases – **Dam** and **Dcm** (3, 4). The methylase encoded by the *dam* gene methylates the N6-position of an adenine residue within the GATC sequence (5, 6). The methylase encoded by the *dcm* gene methylates the C5position of an internal cytosine residue within the CCWGG sequence (4, 7).

In addition to Dam and Dcm methylases, laboratory strains of *E.coli* K12 and B may contain **EcoKI** or **EcoBI** enzymes, respectively, encoded by a type I R-M system. These methyltransferases modify adenine residues within their respective recognition sequences: $AAC(N)_6$ TGC for EcoKI and TGA(N)₈ TGCT for EcoBI (3, 4). DNA from higher eukaryotic organisms possesses modified 5-methylcytosine residues within **CpG** or **CpNpG** contexts (2, 8-10). These tissuespecific methylation patterns are heritable. They participate in regulation of gene expression and cellular differentiation.

Most restriction endonucleases are sensitive to DNA methylation. In the case of overlapping of a restriction endonuclease target site with the methylation site, the following results are possible:

- no effect;
- partial inhibition;
- · complete block.

The ability to cleave methylated DNA is an intrinsic and unpredictable property of each restriction endonuclease. Therefore, isoschizomers and neoschizomers which recognize the same DNA sequences can differ in their sensitivity to DNA methylation (*see* Table 1.11 below). For instance, Mbol (recognition sequence GATC) does not cleave DNA methylated by Dam methylase (11), while the isoschizomer Bsp143I is insensitive to Dam methylation. Also, EcoRII does not cleave DNA at the CCWGG site if it is methylated by Dcm, while its neoisoschizomer Mval will cleave this methylated site (12).

Thus, to achieve effective DNA digestion, it is necessary to take into account both the type of DNA methylation and the sensitivity of the restriction endonuclease to that type of methylation.

All restriction endonucleases produced by Fermentas have been examined for their sensitivity to Dam, Dcm, CpG, EcoKI and EcoBI methylation of substrate DNA. Detailed information is presented in the tables on pp.133-138, as well as in the product descriptions (pp.24-120) and in the Certificates of Analysis supplied with each enzyme.

References

- McClelland, M., The effect of sequence specific DNA methylation on restriction endonuclease cleavage, Nucleic Acids Res., 9, 5859-5866, 1981.
- McClelland, M., et al., Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases, Nucleic Acids Res., 22, 3640-3659, 1994.
- Marinus, M.G., Morris, N.R., Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12, J. Bacteriol., 114, 1143-1150, 1973.
- May, M.S., Hattman, S., Analysis of bacteriophage deoxyribonucleic acid sequences methylated by host- and R-factor-controlled enzymes, J. Bacteriol., 123, 768-770, 1975.
- Hattman, S., et al., Sequence specificity of the P1 modification methylase (M.EcoP1) and the DNA methylase (M.Ecodam) controlled by the *Escherichia coli dam* gene, J. Mol. Biol., 126, 367-380, 1978.
- Geier, G.E., Modrich, P., Recognition sequence of the *dam* methylase of *Escherichia coli* K12 and mode of cleavage of Dpnl endonuclease, J. Biol. Chem., 254, 1408-1413, 1979.
- Buryanov, Ya.I., et al., Site specificity and chromatographic properties of *E.coli* K12 and EcoRII DNA-cytosine methylases, FEBS Letters, 88, 251-254, 1978.
- Waalwijk, C., Flavell, R.A., Mspl, an isochizomer of Hpall which cleaves both unmethylated and methylated Hpall sites, Nucleic Acids Res., 5, 3231-3236, 1978.
- Bird, A.P., et al., Methylated and unmethylated DNA compartments in the sea urchin genome, Cell, 17, 889-902, 1979.
- McClelland, M., The frequency and distribution of methylatable DNA sequences in leguminous plant protein coding genes, J. Mol. Evol., 19, 346-354, 1983.
- Dreiseikelmann, B., et al., The effect of differential methylation by *Escherichia coli* of plasmid DNA and phage T7 and lambda DNA on the cleavage by restriction endonuclease Mbol from *Moraxella bovis*, Biochim. Biophys. Acta, 562, 418-428, 1979.
- Butkus, V. et al., Investigation of restriction-modification enzymes from *M.varians* RFL19 with a new type of specificity toward modification of substrate, Nucleic Acids Res., 13, 5727-5746, 1985.

Table 1.11. Fermentas Isoschizomers and Neoschizomers with Differing Sensitivities to the Target Methylation.

Enzyme couple	Recognition and cleavage sites	Sensitivity to methylation
Acc65I	GJGTACC	Overlapping Dcm or CpG methylation may influence DNA cleavage.
Kpnl	GGTAC↓C	Not influenced by Dcm or CpG methylation.
Apal	GGGCC↓C	Overlapping Dcm or CpG methylation may influence DNA cleavage.
Bsp120II	G↓GGCCC	Blocked by overlapping Dcm or CpG methylation.
Bsp143I	JGATC	Not influenced by Dam, blocked by CpG methylation.
Mbol	JGATC	Blocked by Dam methylated DNA.
Dpnl	GA↓TC	Cleaves only Dam methylated DNA.
Cfr9I	C↓CCGGG	CpG methylation may influence DNA cleavage.
Smal	CCC↓GGG	Blocked by CpG methylation.
Csp6l	G↓TAC	Not influenced by CpG methylation.
Rsal	GT↓AC	Overlapping CpG methylation may influence DNA cleavage.
Ecl136II	GAG↓CTC	Overlapping CpG methylation may influence DNA cleavage.
Sacl	GAGCT↓C	Not influenced by CpG methylation.
EcoRII	↓CCWGG	Blocked by Dcm methylation.
Mval	CC↓WGG	Not influenced by Dcm methylation.
Hpall	C↓CGG	Blocked by CpG methylation.
Mspl	C↓CGG	Not influenced by CpG methylation.

Single letter code

R	= G or A;	Н	= A, C or T;
Y	= C or T;	V	= A, C or G;
W	= A or T;	В	= C, G or T;
Μ	= A or C;	D	= A, G or T;
К	= G or T;	Ν	= G, A, T or C.
S	= C or G		

General Properties of Restriction Endonucleases

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Effect of Dam Methylation on DNA Cleavage by Restriction Enzymes

Enzyme Sequence*

To cleave with a restriction endonuclease which is sensitive to the Dam methylation, DNA should be purified from dam- E.coli strains. E.coli GM2163 dam-, dcm- (#M0099) is available upon request. . Control digestions should be performed with Lambda DNA (*dam⁻*, *dcm⁻*), #SD0021.

		Table 1.12. Complet	ely Overlappir	ng Dam Methylation and	Recognition Sites.
Enzyme	Sequence	Effect	Enzyme	Sequence	Effect
BamHI	GGm6ATCC	\downarrow	BspPI	GGm6ATC	¥
Bcll	T <mark>Gm6ATC</mark> A	*	Mbol	Gm6ATC	¥
BgIII	A <mark>Gm6ATC</mark> T	\checkmark	Psul	R <mark>Gm6ATC</mark> Y	\downarrow
Bsp143I	Gm6ATC	\downarrow	Pvul	CGm6ATCG	\checkmark

Effect

Note

DpnI (Gm6 ATC) – cleaves **only** Dam methylated DNA.

Table 1.13. Partially Overlapping Dam Methylation and Recognition Sites.

٨	lote
*	Recognition sequence is indicated in bold.
	Overlapping methylase sequence is highlighted.
	m6A = N6-methyladenine.

Cleavage not blocked.

Cleavage blocked.

↓ Cleavage rate is slowed significantly by methylation.

? The sensitivity to methylation has not been determined.

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or C.
S = C or G;	

Alol	5' GAAC (N) ₄ Gm6A TCC 3' 3' CTTG (N) ₄ C Tm6AG G5'
Bdal	5' TGm6A TC (N) $_{4}$ TCA3' 3'AC Tm6AG (N) $_{4}$ AGT5'
BseJI	5' Gm6A TC (N) ₃ ATC 3' 3' C Tm6AG (N) ₃ TAG 5'
Bsh1285I	5' CGm6A TCG 3' 3' G<mark>C Tm6AG</mark>C 5' ▼
Bsp68I	5'Gm6A TCGCGm6A TC3' 3'C Tm6AGCGC Tm6AG5' ▼
Bsu15I	5' ATCGm6A TC 3' 3' TAG<mark>C Tm6AG</mark>5 '
Hin4I	5' Gm6A TC (N) $_{4}$ VTC 3' 3' C Tm6AG (N) $_{4}$ BAG 5'
	5' GAY (N) ₄ Gm6ATC 3' 3' CTR (N) ₄ CTm6AG 5'
Hphl	5' GGTGm6A TC3' 3' CCAC Tm6AG5'

Enzyme	Sequence* Effect
Kpn2l	5' TCCG<mark>Gm6A</mark>TC 3' 3' AGGCCT m6AG5'
	5' <mark>Gm6A TCCGGm6A</mark> TC3' 3'C Tm6AGGCC Tm6AG5'
Mboll	5' GAA<mark>Gm6A</mark>TC 3' 3' CTT<mark>CT</mark>m6AG 5'
Pagl	5' TCAT<mark>Gm6A</mark>TC 3' 3' AGTA<mark>C</mark>Tm6AG5'
	5' <mark>Gm6A TCAT<mark>Gm6A</mark> TC3' 3'<mark>C Tm6AGTAC T</mark>m6AG5'</mark>
Pfol	5' TCCNG<mark>Gm6A</mark>TC 3' 3' AGGNC<mark>CT</mark> m6AG5'
Smol	5′ CTYRAG m6A TC3′ 3′ GARYT<mark>C</mark> Tm6AG5′
Taql	5' TCGm6A TC3' 3' AGC T m6AG5'
Xbal	5' TCTAGm6A TC3' 3' AGATC T m6AG5'

(continued on next page)



Effect of Dcm Methylation on DNA Cleavage by Restriction Enzymes

To cleave with a restriction enzyme which is sensitive to Dcm methylation, DNA should be purified from *dcm⁻ E.coli* strains. *E.coli* GM2163 *dam⁻*, *dcm⁻* (#M0099) is available upon request. Control digestions should be performed with Lambda DNA (*dam⁻*, *dcm⁻*), #SD0021.

Table 1.14. Completely Overlapping Dcm Methylation and Recognition Sites.

Enzyme	Sequence	Effect
EcoRII	Cm5CWGG	*
Mval	Cm6CWGG	\downarrow
Pasl	CCm5CWGGG	\downarrow

Table 1.15. Partially Overlapping Dcm Methylation and Recognition Sites.

Enzyme	Sequence*	Effect
MISI	5' TGG<mark>Cm5CA</mark>GG 3' 3' ACCGGTm 5CC5'	\$
Pfol	5' T<mark>Cm5CA GG</mark>A 3' 3' AG GTm5CCT 5'	\$
Psp5II	5' RGGW<mark>Cm5CT</mark>GG 3' 3' YCCW<mark>GGAm5CC</mark>5'	*
Sdul	5' <mark>Cm5CW GGGCCm5CW</mark> 3'G GW m5CCCGG G Wm5	GG3′ 5CC5′ ▼
Sfil	5' CCG GWm 5CCNNCCGC 3' CCG GWm 5CCNNCCGC 5' <mark>Cm5CW GG</mark> CC (N) ₅ GG Cm5CW	∎5′ ⊻ 7 GG3′
	3' <mark>G GWm5CC</mark> GG(N) ₅ CC <mark>G G</mark> W	₩5CC5¥
Sgsl	5′Cm5CW GGCGCGCm5CW 3′G GWm5CCGCGCG GWm	GG3′ 5CC5′ ▼
Tsol	5' TAR<mark>Cm5CA</mark>GG 3' 3' ATY<mark>GGT</mark> m5CC5'	¥
Tstl	5' CAC (N) _e TCm5C W GG 3' GTG (N) _e AG G Wm5CC	
Van91I	5' <mark>Cm5CA GG</mark> (N) ₃ TGG 3' <mark>G GTm5CC</mark> (N) ₃ ACC	
Xagl	5' Cm5CT GG (N) ₃ AGG 3' G GA m5CC (N) ₃ TCC	
	5' <mark>Cm5CT</mark> GGNCm5CA 3' <mark>GGAm5CCNGGTm5</mark>	GG3' GCC5

Note

Recognition sequence is indicated in bold.
 Overlapping methylase sequence is highlighted.
 m5C = 5-methylcytosine.

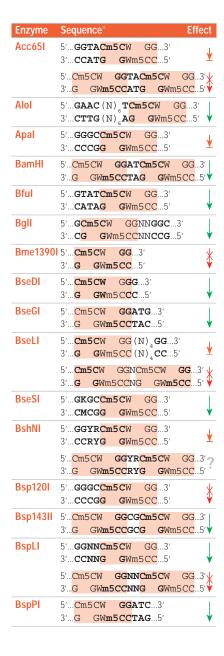
Cleavage not blocked.

- Cleavage blocked.
- Cleavage rate is slowed significantly by methylation.
- ? The sensitivity to methylation has not been determined.

Single letter code

R	= G or A;	Η	= A, C or T;
Y	= C or T;	V	= A, C or G;
W	= A or T;	В	= C, G or T;
М	= A or C;	D	= A, G or T;
К	= G or T;	Ν	= G, A, T or C.
S	= C or G;		(continued or

(continued on next page)



Enzyme	Sequence* Effect
BstXI	5' Cm5CA GG (N) ₄ T GG3' 3' G GTm5CC (N) ₄ ACC 5'
	5' Cm5CA GGNNCm5C T GG3' 3'G GTm5CCNNG GAm5CC5'
BsuRI	5' GG^Cm5C ₩ GG3' 3' CC<mark>G</mark> GWm5CC5'
	5′ <mark>Cm5CW GGCm5CW</mark> GG3′ 3′ <mark>G GWm5CCG GWm5CC</mark> 5′ ▼
Cail	5' CAG NN <mark>Cm5CT GG</mark> 3' 3' GTC NN <mark>G GTm5CC</mark> 5'
Cfrl	5' YGGCm5CW GG3' 3' RCCG GWm5CC5'
Cfr13I	5' GGNCm5CW GG3' 3' CCNG GWm5CC5'
Eco24I	5'Cm5CW GGGCCm5CW GG3' 3'G GWm5CCCGG GWm5CC5
Eco31I	5'Cm5CW GGTCTC3' 3'G GWm5CCAGAG5'
	5' GGWCm5CW GG3' 3' CCWG GWm5CC5'
Eco91I	5′ <mark>Cm5CW GGTNACm5C</mark> W GG3′ 3′ <mark>G GWm5CCANTG GWm5CC</mark> 5♥
Eco57MI	5'Cm5CT GGAG3' 3'G GAm5CCTC5'
Eco147I	5' AGGCm5CT GG3' 3' TCCG GAm5CC5'
Eco0109I	5' RGGNCm5CT GG3' 3' YCCNG GA m5CC5'
Ehel	5′Cm5CW GGCGCm5CW GG3′ 3′G GWm5CCGCG GWm5CC5′▼
Faql	5'Cm5CW GGGAC3' 3'G GWm5CCCTG5' ▼
Gsul	5' CTCm5CA GG 3' 3' GAG GTm5CC 5'
Hin1I	5′ GRCGCm5C ₩ GG3′ 3′ CYGCG G ₩m5CC5′
Hphl	5'Cm5CW GGTGA3' 3'G GWm5CCACT5'
Kpnl	5' GGTACm5C ₩ GG3' 3' CCAT<mark>G</mark> G₩m5CC5'
	5′Cm5CW GGTACm5C W GG3′ 3′ <mark>G GWm5CCATG GWm5CC</mark> 5′♥

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General Properties of Restriction Endonucleases

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Effect of CpG Methylation on DNA Cleavage by Restriction Enzymes

Enzyme	Sequence	Effect	Enzyme	Sequence	Effe
Aatll	GACGTC	¥	Hin1l	GRCGYC	
jil	CACGTC	¥	Hin6I	GCGC	
Baul	CACGAG	\downarrow	Hpall	CCGG	
Bcnl	CCSGG	¥	Kpn2l	TCCGGA	
3sh1236l	CGCG	¥	Mbil	GAGCGG	
3sh12851	CGRYCG	¥	Mlul	ACGCGT	
3shTl	ACCGGT	¥	Mspl	CCGG	
Bsp68l	TCGCGA	¥	Notl	GCGGCCGC	
3sp119l	TTCGAA	*	Nsbl	TGCGCA	
3sp143II	RGCGCY	¥	Paul	GCGCGC	
3su15l	ATCGAT	¥	Pdil	GCCGGC	
Cfr9I	CCCGGG	¥	Pfl23II	CGTACG	
Cfr10I	RCCGGY	¥	Ppu21I	YACGTR	
Cfr42I	CCGCGG	*	Psp1406l	AACGTT	
Cpol	CGGWCCG	*	Pvul	CGATCG	
Csel	GACGC	¥	Sall	GTCGAC	
Co47III	AGCGCT	*	Sgsl	GGCGCGCC	
Eco52I	CGGCCG	¥	Smal	CCCGGG	
Eco72I	CACGTG	¥	Smul	CCCGC	
Eco88I	CYCGRG	¥	Ssil	CCGC	
Eco105I	TACGTA	*	Tail	ACGT	
Ehel	GGCGCC	*	Taql	TCGA	
Esp3I	CGTCTC	¥	Taul	GCSGC	
FspAl	RTGCGCAY	`↓	Xhol	CTCGAG	
Hhal	GCGC	¥			

Methylated DNA substrates were prepared with SssI methyltransferase.



Cleavage not blocked.

Cleavage blocked.

✓ Cleavage rate is slowed significantly by methylation.

Single letter code

9		
R = G or A;	Н	= A, C or T;
Y = C or T;	V	= A, C or G;
W = A or T;	В	= C, G or T;
M = A or C;	D	= A, G or T;
K = G or T;	Ν	= G, A, T or C.
S = C or G:		

Kplizi	ICCGGA	*
Mbil	GAGCGG	¥
Mlul	ACGCGT	¥
Mspl	CCGG	Ļ
Notl	GCGGCCGC	¥
Nsbl	TGCGCA	¥
Paul	GCGCGC	\$
Pdil	GCCGGC	¥
Pfl23II	CGTACG	\$
Ppu21I	YACGTR	*
Psp1406l	AACGTT	*
Pvul	CGATCG	¥
Sall	GTCGAC	*
Sgsl	GGCGCGCC	*
Smal	CCCGGG	\$
Smul	CCCGC	*
Ssil	CCGC	*
Tail	ACGT	*
Taql	TCGA	↓
Taul	GCSGC	*
Xhol	CTCGAG	¥

(continued on next page)



Enzyme	Sequence* Effec	t
Aarl	5' CACCTG<mark>m5C</mark>G3' 3' GTGGAC<mark>Gm5C</mark>5'	Ł
Aasl	5' m5C GAC (N) GTC 3' 3' Gm5C TG (N) CAG 5'	
	5' m5C GAC (N) GTm5C G 3' 3' Gm5C TG (N) CA G m5C5'	
	5' GAm5C G (N) ₅ GTC3' 3'CT Gm5C (N) ₅ CAG5'	Ł
	5' GAm5C G (N) $_{4}$ m5C GTC3' $_{3'$ CT Gm5C (N) $_{4}$ Gm5CAG5'	?
Acc65I	5' GGTACm5C G 3'	Ł
	5' m5C GGTACm5C G 3' 3' Gm5CCATG G m5C5'	Ķ
Adel	E' CAMEC CNNCTC 2'	
		K
Ajul	5' m5C GAA (N) ₇ TTGG 3' 3' Gm5CTT (N) ₇ AACC 5'	
Alfi	5'GCA (N) TGm5C G3' 3'CGT (N) AC Gm5C5'	
Alol	5' m5C GAAC (N) ₆ TCC 3' 3' Gm5CTTG (N) ₆ AGG 5'	K
		ļ
	5' GAA<mark>m5C</mark>G(N)₅TCC 3'	
Alw21I	3' CTT Gm5C (N) _s AGG 5' 5' m5C GWGCWm5C G3'	
Alw26I	3' Gm5CWCGW Gm5C5'	
	3' CAGA G m5C5' 5'm5C GTCTC 3'	Ķ
Alw44I		k K
Apal	5' GGGCC<mark>m5C</mark>G 3'	
		Ł
BamHI	3' Gm5CCCGG Gm5C5' - 5'm5C GGATCm5C G3'	<u>-</u>
Dful	3' Gm5CCTAG Gm5C5'	
Bful	3' Gm5CATAGG5'	
	5' GTATC<mark>m5C</mark>G 3' 3' CATAG<mark>G</mark> m5C5'	
Bgll	5' GCm5C G (N) ₃ m5C GGC 3' 3' CG Gm5C (N) ₃ Gm5CCG 5'	
	5′ GCC (N)₅ GGm5C G3′ 3′ CGG (N)₅ CC Gm5C 5′	Ł
	5' m5C GCC (N) GGm5C G 3' 3' Gm5C GG (N) CC Gm5C 5'	Ķ
Rmo1300	5' Cm5C GGG 3'	k

Enzyme	Sequence* Effe	ct
Boxl	5' GAm5C G (N) 3GTC 3' 3' CT Gm5C (N) 3CAG 5'	⊻
	5'GAm5C GNNm5C GTC3'	\mathbf{Y}
	3' CT G m5CNN G m5C AG5'	Ŷ
	5' GAC (N) ₄ GTm5C G3' 3' CTG (N) ₄ CA Gm5C	¥
Bpil	5' GAAGAm5C G3' 3' CTTCT<mark>G</mark>m5C5'	↓
	5' m5C GAAGAC 3' 3' Gm5CTTCTG5'	↓
Bpll	5' m5C GAG (N) CTm5C G 3 3' Gm5C TC (N) GA G m5C5	′↓
Bpu10I	5' CCTNAGm5C G3' 3' GGANTC Gm5C5'	↓
Bpu1102I	5' <mark>m5C GCTNAGm5C G</mark> 3' 3' Gm5CGANTC Gm5C5'	\downarrow
BseDI	5' Cm5C Gm5C GG3' 3'G Gm5C Gm5CC5'	\downarrow
BseGI	5' m5C GGATG 3' 3' Gm5CCTAC5'	↓
BseJI	5' GAT (N) ₄ ATm5C G3' 3' CTA (N) ₄ TA Gm5C5'	↓
	5' m5C GAT (N) ATm5C G 3 3' Gm5C TA (N) TA Gm5C 5	?
BseLl	5' Cm5C G (N) GG3' 3'G Gm5C (N) CC5'	¥
	5' Cm5C G (N) ₅ m5C GG3' 3'G Gm5C (N) ₅ Gm5CC5'	ᢤ
BseMI	5' m5C GCAATG 3' 3' Gm5CGTTAC5'	↓ ↓ ↓
BseSI	5' m5C GKGCMm5C G 3' 3' G m5CMCGK G m5C5'	↓
BseXI	5' m5C GCAGm5C G3' 3' G m5CGTC Gm5C5'	↓
BshNI	5' GGYRC<mark>m5C</mark>G3 ' 3' CCRYG<mark>G</mark>m5C5'	¥
	5' m5C GGYRCm5C G3' 3' G m5CCRYG G m5C5'	ᢤ
	5' GGm5C GCC3' 3' CC<mark>Gm5C</mark>GG5 '	¥
Bsp120I	5' GGGCC<mark>m5C</mark>G3 ' 3' CCCGG<mark>G</mark>M5C5'	ᢤ
Bsp143I	5' GAT<mark>m5C</mark>G3 ' 3' CTAGm5C 5'	ᢤ
BspLI	5' GGNNC<mark>m5C</mark>G3 ' 3' CCNNG<mark>Gm5C</mark>5'	↓
	5' m5C GGNNCm5C G3' 3' G m5C CNNG Gm5C5'	ᢤ
BspPI	5' GGATm5C G3' 3' CCTA G m5C5'	★ ↓ ↓ ↓ ↓
	5' m5C GGATC 3' 3' Gm5CCTAG5'	↓
Bst1107I	5' GTATAm5C G3' 3' CATAT Gm5C5'	¥
	5' <mark>m5C GTATAm5C G</mark> 3'	ᢤ
	3' Gm5CATAT Gm5C5'	V

Elizyille	Sequence* Eff	ec
BsuRI	5' <mark>m5C GGCm5C G</mark> 3'	
	3' Gm5CCG Gm5C5'	
Bvel	5' ACCTG<mark>m5C</mark>G3'	
	3' TGGAC G m5C5'	
Cfrl	5' YGGC<mark>m5C G</mark>3 '	
	3' RCCG Gm5C 5'	
Cfr13I	5' GGNC<mark>m5C</mark>G 3'	
	3' CCNG G m5C ^{5'}	
Csp6l	5' <mark>m5C G</mark> TAm5C G3'	
	3' G m5CAT G m5C5'	
Dpnl	5' Gm6A T<mark>m5C G</mark>3 '	
	3' C Tm6A Gm5 C5'	
Eam1104I	5' CTCTT<mark>m5C</mark>G3'	
	3' GAGAA G m5C5'	
Eam1105I	5' GAm5C G (N) ₃ m5C GTC	3′
	3' CT G m5C (N) ₃ Gm5CAG	5′
	5' GAC (N) ₅ GT <mark>m5C G</mark> 3'	
	3' CTG (N) ₅ CA G m5C ^{5'}	
	5'm5C GAC(N) ₅ GTm5C G	3′
	$3'$ Gm5C TG (N) $_{5}$ CA Gm5C	5′
Ecl136II	5' GAGCT<mark>m5C</mark>G 3'	
	3' CTCGA G m5C5'	
	5' <mark>m5C G</mark> AGCT <mark>m5C G</mark> 3'	
	3' Gm5CTCGA Gm5C5'	
Eco24I	5' <mark>m5C GRGCY</mark> m5C G3'	
	3' Gm5CYCGR Gm5C5'	
Eco31I	5' GGTCT<mark>m5C</mark>G 3'	
	3' CCAGA G m5C5'	
	5' <mark>m5C GGTCTC</mark> 3'	
	3' Gm5CCAGAG5'	
Eco32I	5' <mark>m5C GATATm5C G</mark> 3'	
	3' Gm5CTATA Gm5C5'	
Eco47I	5' GGWCm5C G3'	
	3' CCWG G m5C ^{5'}	
Eco91I	5'm5C GGTNACm5C G3'	
	3' Gm5CCANTG Gm5C5'	
EcoRI	5' m5C GAATTm5C G3'	
	3' Gm5CTTAA Gm5C5'	
Faql	5' GGGAm5C G3'	
	3' CCCT Gm 5C5'	
	5' m5C GGGAC 3'	
	3' Gm5CCCTG5'	
Hin4l	5' $m5C$ GAY (N) VTC3'	
	3' Gm5CTR (N) ₅ BAG 5'	
	5'GAY (N) $\nabla Tm5C$ G3'	
	3' CTR (N) ₅ BA G m5C5'	
	5'm5C GAY (N) ${}_{5}$ VTm5C G	
lling	3' Gm5CTR (N) ₅ BA Gm5C	J
Hincll	5'GTYRAm5C G3'	
	3' CARYT Gm 5C5'	
	5'm5C GTYRAm5C G3'	
	3' Gm5CARYT Gm5C5'	
	5' GTm5C GAC 3'	

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1. RESTRICTION ENDONUCLEASES —

(continued on next page)

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Sequence³

General Properties of Restriction Endonucleases

Table 1	1 17	CnG	Partially	Overlans	tho	Recognition	Sito
lable	1.1/.	CpG	ганану	Overlaps	uie	Recognition	JIE.

5'...**GAWTm5C** G...3'

3'...**CTWA Gm5C**...5'

5'...**TCm5C GGGA**...3'

3'....AG Gm5CCCT....5'

5'...**m5C GAAC** (N) **CTC**...3' 3'... Gm5CTTG (N) GAG...5' 5'...GAAC (N) CTm5C G...3' 3'...**CTTG** (N) **GA G**m5C...5' 5'...GAAm5C G (N) CTC...3'

3'...CTT Gm5C (N) GAG...5'

5'....**m5C GGATCm5C** G....3' 3'... Gm5CCTAG Gm5C...5'

5'...**GTAm5C** G...3'

3'...CAT Gm5C...5' 5'...m5C GTAm5C G...3'

5'...Gm5C GGC...3' 3'...C Gm5CCG...5' 5'....GCNGm5C G....3'

3'...**CGNC Gm5C**...5'

5'...**GAGTm5C** G...3' 3'...**CTCA Gm5C**...5' 5'...**m5C GAGTC**...3' 3'... Gm5CTCAG...5'

3'... Gm5CAT Gm5C...5'

5'...**m5C GAGCTm5C G**...3' 3'... Gm5CTCGA Gm5C...5'

5'...m5C GDGCHm5C G...3' 3'... Gm5CHCGD Gm5C...5'

3'...CCG Gm5C (N) CCGG...5' 5′...**GGCm5C** G (N) m5C GGCC...3′ 3'...CCG Gm5C (N) Gm5CCGG...5'

5'...**CTm5C** GGT...3'

3'...GA Gm5CCA...5'

5'....**Am5C GAG**....3'

3'...**T** Gm5CTC...5' 5'...**CAm5C** G (N) **TCC**...3'

3'...**GT Gm5C** (N) **AGG**...5'

5'...**CAC** (N) **TCm5C** G...3'

3'...**GTG** (N) **AG G**m5C...5'

5'....m5C GAATTm5C G....3'

3'... Gm5CTTAA Gm5C....5'

5'...**m5C GCATGm5C** G...3'

3'... Gm5CGTAC Gm5C...5'

5'...**RCATG<mark>m5C</mark>G**...3' 3'...**YGTAC Gm5C**...5'

5'....GTm5C GAC....3'

3'...CA Gm5CTG...5'

5'...GTMKAm5C G...3' 3'...CAKMT Gm5C...5'

5'...**m5C GGCC** (N)_**GGCm5C** G...3' 3'... Gm5CCGG (N) CCG Gm5C...5'♥ 5'...**GGCm5C** G (N)₄GGCC...3'

5'...GAm5C GNNGTm5C G...3' 3'...**CT** Gm5CNNCA Gm5C...5'

5'...**GAA** (N) **TTm5C** G...3'

3'...**CTT** (N) **AA G**m5C...5'

5'...**m5C GAA**(N)₄**TTm5C** G...3' 3'... G**m5C**TT(N)₄**AA** Gm5C...5'

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-	o * F((Table 1.17.
Enzyme			Enzyme
Hinfl	5' GANT<mark>m5C</mark>G3' 3' CTNAG m5C5'	¥	Pdml
	5' <mark>m5C GANTm5C G</mark> 3'	¥	
	3' G m5CTNA Gm5C5'	_	
Hphl	5' m5C GGTGA 3' 3' G m5C CACT5'	¥	Pfel
Нру8І	5' GTNNAm5C G3' 3' CANNT Gm5C5'	⊻	Pfol
	5' <mark>m5C GTNNAm5C G</mark> 3'	?	Ppil
HpyF10VI	3' Gm5CA NN T Gm5C 5' 5' GC (N) ₇ Gm5C G 3'	¥	
	3' CG (N) ₇ C G m5 C 5' 5' m 5 C GC (N) ₇ G m5 C G 3'	ᢤ	
	3' $\mathbf{Gm5CG}(\mathbf{N})_{7}^{'}\mathbf{C} = \mathbf{Gm5C}5'$ 5' $\mathbf{Gm5C} = \mathbf{G}(\mathbf{N})_{6}\mathbf{GC}3'$		Psul
	3' C Gm5C (N) ₆ CG5'	¥	
	$5'\mathbf{Gm5C}$ G (N) $_{5}$ m5C GC3' $3'\mathbf{C}$ Gm5C (N) $_{5}$ Gm5CG5'	?	Psyl
Kpnl	5' <mark>m5C GGTACm5C</mark> G3' 3' G m5CCATG G m5C5'	¥	Rsal
KspAl	5' GTTAAm5C G3' 3' CAATT Gm5C5'	¥	
	5' <mark>m5C GTTAAm5C G</mark> 3'	ᢤ	Sacl
Lgul	3' Gm5CAATT Gm5C5' 5'm5C GCTCTTC3'		Satl
	3' Gm5CGAGAAG5' 5'GCTCTTm5C G3'	¥ 	
	3' CGAGAA G m5C5'	♥	Cabl
Lwel	5' GCATm5C G3' 3' CGTA Gm5C5'	⊻	Schl
Mbil	5' <mark>m5C GAGCGG</mark> 3' 3' G m5C TCGCC5'	ᢤ	
Mbol	5'm5C GATm5C G3' 3' Gm5CTA Gm5C5'	\downarrow	Sdul
Mboll	5' m5C GAAGA 3' 3' Gm5CTTCT 5'	\downarrow	Sfil
MnII	5' CCT<mark>m5C</mark>G 3'	j	
Mssl	3' GGA Gm5C 5' 5' GTTTAAAm5C G3'	<u> </u>	
	3' CAAATTT Gm5C 5' 5' m5C GTTTAAAm5C G 3'	*	Smol
	3' Gm5CAAATTT Gm5C5'	¥	51101
Mva1269I	5' GAATG<mark>m5C</mark>G 3' 3' CTTACG m5C5'	V	Taal
	5' m5C GAATGC 3' 3' Gm5CTTACG 5'	Ļ	Tst
Nhel	5' GCTAG<mark>m5C</mark>G 3'	 ⊻	
	3' CGATC Gm5C 5' 5' m5C GCTAGm5C G 3'	*	Xapl
	3' Gm5CGATC Gm5C5'	Ŷ	
NmuCl	5' GTCAm5C G3' 3' CAGT G m5C5'	¥	Xcel
	5' m5C GTCAm5C G3' 3' G m5CAGT G m5C5'	¥	
Olil	5' CAm5C GNNm5C GTG 3' 3' GT Gm 5CNN Gm5CAC 5'	¥	Xmil
Pael	5' <mark>m5C GCATGm5C G</mark> 3'	Ţ	
	3' Gm5CGTAC Gm5C5'		

ISO	ISO
9001	14001

Note

- * Recognition sequence is indicated in bold. Overlapping methylase sequence is highlighted. m5C = 5-methylcytosine.
- Cleavage not blocked.
- Cleavage blocked.
- Cleavage rate is slowed significantly by methylation.
- ? The sensitivity to methylation has not been determined.

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or C.
S = C or G;	

Bulk quantities & custom formulations available on reque	st
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Effect of EcoKI and EcoBI Methylation on DNA Cleavage by Restriction Enzymes

Enzyme Sequence*

Aatll	5'T Gm6ACGTC (N) ₄ TGCT3' 3'A C TGCAG (N) ₄ m6ACGA5'	,
Alul	5'TG m6AGCT (N) ₅ TGCT3' 3'AC TCGA (N) ₅ m6ACGA5'	
Bcll	$ \begin{array}{l} 5'\textbf{TGm6A} \textbf{TCA} (\texttt{N})_{5} \text{TGCT}3'\\ 3'\textbf{AC} \textbf{Tm6AGT} (\texttt{N})_{5}\text{m6ACGA}5' \end{array} $	
BgIII	5'TG m6AGATCT (N) ₃ TGCT3' 3'AC TCTAGA (N) ₃ m6ACGA5'	1
Bpu10I	5' CCTGm6AGC (N) ⁶ TGCT3' 3' GGAC TCG (N) ⁶ m6ACGA5'	,
Bsp143I	5'T Gm6ATC (N) ₆ TGCT3' 3'A C TAG (N) ₆ m6ACGA5'	,
EcoRI	$5'TGm6AATTC(N)_4$ TGCT $3'$ $3'AC$ TTAAG (N)_4m6ACGA $5'$,
Hincll	5' GTTGm6AC (N) ₇ TGCT3' 3' CAAC TG (N) ₇ m6ACGA5'	
HindIII	5'TG m6AAGCTT (N) 3 TGCT3' 3'AC TTCGAA (N) 3m6ACGA5'	1
Hinfl	5'T Gm6ANTC (N) TGCT3' 3'A C TNAG (N) m6ACGA5'	
Hphl	5' GGTGm6A (N) ₈ TGCT3' 3' CCAC T (N) ₈ m6ACGA5'	2
Mbol	5'T Gm6ATC (N) ₆ TGCT3' 3'A C TAG (N) ₆ m6ACGA5'	2
Mboll	5'T Gm6AAGA (N) 5 TGCT3' 3'A C TTCT (N) m6ACGA5'	,
Mlul	$ \begin{array}{llllllllllllllllllllllllllllllllllll$,
MnII	5'T Gm6AGG (N) ⁶ TGCT3' 3'A C TCC (N) ⁶ m6ACGA5'	
Ndel	$5'TGm6A(N)_4$ CATA TG CT3' 3'AC T(N)_4 GTATm6AC GA5'	,
Olil	5'TGm6A (N) $_{4}$ CACG TG CT3' 3'AC T (N) $_{4}$ GTGCm6AC GA5'	
Pael	5'TGm6A (N) ₅ GCA TGCT3'	2

Methylated DNA substrates were purified from E.coli K12 or E.coli B.

Effect

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Table 1.18. EcoBI Overlapping Methylation.

Enzyme	Sequence* Effect
Pagl	5' TCATGm6A (N) ₈ TGCT3' 3' AGTAC T (N) ₈ m6ACGA5'
Sacl	5'TGm6AGCTC (N) $_4$ TGCT3' 3'AC TCGAG (N) $_4$ m6ACGA5'
Scal	5'TG m6AGTACT (N) ₃ TGCT3' 3'AC TCATGA (N) ₃ m6ACGA5'
Sspl	5'TG m6AATATT (N) ₃ TGCT3' 3'AC TTATAA (N) ₃ m6ACGA5'
Tasl	5'TG m6AATT (N) ₅ TGCT3' 3'AC TTAA (N) ₅ m6ACGA5'

Table 1.19. EcoKI Overlapping Methylation.				
Enzyme	Sequence* Effe	ct		
Alw21I	5'Am6AC (N) ₆ G TGCWC 3' 3'T TG (N) ₆ Cm6ACGWG 5'	↓		
Alw44I	5'Am6AC (N) ₆ G TGCAC 3' 3'T TG (N) ₆ Cm6ACGTG 5'	↓		
BseSI	5'Am6AC (N) ₆ G TGCMC 3' 3'T TG (N) ₆ Cm6ACGKG 5'	¥		
Dral	5′ TTTAAm6AC (N) ₆ G TGC3′ 3′ AAATT TG (N) ₆ Cm6ACG5′	ᢤ		
Hincll	5' GTYAm6AC (N) ₆ G TGC3' 3' CART TG (N) ₆ Cm6ACG5'	ᢤ		
KspAl	5' GTTAm6AC (N) ₆ G TGC3' 3' CAAT TG (N) ₆ Cm6ACG5'	ᢤ		
Mlul	5'Am6ACGCGTNNG TGC3' 3'T TGCGCA NNCm6ACG5'	↓		
Mssl	5' GTTTAAm6AC (N) ₆ G TGC3' 3' CAAATT TG (N) ₆ Cm6ACG5'	ᢤ		
Nsbl	5'Am6AC (N) ₆ G TGCGCA 3' 3'T TG (N) ₆ Cm6ACGCGT 5'	↓		
Olil	5'Am6A CAC (N) ₄ G TG C3' 3'T T GTG (N) ₄ Cm6AC G5'	ᢤ		
Tru1l	5' TTAm6A C (N) ₆ G TGC3' 3' AAT T G (N) ₆ Cm6ACG5'	ᢤ		

Note

1. RESTRICTION ENDONUCLEASES

- * Recognition sequence is indicated in bold. m6A = N6-methyladenine.
- Cleavage not blocked.
- Cleavage blocked.
- eq Cleavage rate is slowed significantly by meth

The recognition sequences of the following ende ases may also overlap with DNA sequences met by EcoBI and EcoKI.

The following enzymes have not been test sensitivity to EcoBI methylation: Aarl, Ade Alw21I, Baul, Bcul, Bfil, Bglll, Boxl, Bpu1102I, BseJI, BseGI, BseMI, BseNI, BshTI, Bsp143II, BspPI, Bsu15I, Bvel, Cfr10I, Csel, Dpnl, Eam1104I, Ecl136II, E Eco31I, Eco32I, Eco47III, Eco57I, Eco Eco72I, Eco81I, Eco91I, Eco147I, Eco Esp3I, FspAI, HindIII, Hin1I, Hin1II, Hin4I, HpyF3I, Lgul, Lwel, Mbil, Mph1103I, Munl, Mva1269I, Ndel, NmuCl, Pdml, Pfel, Ppu21I, Pscl, Psp5II, Psp1406I, Psul, Psyl, Pvull, Schl, Sdul, Smil, Smol, Sspl, Taal, Tail, Tatl, Tstl, Vspl, Xagl, Xapl and Xcel.

The following enzymes have not been tested for sensitivity to EcoKI methylation: Adel, Ajil, Baul, Bcul, Bfil, BseNI, BshNI, BshTI, Bsp119I, Bvel, Cfr10I, Eco72I, Eco91I, FspAI, Hpy8I, Pdml, Ppu21I, Pscl, Psp1406l, Sdul, Taal, Tail, Tsol and Xcel.

Single letter code

R	= G or A;	Н	= A, C or T;
Y	= C or T;	V	= A, C or G;
W	= A or T;	В	= C, G or T;
Μ	= A or C;	D	= A, G or T;
К	= G or T;	Ν	= G, A, T or C.
S	= C or G;		

		SGOAC ICO (II) IIIOACOA
	Bsp143I	$ \begin{array}{llllllllllllllllllllllllllllllllllll$
	EcoRI	$5'TGm6AATTC(N)_4$ TGCT 3'AC TTAAG(N) ₄ m6ACGA
	Hincll	5' GTTGm6AC (N) ₇ TGCT3 3' CAAC TG (N) ₇ m6ACGA5
	HindIII	5'TGm6AAGCTT (N) ₃ TGCT 3'AC TTCGAA (N) ₃ m6ACGA
	Hinfl	5'T Gm6ANTC (N) ₅ TGCT3 3'A C TNAG (N) ₅ m6ACGA5
	Hphl	5′ GGTGm6A (N) ₈ TGCT3′ 3′ CCAC T (N) ₈ m6ACGA5′
	Mbol	5'T Gm6ATC (N) ₆ TGCT3' 3'AC TAG (N) ₆ m6ACGA5'
hylation.	Mboll	5'T Gm6AAGA (N) ₅ TGCT3 3'AC TTCT (N) ₅ m6ACGA5
donucle- thylated	Mlul	5'TG m6ACGCGT (N) ₃ TGCT 3'AC TGCGCA (N) ₃ m6ACGA
sted for el, Ajil, I, Bpil,	Mnll	5'T Gm6AGG (N) GCT3' 3'AC TCC (N) 6m6ACGA5'
	Ndel	$5'TGm6A(N)_4$ CATA TG CT $3'AC$ T(N) $_4$ GTATm6AC GA
BseXI, I, Cail,	Olil	5'TGm6A (N) ₄ CACG TG CT 3'AC T (N) ₄ GTGCm6AC GA
Eco24I, :o57MI, :o0109I,	Pael	5'TGm6A (N) ₅ GCA TGC T3 3'AC T (N) ₅ CGTm6ACG A5
Нру8І,		

General Properties of Restriction Endonucleases

Cleavage of Restriction Targets Located in Close Vicinity within pUC19 Multiple Cloning Site

Double digestions within multiple cloning sites (MCS) are often ineffective when the DNA target sequences are in close vicinity, or they are too close to the end of a DNA molecule (*see* Table 1.21 on p.141). Nevertheless, it is often necessary to perform effective double digestions within the cloning sites in which restriction targets are in close vicinity. Experimental guidelines for these applications are presented in the table below. The data were generated using a linearized pUC19

plasmid. The plasmid was initially cleaved with a primary restriction enzyme (the first cut), and was then end-labeled with [³²P] by T4 Polynucleotide Kinase (#EK0031). This DNA was digested for one hour with varying amounts (2, 5 and 10 units) of a second restriction enzyme in an optimal reaction buffer (the second cut). Reaction products were separated by PAGE, and the amount of the label left on the DNA was determined by autoradiography. A decrease in radioactivity

reflects the cleavage by the second restriction enzyme. The results presented in the table below should be used to choose the optimal order of DNA digestions.

Thus, the first reaction should be performed with a restriction enzyme that cleaves inefficiently close to the end of DNA, while the second digestion should be performed with a restriction endonuclease which tolerates a close proximity to the DNA end.

pUC19 multiple cloning site

			EcoRI		ci13611 .co241	A	cc65	Cfr9l Eco88l						incll Sall		Pstl					
		396	Xapl	_ `	Sacl		Kpnl 🔔	Smal		BamHI		Xbal)	(mil	Bvel	Sd	al —	Pael		lindIII	452
5′	C CAG	т GA	ATT	CGA	GCT	CGG	TAC	CCG	GGG	ATC	CTC	TAG	AGT	CGA	CCT	GCA	GGC	ATG	CAA	GCT	¶GG C 3′
31	G GTC	ACT	TAA	GCT	CGA	GCC	ATG	GGC	CCC	TAG	GAG	ATC	TCA	GCT	GGA	CGT	CCG	TAC	GTT	CGA	Acc g 5′

Enzyme pair	First cut	Second cut	Efficiency of the 2nd cut, %	bp from the 1st cut*
Acc65I/EcoRI	Acc65I	EcoRI	100	7
	EcoRI	Acc65I	100	7
Acc65I/Xapl	Acc65I	Xapl	50-100	7
	Xapl	Acc65I	50-100	7
Acc65I/BamHI	Acc65I	BamHI	100	4
	BamHI	Acc65I	100	4
Acc65I/Ecl136II		Ecl136II	50-100	3
	Ecl136II	Acc65I	100	1
Acc65I/Eco24I	Acc65I	Eco24I	0	1
	Eco24I	Acc65I	100	1
Acc65I/Sacl	Acc65I	Sacl	0-20	1
	Sacl	Acc65I	50-100	1
BamHI/Cfr9I	BamHI	Cfr9I	50-100	0
	Cfr9I	BamHI	50-100	0
BamHI/Eco88I	BamHI	Eco88I	50-100	0
	Eco88I	BamHI	100	0
BamHI/HincII	BamHI	Hincll	100	9
	Hincll	BamHI	50-100	7
BamHI/KpnI	BamHI	Kpnl	100	4
	Kpnl	BamHI	100	4
BamHI/Sall	BamHI	Sall	100	7
	Sall	BamHI	50-100	7
BamHI/Xbal	BamHI	Xbal	100	1
	Xbal	BamHI	20-50	1
Cfr9I/Ecl136I	Cfr9I	Ecl136II	100	7
	Ecl136II	Cfr9I	50-100	5
Cfr9I/Eco24I	Cfr9I	Eco24I	100	5
Cfr9I/Sacl	Eco24I	Cfr9I	95	5
CILAI/29CI	Cfr9I	Sacl	50-100	5
Cfr9I/Xbal	Sacl	Cfr9I	50-100	~
CILAI/XD91	Cfr9I	Xbal	50-100	6
Ecl136I/Eco88I	Xbal Ecl136II	Cfr9I Eco88I	50-100	6 5
LU1301/EU0881	ECOSSI	EC0881 Ecl136II	50-100 100	5 7
Ecl136I/EcoRI	Ecl136II	EcoRI	100	1
LUISUILLUKI	ECORI	ECORI ECI136II	100	3
Ecl136I/Xapl	Ecl136II	Xapl	50-100	1
	Xapl	Ecl136II	50-100	3
				-

 Table 1.20. Cleavage of Restriction Targets Located in Close Vicinity within pUC19 Multiple Cloning Site.

		-		0
Enzyme pair	First cut	Second cut	Efficiency of the 2nd cut, %	bp from the 1st cut*
Eco24I/Eco88I	Eco24I	Eco88I	50-100	5
2002 11 200001	Eco88I	Eco24I	100	5
Eco24I/EcoRI	Eco24I	EcoRI	100	1
LOOL IN LOOK	EcoRI	Eco24I	100	1
Eco24I/Kpnl	Eco24I	Kpnl	50-100	1
LOOL IN ROM	Kpnl	Eco24I	0	1
Eco24I/Xapl	Eco24I	Xapl	50-100	1
LOOL III Mapi	Xapl	Eco24I	20-50	1
Eco88I/Sacl	Eco88I	Sacl	100	5
200001100001	Sacl	Eco88I	50-100	5
Eco88I/Xbal	Eco88I	Xbal	100	6
LCOUGHADAI	Xbal	Eco88I	100	6
EcoRI/Cfr9I	EcoRI	Cfr9I	50-100	11
LCOKI/CII 71	Cfr9I	EcoRI	100	11
EcoRI/Eco88I	EcoRI	Eco88I	50-100	11
LUKIILUOOI	Eco88I	EcoRI	100	11
EcoRI/KpnI	EcoRI	Kpnl	100	7
сокихри	Kpnl	EcoRI	100	7
EcoRI/Sacl	EcoRI	Sacl	30-50	1
LUCKI/Saci	Sacl	EcoRI	50-100	1
Hincll/Pael	Hincl	Pael	100	7
nincii/Paei	Pael	Hincll	100	9
Hincll/Pstl	Hincl	Pstl	20-50	1
nincii/rsu	Pstl	Hincll	20-50	3
Hincll/Sdal	Hincl	Sdal	50	1
HINCH/Sual	Sdal			2
Iller all Mh al		Hincll	0-20	
Hincll/Xbal	Hincll	Xbal	50-100	1 3
HindIII/Pael	Xbal HindIII	Hincll Pael	50-100	3 1
Hindili/Pael			0	· ·
Hindlll /Detl	Pael	HindIII	50-100	1
HindIII/PstI	HindIII	Pstl	100	
	Pstl	HindIII	100	7
HindIII/Sdal	HindIII	Sdal	50-100	7
16 1/E 140 (**	Sdal	HindIII	50-100	6
Kpnl/Ecl136II	Kpnl	Ecl136II		1
	Ecl136II		100	3
Kpnl/Sacl	Kpnl	Sacl	100	1
	Sacl	Kpnl	50-100	1

Enzyme pair	First cut	Second cut	Efficiency of the 2nd cut, %	bp from the 1st cut*
Kpnl/Xapl	Kpnl	Xapl	50-100	7
	Xapl	Kpnl	100	7
Pstl/BamHI	Pstl	BamHI	50-100	13
	BamHI	Pstl	50-100	13
Pael/Pstl	Pael	Pstl	20-50	1
	Pstl	Pael	0	1
Pael/Sall	Pael	Sall	100	7
	Sall	Pael	50-100	7
Pael/Sdal	Pael	Sdal	20-50	1
	Sdal	Pael	0	0
PstI/Sall	Pstl	Sall	50-100	1
	Sall	Pstl	0	1
Pstl/Xbal	Pstl	Xbal	50-100	7
	Xbal	Pstl	100	7
Sacl/Smal	Sacl	Smal	50-100	7
	Smal	Sacl	100	5
Sacl/Xapl	Sacl	Xapl	50-100	1
	Xapl	Sacl	0-20	1
Sall/Sdal	Sall	Sdal	20-50	1
	Sdal	Sall	0	0
Sall/Xbal	Sall	Xbal	0-20	1
	Xbal	Sall	50-100	6
Sdal/Xbal	Sdal	Xbal	50-100	6
	Xbal	Sdal	100	7
Smal/Acc65I	Smal	Acc65I	0-20	1
	Acc65I	Smal	0	-1
Smal/BamHI	Smal	BamHI	50-100	2
	BamHI	Smal	0-20	0
Smal/Ecl136II	Smal	Ecl136II		7
	Ecl136II	Smal	100	7
Smal/Eco24I	Smal	Eco24I	50-100	7
	Eco24I	Smal	100	5
Smal/Kpnl	Smal	Kpnl	0	1
	Kpnl	Smal	0	-1
Smal/Xbal	Smal	Xbal	50-100	8
	Xbal	Smal	100	6

Only double-stranded portion of DNA are included, not the overhangs.

190-90



Digestion of PCR Products

Cleavage of PCR Products Directly After Amplification

The most convenient option for digestion of PCR-amplified DNA is the addition of a restriction endonuclease directly to the reaction tube after completion of PCR. All Fermentas restriction enzymes have been assayed in PCR buffers supplemented with all PCR components. The majority of Fermentas restriction enzymes are active in the Fermentas buffers used for PCR.

However, according to our observations, digestion of PCR products is often inefficient, even though the restriction enzymes are 100% active in the PCR mixture prior to any amplification reactions.

Therefore, we recommend dilution of the PCR product at least 3-fold with 1X recommended restriction enzyme buffer prior to digestion.

Protocol for Digestion of PCR Products

Reaction Contents:

PCR Reaction Mixture	10µl (~0.1-0.5µg of DNA)
Water, nuclease-free (#R0581)	18µI
10X recommended buffer	2µI
Restriction Endonuclease	1-2µl (10-20u)

Ø Mix gently.

Incubate at optimal temperature for 1-16 hours.

Note

- If the diluted PCR products are incompletely digested or not digested at all, purify the PCR products with the DNA Extraction Kit (#K0513), then digest the purified DNA.
- For cloning applications, purification of PCR products prior to digestion is highly recommended to remove the active thermophilic DNA polymerase still present in the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield.
- If a restriction endonuclease requires special additives (e.g., SAM), reduce the amount of Water, nuclease-free (#R0581) appropriately.

Cleavage Efficiency Close to the Termini of PCR Fragments

Some restriction enzymes cleave DNA poorly when their recognition sites are located near the end of a DNA strand. The following Table 1.21 presents activities of Fermentas restriction enzymes when their target sites are located close to the end of a PCR product.

Experiments were performed as follows:

PCR primers were designed with 1-5 extra nucleotides at their 5'-end adjacent to the recognition site for the restriction enzyme. The 5'-end was labeled with [³²P] by T4 Polynucleotide Kinase (#EK0031) and these labeled primers were used in the PCR reaction. PCR products were purified with the DNA Extraction Kit (#K0513), and precipitated with ethanol. DNA aliquots (0.5μ g) were incubated with 10 units of restriction enzymes in its optimal buffer (40µl) for 1 hour at the recommended temperature. Reaction products were separated on 10% PAGE and the percentage of their cleavage was determined using OptiQuant Image Analysis software.

(continued on next page)

ISO ISO 9001 14001

1. RESTRICTION ENDONUCLEASES

General Properties of Restriction Endonucleases

Enzyme	bp from 1	the recogi 2	nition site 3	to fragment end 4 5
Aarl	20-50		50-100	
Aasl	50-100			
Aatll	0	0-20	20-50	50-100
Acc65I	0-20	50-100		
Adel	50-100			
Ajil	50-100			
Alul	0-20	20-50	50-100	
Alw21I	50-100			
Alw26I	50-100			
Alw44I	0	20-50	50-100	
Apal	50-100			
BamHI	50-100			
Baul	0-20	20-50	50-100	
Bcnl	20-50		50-100	
Bcll	0	50-100		
Bcul	50-100			
Bfil	50-100			
Bfml	50-100			
Bful	50-100	50.400		
Bgll	20-50	50-100		
BgIII	0	50-100	50 100	
Bme13901 Boxl	20-50	F0 100	50-100	
	0 50-100	50-100		
Bpil Bpu101	20-50	50-100		
Bpu10I Bpu1102I		50-100		
BseDI	50-100 0	50-100		
BseGl	50-100	50-100		
BseJI	0	50-100		
BseLI	0	50-100		
BseMI	0-20	50-100		
BseMII	50-100	30-100		
BseNI	0	50-100		
BseSI	50-100			
BseXI	20-50	50-100		
Bsh1236l	50-100			
Bsh12851	0-20	50-100		
BshNI	50-100			
BshTl	20-50	50-100		
Bsp68I	0	50-100		
Bsp119I	50-100			
Bsp120I	20-50	50-100		
Bsp143I	50-100			
Bsp143II	0	50-100		
Bsp1407I	20-50	50-100		
BspLI	50-100			
BspPI	0	50-100		
BspTI	0	0-20	50-100	
Bst11071	0-20	50-100		
BstXI	0		50-100	
Bsu15I	50-100			
BsuRI	0-20	20-50	50-100	
Bvel	0-20		50-100	
Cail	0	0-20	50-100	
Cfrl	0	50-100		
Cfr9I	20-50	50-100		
Cfr10I	20-50	50-100		
Cfr13I	50-100			
Cfr42I	50-100			
Сроі	50-100			

				Table	e 1.2′
Enzyme	bp from	the recog	nition site	to fragm	ent er
	1	2	3	4	5
Csel	50-100				
Csp6l	50-100				
Dpnl Drol	50-100	0.00	50.100		
Dral Eam1104	0	0-20	50-100		
	0	50-100			
Eam11051	0	50-100			
Ecl136II Eco24I	50-100				
Eco31I	50-100 20-50		50-100		
Eco32I	20-50	50-100	30-100		
Eco47I	50-100	50 100			
Eco47III	0	0-20	50-100		
Eco52I	0-20	50-100			
Eco57I	50-100				
Eco57MI	50-100				
Eco72I	0-20	50-100			
Eco81I	50-100				
Eco88I	50-100				
Eco91I	20-50	50-100			
Eco105I	20-50	50-100			
Eco130I	0		50-100		
Eco147I	0	50-100			
Eco0109I	50-100				
EcoRI	50-100				
EcoRII	0	0-20	20-50	50-100	
Ehel	20-50		50-100		
Esp3I	50-100				
Faql	0-20	50-100			
FspAl	0-20	20-50	50-100		
FspBl	20-50	50-100			
Gsul	50-100				
Hhal Hin1l	50-100				
Hin1ll	50-100 0	0-20	50-100		
Hin6l	0	0-20	0-20	50-100	
Hincl	50-100		0-20	30-100	
HindIII	0	0-20	50-100		
Hinfl	50-100				
Hpall	0-20	50-100			
Hphl	0	50-100			
Hpy8I	0-20	50-100			
HpyF3I	20-50	50-100			
HpyF10VI	50-100				
Kpnl	50-100				
Kpn2l	0	50-100			
KspAl	20-50	50-100			
Lgul	50-100				
Lwel	20-50	50-100			
Mbil	0	0-20	50-100		
Mbol	50-100				
Mboll	50-100	50.400			
MISI	0-20	50-100	F0 600		
Mul	20-50	E0 100	50-100		
Mnll Mpb11031	0	50-100			
Mph1103I Mspl	20-50 0-20	50-100			
Mssl	20-50	50-100 50-100			
Munl	20-50	50-100			
Mval	0	20-50	50-100		
Mva1269I	0	50-100	30 100		
	2	50 .00			

ent end	Enzyme	bp from	the recog	nition site	to fragment en
5		1	2	3	4 5
	Ncol	0	50-100		
	Ndel*	0-20	20-50	50-100	
	Nhel	0	20-50		50-100
	NmuCl	20-50	50-100		
	Notl	20-50	50-100		
	Nsbl	0	0-20	50-100	
	Olil	0-20	20-50	50-100	
	Pael	0	0-20		20-50 50-10
	Pagl	20-50	50-100		
	Pasl	50-100			
	Paul	0	50-100		
	Pdil	0-20	50-100		
	Pdml	50-100			
	Pfel	50-100	00.50	50 100	
	Pfl23II	0-20	20-50	50-100	
	Pfol Deu 211	0	20-50	50-100	
	Ppu21I	50-100	50.100		
	Pscl	0	50-100		
	Psp5II Psp14061	0	50-100		
	Psp1406l Psul	0-20	50-100		
	Pstl	0-20 0-20	50-100 50-100		
	Psul	0-20			
		0-20	50-100 50-100		
	Psyl Pvul	20-50	50-100	50-100	
	Pvull	50-100		30-100	
	Rsal	50-100			
	Sacl	50-100			
	Sall	20-50		50-100	
	Satl	0	50-100	30-100	
	Scal	0-20	50-100		
	Schl	50-100	50 100		
	Sdal	0-20	50-100		
	Sdul	50-100			
	Sfil	50-100			
	Sgsl	50-100			
	Smal	50-100			
	Smil	0-20	50-100		
	Smol	0	50-100		
	Smul	50-100			
	Ssil	50-100			
	Sspl	0-20	50-100		
	Taal	20-50	50-100		
	Tail	50-100			
	Taql	0	20-50		50-100
	Tasl	0	20-50	50-100	
	Tatl	0-20		20-50	50-100
	Taul	20-50	50-100		
	Tru1l	0	0-20		50-100
	Tsol	20-50	50-100		
	Van91I	0	50-100		
	Vspl	50-100			
	Xagl	20-50	50-100		
	Xapl	20-50		50-100	
	Xbal	20-50	50-100		
	Xcel	0	50-100		
	1001				
	Xhol	0-20	50-100		
		0-20 50-100	50-100		





Note

* Incubation was performed for 16 hours.



Fermentas Guide for Successful Digestions

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1	No di or ind diges
I. RESTRICTION ENDONUCLEASES	

		Table 1.22. Fermentas Guide for Successful Digestions.		
olem	Possible cause	Recommended solution		
digestion ncomplete estion	The DNA sample contains impurities that inhibit restric- tion enzymes.	 <i>To check if these contaminants inhibit restriction enzymes, digest th control DNA. In parallel, digest your sample supplemented with the contr DNA.</i> If PCR products are used directly after amplification, dilute the sample 3-fo with the recommended buffer prior digestion (see p. 140 for more details). If DNA is purified using silica or resin suspensions, re-centrifuge you sample (10min at 10,000rpm) to remove any remnant particles. Consider re-purification of the sample DNA. 		
	Some restriction enzymes cleave supercoiled plasmid DNA with lower efficiency.	Add more of the restriction enzyme (5-10u of restriction enzyme per 1 μ g of DNA).		
	The DNA sequence context influences the efficiency of digestion. Therefore, some DNA sites are cleaved slowly, or they are not cleaved at all (for more details <i>see</i> Site Preferences by Restriction Endonucleases on p.130).	 Add 5-10u of restriction enzymes per 1µg of DNA. Try another isoschizomer (<i>see</i> Table 1.3 on pp.6-15). 		
	Some restriction enzymes, like Aarl, Bvel, Cfr10I, Eam1104, Eco57I, EcoRII, Sfil, require at least two target sites per DNA molecule for efficient cleavage (for more details <i>see</i> Site Preferences by Restriction Endonucleases on p.130).	 Evaluate the number of recognition sites per DNA molecule. If there is only one recognition site per DNA molecule, add an activator DNA containing the same enzyme-specific recognition site (Fermentas restriction enzymes such as Aarl (#ER1581) and Bvel (#ER1741) are supplied with activating oligonucleotides). 		
	Some restriction enzymes cleave DNA poorly, if the recognition site is too close to the end of the DNA molecule.	 Refer to Tables 1.20 (p.139) and 1.21 (p.141) to check the effectiveness of restriction endonucleases at the ends of DNA. Consider direct cloning of your PCR product into Fermentas vectors for blunt and TA cloning (GeneJET[™] PCR Cloning Kit, #K1221 or InsTAclone[™] PCR Cloning Kit*, #K1213). * Available in certain countries only. 		
	The recognition site for the restriction enzyme is not present in the DNA molecule.	Re-check the DNA sequence and cloning strategy.		
	The DNA molecule is methylated at the site which is recognized by a methylation-sensitive restriction endonuclease.	 Identify which type of DNA methylation can occur (<i>see</i> Digestion of Me- thylated DNA on p.132). Use the Tables 1.12-1.19 on pp.133-138 to check if methylation could influence DNA digestion. 		
	<i>Note</i> PCR products are NOT methylated when the PCR is carried out with standard dNTPs and non-methylated primers.	 3. If methylation is the reason for impaired DNA cleavage, we suggest the following: propagate your plasmid in <i>E.coli dam</i>-, <i>dcm</i>- strain. (<i>E.coli</i> GM2163 <i>dam</i>-, <i>dcm</i>-, strain is available for free upon request under #M0099), use the REsearch[™] engine on <u>www.fermentas.com/research</u> or check the Fermentas catalog for availability of a restriction endonuclease isoschizomer which is not sensitive to DNA methylation. 		
	Restriction enzyme Dpnl was used to digest DNA containing unmethylated targets.	 If the cleavage site is not important for your experiment use other neo-schizomer: Bsp143I or Mbol. If the cleavage site must be retained, propagate your plasmid in <i>E.coli dam⁺</i> strains. 		
	Suboptimal reaction conditions.	 Digest your DNA under the specific conditions indicated in the product's Certificate of Analysis (supplied with each enzyme). Use the Fermentas buffer supplied with the restriction endonuclease. Use additives where required. Perform digestion at the optimal temperature; refer Table 1.10 on p.130 for data on the activity of thermophilic enzymes at 37°C. Ensure the volume of the reaction mixture was not reduced due to evaporation during incubation; the resulting increase in salt concentration may reduce enzyme activity. 		

Fermentas Guide for Successful Digestions

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 Table 1.22.
 Fermentas Guide for Successful Digestions.

Problem	Possible cause	Recommended solution
No digestion or incomplete digestion (continued)	The restriction enzyme has been diluted improperly.	 Never dilute enzyme in water or 10X reaction buffer. Avoid dilution in 1X reaction buffer in the absence of DNA. Dilute restriction enzymes with Fermentas Dilution Buffer (#B19). Restriction enzymes diluted with this buffer are stable for at least 3-4 weeks at -20°C (for more information <i>see</i> p.123).
	The restriction enzyme was added to a reaction buffer with low ionic strength in the absence of stabilizing agents.	
	The glycerol concentration in the reaction mixture is too high.	 The glycerol concentration in the reaction mixture should not exceed 5%. Thus, the volume of the restriction enzyme added to the mixture should not exceed 1/10 of the total volume. Alw211, Bpil, Bsp68I, BspTI, Eco32I, Eco91I, EcoRI, Hin6I, HinfI, Mph1103I, Mva1269I and NcoI are especially sensitive to the high glycerol concentration in the reaction mixture.
	The DNA concentration in the reaction mixture is too high or too low	The optimal range of DNA concentration in the reaction mixture is 0.02-0.1 $\mu g/\mu l.$
	The restriction enzyme has been inactivated due to improper storage or handling.	 Check the expiration date. Check if the enzyme has been stored at -20°C. Perform a digestion of control DNA.
Atypical cleavage pattern	Incomplete digestion of DNA (see p.123 for more details).	Add more enzyme or prolong the incubation.
	Star (relaxed) activity of restriction enzyme (<i>see</i> p.131 for more details).	 Add less restriction enzyme (not more than 10u of restriction enzyme per 1µg DNA). Digest DNA in the recommended buffer. Ensure that the glycerol concentration in the reaction mixture does not exceed 5%. Shorten the incubation time. Ensure the volume of the reaction mixture was not reduced due to evaporation during incubation; the resulting increase in glycerol concentration may cause star activity.
	Some newly generated target sites in constructed DNA were overseen.	Recheck your DNA sequence and cloning strategy to identify all target sites.
	If an atypical pattern of DNA digestion persists, the restriction enzyme or buffer could be contaminated with another restriction enzyme due to improper handling.	Use a new tube of enzyme or/and buffer.
	The sample DNA preparation is a mixture of two different DNAs.	Prepare non-contaminated DNA.
Diffused DNA zones, gel shift	Contaminated substrate.	 Purify the DNA sample by phenol/chloroform extraction and ethanol precipitation (<i>see</i> p.123). Perform two control reactions: one without a restriction enzyme and one with another restriction enzyme.
	The enzyme was contaminated due to improper handling.	 Use a new tube of enzyme. Verify enzyme activity with the substrate indicated in the product's Cer- tificate of Analysis.
	Bacterial growth within the buffer(s).	Use a new tube of buffer.Store all buffers at -20°C.
	Protein binding to the substrate DNA affects the elec- trophoretic mobility of digestion products (gel shift). Restriction endonucleases Aarl, Alol, Bdal, BspPI, EcoRII, Eco57I, Gsul, Taul, Tsol are particularly prone to bind their substrate DNA.	Use the 6X Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

(continued on next page)



Table 1.22. Fermentas Guide for Successful Digestions.

Problem	Possible cause	Recommended solution
Low efficiency of restriction frag- ment ligation	Restriction enzyme is still active in the ligation mix- ture.	 Check the thermostability of the restriction enzyme in the product description, Certificate of Analysis or Table 1.8 on pp.124-126. Purify the digested DNA by phenol/chloroform extraction and ethanol precipitation.
	Restriction enzyme did not cut target sites situated close to the DNA termini.	 Refer to Tables 1.19 (p.139) and 1.20 (p.141) to check effectiveness of DNA cleavage by restriction endonucleases close to the ends of DNA. Consider direct cloning of your PCR product into Fermentas vectors for blunt and TA cloning (GeneJET[™] PCR Cloning Kit, #K1221 or InsTAclone[™] PCR Cloning Kit*, #K1213). * Available in certain countries only.
	Restriction fragments with blunt ends are more dif- ficult to ligate.	 Use 100-500u/ml of ligase for ligation (final concentration). Add 10% of polyethylene glycol (supplied with ligase) to the reaction mixture.

REsearch[™] is a unique online tool designed to assist in the selection of a Fermentas restriction endonuclease(s) for your experiments using either the enzyme name or the recognition sequence. This tool also helps you to identify commercially available isoschizomers and choose the optimal buffer for double digestion. It also contains important information regarding restriction enzyme stability during prolonged incubations, conditions for their thermal inactivation, and guidelines on how to generate DNA ends, including cleavage close to the termini of PCR products. Information about new cleavage sites generated by ligation of blunt or compatible sticky ends is also presented together with data about the sensitivity of the restriction enzymes to DNA methylation. The REsearch[™] tool is regularly updated to include all necessary information regarding the newly discovered restriction enzymes.

Use **REsearch**[™] at <u>www.fermentas.com/research</u>, **DoubleDigest**[™] at <u>www.fermentas.com/doubledigest</u> to plan your experiments.

Newly Generated Recognition Sequences Resulting from the Removal of a 3'-overhang and Self-ligation

Note

•	[] denotes	the enzymes that cleave the target both	
	before and	after the ligation.	

• Enzymes produced by Fermentas are shown in orange.

Single letter code

R Y W

M K S

= G or A;	H = A, C or T;
= C or T;	V = A, C or G;
= A or T;	B = C, G or T;
= A or C;	D = A, G or T;
= G or T;	$N \ = G, A, T \text{ or } C.$
= C or G;	

Restriction enzyme	Recognition sequence	Newly generated sequence after reaction	Restriction enzymes that cleave the newly generated recognition sequence
Aasl	GACNNNN↓NNGTC	GACNNNNGTC	BoxI
Adel	CACNNN↓GTG	CACGTG	Eco72I, Maell, Ppu21I, Tail
Bgll	GCCNNNN↓NGGC	GCCNNGGC	BseDI
Bsh12851	CGRY↓CG	CGCG	Bsh1236l
Cail	CAGNNN↓CTG	CAGCTG	Alul, CviJI, MspA1I, Pvull
Cfr42I	CCGC↓GG	CCGG	Hpall, Mspl
Eam1105I	GACNNN↓NNGTC	GACNNNNGTC	Boxl
Fsel	GGCCGG↓CC	GGCC	[BsuRI], [CviJI]
Hpy188I	TCN↓GA	TCGA	Taql
Pacl	TTAAT↓TAA	TTATAA	Psil
Pvul	CGAT↓CG	CGCG	Bsh1236l
Sdal	CCTGCA↓GG	CCGG	Hpall, Mspl
Sfil	GGCCNNNN↓NGGCC	GGCCNNGGCC	BseDI, [BsuRI], [CviJI]
Sgfl	GCGAT↓CGC	GCGCGC	Bsh1236I, Cac8I, Hhal, Hin6I, Paul
Taal	ACN↓GT	ACGT	Maell, Tail

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Information on New Cleavage Sites

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REsearch™ is a unique online tool designed to assist in the selection of a Fermentas restriction endonuclease(s) for your experiments using either the enzyme name or the recognition sequence. This tool also helps you to identify commercially available isoschizomers and choose the optimal buffer for double digestion. It also contains important information regarding restriction enzyme stability during prolonged incubations, conditions for their thermal inactivation, and guidelines on how to generate DNA ends, including cleavage

close to the termini of PCR products. Information about new cleavage sites generated by ligation of blunt or compatible sticky ends is also presented together with data about the sensitivity of the restriction enzymes to DNA methylation. The REsearch[™] tool is regularly updated to include all neccessary information regarding the newly

discovered restriction enzymes. Use REsearch™ at www.fermentas.com/research,

DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> to plan your experiments.

Newly Generated Recognition Sequences Resulting from the Fill-in of a 5'-overhang and Self-ligation

Note

- Restriction enzymes that have degenerate recognition sequences (i.e., recognize more than one sequence) are indicated by an asterisk (*). Be aware that these restriction endonucleases will cleave sequences in addition to the one listed.
- [] denotes the enzymes that cleave the target both before and after the ligation.
- Enzymes produced by Fermentas are shown in orange.

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or G
S = C or G	

Table 1.24. Newly Generated Recognition Sequences Resulting from the Fill-in of a 5'-overhang and Self-ligation.

Restriction	Recognition sequence	Newly generated sequence	Restriction enzymes that cleave the newly generated
enzyme		after reaction	recognition sequence
Acc65I	G↓GTACC	GGTACGTACC	[Csp6I], Eco105I, Maell, Ppu21I, [RsaI], Tail
AfIIII*	A↓CACGT	ACACGCACGT	[Maell], [Tail]
AfIIII*	A↓CATGT	ACATGCATGT	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I, [XceI]
AfIIII*	A↓CGCGT	ACGCGCGCGT	[Bsh1236I], Cac8I, Hhal, Hin6I, Paul
AfIIII*	A↓CGTGT	ACGTGCGTGT	[Maell], [Tail]
Alw44I	G↓TGCAC	GTGCATGCAC	Cac8I, CviAII, FatI, Hin1II, [HpyCH4V], Pael, Xcel
BamHI	G↓GATCC	GGATCGATCC	[Bsp143I], [BspPI], Bsu15I, [DpnI], [MboI], Taql
BbvCl	CC↓TCAGC	CCTCATCAGC	[MnII]
Bcll	T↓GATCA	TGATCGATCA	[Bsp143I], Bsu15I, [DpnI], [Mbol], Taql
Bcnl	CC↓SGG	CCSSGG	BseDI
Bcnl*	CC↓CGG	CCCCGG	[Bcnl], [Bme1390I], BseDI, [BssKI], [Hpall], [Mspl]
Bcnl*	CC↓GGG	CCGGGG	[Bcnl], [Bme1390I], BseDI, [BssKI], [HpalI], [Mspl]
Bcul	A↓CTAGT	ACTAGCTAGT	Alul, CviJI, [FspBI]
Bfml*	C↓TGCAG	CTGCATGCAG	Cac8I, CviAII, FatI, Hin1II, [HpyCH4V], PaeI, XceI
BgIII	A↓GATCT	AGATCGATCT	[Bsp143], Bsu15I, [Dpnl], [Mbol], Taql
Bme1390I	CC↓NGG	CCNNGG	BseDI
Bme1390I*	CC↓AGG	CCAAGG	BseDI, Eco130I
Bme1390I*	CC↓CGG	CCCCGG	[Bcnl], [Bme1390I], BseDI, [BssKI], [HpalI], [Mspl]
Bme1390I*	CC↓GGG	CCGGGG	[Bcnl], [Bme1390I], BseDI, [BssKI], [HpalI], [Mspl]
Bme1390I*	CC↓TGG	CCTTGG	BseDI, Eco130I
Bpu10I*	CC↓TCAGC	CCTCATCAGC	[MnII]
BsaWI	W↓CCGGW	WCCGGCCGGW	Bsh1285I, BsuRI, CfrI, CviJI, Eco52I, [Hpall], [Mspl]
BseYl	C↓CCAGC	CCCAGCCAGC	[BseYI], Cac8I, CviJI
BshNI*	G↓GCACC	GGCACGCACC	Cac8l
BshNI*	G↓GCGCC	GGCGCGCGCC	Bsh1236I, Cac8I, [Hhal], [Hin6I], Paul
BshNI*	G↓GTACC	GGTACGTACC	[Csp6I], Eco105I, Maell, Ppu21I, [Rsal], Tail
BshNI*	G↓GTGCC	GGTGCGTGCC	Cac8l
BshTl	A↓CCGGT	ACCGGCCGGT	Bsh1285I, BsuRI, [Cfr10I], CfrI, CviJI, Eco52I, [HpaII], [MspI]
Bsp119I	TT↓CGAA	TTCGCGAA	Bsh1236I, Bsp68I, Hpy188III
Bsp120I	G↓GGCCC	GGGCCGGCCC	[BsuRI], Cac8I, Cfr10I, [Cfr13I], [CviJI], Fsel, HpalI, MspI, NgoMIV, Pdil
Bsp1407I	T↓GTACA	TGTACGTACA	[Csp6I], Eco105I, Maell, Ppu21I, [Rsal], Tail
Bsp143I	↓GATC	GATCGATC	[Bsp143I], Bsu15I, [Dpnl], [Mbol], Taql
BspTI	C↓TTAAG	CTTAATTAAG	Pacl, Tasl, [Tru1I]
BssKl	↓ CCNGG	CCNGGCCNGG	[Bme1390I], [BssKI], BsuRI, CviJI
BssKI*	↓CCAGG	CCAGGCCAGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]



Table 1 24 Newly G	enerated Recognition	Securences	Resulting from t	he Fill-in of a 5'	-overhand and Sc	lf_ligation
Table 1.24. Newly C	lenerateu Necogrittion	Sequences	Resulting norm t		-overnany and Se	ii-iiyation.

enzyme BssKI* BssKI* BssKI* Bsu15I	↓cccgg	after reaction	recognition sequence
BssKI* BssKI*	↓UUUUU	CCCGGCCCGG	[Bcnl], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpalI], [Mspl]
BssKI*	↓CCGGG	CCGGGCCGGG	
		CCTGGCCTGG	[Bcnl], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [Hpall], [Mspl]
			[Bme1390], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
	ATUCGAT	ATCGCGAT	Bsh1236I, Bsp68I, Hpy188III
Btgl*	C↓CATGG	CCATGCATGG	BfrBI, [CviAII], [Fatl], [Hin1II], HpyCH4V, Mph1103I
Btgl*	C↓CGCGG	CCGCGCGCGG	[Bsh1236I], Cac8I, Hhal, Hin6I, Paul, [Ssil]
Cfr10I	RUCCGGY	RCCGGCCGGY	Bsh1285I, BsuRI, [Cfr10I], CfrI, CviJI, Eco52I, [HpalI], [MspI]
Cfr13I*	GLGCCC	GGCCGCCC	[BsuRI], [CviJI], SatI, SsiI, Taul
Cfr13I*	GUGGCC	GGGCGGCC	[BsuRI], [CviJI], SatI, Taul
Cfr9I	C↓CCGGG	CCCGGCCGGG	[Bcnl], [Bme1390I], Bsh1285I, [BssKl], BsuRI, Cfrl, CviJI, Eco52I, [Hpall] [Mspl]
Cfrl	Y↓GGCCR	YGGCCGGCCR	[BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], Fsel, Hpall, MspI, NgoMIV, Pdil
Cpol*	CG↓GACCG	CGGACGACCG	Bsh1285I
Cpol*	CG↓GTCCG	CGGTCGTCCG	Bsh1285I
Csp6l	G↓TAC	GTATAC	Bst1107I, Hpy8I, Xmil
CviAll	C↓ATG	CATATG	Ndel
Eco130I*	C↓CATGG	CCATGCATGG	BfrBI, [CviAll], [Fatl], [Hin1II], HpyCH4V, Mph1103I
Eco130I*	C↓CTAGG	CCTAGCTAGG	Alul, CviJI, [FspBI]
Eco52I	C↓GGCCG	CGGCCGGCCG	[Bsh1285I], [BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], [Eco52I], Fsel, Hpall,
200321	00000	000000000	Mspl, NgoMIV, Pdil
Eco81I*	CCUTCAGG	CCTCATCAGG	[MnII]
Eco88I	C↓YCGRG	CYCGRYCGRG	Bsh1285I
Eco88I*	C↓CCGAG	CCCGACCGAG	Bsh12851
Eco88I*	C↓CCGGG	CCCGGCCGGG	[Bcnl], [Bme1390I], Bsh1285I, [BssKl], BsuRI, Cfrl, CviJI, Eco52I, [Hpall] [Mspl]
Eco88I*	C↓TCGAG	CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, Mbol, Pvul, [Taql]
Eco88I*	C↓TCGGG	CTCGGTCGGG	Bsh1285I
Eco91I	G↓GTNACC	GGTNACGTNACC	Maell, [Maelll], <mark>Tail</mark>
Eco91I*	GUGTAACC	GGTAACGTAACC	Maell, [Maelll], Tail
Eco91I*	G↓GTCACC	GGTCACGTCACC	Ajil, Maell, [Maell], [NmuCl], Tail
Eco911*	G↓GTGACC	GGTGACGTGACC	[Hph], Maell, [Maell], [NmuCl], Tail
Eco911*	G↓GTTACC	GGTTACGTTACC	Maell, [Maell], Tail
Eco0109I*	RGJGCCCY	RGGCCGCCCY	[BsuRI], [CviJI], Satl, Ssil, Taul
Eco01091*	RGJGGCCY	RGGGCGGCCY	[BsuRI], [CviJI], Sati, Taul
EcoRI	GUAATTC	GAATTAATTC	Pdml, [Tasl], Tru1l, Vspl
EcoRII	↓CCWGG	CCWGGCCWGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
EcoRII*		CCAGGCCAGG	[DITET 3 901], [DSSNI], DSURI, CVIJI, [CORTI], [Wivel]
			[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
EcoRII*	↓CCTGG	CCTGGCCTGG	[Bme1390], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
Fatl	CATG	CATGCATG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
FspBI	C↓TAG	CTATAG	Bfml
Hin1I	GR↓CGYC	GRCGCGYC	Bsh1236l
Hin6l	GUCGC	GCGCGC	Bsh1236I, Cac8I, [Hhal], [Hin6I], Paul
HindIII	AJAGCTT	AAGCTAGCTT	[Alul], Bmtl, Cac8l, [CviJI], FspBl, Nhel
Hpall	C↓CGG	CCGCGG	BseDI, Bsh1236I, BtgI, Cfr42I, MspA1I, Ssil
Kasl	G↓GCGCC	GGCGCGCGCC	Bsh1236I, Cac8I, [Hhal], [Hin6I], Paul
Kpn2I	T↓CCGGA	TCCGGCCGGA	Bsh1285I, BsuRI, CfrI, CviJI, Eco52I, [Hpall], [Mspl]
Maell	A↓CGT	ACGCGT	Afilli, Bsh1236I, Mlul
Maelli	↓GTNAC	GTNACGTNAC	Maell, [MaellI], Tail
Maelli*	↓GTAAC	GTAACGTAAC	Maell, [Maell], Tail
Maelll*	JGTCAC	GTCACGTCAC	Ajil, Maell, [Maell], [NmuCl], Tail
Maelli*	↓GTGAC	GTGACGTGAC	Maell, [Maell], [NmuCl], Tail
Maelli*	GTTAC	GTTACGTTAC	Maeil, [Maeili], Tail
Mbol	↓GATC	GATCGATC	[Bsp143I], Bsu15I, [DpnI], [MboI], Taql
Mul		ACGCGCGCGT	[Bsh1236], Cac8l, Hhal, Hin6l, Paul
Mspl		CCGCGG	BseDI, Bsh1236I, BtgI, Cfr42I, MspA1I, Ssil
Muni	CUAATTG	CAATTAATTG	Tasi), Tru1i, Vspi
Mval	CCVWGG	CCWWGG	BseDI, Eco130I
Mval*	CCJAGG	CCAAGG	BseDI, Eco130I
Mval*	CC↓TGG	CCTTGG	BseDI, Eco130I
Narl	GG↓CGCC	GGCGCGCC	Bsh1236I, Cac8I, [Hhal], [Hin6I], Paul, Sgsl
Ncol	C↓CATGG	CCATGCATGG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
NgoMIV	G↓CCGGC	GCCGGCCGGC	Bsh1285I, BsuRI, [Cac8I], [Cfr10I], Cfr1, CviJI, Eco52I, [HpaII], [MspI], [NgoMIV], [PdiI]
Nhol	G↓CTAGC	CCTACCTACC	
Nhel		GCTAGCTAGC	Alul, [Bmtl], [Cac8l], CviJI, [FspBI], [Nhel]
NmuCl	↓GTSAC	GTSACGTSAC	Maell, [Maell], [NmuCl], Tail

ISO	ISO
9001	14001

Information on New Cleavage Sites

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Table 1.24. Newly Generated Recognition Sequences Resulting from the Fill-in of a 5'-overhang and Self-ligation.

Restriction	Recognition sequence	Newly generated sequence	Restriction enzymes that cleave the newly generated
enzyme		after reaction	recognition sequence
NmuCI*	GTCAC	GTCACGTCAC	Ajil, Maell, [Maelll], [NmuCl], Tail
NmuCI*	↓ GTGAC	GTGACGTGAC	Maell, [MaellI], [NmuCl], Tail
Notl	GCJGGCCGC	GCGGCCGGCCGC	[Bsh1285I], [BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], [Eco52I], Fsel, Hpall,
			Mspl, NgoMIV, Pdil, [Satl], [Ssil], [Taul]
Pagl	T↓CATGA	TCATGCATGA	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
Pasl*	CC↓CAGGG	CCCAGCAGGG	BseYI, EcoP15I
Paul	G↓CGCGC	GCGCGCGCGC	[Bsh1236I], [Cac8I], [Hhal], [Hin6I], [Paul]
Pfl23II	C↓GTACG	CGTACGTACG	[Csp6I], Eco105I, Maell, [PfI23II], Ppu21I, [Rsal], Tail
Pfol	T↓CCNGGA	TCCNGGCCNGGA	[Bme1390I], [BssKI], BsuRI, CviJI
Pfol*	T↓CCAGGA	TCCAGGCCAGGA	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
Pfol*	T↓CCCGGA	TCCCGGCCCGGA	[Bcnl], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [Hpall], [Mspl]
Pfol*	T↓CCGGGA	TCCGGGCCGGGA	[Bcnl], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpalI], [Mspl]
Pfol*	T↓CCTGGA	TCCTGGCCTGGA	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
Pscl	A↓CATGT	ACATGCATGT	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I, [XceI]
Psp1406l	AAJCGTT	AACGCGTT	Afilli, <mark>Bsh1236</mark> I, <mark>Mlul</mark>
PspXI	VC	VCTCGATCGAGB	Bsh1285I, Bsp143I, Dpnl, Mbol, Pvul, [Taql]
Psul	R↓GATCY	RGATCGATCY	[Bsp143], Bsu15I, [Dpnl], [Mbol], Taql
Psyl	GACN	GACNNNNGTC	BoxI
Psyl*	GACN	GACNAANGTC	BoxI
Psyl*	GACN↓CNGTC	GACNCCNGTC	BoxI
Psyl*	GACN	GACNGGNGTC	BoxI
Psyl*	GACN	GACNTTNGTC	BoxI
Sall	G↓TCGAC	GTCGATCGAC	Bsh1285I, Bsp143I, Dpnl, Mbol, Pvul, [Taql]
SanDI*	GGUGACCC	GGGACGACCC	[FaqI]
Satl	GCUNGC	GCNNGC	Cac8l
Satl*	GCLAGC	GCAAGC	Cac8l
Satl*	GCUCGC	GCCCGC	Cac8I, <mark>Smul</mark> , [<mark>Ssil</mark>]
Satl*	GCUGGC	GCGGGC	Cac8l
Satl*	GCUTGC	GCTTGC	Cac8l
SexAl	AUCCWGGT	ACCWGGCCWGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
SexAI*	AUCCAGGT	ACCAGGCCAGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
SexAI*	AUCCTGGT	ACCTGGCCTGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [Mval]
SgrAl	CRUCCGGYG	CRCCGGCCGGYG	Bsh1285I, BsuRI, [Cfr10I], CfrI, CviJI, Eco52I, [Hpall], [Mspl]
Sgsl	GGUCGCGCC	GGCGCGCGCGCC	[Bsh1236I], [Cac8I], [Hhal], [Hin6I], [Paul]
Smol*	C TCGAG	CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, Mbol, Pvul, [Taql]
Smol*	C↓TTAAG	CTTAATTAAG	Paci, Tasi, [Tru1i]
Ssil		CCGCGC	Bsh1236l, Hhal, Hin6l, [Ssil]
Taql		TCGCGA	Bsh1236I, Bsp68I, Hpy188III
Tasl		AATTAATT	[Tasi], Tru1i, Vspl
Tatl	W GTACW	WGTACGTACW	[Csp6I], Eco105I, Maell, Ppu21I, [Rsal], Tail
Tru1I		TTATAA	Psil
Tsel	G CWGC	GCWGCWGC	[Sati], [Tsel]
Tsel*	GUCAGC	GCAGCAGC	[BseXI], EcoP15I, [SatI], [Tsel]
Tsel*	GUCTGC	GCTGCTGC	[Sati], [Tsel]
Vspl		ATTATAAT	Psil
Xapl	RUAATTY	RAATTAATTY	[Tasl], Tru1I, Vspl
Xbal		TCTAGCTAGA	Alul, CviJI, [FspBI]
Xhol		CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, Mbol, Pvul, [Taql]
XmaJI		CCTAGCTAGG	Alul, CviJI, [FspBI]
Xmil*		GTCGCGAC	Bsh1236I, Bsp68I, Hpy188III
Xmil*	GTJCTAC	GTCTCTAC	Alw26l



REsearch[™] is a unique online tool designed to assist in the selection of a Fermentas restriction endonuclease(s) for your experiments using either the enzyme name or the recognition sequence. This tool also helps you to identify commercially available isoschizomers and choose the optimal buffer for double digestion. It also contains important information regarding restriction enzyme stability during prolonged incubations, conditions for their thermal inactivation, and guidelines on how to generate DNA ends, including cleavage close to the termini of PCR products. Information about new cleavage sites generated by ligation of blunt or compatible sticky ends is also presented together with data about the sensitivity of the restriction enzymes to DNA methylation.

The REsearch[™] tool is regularly updated to include all neccessary information regarding the newly discovered restriction enzymes.

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Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends

Note

- Restriction enzymes that have degenerate recognition sequences (i.e., recognize more than one sequence) are indicated by an asterisk (*). Be aware that these restriction endonucleases will cleave sequences in addition to the one listed.
- Enzymes produced by Fermentas are shown in orange.

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or (
S = C or G:	

(continued on next page)

Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme	enzyme	newly generated recognition sequence
AjiI* (CAC↓GTC)	EcI136II (GAG↓CTC), Mbil* (CCG↓CTC)	MnII
	Eco105I (TACJGTA), Ppu21I* (YACJGTA)	Maell, Ppu21I, Tail
	Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTG)	Eco72I, Maell, Ppu21I, Tail
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	BceAl
	Zral (GAC J GTC)	Ajil, Maell, Tail
AjiI* (GAC↓GTG)	Dpnl (GAUTC)	Hinfl
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mnll
	Eco105I (TAC↓GTA), Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTA), Ppu21I* (YAC↓GTG)	Maell, Tail
	Eco47III (AGCJGCT), FspAI* (RTGCJGCAC), FspAI* (RTGCJGCAT), NsbI (TGCJGCA)	Csel
	Ehel (GGC↓GCC)	Csel, Hin1I
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCCJGGC)	BceAl
	Zral (GAC J GTC)	Aatll, Hin1l, Maell, Tail, Zral
Alul (AG↓CT)	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MISI (TGG↓CCA)	CviJI
	CviJI* (RG↓CT), Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC), MspA1I* (CMG↓CTG), PvuII (CAG↓CTG)	Alul, CviJI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, Mbol
	Ehel (GGCJGCC)	BsuRI, CviJI
BfrBI (ATG↓CAT)	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	Dral (TTT↓AAA), Hincll* (GTY↓AAC), KspAI (GTT↓AAC), MssI (GTTT↓AAAC), Smil (ATTT↓AAAT)	TspDTI
	Eco32I (GAT_JATC)	Bsp143I, DpnI, Mbol
	Eco47III (AGCJGCT)	CviJI
	Ehel (GGC↓GCC)	BsuRI, CviJI
	HpyCH4V (TG↓CA)	HpyCH4V
Bsh1236I (CG↓CG)	Bsp68I (TCG↓CGA), Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Bsh1236l
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, Mbol
	Eco47III (AGC↓GCT)	CviJI
	Ehel (GGC↓GCC)	BsuRI, CviJI
Bsp68I (TCG↓CGA)	Bsh1236I (CG \downarrow CG), Mbil* (GAG \downarrow CGG), MspA1I* (CMG \downarrow CGG)	Bsh1236I
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	Dral (TTT↓AAA), Hincll* (GTY↓AAC), KspAI (GTT↓AAC), MssI (GTTT↓AAAC), RsaI (GT↓AC),	Tagl
	Scal (AGT \downarrow ACT), Smil (ATT \downarrow AAAT), Sspl (AT \downarrow ATT)	
	Eco32I (GATJATC)	Bsp143I, DpnI, Mbol, Taql
	Eco47III (AGC↓GCT)	CviJI
	Ebel (GGC↓GCC)	BsuRI, CviJI

Information on New Cleavage Sites

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 Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

First restriction enzyme	Second restriction enzyme	Restriction enzymes that cleave the newly generated recognition sequence
	Hincll* (GTY↓GAC)	Hpy188I
<mark>Bst1107I</mark> (GTA↓TAC)	Alul ($AG\downarrow$ CT), BfrBI ($ATG\downarrow$ CAT), Bsh1236I ($CG\downarrow$ CG), Bsp68I ($TCG\downarrow$ CGA), BsuRI ($GG\downarrow$ CC), CviJI* ($RG\downarrow$ CC), CviJI* ($RG\downarrow$ CT), Ecl136II ($GAG\downarrow$ CTC), Eco147I ($AGG\downarrow$ CCT), HpyCH4V ($TG\downarrow$ CA), MbiI* ($CCG\downarrow$ CTC), MbiI* ($GAG\downarrow$ CGG), MISI ($TGG\downarrow$ CCA), MspA1I* ($CMG\downarrow$ CGG), MspA1I* ($CMG\downarrow$ CTG), PvuII ($CAG\downarrow$ CTG)	Csp6l, Rsal
	Eco47III (AGC↓GCT)	Alul, CviJI
	Ehel (GGCJGCC)	CviJI
	Hincll* (GTY JAAC), KspAI (GTT JAAC)	Нру8І
	HincII* (GTY↓GAC)	Hpy8I, Xmil
	Rsal (GT↓AC), Scal (AGT↓ACT)	Maelli
	SspI (AAT↓ATT)	Tasl
BsuRI (GG↓CC)	Alul (AG↓CT), CviJI* (RG↓CT), Ecl136II (GAG↓CTC), Eco47III (AGC↓GCT), MbiI* (CCG↓CTC), MspA1I* (CMG↓CTG), PvuII (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	CviJI* (RG↓CC), <mark>Eco147I</mark> (AGG↓CCT), <mark>MIsI</mark> (TGG↓CCA)	BsuRI, CviJI
	Eco32I (GAT JATC)	Bsp143I, BspPI, DpnI, Mbol
	Ehel (GGC↓GCC)	BsuRI, Cfr13I, CviJI
	FspAI* (RTGC↓GCAC)	BseSI, Sdul
	Hincll* (GTY↓GAC)	Faql
CviJI* (AG↓CY)	Alul (AGJCT), Ecl136II (GAGJCTC), Mbil* (CCGJCTC), MspA1I* (CMGJCTG), Pvull (CAGJCTG)	Alul, CviJI
		Csp6l, Rsal
	BSuRI (GG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MISI (TGG↓CCA)	CviJI
	Eco32I (GAT JATC)	Bsp143I, DpnI, Mbol
		BsuRI, CviJI
CviJI* (GG↓CY)	Alul (AG↓CT), EcI136II (GAG↓CTC), Eco47III (AGC↓GCT), Mbil* (CCG↓CTC), MspA1I* (CMG↓CTG), Pvull (CAG↓CTG)	
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	BsuRI (GG↓CC), Eco147I (AGG↓CCT), MISI (TGG↓CCA)	BsuRI, CviJI
	Eco32I (GAT JATC)	Bsp143I, BspPI, DpnI, Mbol
	Ehel (GGC↓GCC)	BsuRI, Cfr13I, CviJI
	FspAI* (RTGC↓GCAC)	BseSI, Sdul
	Hincli* (GTY↓GAC)	Faql
<mark>DpnI</mark> (GA↓TC)	Ajil* (CAC↓GTC), Zral (GAC↓GTC)	Hinfl, Plel, Schl
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	Hinfl Hinfl Dfal
	Eco32I (GAT↓ATC)	Hinfl, Pfel
	Eco47III (AGC↓GCT)	Alul, CviJI CviJI
	Ehel (GGC↓GCC) FspAI* (RTGC↓GCAC)	
		Alw211, Sdul Tasl
Dral (TTT↓AAA)	Sspl (AAT↓ATT) Bsp68I (TCG↓CGA)	Tagl
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	HpyCH4V
	Hincli* (GTY_AAC), KspAI (GTT_AAC), Psil (TTA_TAA)	Tru1I
	Minor (GTTVAAC), KSPAI (GTTVAAC), FSII (TAVTAA) MSSI (GTTTVAAC), Smil (ATTTVAAC)	Dral, Tru1l
EcI136II (GAG↓CTC)	Alul (AG↓CT), CviJI* (RG↓CT), MspA1I* (CMG↓CTG), PvuII (CAG↓CTG)	Alul, CviJI
	Bst1107I ($GTA\downarrowTAC$)	Csp6l, Rsal
	BSCHOFI (GIA⊄IAC) BSURI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MISI (TGG↓CCA)	CviJI
	Dpnl (GA \downarrow TC)	Hinfl, Plel, Schl
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, Mbol
	Ecose (Garvaro)	BsuRI, CviJI
	Mbil* (CCG↓CTC)	Alul, Alw21I, CviJI, Ecl136II, Eco24I,
		Saci, Sdul
Eco105I (TAC↓GTA)	Ajil* (CAC↓GTC), Zral (GAC↓GTC)	Maell, Tail
	Ajil* (GAC↓GTG), Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTG)	Maell, Ppu21I, Tail
	Ecl136II (GAG \downarrow CTC), Mbil* (CCG \downarrow CTC)	Macii, i paziri, ian
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	BceAl
	Ppu21I* (YAC↓GTA)	Eco105I, Maell, Ppu21I, Tail
Eco147I (AGG↓CCT)	Alul (AG↓CT), CviJI* (RG↓CT), Ecl136II (GAG↓CTC), Eco47III (AGC↓GCT), MbiI* (CCG↓CTC), MspA1I* (CMG↓CTG), PvuII (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	Bstrion (GrA⊊rac) BsuRI (GG↓CC), CviJI* (RG↓CC), MISI (TGG↓CCA)	BsuRI, CviJI
	ECO32I (GAT \downarrow ATC)	Bsp143I, BspPI, DpnI, Mbol
	Ecose (GAT CAT CAT CAT CAT CAT CAT CAT CAT CAT C	BsuRI, Cfr13I, CviJI
	FspAI* (RTGC↓GCAC)	BseSI, Sdul

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Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

(continued on next page)

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme		newly generated recognition sequence
Eco32I (GAT↓ATC)	Alul (AG \downarrow CT), BfrBI (ATG \downarrow CAT), Bsh1236I (CG \downarrow CG), BsuRI (GG \downarrow CC), CviJI* (RG \downarrow CC), CviJI* (RG \downarrow CT), Ecl136II (GAG \downarrow CTC), Eco147I (AGG \downarrow CCT), HpyCH4V (TG \downarrow CA), MbiI* (CCG \downarrow CTC), MbiI* (GAG \downarrow CGG), MISI (TGG \downarrow CCA), MspA1I* (CMG \downarrow CGG), MspA1I* (CMG \downarrow CGG), PvuII (CAG \downarrow CTG)	Bsp143I, DpnI, Mbol
	Bsp68I (TCG↓CGA)	Bsp143I, DpnI, Mbol, Taql
	DpnI (GA↓TC)	Hinfl, Pfel
	FspAI* (RTGC↓GCAC), NsbI (TGC↓GCA)	НруСН4V
	FspAI* (RTGC↓GCAT)	BfrBI, HpyCH4V, Mph1103I
		Tru1I
Eco47III (AGC↓GCT)	Alul (AG \downarrow CT), BfrBI (ATG \downarrow CAT), Bsh1236I (CG \downarrow CG), Bsp68I (TCG \downarrow CGA), BsuRI (GG \downarrow CC), CviJI* (RG \downarrow CC), CviJI* (RG \downarrow CT), Eco147I (AGG \downarrow CCT), HpyCH4V (TG \downarrow CA), MISI (TGG \downarrow CCA), MspA1I* (CMG \downarrow CTG), PvuII (CAG \downarrow CTG)	CviJI
	Bst1107I (GTA↓TAC), DpnI (GA↓TC), Psil (TTA↓TAA)	Alul, CviJI
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	CviJI, MnII
	Eco32I (GAT↓ATC)	Lwel
	Ehel (GGC↓GCC)	Bsp143II, Hhal, Hin6I
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	Hhal, Hin6l
	Mbil* (GAG JCGG), MspA1I* (CMG JCGG)	CviJI, Hpall, Mspl
	Pdil (GCC↓GGC)	Satl, Taul
Eco72I (CAC↓GTG)	Srfl (GCCC↓GGGC) Ajil* (CAC↓GTC), Zral (GAC↓GTC)	Cac8l Ajil, Maell, Tail
	Ajil* (GAC↓GTG), Ppu21I* (YAC↓GTG)	Eco72I, Maell, Ppu21I, Tail
	Ec136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mnll
	Eco105I (TAC \downarrow GTA), Ppu21I* (YAC \downarrow GTA)	Maell, Ppu21I, Tail
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	BceAl
Ehel (GGC↓GCC)	AjiI* (CAC↓GTC), Zral (GAC↓GTC)	Hin1I
	Alul (AG \downarrow CT), BfrBI (ATG \downarrow CAT), Bsh1236I (CG \downarrow CG), Bsp68I (TCG \downarrow CGA), CviJI* (RG \downarrow CT),	BsuRI, CviJI
	HpyCH4V (TG↓CA), MspA1I* (CMG↓CTG), Pvull (CAG↓CTG)	
	Bst1107I (GTAJTAC), DpnI (GAJTC), Psil (TTAJTAA)	CviJI
	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), MISI (TGG↓CCA)	BsuRI, Cfr13I, CviJI
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	BsuRI, CviJI, MnII
	Eco32I (GAT↓ATC) Eco47III (AGC↓GCT)	Lwel Bsp143II, Hhal, Hin6I
	FSPAI* (RTGC \downarrow GCAC), FSPAI* (RTGC \downarrow GCAT), NSbI (TGC \downarrow GCA)	Hhal, Hin6l
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	BsuRI, CviJI, Hpall, Mspl
	Pdil (GCCJGGC)	Satl, Taul
	Srfl (GCCC↓GGGC)	Cac8l
FspAI* (ATGC↓GCAY)	Dral (TTT↓AAA), Hincll* (GTY↓AAC), KspAl (GTT↓AAC), MssI (GTTT↓AAAC), Rsal (GT↓AC), Scal (AGT↓ACT), Smil (ATTT↓AAAT)	НруСН4V
	EcI136II (GAG↓CTC), MbiI* (CCG↓CTC)	MnII
	Eco32I (GAT↓ATC)	BfrBl, HpyCH4V, Lwel, Mph1103I
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC)	Hhal, Hin6l
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Nsbl (TGC↓GCA)	Hhal, Hin6l, Nsbl
	Pdil (GCC↓GGC) Srfl (GCC↓GGGC)	Satl, Taul Cac8l
	Sspl (AAT↓ATT)	BfrBI, HpyCH4V, Mph1103I
FspAI* (GTGC↓GCAY)	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), MISI (TGG↓CCA)	BseSI, Sdul
	DpnI (GA↓TC)	Alw21I, Sdul
	Dral (TTT↓AAA), Hincll* (GTY↓AAC), KspAl (GTT↓AAC), MssI (GTTT↓AAAC), Smil (ATTT↓AAAT),	HpyCH4V
	SspI (AAT↓ATT)	
	EcI136II (GAG↓CTC), MbiI* (CCG↓CTC)	MnII
	Eco32I (GAT JATC)	HpyCH4V, Lwel
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC)	Hhal, Hin6l
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Nsbl (TGC↓GCA)	Hhal, Hin6l, Nsbl
	Pdil (GCC↓GGC) Rsal (GT↓AC), Scal (AGT↓ACT)	Sati, Taul Alw21I, Alw44I, BseSI, Hpy8I, HpyCH4V, Sdul
	Srfl (GCCC↓GGGC)	Cac8l
Hincll* (GTC↓RAC)	Bsp68I (TCG↓CGA)	Hpy188I
(========)	Bst1107I (GTA↓TAC)	Hpy8I, Xmil
	DpnI (GA↓TC)	Alw26I
	EcI136II (GAG↓CTC), Mbil* (CCG↓CTC)	MnII
	KspAI (GTT↓AAC)	Hincll, Hpy8l

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Information on New Cleavage Sites

 Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

First restriction enzyme	Second restriction enzyme	Restriction enzymes that cleave the newly generated recognition sequence
,	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Rsal (GT↓AC), Scal (AGT↓ACT)	Maelli, NmuCl
HincII* (GTT↓RAC)	Bsp68I (TCG↓CGA)	Taql
	Bst1107I (GTA↓TAC)	Нру8І
	Dral (TTT↓AAA), MssI (GTTT↓AAAC), Psil (TTA↓TAA), Smil (ATTT↓AAAT)	Tru1I
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	НруСН4V
	KspAI (GTT JAAC)	Hincll, Hpy8I, KspAI, Tru1I
	Rsal (GT↓AC), Scal (AGT↓ACT)	Maelli
HpyCH4V (TG↓CA)	BfrBI (ATG↓CAT)	HpyCH4V
	Bst1107I (GTA↓TAC) Eco32I (GAT↓ATC)	Csp6l, Rsal
	EC0321 (GAT VATC) Ec047III (AGC J GCT)	Bsp143I, DpnI, Mbol CviJI
	Ehel (GGC↓GCC)	BsuRI, CviJI
KspAI (GTT↓AAC)	Bsp68I (TCG↓CGA)	Tagl
	Bst1107I (GTA↓TAC)	Hpy8l
	Dral (TTT↓AAA), MssI (GTTT↓AAAC), Psil (TTA↓TAA), Smil (ATTT↓AAAT)	Tru1l
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	HpyCH4V
	Hincll* (GTY JAAC)	Hincll, Hpy8l, KspAl, Tru1l
	Hincll* (GTY JGAC)	Hincll, Hpy8l
	Rsal (GT↓AC), Scal (AGT↓ACT)	Maelli
Mbil* (CCG↓CTC)	Ajil* (CAC↓GTC), Ajil* (GAC↓GTG), Eco105I (TAC↓GTA), Eco72I (CAC↓GTG),	Hpall, Mspl
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), HincII* (GTY↓GAC), NsbI (TGC↓GCA), Ppu21I* (YAC↓GTA), Ppu21I* (YAC↓GTG), ZraI (GAC↓GTC)	
	Alul (AG \downarrow CT), BfrBI (ATG \downarrow CAT), BsuRI (GG \downarrow CC), CviJI* (RG \downarrow CC), CviJI* (RG \downarrow CT), Eco147I (AGG \downarrow CCT), HpyCH4V (TG \downarrow CA), MISI (TGG \downarrow CCA)	Ssil
	Bsh1236I (CG↓CG), Bsp68I (TCG↓CGA)	Bsh1236l, Ssil
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	EcI136II (GAGJCTC)	Mbil, Ssil
	Eco32I (GAT JATC)	Bsp143I, DpnI, Mbol
	Eco47III (AGC↓GCT)	CviJI, HpalI , Mspl
	Ehel (GGC↓GCC)	BsuRI, CviJI, Hpall, Mspl
	MspA1I* (CMG↓CGG)	BseDI, Bsh1236I, Btgl, Cfr42I, MspA1I, Ssil
	MspA1I* (CMG↓CTG), Pvull (CAG↓CTG)	MspA1I, <mark>Ssil</mark>
	Pdil (GCC↓GGC)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
	Smal (CCC↓GGG), Srfl (GCCC↓GGGC)	Bcnl, Bme1390I, BseDI, BssKI, Hpall, Mspl
<mark>VIbiI</mark> * (GAG↓CGG)	Alul (AG↓CT), CviJI* (RG↓CT), MspA1I* (CMG↓CTG), Pvull (CAG↓CTG)	Alul, CviJI
	Bst1107I (GTAJTAC)	Csp6l, Rsal
	Bsuri (GG \downarrow CC), CviJI* (RG \downarrow CC), Eco147I (AGG \downarrow CCT), Eco47III (AGC \downarrow GCT), Misi (TGG \downarrow CCA)	CviJI
	DpnI (GA↓TC)	Hinfl, Plel, Schl
	Ecl136II (GAG↓CTC)	Alul, Alw211, CviJl, Ecl13611, Eco241, Sacl, Sdul
	Eco32I (GAT JATC)	Bsp143I, DpnI, Mbol
	Ehel (GGC↓GCC)	BsuRI, CviJI
VIISI (TGG↓CCA)	Alul (AG↓CT), CviJI* (RG↓CT), Ecl136II (GAG↓CTC), Eco47III (AGC↓GCT), MbiI* (CCG↓CTC),	CviJI
	MspA1I* (CMG↓CTG), Pvull (CAG↓CTG)	
		Csp6l, Rsal
	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT)	BsuRI, CviJI
	Eco32I (GAT↓ATC) Ehel (GGC↓GCC)	Bsp143I, BspPI, DpnI, Mbol BsuRI, Cfr13I, CviJI
	FspAI* (RTGC↓GCAC) HinclI* (GTY↓GAC)	BseSI, Sdul Fagl
VISPA1I* (CAG↓CKG)	Alul (AG↓CT), CviJI* (RG↓CT), Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC)	Alul, CviJI
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	BSUTION (GTAVIAC) BSURI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MISI (TGG↓CCA)	CviJI
	Eco32I (GATUATC)	Bsp143I, DpnI, Mbol
	Ehel (GGC↓GCC)	BsuRI, CviJI
	Mbil* (GAG↓CGG)	MspA1I
	Pvull (CAG↓CTG)	Alul, CviJI, MspA1I, Pvull
MspA1I* (CCG↓CKG)	Ajil* (CAC \downarrow GTC), Ajil* (GAC \downarrow GTG), Eco105I (TAC \downarrow GTA), Eco72I (CAC \downarrow GTG),	Hpall, Mspl
	FspAI* (RTGC \downarrow GCAC), FspAI* (RTGC \downarrow GCAT), HincII* (GTY \downarrow GAC), NsbI (TGC \downarrow GCA), Ppu21I* (YAC \downarrow GTA), Ppu2II* (YAC \downarrow GTA), PpuII* (YAC \downarrow GTA), PpuI	F contraction
	Alul (AG \downarrow CT), BfrBI (ATG \downarrow CAT), BsuRI (GG \downarrow CC), CviJI* (RG \downarrow CC), CviJI* (RG \downarrow CT),	Ssil

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Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme	enzyme	newly generated recognition sequence
	Bsh1236I (CG↓CG), Bsp68I (TCG↓CGA)	Bsh1236l, Ssil
		Csp6l, Rsal
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mbil, Ssil
	Eco32I (GATJATC)	Bsp143I, DpnI, Mbol
	Eco47III (AGC↓GCT)	CviJI, Hpall, Mspl
	Ehel (GGC↓GCC)	BsuRI, CviJI, Hpall, Mspl
	Mbil* (GAG↓CGG)	BseDI, Bsh1236I, Btgl, Cfr42I, MspA1I,
	Pdil (GCC↓GGC)	Ssil Bcnl, Bme1390I, BssKl, Hpall, Mspl
	Pull (CCC↓GGC) Pvull (CAG↓CTG)	MspA1I, Ssil
	Smal (CCC↓GGG), Srfl (GCCC↓GGGC)	Bcnl, Bme1390l, BseDl, BssKl, Hpall,
		Mspl
<mark>VISSI</mark> (GTTT↓AAAC)	Bsp68I (TCG↓CGA)	Taql
	Dral (TTT↓AAA), Smil (ATTT↓AAAT)	Dral, Tru1l
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	НруСН4V
	HincII* (GTY↓AAC), KspAI (GTT↓AAC), Psil (TTA↓TAA)	Tru1l
	Rsal (GT \downarrow AC), Scal (AGT \downarrow ACT)	Hpy8I
<mark>VsbI</mark> (TGC↓GCA)	Dral (TTT \downarrow AAA), Hincll* (GTY \downarrow AAC), KspAI (GTT \downarrow AAC), MssI (GTT \downarrow AAAC), RsaI (GT \downarrow AC),	HpyCH4V
	Scal (AGT \downarrow ACT), Smil (ATTT \downarrow AAAT), Sspl (AAT \downarrow ATT)	10,0111
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	MnII
	Eco32I (GAT↓ATC)	HpyCH4V, Lwel
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC)	Hhal, Hin6l
	FspAI * (RTGC \downarrow GCAC), FspAI * (RTGC \downarrow GCAT)	Hhal, Hin6l, Nsbl
	Mbil* (GAG \downarrow CGG), MspA1I* (CMG \downarrow CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	Satl, Taul
	Srfl (GCCC LGGGC)	Cac8l
Pdil (GCC↓GGC)	DpnI (GA↓TC), EcI136II (GAG↓CTC), MbiI* (CCG↓CTC)	MnII
	Eco32I (GAT↓ATC)	Bccl
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC), FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT),	Satl, Ssil, Taul
	Nsbl (TGC↓GCA)	
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG), Smal (CCC↓GGG), Srfl (GCCC↓GGGC)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
Ppu21I* (CAC↓GTR)	Ajil* (CAC↓GTC), Zral (GAC↓GTC)	Ajil, Maell, Tail
	Ajil* (GAC↓GTG), Eco72I (CAC↓GTG)	Eco72I, Maell, Ppu21I, Tail
	EcI136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mnll
	Eco105I (TACJGTA)	Maell, Ppu21I, Tail
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	BceAl
Ppu21I* (TAC↓GTR)	AjiI* (CAC↓GTC), Zral (GAC↓GTC)	Maell, Tail
	AjiI* (GAC↓GTG), <mark>Eco72I</mark> (CAC↓GTG)	Maell, Ppu21I, Tail
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	MnII
	Eco105I (TAC↓GTA)	Eco105I, Maell, Ppu21I, Tail
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Hpall, Mspl
	Pdil (GCC↓GGC)	BceAl
Psil (TTA↓TAA)	Dral (TTT↓AAA), Eco32I (GAT↓ATC), HincII* (GTY↓AAC), KspAI (GTT↓AAC), MssI (GTTT↓AAAC),	Tru1I
	Rsal (GT↓AC), Scal (AGT↓ACT), Smil (ATTT↓AAAT)	
	Eco47III (AGC↓GCT)	Alul, CviJI
	Ehel (GGC↓GCC)	CviJI
	Sspl (AAT↓ATT)	Tasl, Tru1l
Pvull (CAG↓CTG)	Alul (AG↓CT), CviJI* (RG↓CT), Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC)	Alul, CviJI
	Bst1107I (GTA↓TAC)	Csp6l, Rsal
	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MISI (TGG↓CCA)	CviJI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, Mbol
	Ehel (GGC↓GCC)	BsuRI, CviJI
	Mbil* (GAGJCGG), MspA1I* (CMGJCGG)	MspA1I
	MspA1I* (CMG CTG)	Alul, CviJl, MspA1l, Pvull
<mark>Rsal</mark> (GT↓AC)	Bsp68I (TCG↓CGA)	Taql
	Bst1107I (GTA JTAC), HincII* (GTY JAAC), KspAI (GTT JAAC)	Maelli
	Ec136II (GAG↓CTC), Mbil* (CCG↓CTC)	Alw261
	FspAI* (RTGC↓GCAC)	Alw21I, Alw44I, BseSI, Hpy8I, HpyCH4
		Sdul
	FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	HpyCH4V
	Hincll* (GTV JGAC)	Maelli, NmuCl
	MssI (GTTT↓AAAC)	Hpy8I
		Tru1l
	Scal (AGT↓ACT)	Csp6l, Rsal

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Information on New Cleavage Sites

Table 1.25. Newly Generated Recognition Sequences Resulting from the Ligation of Blunt DNA Ends.

First restriction enzyme	Second restriction enzyme	Restriction enzymes that cleave the newly generated recognition sequence
Scal (AGT↓ACT)	Bsp68I (TCG↓CGA)	Tagl
	Bst1107I (GTA↓TAC), HincII* (GTY↓AAC), KspAI (GTT↓AAC)	MaeIII
	Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	Alw26I
	FspAI* (RTGC↓GCAC)	Alw21I, Alw44I, BseSI, Hpy8I, HpyCH4V, Sdul
	FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	HpyCH4V
	Hincll* (GTY JGAC)	Maelli, NmuCl
	MssI (GTTT JAAAC)	Hpy8I
	Psil (TTAJ TAA)	Tru1I
	Rsal (GT↓AC)	Csp6l, Rsal
Smal (CCC↓GGG)	Dpnl (GA↓TC), Ecl136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mnll
	Eco32I (GAT JATC)	Bccl
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC), FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), Nsbl (TGC↓GCA)	Smul, Ssil
	Mbil* (GAG↓CGG), MspA1I* (CMG↓CGG)	Bcnl, Bme1390I, BseDI, BssKI, Hpall, Mspl
	Pdil (GCC↓GGC)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
	Srfl (GCCC↓GGGC)	Bcnl, Bme1390l, BseDl, BssKl, Cfr9l, Eco88l, Hpall, Mspl, Smal
Smil (ATTT↓AAAT)	Bsp68I (TCG↓CGA)	Tagl
	Dral (TTT↓AAA), MssI (GTTT↓AAAC)	Dral, Tru1l
	FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓AAC), KspAI (GTT↓AAC), Psil (TTA↓TAA)	Tru1I
SrfI (GCCC↓GGGC)	DpnI (GA↓TC), EcI136II (GAG↓CTC), Mbil* (CCG↓CTC)	Mnll
· · ·	Eco32I (GAT JATC)	Bccl
	Eco47III (AGC↓GCT), Ehel (GGC↓GCC), FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), Nsbl (TGC↓GCA)	Cac8l, <mark>Smul, Ssil</mark>
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	Bcni, Bme1390i, BseDi, BssKi, Hpali, Mspi
	Pdil (GCC↓GGC)	Bcnl, Bme1390I, BssKl, Hpall, Mspl
	Smal (CCC↓GGG)	Bcnl, Bme1390I, BseDI, BssKI, Cfr9I,
		Eco88I, Hpall, Mspl, Smal
SspI (AAT↓ATT)	Bsp68I (TCG↓CGA)	Taql
	Bst1107I (GTA↓TAC), DpnI (GA↓TC)	Tasl
	FspAI* (RTGC↓GCAC), NsbI (TGC↓GCA)	HpyCH4V
	FspAI* (RTGC↓GCAT)	BfrBI, HpyCH4V, Mph1103I
	Psil (TTAJTAA)	Tasl, Tru1l
Zral (GAC↓GTC)	Ajil* (CAC↓GTC)	Aatll, Hin1I, Maell, Tail, Zral
	Ajil* (GAC↓GTG), Eco105I (TAC↓GTA), Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTA), Ppu21I* (YAC↓GTG)	Maell, Tail
	DpnI (GA↓TC)	Hinfl
	EcI136II (GAG↓CTC), Mbil* (CCG↓CTC)	MnII
	Eco47III (AGC↓GCT), FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	Csel
		0301
	Ehel (GGC↓GCC)	Csel, Hin1I

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REsearch[™] is a unique online tool designed to assist in the selection of a Fermentas restriction endonuclease(s) for your experiments using either the enzyme name or the recognition sequence. This tool also helps you to identify commercially available isoschizomers and choose the optimal buffer for double digestion. It also contains important information regarding restriction enzyme stability during prolonged incubations, conditions for their thermal inactivation, and guidelines on how to generate DNA ends, including cleavage close to the termini of PCR products. Information about new cleavage sites generated by ligation of blunt or compatible sticky ends is also presented together with data about the sensitivity of the restriction enzymes to DNA methylation.

The REsearch[™] tool is regularly updated to include all neccessary information regarding the newly discovered restriction enzymes.

Use REsearch[™] at www.fermentas.com/research, DoubleDigest[™] at <u>www.fermentas.com/doubledigest</u> to plan your experiments.

Newly Generated Recognition Sequences Resulting from the Ligation of Protruding Compatible DNA Ends

Note

- · Restriction enzymes that have degenerate recognition sequences (i.e., recognize more than one sequence) are indicated by an asterisk (*). Be aware that these restriction endonucleases will cleave sequences in addition to the one listed.
- · Enzymes produced by Fermentas are shown in orange.

Single letter code

R = G or A;	H = A, C or T;
Y = C or T;	V = A, C or G;
W = A or T;	B = C, G or T;
M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or G
S = C or G;	

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme	enzyme	newly generated recognition sequence
AatII (GACGT↓C)	Tail (ACGT↓)	Maell, <mark>Tail</mark>
Acc65I (G↓GTACC)	BshNI* (G↓GTACC)	Acc65I, BshNI, BspLI, Csp6I, KpnI,
		Rsal
	Bsp1407I (T↓GTACA), PfI23II (C↓GTACG), TatI* (W↓GTACA), TatI* (W↓GTACT)	Csp6l, Rsal
AfIIII* (A↓CATGT)	BtgI* (C↓CATGG), Eco130I * (C↓CATGG), Fatl (↓CATG), <mark>Ncol</mark> (C↓CATGG), <mark>PagI</mark> (T↓CATGA)	CviAll, Fatl, Hin1ll
	PscI (A↓CATGT)	AfIIII, CviAII, FatI, Hin1II, PscI, XceI
AfIIII* (A↓CGCGT)	BtgI* (C↓CGCGG)	Bsh1236l
	Miul (A↓CGCGT)	Afilli, Bsh1236I, Mlul
	Paul (G↓CGCGC), Sgsl (GG↓CGCGCC)	Bsh1236I, Hhal, Hin6I
AfIIII* (A↓CGTGT)	Btgl* (C↓CGTGG)	Maell, Tail
Alw21I* (GAGCA↓C)	Sdul* (GAGCA↓C)	Alw21I, Sdul
Alw21I* (GAGCT↓C)	Eco24I * (GAGCT \downarrow C), SacI (GAGCT \downarrow C), SduI * (GAGCT \downarrow C)	Alul, Alw21I, CviJI, Ecl136II, Eco24I,
		Sacl, Sdul
Alw21I* (GTGCA↓C)	BseSI* (GTGCA↓C), SduI* (GTGCA↓C)	Alw21I, Alw44I, BseSI, Hpy8I, HpyCH4V
· · · ·		Sdul
	Mph1103I (ATGCA↓T)	HpyCH4V
	Pstl (CTGCA↓G), Sdal (CCTGCA↓GG)	Bsgl, HpyCH4V
Alw21I* (GTGCT↓C)	Sdul* (GTGCT↓C)	Alw21I, Sdul
Alw44I (G↓TGCAC)	Bfml* (C↓TGCAG)	Bsgl, HpyCH4V
Apal (GGGCC↓C)	BseSI [*] (GGGCC↓C), Eco24I [*] (GGGCC↓C), SduI [*] (GGGCC↓C)	Apal, BseSI, Bsp120I, BspLI, BsuRI,
• • • •		Cfr13I, CviJI, Eco24I, Sdul
BamHI (G↓GATCC)	BcII (T↓GATCA), Bsp143I (↓GATC), MboI (↓GATC)	Bsp143I, BspPI, DpnI, Mbol
. ,	BgIII (AJGATCT), Psul* (RJGATCT)	Bsp143I, BspPI, DpnI, Mbol, Psul
	Psul* (R↓GATCC)	BamHI, Bsp143I, BspLI, BspPI, DpnI,
		Mbol, Psul
Bbel (GGCGC↓C)	Bsp143II* (RGCGC↓C)	Bbel, BshNI, Bsp143II, BspLI, Ehel,
		Hhal, Hin1I, Hin6I, Kasl, Narl
	Bsp143II* (RGCGC↓T)	Bsp143II, Hhal, Hin6I
BcII (T↓GATCA)	BamHI (GJGATCC), BgIII (AJGATCT), Bsp143I (JGATC), MboI (JGATC), Psul* (RJGATCC),	Bsp143I, DpnI, Mbol
(, , , , , , , , , , , , , , , , , , ,	Psul* (R↓GATCT)	
Bcul (A↓CTAGT)	Eco130I* (C↓CTAGG), Nhel (G↓CTAGC), Xbal (T↓CTAGA), XmaJI (C↓CTAGG)	FspBl
Bfml* (C↓TGCAG)	Alw44I (GJTGCAC)	HpyCH4V
BgIII (A↓GATCT)	BamHI (GJGATCC), Psul* (RJGATCC)	Bsp143I, DpnI, Mbol, Psul
	BcII (TJGATCA), Bsp143I (JGATC), Mbol (JGATC)	Bsp143I, DpnI, Mbol
	Psul* (R↓GATCT)	Bglll, Bsp143I, DpnI, Mbol, Psul
BsaWI* (A↓CCGGW)	BshTI (A↓CCGGT), Cfr10I* (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, BshTI, Cfr10I, Hpall, Mspl
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SqrAI* (CR↓CCGGCG)	Cfr10l, Hpall, Mspl
	Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
		(continued on next page

Information on New Cleavage Sites

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Table 1.26. Newl	y Generated Recognition	Sequences Resulting	from the Ligation of Protru	uding Compatible DNA Ends.

Spr:// Spr://<	First restriction	Second restriction	Restriction enzymes that cleave the newly generated recognition sequence
Safe (FLCCGW) Bant (ALCCGGT), Chrild'r (ALCCCG), Safe' (CALCCGCC) Bank (Maple) Chrift (CACCGG), Ecc881 (CLCCGG), Safe' (CALCCGCC) Bank (Maple) Sest ¹ (GGCCL) Safe (GGCCL) Bask (Maple) Sest ¹ (GGCCL) Safe (GGCCL) Bask (Maple) Sest ¹ (GGCCL) Apal (GGCCL), Ecc241 (GGCCL), Sdut' (GGCCL) Apal (GGCCL) Sest ¹ (GGCCL) Apal (GGCCL), Ecc241 (GGCCL), Sdut' (GGCCL) Apal (GGCCL) Sest ¹ (GGCCL) Apal (GGCCL), Ecc241 (GGCCL), Sdut' (GGCCL) Apacle (GGCL), Sdut' (GGCL) Sest ¹ (GGCCL) Apacle (GGCCL), Sdut' (GGCCL) Apacle (GGCL), Sdut' (GGCL) Sest ¹ (GGCCL) Apacle (GGCCL), Sdut' (GGCCL) Bask (GLL), Sdut (GGCL), Sdut' (GGCCL) Sest ¹ (GGCCCL) Apacle (GGLC), Sdut' (GGCCL) Bask (GLL), Sdut (GGCCL) Sest ¹ (GGCCCL) Sest ¹ (GGCCCL), Sdut' (GGCCL) Bask (GLL), Sdut (GGCCL), Sdut' (GGCCL), Sdut' (GGCCL), Sdut' (GGCCL), Sdut' (GGCCL), Sdut' (GGCCL), Sdut', Sdut Sest ¹ (GGCCCC) Sest ¹ (GGCCCC), Sdut' (GGCCL), Sdut' (GGCCL), Sdut', NUCLAN, Hall, Mapl, Ma	enzyme	enzyme	
Christ Pill Cloccol, NepMit (CLOCCO, SyAF (CRECOCCO) Hpail, Mayle Christ Pill Cloccol, NepMit (CLOCCO, SyAF (CRECOCCO) Bene 1390, Bessi, Hpail, Mayle Sest Picococci, Sali (Soccol, Cloccol) Bessi, Sali (Soccol, Cloccol) Bessi, Sali (Soccol, Cloccol) Sest Picococci, Sali (Soccol, Cloccol) Apal (Soccol, Cloccol) Apal (Soccol, Cloccol) Apal (Soccol, Social) Sest Picococci, Sali (Soccol, Cloccol, Sali (Soccol, Cloccocc) Alv21, Mavdi, Bessi, Hypel, Hypel, Mayle Mph1103 (AlcCol, Sali (Cloccol, Sali (Socol, Cloccocc) Bessi, Sali (Soccol, Sali (Cloccol, Sali (Cloccol, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Sali (Cloccol, Sali (Cloccol, Sali (Socol, Cloccocc) Shift (Cloccocc) Bessi, Sali (Soccol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Shift (Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Shift (Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Shift (Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Shift (Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessi, Sali (Socol, Cloccocc) Bessil (Socol, Cloccocc) Shift (Cl			
Christ (C-CCCC), EcoBar (C-CCCC) Ben, Ben, Ben, Ben, Ben, Ben, Ben, Ben,	SSAMI" (I↓CCGGW)		
Kmp2 (L100CCA) Beaver, Hapit (L100CCA) Beaver, Hapit (H100CCA) Beaver, Hapit (H100CCA) Beaver, Hapit (H100CCA) Beaver, Hapit (H100CCA) Chapit (Beaver, Hapit			
Self (GGCALC) Stul (GGCALC) BesCl Stul (GGCALC) Self (GGCALC) Apal (GGCCLC), Eco24' (GGCCLC), Stul' (GGCCLC) Apal (GGCCLC), Eco24' (GGCCLC) Self (GGCCLC) Apal (GGCCLC), Eco24' (GGCCLC), Stul' (GGCCLC) Apal (GGCCLC), Eco24', Stul (GGCCLC) Self (GGCCL) Apal (GGCCLC), Eco24' (GGCCLC) Apal (GGCCLC), Eco24', Stul (GGCCLC) Self (GGCCL) Besl, Stul (GGCCLC), Eco24' (GGCCLC) Besl, Stul (GGCCLC), Eco24', Stul (GGCCCLC), Eco24', Stul (GGCCLC), Eco24', Stul (GGCCC), Eco24', Stul (GGCC), Eco24', Stul (GGCCC), Eco24', Stul (GGCCC), Eco24', Stul (GGCCC), Eco24', Eco26', Stul (GGCC), Eco24', Eco26', Stul (GGCCC), Eco24', Eco26', Stul (GGCCC), Eco24', Eco26', Stul (GGCCC), Eco24', Eco26', Stul (GGCCC), Eco24', Eco26', Stul (GGCC), Eco24', Eco26', Stul (GGCC), Eco24', Eco26', Stul (GGCC), Eco26', Eco24', Eco26', Stul (GGCC), Eco26', Eco			
ses51* [GGGCC1C] Apal (GGGCC1C), Eco24* (GGGCC1C), Sdul* (GGGCC1C) Apal, Bess1, Bep201, Bep21, Bep14, Bep31, Mp21,			
cbr131_C0U_0_AVx11* (GIGCALC), Sdu* (GIGCALC) Chr131_C0U_0_AVx11* (GIGCALC), Sdu* (GIGCALC) Mph11031_(GIGCALC), Sdu* (GIGCALC) BypCHV Self* (GIGCCL) BypCHV Self* (GIGCALC), Sdu* (GIGCATICC) BypCHV Self* (GIGCALC) BypCHV Shift* (GLGCAC) Hall, Hin1, Hin6, Ka, Hall Shift (GLGCAC) Hall, Hin1, Hin6, Ka, Hall Shift (GLGCAC) Hall			
SesS* (GTGCALC) Alw21* (GTGCALC) Alw21* (GTGCALC) Alw21* (GTGCALC) Mph1103 (ATGCALT) HypOH HypOH PS11 (GTGCALC) Sale (GTGCALC) Bs31, HypOH SeST* (GTGCCC) Sale (GTGCCLC) Bs35, Sale SeST* (GTGCCC) Sale (GTGCCC) Bs31, ByDL, End NP11 (GLGCCC) Sale (GTGCCC) Bs31, ByDL, End SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, End SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, End SeST* (GTGCCC) Sale (GLGCCCC) Bs31, ByDL, End SeST* (GTGCCC) Sale (GLGCCCC) Bs31, ByDL, End SeST* (GTGCCC) Sale (GLGCCCC) Bs31, ByDL, ByDL SeST* (GTGCCC) Sale (GLGCCCC) Bs31, ByDL, ByDL SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, ByDL, ByDL SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, ByDL, ByDL SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, ByDL SeST* (GTGCCC) Bs31, ByDL, ByDL, ByDL Bs31, ByDL, ByDL SeST* (GTGCCC) Sale (GLGCCC) Bs31, ByDL, ByDL SeST* (GTGCC, ByDNY,	55631 (0000040)		
Mph1103 (ATCOALT) HypCHV PstI (CTCOALG, Sal (CTCOALGC) BigH, HypCHV seri (TSCCALC) Bask, Salu PstI (CTCOALG, Sal (CTCOALGC) Bask, Salu sh128F (CATLOQ Pad (TRATLTA) Tru1 Pvul (CATLOQ, Pad, TTALTA) Tru1 Pvul (CATLOQ, Pad, TTALTA) Tru1 Sh1725F, Bask, Salu Bash, Zash, Salu Sh1725F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp13, Dpnt, Mbol, Pvul Bash, Zash, Salu Sh17125F, Bash, Bsp14, TCDH, Pad, Msp1 Bash, Zash, Salu Sh17125F, Bash, Bsp14, TDDH, Pad, Msp1 Bash, Zash, Salu Sh17125F, Bash, Bsp14, TDH, Pad, Msp1 Bash, Zash, Salu Sh17125F, Bash, Bash, Tal, TCOA, Pad, Mash, Bash, Zash, Salu, Talu Dash, Zash, Za			
Mph1102 (ATCCALT) PETI (CTCALG) Safe (CCTCALGC) HsyCHW sest*i (CTCCLC) Safe (CTCCALG) HsyCHW sest*i (CTCCLC) Safe (CTCCALG) HsyCHW sest*i (CTCCLC) Safe (CTCCALG) HsyCHW sh128*i (CALCCALG) HsyLESS (CALLGALG) HsyLESS (CALLGALGALGALGALGALGALGALGALGALGALGALGALG			
Pstl (CTCAL)G, Sdal (CTCCALGG) Bigl, Hypol/HW Sel1 (GTCCC)G) BaseSI, Sdul Tru1 Pvul (CALCC) Pad (TMAL LA) Tru1 Pvul (CALCC) Bash (ZSR, Sdul) Tru1 Pvul (CALCC) Bash (ZSR, Sdul) Bash (ZSR, Sdul) shNW* (GLGCCC) Kasi (GLGCCC) Bash (ZSR, Sdul) Bash (ZSR, Sdul) shNW* (GLGCCC) Acc651 (GLGTACC) Acc651 (SLGTACC) Acc651 (SLGTACC) shNW* (GLGCCCG) Bash (ZSR, Sdul) Bash (ZSR, Sdul) Bash (ZSR, Sdul) shNW* (GLGCCCG) Acc651 (SLGTACC) Bash (ZSR, Sdul) Bash (ZSR, Sdul) shNW* (GLGCCCG) Bash (ZSR, ZSR, ZSR, ZSR, ZSR, ZSR, ZSR, ZSR,			
sel" (GTGCCL) Sdu" (GTGCCL) sel" (GTGCCL) Sdu" (GTGCCL) Put (CCAT-LCG), Sdu (CCAT-LCQ) shart (GLGCGC), Asia (GLGCGCT)-CCC) shart (GLGCGCC), Asia (GLGCGCC)-CCC) shart (GLGCGCC), Asia (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC) shart (GLGCGCC), Catol (CLGCGC), Sdu" (CLGCGCG) shart (GLGCGCC), Catol (GLGCGCC), Sdu" (CLGCGCG) shart (GLGCGCC), Catol (GLGCGCC), Sdu" (CLGCGCC) shart (GLGCCC), Catol (GLGCGCC), Catol (GLGCGCC), Catol (GLGCCCC) shart (GLGCCC), Catol (GLGCGCC), Catol (GLGCCCC) shart (GLGCCC), Catol (GLGCCC), Catol (GLGCCC), Catol (GLGCCC), Catol (GLGCCC), Catol (GLGCCC), Catol (GLGCCCC), Catol (GLGCCC), Satol (GLGCCCC), Satol (GLGCCC), Satol (GLGCCC), Satol (GLGCCCC),			
sh1285F (CGATLCG) Pact (TAAT_TAA) Put (CGATLCG) Set(CGATLCGC) Boh1285 (Sep1431, Dept, Mob, Pvut Pvut (CGATLCG), Set(CGATLCGC) Boh1285 (Sep1431, Dept, Mob, Pvut Pvut (CGATLCG, Set(CGATLCGC) Boh1285, Sep1431, Dept, Mob, Pvut Pvut (CGATLCG, Set(CGATLCGC) Boh1285, Sep1431, Dept, Mob, Pvut Pvut (CGATLCG, Set(CGATLCGC) Boh1285, Sep1431, Dept, Mob, Pvut Boh1285, Sep1431, CGATLCGC, Set(CGATLCGC) Boh1285, Sep1431, CGATLCGC, Set(CGATLCGC) Boh1285, Sep1431, CGATLCGC, Sep4, FCatLCGCGCG) Boh1285, Sep1431, CGATLCGCG, LCGCG, LCGCGC, CGATLCGCCGCG, CGATLCGCGCG, CGATLCGCGCG, Boh14, Mopl CGATUR FLCCGCG, LCGCG, LCGCGC, LCGCCG, CGATLCGCGCG, CGATLCGCGCG, CGATLCGCGCG, CGATLCGCGCG, Boh14, Mopl CGATUR FLCCGCG, LCGCG, LCGCCG, LCGCCG, CGATLCGCGCG, CGATLCGCGCG, Boh14, CGATLCGCGCG, Boh14, CGATLCGCGC, CGATLCGCGCG, Boh14, CGATLCGCGC, CGATLCGCGCG, Boh14, CGATLCGCGC, CGATLCGCGCG, Boh14, CGATLCGCGC, CGATLCGCGCG, Boh14, CGATLCGCG, LCGCCG, Boh14, CGATLCGCG, LCGCCG, Boh14, CGATLCGCG, LCGCCG, Boh14, CGATLCGCG, LCGCCG, Boh14, CGATLCG, LCGCCGC, CGATLCGCG, CGATLCGCG, Boh14, CGATLCG, LCGCCGC, Boh14, CGATLCG, LCGCCGC, CGATLCGCG, LCGCCG, CGATLCGCG, Boh14, CGATLCG, LCGCCGC, Boh14, CGATLCG, LCGCCGC, Boh14, CGATLCG, LCGCCGC, Boh14, CGATLCG, LCGCCG, Boh14, CGATLCG, LCGCCG, Boh14, CGATLCG, LCGCCG, Boh14, CGATLCG, LCGCGC, Boh14, CGATLCG, LCGCGC, Boh14, CGATLCG, LCGCGC, Boh14, CGATLCG, LCGCGC, Boh14, CGATLCG, LCGCG, SANT, CGATLCGC, LCGCG, Boh14, CGATLCG, LCGCG, SANT, CGATLCGC, SANT, CGATLCGCG, Boh14, LCGCGG, LCGCG, Boh14, CGATLCG, LCGCGG, Boh14, LCGCGG, LCGCGG, Boh14, LCGCGG, LCGCG, Boh14, LCGCGG, LCGCG, Boh14, LCGCGG, LCGCG, Boh14, LCGCGG, LCGCG, Boh14, LCGCGG, LCGCGG, Boh14, LCGCGG, SANT, CRLCGCGG, Boh14, LCGCGG, SANT, CRLCGCGGG, Boh14, LCGCGG, Boh14, LCGCGG, SANT, CRLCGCGGG, Boh14, LCGCGG, Boh			5.15
Pvul (CCAT-LCG), Sgf) (CCAT-LCCC) Beh, BShN, BSp1431, Bsp14, Ehel, Hah, Hin1, Hind, Kasi, Narl ShW* (GLGCCC) Kasi (GLGCGCC) Hah, Hin1, Hind, Kasi, Narl ShW* (GLGCCC) Acc65i (GLGTACC) Acc65i, RShN, ShN, Hind, Kasi, Narl ShW* (GLGCCC) Bsp14071 (TLGTACA), PH23II (CLGTACG), Tait* (WLGTACT) Csp64, Rsal SshW* (WLCCGGR), Kpr21 (TLCCGGA) BswW* (WLCCGGR), Kpr21 (TLCCGGA) BswW, BShT, CHTOH, Hapl, Msp1 SshW* (WLCCGGR), MgWC (GLGCCGC), SgA* (CRLCCGCGC) Cr101 (Hapl, Msp1 Ksp11 ChTOP (RLCCGGA), Tag1 (TLCGA), Xmii* (GLCCGC) BswW, ML, Msp1 Ksp11 Sp1201 (GLGCCC) Cr14 (Hapl, Msp1 Ksp11 Sp1201 (GLGCCC) Bsp1481 Ksp11 Ksp11 Sp1201 (GLGCCC) Bsp1481 Sp1431 Sp1431 Sp1431 Sp141 (GLCCC) Bsp1481 Sp1431 Sp1431 Sp1431 Sp1431 Sp1431 Sp1431 <			
ShNT (GLECCC) Kasi (GLECCC) Bbbl. (Esp143), (Esp1			
Heal, Hintl, Hind, Kasl, Rait Heal, Hintl, Hind, Kasl, Rait ShN* (CETACC) Acc651 (CETACC) Acc651 (CETACC) Acc651 (CETACC) Bsp14071 (TETACA), P123II (CETACG), Tatt* (WEGTACA), Tatt* (WEGTACT) Csp64, Rsal Csp64, Rsal IshTI (AECCGG) BsdW1 (WECCGGA), Kpn21 (TECCGGA) BsdW1 (Hpa11, Msp1 Csp64, Rsal BsdW1 (WECCGGA), Contor* (RECCGGA), SgA** (CRECCGGC) Cr1010 (RECCGGA), Tag1 (TECCGGA) BsdW1, Hpa11, Msp1 Sp1101 (TECCAA), Tag1 (TECCGGA), Xmit* (GETCGCAC) Tag1 BsdW1, (F131, CVU), Tag1, SsX, Hpa11, Msp1 Sp1201 (EEGCCCA), Cr17 (VEGCCGA), Tag1 (TECCACC) BsdW1, (F131, CVU), Tag1, SsX, Tag1 Sp14071 (TEGTACA) Sp14071 (TEGTACA) Acc651 (EEGTACC), Seb1* (GEGTACC), P1231 (CEGTACC) BsdW1, (F131, CVU), SsX, Hpa11, Msp1 Sp14071 (TEGTACA) Acc651 (EEGTACC), Sp143, Tag1 Csp64, Rsal Tag1 Sp14071 (TEGTACA) Acc651 (EEGTACC), P1231 (CEGTACC) Bsp1431, P131, P141, P141, P141 Sp14071 (TEGTACA) BsdW1 (RECCAC) Bsp1431, P141, P141, P141 Sp1431 (FEGCAC) Bsp1431, P141, P141, P141 P141,	SchNI* (G.LGCGCC)		
ShN* (G.JGTACC) Acc651 (G.JGTACC) Rec61 Bsp14071 (T.JGTACA) PR2311 (C.JGTACA), Tatl* (W.JGTACA), Tatl* (W.JGTACA) Bsa4W Acc651, Bsh1W, Bsh1, Csp61, Ksp1 ShTI (ALCCGGT) Bsa4W Mod 1. Msp1 Csp60, Rsa1 ShTI (ALCCGGT) Bsa4W Mod 1. Msp1 Bsa4W, Mpa1, Msp1 Cr100 (R.LCCGGC), SgrA1 (R.LCCGGC), SgrA1 (R.LCCGCG) Bsa4W, Mpa1, Msp1 Grant 1. Msp1 Cr101 (R.LCCGGC), C.GCGB (C.CCGG) Bsa4W, Mpa1, Msp1 Tatl Msp120 (I.GCCGC, C.GCG), C.CCGG (C.GCGCG) Bsa4W, Mpa1, Msp1 Sp1201 (G.LGCCC, C.G., Cr11 (Y.LCCGGC), Eco52 (I.GLGSCCC) Csp64, Rsa1 Tatl Sp1201 (G.LGCCC, C.G., Cr11 (Y.LCCGCG), Eco52 (I.GLGSCCC) Bss147, Cr13, C.V.II Ss147, Cr13, C.V.II Sp14071 (T.LGTAA) Acc651 (I.GLGTAC), Bs141 (G.LGTAC), PD231 (I.GLGTAC) Bs91431, Cla34, Cla34, Ss1, Tau1 Sp14071 (T.LGTAC) Bs91431 (I.GATCC) Bs91431, Mb1, Mb1 Ss1431, Dp1, Mb2 Sp14071 (T.LGTAC) Bs141 (I.GLGATCC), Bs14 (I.GLGTAC), Bs141 (I.GLGTAC), Bs1431, Dp1, Mb2 Bs1433, Dp1, Mb2 Sp14071 (T.LGTAC) Bs141 (I.GLGATCC) Bs1431, Mb1, Mb2 Bs1433, Dp1, Mb2 Sp14131 (GLGATC) Bs1433, Dp1, Mb141 (GLGATC) Bs1433, Dp1, Mb2			
Bsp14071 (T_LGTACA), PfI23II (CLGTACG), Talt* (WLGTACA), Talt* (WLGTACT) Csp6/Rail IshTI (ALCCGGT) BsdWT (WLCCGGA), Kpn21 (T_LCCGGA) BsdWT (WLGCGGA), Kpn21 (T_LCCGGA), SgrA* (CR_LCCGCCG) BsdWT, Hpall, Msp1 IshTI (ALCCGGT) BsdWT (WLCCGGA), Kpn21 (T_LCCGGA), SgrA* (CR_LCCGCCG) Cfr01 (Hpall, Msp1 Serving (T_LCGGA), Tag1 (T_LCCGGA), SgrA* (CR_LCCGCCG) Cfr01 (Hpall, Msp1 Serving (T_LGTACA), Tag1 (T_LCCGCA), Xmit* (GT_LCGAC) Tag1 Serving (T_LGTACA), Tag1 (T_LCGCA), Xmit* (GT_LCGAC) BsurR, (Tr13, Cvull, Satl, SstI, Tau1 Sp14071 (T_LGTACA), Tag1 (T_LCGAC), Xmit* (GT_LCGAC) Csp6/, Rsa1 Sp14071 (T_LGTACA), Tag1 (T_LCGAC), Sp1401 (LGTACC), P12311 (CLGTACC) Csp6/, Rsa1 Sp14071 (T_LGTACA), Tag1 (T_LCGAC), Sp1401 (LGTACC), P12311 (CLGTACC) Csp6/, Rsa1 Sp14071 (T_LGTACA), Tag1 (T_LCGAC), Sp1401 (LGATCT), Mbo1 (LGATC), P301* (R_LGATCC), P301* (R_LGATCC)) Sp1401* (Sp1401, Sp1401* (Sp140* (S	SchNI* (G. GTACC)		
Bsp1407(TLGFACA).PH23II (CLGTACG), Tath" (WLGTACA), Tath" (WLGTACT) Csp6I, Rsal shTI (ALCCGCT) BsaWI (WLCCGGT), Chr10* (RLCCGGT), SgAI" (CRLCCGGTG) BsaWI, Bsp1I, Sp1I, SgAI" (WLGTACG), SgAI" (CRLCCGGTG) BsaWI, Bsp1I, Sp1I, SgAI" (SLCGGC), SgAI" (CRLCCGGCG) Chr10, HpaI, Msp1 sp1191 (TLGCAA) BsuISI (ALCGGT), SgAI" (CRLCCGGC) BsuIR, Chr13, CWJI Tatl sp1191 (TLGCAA) BsuISI (ALCGGT), Chr14" (SLCGGC), SgAI" (CRLCCGCG) BsuR, Chr13, CWJI SgAIN, SgAI, SgAI, SgAI, SgAI (SLCGCG) BsuR, Chr13, CWJI sp1191 (TLGCAA) BsuISI (ALGCAC), Chr14" (SLGCGCG) CSgAI, RsaI SgAIN, Chr13, CWJI SgAIN,			
shTl (ALCCGGT) BsaWr (VLCCGGA), Kpp21 (°LCCGGA) BsaWr (VLCCGGA), Kpp21 (°LCCCGGA) BsaWr (VLCCGGA), Cort (VLGCCGGA), SgrAr (°CR/CCCGCCG) BsaWr (VLCCGGA), Cort (NLB), Kpp1 Sp119 (CTLCCGGA), Cort (NLGCCGGA), SgrAr (°CR/CCCGCCG) Cfr101, Hpa1, Msp1 Sp120 (CLCCGGA), Toaq (°LCCCGGA), SgrAr (°CR/CCGCGC) Taq1 Sp120 (CLCCGGA), Cort (°LCCGGA), Xm11 (°GLCGAC, C) Taq1 Sp120 (CLCCGA), Crit (°LGCCCA), Crit (°LGCAC, C) BsuN1, (Cr13), CVII, SaI, SsI, Tau1 Sp120 (CLCACA), Crit (°LGCCCA), Crit (°LGCACC) BsuN1, (Cr13), CVII, SaI, SsI, Tau1 Sp1407 (CLCACA), Accocc, Del CLCACC, Del CLCACCC) BsuN1, (Cr13), CVII, SaI, SsI, Tau1 Sp1407 (CLCACA), Caccocc, Del CLCACC, Del CLCACCAC) Bsp1431, Han1 Sp1431 (CLCACC), Bash (°LGATCC), Bg11 (LGATCC), Mbol (LGATC), PsuI* (RLGATCC), Bsp1431, Han1, Hin61 Bsp1131, CRCACCA Sp1431 (CLCACC), Bele (CGCCCCL) Bsp1431, Han1, Hin61 Bsp1131, CRCACA Sp1431 (CLCACC), Bele (CGCCCCL) Bsp1431, Han1, Hin61 Bsp131, CR11A, Hin61 Sp1431 (CCCCCLA) Bsp119, (TTLCGAA), Taq1 (°LCAGA), Taq1 (°LCAGCA) Bsp1131, CR11A, Hin61 Sp1131 (CLCCCCLA) Bsp1131, CR11A, Hin61 Bsp1331, Bsp1341, Hin61 Sp1131 (CLCCCCCLA) <t< td=""><td></td><td>Bsp14071 (TJGTACA) Pf12311 (CJGTACG) Tatl* (WJGTACA) Tatl* (WJGTACT)</td><td></td></t<>		Bsp14071 (TJGTACA) Pf12311 (CJGTACG) Tatl* (WJGTACA) Tatl* (WJGTACT)	
BsaWr (WLCCGC), Cirt10* (R_LCCGC), SgrA* (CR_LCCGCGC) Cirt10* (RLCCGC), Unpaul, Mspl Cirt10* (RLCCGC), Unpaul, Mspl Sexta, Baski, Hpail, Mspl sp119* (ITLGCA) BsuN, Cirt13; CirU, Hpail, Mspl sp119* (ITLGCA) BsuN, Cirt13; CirU, SsiX, Hpail, Mspl sp119* (ITLGCA) BsuN, Cirt13; CirU, SsiX, Hpail, Mspl sp110* (ITLGCA) CirCCGC, CirCC, CirC	SchTI (ALCCGGT)		
Chr101* RelCCCGC) Synth* CRF2CCCGCCC Bant1390 Bant141 Chr101			
Chr91 (CLCCGGG) Eco881* (CLCGGGG) Bent, Bme1390, Basts (H, Hpall, Mspl sp191 (TLCAGA) TLCAGA), Taql (LCCG, Xmit* (CLCGAC) Taql sp110 (GLGGCCC) Chr1* (YLGGCCG), Eco521 (CLGGCCG) BsuRl, Chr13), CAUL, Sall, Sall, Taul sp110 (GLGGCCC) Chr4* (YLGGCCG), Eco521 (CLGGCCG) BsuRl, Chr13), CAUL, Sall, Sall, Taul sp110 (TLCAGA) Acc651 (GLGTACC), Bsh1* (GLGTACC), PH23II (CLGTACC) CSp6), Rsal, Taul sp1407 (TLCAGA) CC560, Rsal, Taul Sp1407, CSp60, Rsal, Taul sp1407 (GLGACC) Bsp1407, CSp60, Rsal, Taul Sp1407, CSp60, Rsal, Taul sp1407 (GLGACC) Bsp141, Hal, Hin61 Bsp141, Hal, Hin61 sp1431* (ACCCCL) Bsp141, GLGACC), Bsp1431* (ACCCCL) Bsp1431, Hal, Hin61 sp1431* (GCGCCL) Bsp141, GLGACC, CS Hal, Hin14, Hin61 sp1431* (GCGCCL) Bsp141, GLGACC, CS Hal, Hin14 sp11 (GLCACG) Bsp141, Fall Hal, Hal, Hin61 sp1431* (GCGCCL) Bsp141, Fall Hal, Hin14, Hin61 sp1431* (GCGCCL) Bsp141, Hal, Hin61 Ss1 sp11 (GLCACG) Ssp141, Hal, Hin61 Ss1 sp11 (GLCACGC) Hal, Hin11 Hal, Hin11			
sp119 (IT_LCGA) Bastls (ATLCGA), Taql (T_LCGA), Xmit* (GTLCGAC) Taql sp120 (GLGGCCC) Cfrt* (V-LGGCCG), Ecrit* (V-LGGCCG), EcoS21 (CLGGCCG) BsuRl, Cfr13], Oull, Satl, Ssil, Taul sp1407 (T_LGTACA) AcceSi (GLGTACC), Bshit* (GLGTACC), Pf123I (CLGTACG) Csp6i, Rsal sp1407 (T_LGTACA) AcceSi (GLGTACC), Bshit* (GLGTACC), Pf123I (CLGTACG) Csp6i, Rsal, Tatl sp1407 (T_GTACA) Bsp1407I, Csp6i, Rsal, Tatl Csp6i, Rsal, Tatl sp1403 (LGATC) Bsp1407I, Csp6i, Rsal, Tatl Csp6i, Rsal, Tatl sp1403 (LGATC) Bsp143I, Hal, Hin6I Bsp143I, Hal, Hin6I sp1431* (ACCCCL) Bsp1431, Hal, Hin6I Bsp1431, Bsp1431			
sp1201 (GLGGCC) Ch1* (YLGGCCG), Eco521 (CLGGCCG) BsuR), Ch131, CvJJ Nott (GCLGGCCC) BsuR), Ch131, CvJJ BsuR), Ch131, CvJJ sp14071 (TLGTACA) Acc651 (GLGTACC), Bsh1* (GLGTACC), PH2311 (CLGTACG) Csp61, Rsa1, Tatl sp14071 (TLGTACA) Acc651 (GLGTACC), Bsh1* (GLGTACC), PH2311 (CLGTACG) Csp61, Rsa1, Tatl sp1431 (LGATC) Bsm141 (GLGATCC), Bcl (TLGATCA), Bg11 (ALGATCT), Mbol (LGATCC), Psu* (RLGATCC), Bsp14311, Hal, Hin61 sp1431* (ACCCCL*) Bse1 (GGCGCL-C) Bsp14311, Hal, Hin61 sp1431* (GGCGCL*) Bse1 (GGCGCL-C) Bsp14311, Hal, Hin61 sp1431* (GGCGCL*) Bse1 (GGCGCL-C) Bsp1431, Hal, Hin61 sp1431* (GGCGCL*) Bse1 (GGCGCL-C) Bsp1431, Hal, Hin61 sp1431* (GGCGCL*) Bse1 (GGCGCL-C) Bsp1431, Hal, Hin61 sp1431* (GGCGCL*) Bsp1431, Hal, Hal, Hal, Hal, Hal, Hal, Hal, Hal	Ssn1191 (TT CGAA)	Bsu151 (AT \downarrow CGAT) Tagl (T \downarrow CGA) Xmil* (GT \downarrow CGAC)	
Noti (GCLGGCCGC) BsuRt, Crt 31, CvIJ, Sati, Sati, Taul isp14071 (LGTACA) Acc651 (GLGTACC), BshN* (GLGTACC), P123II (CLGTACG) Csp6l, Rsal, Taul Taut* (WLGTACT) Csp6l, Rsal, Taul Csp6l, Rsal, Taul isp1431 (LGATC) BamH (GLGACC), Sell (TLGATCA), BgIII (ALGATCT), Mbol (LGATC), PsuI* (RLGATCC), PsuI* (RLGATCT) Bsp1431 (LGATC) Bsp1431 (LGATCA) isp1431 (LGATC) Bsp1431 (GCGCCL*) Bsp1431 (GCGCCL*) Bsp1431 (GCGCCL*) isp1431 (LGCCCCL*) Bsp1431 (GCGCCL*) Bsp1431 (GCGCCL*) Bsp1431 (GCGCCL*) isp1431 (GCCCCL*) Bsp1431 (GCGCCL*) Bsp1431 (GCCCCL*) Bsp1431 (GCCCCL*) isp1431 (GCCCCL*) Bsp113 (TTLCGA), Taul (LCATG), Taul (LCATG), Taul (LCATG) Maell, Tail igi* (CLCATGC) Anili* (ALCATGT), Faul (LCATG), Pagi (TLCATGA), Psci (ALCATGT) CvAll, Fail, Hin11 igi* (CLCCGCG) Anili* (ALCATGT), Raul (LCATG) Bsb12331 (Hal, Hin16, Ssil igi* (CLCCCCG) Anili* (ALCATGT), Raul (LCATG) Bsb12331 (Hal, Hin16, Ssil igi* (CLCCCCG) Anili* (ALCATGT), Raul (LCATG) Bsb12331 (Hal, Hin16, Ssil igi* (CLCCCCG) Anili* (ALCATGT), Raul (LCATG) Bsb12331 (Hal, Hin16, Ssil igi* (CLCCCCG) <td< td=""><td></td><td></td><td></td></td<>			
sp1407I ([LGTACA) Acc6st (c]c1ACA) Bs71407I (C]GTACA) Csp64, Rsal Tatt' (WJGTACA) Csp64, Rsal, Tatt Csp64, Rsal, Tatt sp1431 (LGATC) BamHI (G]CACCO, Bcl ([LGATCA), BgIII (AJGATCT), Mbol (JGATC), Psuf* (RJGATCC), Bsp1431, Mbol sp1431 (LGATC) BsmHI (G]CGCCLO, Bcl ([LGATCA), BgIII (AJGATCT), Mbol (JGATC), Psuf* (RJGATCC), Bsp1431, Mbol sp1431 (GCCCCLY) Bbel (GCCCCLC) Bbel (GCCCCLC) Bbel (GCCCCLC) sp1431 (GCCCCLY) Bbel (GCCCCCC) Bbel (GCCCCCC) Bbel (GCCCCCC) sp11 (C]TLCGAA), Taql (TLCGA), Xmii* (GTLCGAC) Taql Taql sp11 (C]CLACCGC) Affilir (ALCACGT) Maell, Tail sp1 (C]CLACGGC) Affilir (ALCACGT) Maell, Tail sp1 (C]CLACGGC) Affilir (ALCACGT) Maell, Tail sp1 (C]CLACGGC) Affilir (ALCACGT) Maell, Tail sp1 (C]CCCGCG) Sp1 (GLCCGCC) Bsp193 (GLCGCCC) sp1 (GLCGCGC) Affilir (ALCAGGT) Maell, Tail sp1 (C]CCCGCG) Affilir (ALCAGGT) Maell, Tail sp1 (C]CCCGCG) Affilir (LCCGGT), Sp1 (CLCATGG) Bse10, Bj1, Mall, AlLCAGGT) sp1 (C]CCCG			
Tatl* (WJ-GTACA) Bsp14071, Csp61, Rsa1, Tatl sp1431 (JGATC) Barthil (GJCATC), Bell (TJCATCA), Bgill (AJCATCT), Mbol (JCATC), Psul* (RJCATCC), Psul* (RJCATCT) Bsp1431, GGCCCLV) Bsp1431, GGCCCCLV) Bsp1431, Bsp1431, GGCCCCLV) Bsp1431, Bsp1431, Bsp1431, Bsp14, Bsp1431, Bsp14, Bsp1431, Bsp14, Bsp1431, Bsp14, Bsp1431, Sp11431* (GCCCCLV) Bbel (GCCCCLC) Bsp1431* (GCCCCLV) Bbel, BshNI, Bsp1431, Bsp14, Bsp14, Bsp14, Bsp14, Bsp14, Bsp14, Bsp1431* (GCCCCLV) Haal, Hin11, Hin61 sp1131 (TJCCAAC) Bsp1191 (TJCCAA), Taq1 (TJCGA), Xmil* (GTJCGAC) Taq1 Taq1 sup131 (GLCACCG) Amili* (AJCACGT) Maeli, Tail Taq1 sup131 (GLCACCG) Amili* (AJCACGT), Mul (ALCGCGT) Bsp112, Earl, Hin11 sup131 (GLCACCG) Sup141, CLCACGG), Sup1 (GLCCGCG) Bsh12236, Sil Bsh12236, Sil sup14 (GLCCGCG), Sup1 (GLCCGCG) Bsh12236, Haal, Hin61, Ssil Far236, Sil Far236, Sil sup14 (GLCCGCG), Sup1 (GLCCGGC) Bsh12326, Haal, Hin64, Ssil Far236, Sil Far236, Sil sup14 (GLCCGCG), Sup14 (CCCGGT), Sup14* (CRLCCGGGG) Bsh14, Min64, Ssil Far236, Sil Far236, Sil sup14 (GLCCGCG), Sup14* (CRLCCGGG) Bsh14, Map1 Sup14 Sup14, Sup1 Sup14 Sup14 Sup14	BSD1407I (TUGTACA)		
Tatl* (WJ-GTACT) Csp6l, Rsal, Tatl isp1431 (JGATC) BamHI (GJ-GATCC), Bcll (TJ-GATCA), BgllI (AJ-GATCT), Mbol (JGATC), Psu* (RJ-GATCC), Psu* (RJ-GATCC) Bsp1431, Dpnl, Mbol sp1431* (AGCCCL/Y) Bbel (GGCCGLC) Bsp1431, Hnal, Hin61 sp1431* (GGCCCL/Y) Bbel (GGCCGLC) Bsp1431, Sp11, Ehel, Hhal, Hin11, Hin61, Kasl, Natl sp115 Sm0* (CJ-TTAAG) Bsp11; Sm01, Tru11 sp116 CJ-GATGG) Hal, Hin11, Hin61, Kasl, Natl tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Maell, Tail tg1* (CJ-CACGG) Aflil* (AJ-CACGT), Fall (JCATGA), Pscl (AJ-CATGT) CvAll, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT), Mall (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, CvAll, Eco130, Fall, Hin11 tg1* (CJ-CACGG) Aflil* (AJ-CACGT) Bscl (Bg1, Cv			
Sp1431 (JGATC) BamH1 (GJGATCC), BCII (TJGATCA), BgIII (AJGATCT), Mbol (JGATC), Psul* (RJGATCC), Psul* (RJGATCT) Bsp1431, Bpn1, Mbol Sp14311* (AGCCCL') Bbel (GGCCCLC) Bbel, BShNI, BSp143II, BSp11, Ehel, Hhal, Hin11, Hin6i, Kasl, Narl Sp11311* (GGCCCLY) Bbel (GGCCCLC) Bbel, ISSNI, BSp113II, BSp11, Ehel, Hhal, Hin11, Hin6i, Kasl, Narl Sp11 (CJTTAAG) Smol* (CLTTAAG) Bsp1179 (TJCCAA), Taql (TJCCAA), Xmil* (GTJCCAC) Taql Igi* (CLATGG) Afilii* (ALCAGT) Maeli, Tail Nool Igi* (CLATGG) Afilii* (ALCAGT) Kol (LCATGG) Bse112361, Stail Igi* (CLATGG) Afilii* (ALCAGCT), Mul (ALCGCGT) Bse112361, Stail Nool Igi* (CLATGG) Afilii* (ALCGCGT), Mul (ALCGCGT) Bsh12361, Stail Nool Igi* (CLCCGCG) Savit* (MLCCGGA), Savit* (CRLCCGGA) Bsavit* (MLCGGA), Kavit Savit* (MLCGGA), Kavit* (CRLCCGGA) Baswit* (MLCCGGG), Savit* (CRLCCGGCG) Cr101 (Hpail, Msp1 Msp1 Msp1 Mul (GLCCGCC), Savit* (CRLCCGGCG) Cr101 (Hpail, Msp1 Msp1 Mul (GLCCGCC), Savit* (CRLCCGGCG) Cr101 (Hpail, Msp1 Msp1 Mul (GLCCGCC), Savit* (CRLCCGGCG) Cr101 (Hpail, Msp1 Msp1			
Psul* (RLGATCT) Babel (GCCGCLC) Bsp143II; MacGCCLY) Bbel (GCCGCLC) sp113II* (GCCGCLY) Bbel (GCCGCLC) Bbel, BshNI, Bsp143I, BspLI, Ehel, Hhal, Hin1I, Hin6(, Kasi, Narl sp113II* (GCCGCLY) Bbel (GCCGCLC) Bsp11, Smol* (CLTTAAG) Bsp11, Smol* (CLTTAAG) sp115 (AT-LGCA) Bsp119 (TT-LCGAA), Taql (TLCCGA), Xmil* (GT_LCGAC) Taql tg1* (CLCACGG) Atlin* (ALCACGT) Maell, Tail tg1* (CLCACGG) Atlin* (ALCACGT) CvAil, Fall, Hin1I Ecc1301* (CLCATGG), Neol (CLCATG), Pagl (TLCATGA), Pscl (ALCATGT) CvAil, Fall, Min1I tg1* (CLCACGG) Atlin* (ALCGCGT) Bsh1236, Issil tg1* (CLCACGG) Ssil Bsh1236, Issil paul (GLCCCGC), Sg3; (GCLCCCCC) Bsh1236, Issil Bsh1236, Issil g1* (CLCCGGY) BsaW* (WLCCGGA), Bsn11 (ALCGCGT) BsaW BsaW* (WLCCGGA), Bsn11 (ALCCGCG) g1* (CLCCCGC), Sg3; (GCLCCCGC) Brh138, Issp1, Cr10, Hpall, Msp1 BsaW* (WLCCGGA), Bsn11 (ALCCGCG) Brh138, Issp1, Msp1 g1* (CLCCGC), Sg3; (GCLCCCGC) Brh138, Issp1, Cr10, Hpall, Msp1 BsaW* Hpall, Msp1 g1* (C1* (GCCGC)) BsaW* (WLCCGGA), Bsn11 (ALCCCGGT), Cr10* (RLCCGGC), Cr10+ (RLCCGGC), Cr10+ Rpall, Ms	BSD143I (JGATC)		
sp143II* (AGCGCL/) Bbel (GCGCCL/C) Bsp143II, Hhal, Hin61 sp143II* (GCCCCL/) Bbel (GCCCCL) Bbel, BshNI, Bsp143II, Bsp14, Bsp1,			
sp143II* (GGCGCLV) Bbel (GGCGCLC) Bbel (SGCGCLC) sp11 (CJTRAG) Sm01* (CJTRAG) Bsp119 (TJCGCA), Taql (TJCGA), XmiI* (GTJCGAC) Taql su151 (ATJCGAT) Bsp1191 (TJCGCA), Taql (TJCGA), XmiI* (GTJCGAC) Taql ight* (CJCACGG) Afilli* (AJCATGT), Fall (JCATG), Pagl (TJCATGA), Pscl (AJCATGT) CVAII, Fall, Hin11 Eco1301* (CJCATGG) BseDI, Bigl, CVAII, Fall, Hin11 Eco1301, Fall, Hin11 Eco1301* (CJCATGG), Ncol (CJCATGG) BscDI, Bigl, CVAII, Fall, Hin11 Ncol Ight* (CJCGCGG) Afilli* (AJCGCGT), Miul (AJCGCGT) Bsh12361, Ssil Bsh12361, Ssil Ight* (CJCGCGG) Sayli* (WJCCGGGA, Sp1 (GLCGCGCC) Bsh12361, Hal, Hin61, Ssil Ssil Ifr101* (AJCCCGGY) Bsh11 (AJCGCGT), SgrAi* (CRLCCGGGF) Bsh12361, Hal, Hin61, Ssil Ssil Mill* (WJCCGGA), Kpn2 (TJCCGGA) Bsh11, AJEGA BsaWi* (WJCCGGA), SgrAi* (CRLCCGCGF) BsaWi* Bsh11, AJEGA MopMIV (GLCCGGC), SgrAi* (CRLCCGCGC) Cfr101, Hpall, Msp1 Sp11 Msp1 Sp11 MopMIV (GLCCGGC), SgrAi* (CRLCCGCGA) Hpall, Msp1 Sp11 Sp124(CCGCGGA) Hpall, Msp1 MopMIV (GLCCGGC), SgrAi* (CRLCCGCGA) Cfr101, Hpall, Msp1 Sp14 Sp14 Sp14 Sp14	BSD143II* (AGCGCJY)		Bsp143II Hhat Hin6I
Hhal, Hin1I, Hin6I, Kasi, Nari SpTI (C↓TTAAG) Smol* (C↓TTAAG) SpTI, Smol, Tru11 SpTI (C↓CACGG) BspTI 9[(T↓CGAA), Taql (T↓CGA), Xmil* (GT↓CGAC) Taql Igl* (C↓CACGG) Anili* (A↓CACGT) Maeli, Tail Igl* (C↓CACGG) Anili* (A↓CACGT) Maeli, Tail Igl* (C↓CACGG) Anili* (A↓CACGT) Waeli, Tail Igl* (C↓CATGG) Noli (C↓CATGG), Pagl (T↓CATGA), Pscl (A↓CATGT) Cv/All, Eco130i, Fait, Hin11 Victore Bsabl, Bigl, CviAll, Eco130i, Fait, Hin11 Neol Igl* (C↓CACGG) Null (A↓CCGCT) Bsabl, Bigl, CviAll, Eco130i, Fait, Hin11 VictoreGG Bsabl Bigl, CviAll, Eco130i, Fait, Hin11 VictoreGG Stati (A↓CCGCGT) Bsh1236i, Hait, Hin6i, Sail Bigl* (C↓CCGCG), Sgsl (GG↓CCGGA) Bsabl Bsabl Migl Cfr91 (C↓CCGGG), Eco81* (C↓CCGGA) Bsabl Bsabl Migl My (V↓CCGGA), Kpn21 (T↓CCGGA) Bsabl Bsabl Bsabl My (V↓CCGGA), SgrA1* (CR↓CCGGC) Cfr101, Hpa1, Msp1 Msp1 Cfr91 (C↓CCGGG), SgrA1* (CR↓CCGGC) Cfr101, Hpa1, Msp1 Msp1 My (V↓CCGGA), Sgr			
SpTI (C↓TTAAG) Smol* (C↓TTAAG) BspT1, Smol, Tru1l Su15i (A1↓CCAT) Bsp119 (T1↓CCAA), Taqi (T↓CGA), Xmil* (C1↓CGAC) Taqi Igi* (C↓ACGC) Afilli* (A↓CACGT) Maell, Tail Igi* (C↓ACGG) Afilli* (A↓CACGT) Eco1301* (C↓CATGG), Pagi (T↓CATGA), Psci (A↓CATGT) CviAll, Fatl, Hin1li Igi* (C↓ACGCG) Afilli* (A↓CACGT) Bsch12, Sill Eco1301* (C↓CATGG), Nool (C↓CATGG) Igi* (C↓ACGCG) Afilli* (A↓CGCGT), Mult (A↓CGCGT) Bsh1236i, Ssil Nool Igi* (C↓ACGCG) Afilli* (A↓CGCGT), Mult (A↓CGCGT) Bsh1236i, Hhal, Hin6l, Ssil Ssill Igi* (C↓ACGCG) BsaWr W↓CCCGGA), EshT1 (A↓CCCGGT) BsaWr BsaWr Ssill Cfr9l (C↓CCGCGG), EshT1 (A↓CCCGGT) BsaWr W↓CCCGGA), Kpn2l (T↓CCGGA) BsaWr BsaWr BsaWr Ssill Ssill ffr10* (G↓CCGGC) BsaWr W↓CCCGGT), SgrAI* (CR↓CCGGGG) Cfr10i, Hpall, Mspl Ssill			
Su15I ATUL Bsp119I (TI_CGA), Taql Taql Ight CLCACGG ATIIII* (ALCACGT) Maell, Tail Ight CLCACGG ATIIII* (ALCATGT), Fail (LCATG), Pagl (TLCATGA), Pscl (ALCATGT) CVAIL, Fail, Hin11 Eco1301* CLCATGG ATIIII* (ALCGCGT), Fail, (LCATGG), Ncol (CLCATGG) BscD1, Bigl, CvAII, Eco1301, Fail, Hin11 Itagl* (CLCCGCG) Affilit* (ALCCGCT), Fail (LCATGG), Ncol (CLCATGG) BscD1, Bigl, CvAII, Eco1301, Fail, Hin11 Itagl* (CLCCGCG) Affilit* (ALCCGCGT), Mlui (ALCGCGT) BscD13261, Ssil Itagl* Paul (GLCCCCC), Sgsl (GL-CCCCCC) BscD13261, Ssil, Hpail, Mspl BscD1 BsaWi* (WLCCGGT), BshTI (ALCCGGT), SgrAI* (CR_LCCGGC) Bcnl, Bme13901, Bsski, Hpail, Mspl Cfr91 (CL2CGGC), SgrAI* (CR_LCCGGC) Cfr10, Hpail, Mspl MgoMIV (GLCCGGC), SgrAI* (CR_LCCGGCG) Ssil	BSpTI (C↓TTAAG)	Smol* (C↓TTAAG)	
tgt* (C_LCACGG) Afilit* (A_LCACGT) Maell, Tail tgt* (C_LCATGG) Afilit* (A_LCATGT), Fall (LCATG), Pagl (T_LCATGA), Pscl (A_LCATGT) CvAll, Fall, Hin11 tgt* (C_LCATGG) Afilit* (A_LCGCGT), Nol (C_LCATGG) BseDI, Btgl, Cviall, Eco130I, Fall, Hin11 tgt* (C_LCGCGG) Afilit* (A_LCGCGT), Nol (C_LCATGG) BseDI, Btgl, Cviall, Eco130I, Fall, Hin11 tgt* (C_LCGCGG) Afilit* (A_LCGCGT), Nol (A_LCGCGT) Bsh1236I, Sail Paul (G_LCGCGC), Sgsl (GG_LCCGCCC) Bsh1236I, Sail BsaWI tgt* (L_LCGGG) ResaWI* (W_LCCGGG), SgrAI* (CR_LCCGGTG) BsaWI, Bsh1, Cfr10I, Hpall, Mspl Cfr91 (C_LCCGGG), Eco88I* (CLCCGGC) Cfr10I, Hpall, Mspl BsaWI* (W_LCCGGG), SgrAI* (CR_LCCGGCG) fr101* (G_LCCGGY) BsaWI* (W_LCCGGG), SgrAI* (CR_LCCGGCG) Cfr10I, Hpall, Mspl Cfr91 (C_LCCGGG), Eco88I* (CLCCGGC) Cfr10I, Hpall, Mspl BsaWI* (W_LCCGGG), SgrAI* (CR_LCCGGCG) Cfr91 (C_LCCGGG), Eco88I* (CLCCGGC) Cfr10I, Hpall, Mspl Ssil fr91 (C_LCCGGG) BsaWI* (W_LCCGGGI), SgrAI* (CR_LCCGGGC) Cfr10I, Hpall, Mspl, NgoMIV, Pd fr12 (CCCCLGG) BsaWI* (W_LCCGGI), SgrAI* (CR_LCCGGC), SgrAI* (CR_LCCGGCC), SgrAI* (CR_LCCGGC), SgrAI* (CR_LCCGGC), SgrAI* (CR_LCCGGC), SgrAI* (CR_LCCGGCC), SgrAI* (CR_LCCGGC), SgrAI* (CR_LCCGGC), SgrAI* (C			
tgl* (CLCATGG) Aflill* (ALCATGT), Fall (LCATG), Pagl (TLCATGA), Pscl (ALCATGT) CviAll, Fall, Hin111 Eco1301* (CLCATGG), Ncol (CLCATGG) Bscl, Reol (CLCATGG) Ncol (CLCATGG) Sgsl (GLCCCCCC) Bsh12361, Ssil (CLCCGCG) Bsh12361, Ssil (CLCCGCG) Bsh12361, Ssil (CLCCGCG) BsaWt* (WLCCGGA), Kpn21 (TLCCGGA) Bsh2Wt (RLCCGGA) BsaWt* (WLCCGGA), Bsh12 (ALCCGGC) BsaWt* (WLCCGGA), Bsh12 (ALCCGGC) BsaWt* (WLCCGGA), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr91 (CLCCGGG), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Mspl (GLCCGG), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr01* (GLCCGG), BsaWt* (WLCCGGA), Bsh12 (ALCCGGC), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr10* (GLCCGG), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr91 (CLCCGGG), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr91 (CLCCGGG), SgrA1* (CRLCCGGCG) Cfr10, Hpall, Mspl (Cfr91 (CLCCGGG), SgrA1* (CRLCCGGCG) Cac81, Cfr10, Hpall, Mspl (Cfr91 (CLCCGGG), SgrA1* (CRLCCGGCG), SgrA1* (CRLCCGGC), SgrA1, Sg	Btgl* (C↓CACGG)	Afilit* (A↓CACGT)	
Eco1301* (C_LCATGG), Ncol (C_LCATGG) BseDl, Btgl, CviAll, Eco1301, Fatl, Hin11 Intervention Ncol tgl* (C_LCGCGG) Attilit* (A_LCGCGT), Miul (A_LCGCGT) Bsh12361, Ssil Paul (G_LCGCGC), Sgsl (GG_LCGCGCC) Bsh12361, Hal, Hin61, Ssil Bsh12361, Hal, Hin61, Ssil fr101* (A_LCCGGT) BsaWi* (W_LCCGGA), Kpn21 (T_LCCGGA) BsaWi (W_LCCGGG), BshT1 (A_LCCGGT), SgrA1* (CR_LCCGGGG) Bcnl, Bme13901, BssK1, Hpall, Mspl Cfr91 (C_LCCGGC), SgrA1* (CR_LCCGGCG) Cfr101, Hpall, Mspl BsaWi* (W_LCCGGT), SgrA1* (CR_LCCGGCG) Cfr101, Hpall, Mspl fr101* (G_LCCGGC) BsaWi* (W_LCCGGT), SgrA1* (CR_LCCGGCG) Cfr101, Hpall, Mspl Cfr101, Hpall, Mspl cfr91 (C_LCCGGC), SgrA1* (CR_LCCGGC) Cfr101, Hpall, Mspl SgrA1* (CR_LCCGGC), SgrA1* (CR_LCCGGCG) SgrA1* (M_LCCGGT), SgrA1* (CR_LCCGGC) fr121 (CCGC_LGG) BsaWi* (W_LCCGGC), SgrA1* (CR_LCCGGC), Cfr101* (R_LCCGGC), Cfr101* (R_LCCGGC), SgrA1* (CR_LCCGGC), SgrA1* (
Ncol tgl* (CLCGCGG) Afilli* (ALCGCGT), Miul (ALCGCGT) Bsh1236i, Ssil Paul (GLCGCGC), Sgsl (GGLCGCCCC) Bsh1236i, Ssil BsaWi* (WLCCGGA), Kpn21 (TLCCGGA) BsaWi* (WLCCGGT), BshT1 (ALCCGGT), SgrAi* (CRLCCGGTG) BsaWi, BshT1, Cr101, Hpall, Mspl BsaWi* (WLCCGGA), Kpn21 (TLCCGGA) BsaWi* (WLCCGGT), SgrAi* (CRLCCGGCG) Cr101, Hpall, Mspl NgoMiv (GLCCGC), SgrAi* (CRLCCGGCG) Cr101, Hpall, Mspl Mspl NgoMiv (GLCCGGC), SgrAi* (CRLCCGGCG) Cr101, Hpall, Mspl Cfr91 (CLCCGGG), Eco881* (CLCCGGG) Bcnl, Bme13901, BssKI, Hpall, Mspl ClcCCGCJCG) BsaWi* (WLCCGGT), SgrAi* (CRLCCGGCG) Cr101, Hpall, Mspl ClcCCGCGG) Bcnl, Bme13901, BssKI, Hpall, Mspl Mspl MgoMIV (GLCCGGC), SgrAi* (CRLCCGGG) Cr101, Hpall, Mspl Ssil MgoMIV (GLCCGGC), SgrAi* (CRLCCGGG) Cac8I, Cfr101, Hpall, Mspl Ssil MgoMIV (GLCCGGC), SgrAi* (CRLCCGGG) Ssil Ssil fr191 (CLCCGGG) BsaWi* (WLCCGGT), SgrAi* (CRLCCGGC), SgrAi* (CRLCCGGC)			BseDI, Btgl, CviAll, Eco130I, Fatl, Hin1II,
Paul (G↓CGGG), Sgsl (GG↓CGCGCC) Bsh1236i, Hhal, Hin6i, Ssil fr101* (A↓CCGGY) BsaWI* (W↓CCGGA), Kpn21 (T↓CCGGA) BsaWI, Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) BsaWI, BshTI, Cfr10i, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGG) Bcnl, Bme1390i, BssKi, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGG) Cfr10i, Hpall, Mspl BsaWI* (W↓CCGGA), Kpn21 (T↓CCGGA) Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) Cfr10i, Hpall, Mspl Cfr9i (C↓CCGGC), Eco881* (C↓CCGGGC) Bcnl, Bme1390i, BssKi, Hpall, Mspl Cfr9i (C↓CCGGC), Eco881* (C↓CCGGGC) Bcnl, Bme1390i, BssKi, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGGC) Cac8i, Cfr10i, Hpall, Mspl, NgoMIV, Pall, Mspl (CCCC↓GG) BsaWi* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10i* (R↓CCGGC), Cfr10i* (R↓CCGGC), SgrAI* (CR↓CCGGC), S			0
Paul (G↓CGGG), Sgsl (GG↓CGCGCC) Bsh1236i, Hhal, Hin6i, Ssil fr101* (A↓CCGGY) BsaWI* (W↓CCGGA), Kpn21 (T↓CCGGA) BsaWI, Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) BsaWI, BshTI, Cfr10i, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGG) Bcnl, Bme1390i, BssKi, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGG) Cfr10i, Hpall, Mspl BsaWI* (W↓CCGGA), Kpn21 (T↓CCGGA) Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) Cfr10i, Hpall, Mspl Cfr9i (C↓CCGGC), Eco881* (C↓CCGGGC) Bcnl, Bme1390i, BssKi, Hpall, Mspl Cfr9i (C↓CCGGC), Eco881* (C↓CCGGGC) Bcnl, Bme1390i, BssKi, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGGC) Cac8i, Cfr10i, Hpall, Mspl, NgoMIV, Pall, Mspl (CCCC↓GG) BsaWi* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10i* (R↓CCGGC), Cfr10i* (R↓CCGGC), SgrAI* (CR↓CCGGC), S	Btgl* (C↓CGCGG)	Afilit* (A↓CGCGT), Miul (A↓CGCGT)	Bsh1236l, Ssil
fr101* (A↓CCGGY) BsaWI* (W↓CCGGA), Kpn2I (T↓CCGGA) BsaWI* (W↓CCGGA), Kpn2I (T↓CCGGA) BsaWI Cfr10I, HpaII, MspI BsaWI BsaWI Cfr10I, HpaII, MspI BsaWI Cfr10I, HpaII, MspI BsaWI Cfr10I, HpaII, MspI Cfr10I Cfr10I, HpaII, MspI Cfr10I BsaWI* W↓CCGGA) SpIII A↓CCGGA HpaII, MspI SpIII SpIII Cfr10I Cfr10I Cfr10I, HpaII, MspI SpIII SpIII Cfr10I Cfr10I HpaII, MspI SpIII SpI	5 ()	Paul (GUCGCGC), Sgsl (GGUCGCGCC)	Bsh1236l, Hhal, Hin6l, Ssil
BsaWl* (W↓CCGGT), BshTi (A↓CCGGT), SgrAl* (CR↓CCGGTG) BsaWl, BshTi, Cfr10I, Hpall, Mspl Cfr9I (C↓CCGGG), Eco881* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAl* (CR↓CCGGC) Cfr10I, Hpall, Mspl fr101* (G↓CCGGG), BsaWl* (W↓CCGGT), BshTi (A↓CCGGT), SgrAl* (CR↓CCGGTG) Cfr10I, Hpall, Mspl BsaWl* (W↓CCGGC), Eco881* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl Cfr9I (C↓CCGGG), Eco881* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAl* (CR↓CCGGG) Cac81, Cfr10I, Hpall, Mspl NgoMIV (G↓CCGGC), Eco881* (C↓CCGGG) Cac81, Cfr10I, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAl* (CR↓CCGGG) Cac81, Cfr10I, Hpall, Mspl, NgoMIV, Pd fr421 (CCGC↓GG Bsh12851* (CGGC↓CG Ssil fr91 (C↓CCGGG) BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGC), SgrAI* (CR↓CCGGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco881* (C+I, CviJI, Eco521 Notl (GC↓GGCCC) BsuRI, Cfr13, CviJI SatI, SsiI, Taul SatI, SsiI, Taul fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul NotI (GC↓GGCCG) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul	Cfr10I* (A↓CCGGY)		
NgoMIV (G↓CCGGC), SgrAl* (CR↓CCGGCG) Cfr10I, Hpall, Mspl fr101* (G↓CCGGY) BsaWI* (W↓CCGGA), Kpn2I (T↓CCGGA) Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) Cfr10I, Hpall, Mspl Cfr9I (C↓CCGGC), Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), Eco88I* (C↓CCGGG) Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pd NgoMIV (G↓CCGGC), Eco88I* (C↓CCGGG) Ssil NgoMIV (G↓CCGGA), BsaWI* (W↓CCGGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), SgrAI* (CR↓CCGGA), SaWI* (W↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGC), SgrAI* (CR↓CCGGGG) Ssil fr19 (C↓CCCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl Ssil fr14* (C↓GGCCC) Bspl20I (G↓GGCCC) Bspl20I (G↓GGCCC) BsuRI, Cfr13I, CviJI fr1* (C↓GGCCR) Bspl20I (G↓GGCCC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul SatI, SsiI, Taul fr1* (T↓GGCCR) BsuRI, Cfr1, CviJI Eco52I (C↓GGCCC) BsuRI, Cfr1, CviJI fr1* (T↓GGCCR) Bspl20I (G↓GGCCC) BsuRI, Cfr1, CviJI SatI, SsiI, Taul fr1* (T↓GGCCR) BsuRI, Cfr1, CviJI BsuRI, Cfr1, CviJI SatI, SsiI, Taul		BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG)	
fr101* (G↓CCGGY) BsaWI* (W↓CCGGA), Kpn2I (T↓CCGGA) Hpall, Mspl BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) Cfr10I, Hpall, Mspl Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGCG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG) Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pd fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGTG) Bcnl, Bme1390I, BssKI, Hpall, Mspl frI* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13, CviJI Eco52I (C↓GGCCG) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul SsiI, Taul frI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13, CviJI Kfr1* (T↓GGCCR) BsuRI, Cfr1, CviJI, Eco52I, C↓GGCCG) SsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul		Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKl, Hpall, Mspl
BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG) Cfr10I, Hpall, Mspl Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG) Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pd fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) Bsh1285I* (CGGC↓CG) Ssil gravit* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), SgrAI* (CR↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) Bcnl, Bme1390I, BseDI, BssKI, Cfr9I, Eco88I* (C↓CCGGC) BsuRI, Cfr13I, CviJI fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI, Eco52I StatI, SsiI, Taul fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, CJ↓GGCCG) StatI, SsiI, Taul fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI StatI, SsiI, Taul			Cfr10I, Hpall, Mspl
Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG) Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pd fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) Bsh1285I* (CGGC↓CG) Ssil gray BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), SgrAI* (CR↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGGG) Bcnl, Bme1390I, BssKI, Hpall, Mspl fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) Bsn12, Smal fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) Bsh1285I, BsuRI, Cfr13, CviJI fr1* (C↓GGCCR) Bsp120I (G↓GGCCC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I Notl (GC↓GGCCC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, SaII, SaiI, Taul fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13, CviJI fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13, CviJI fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13, CviJI fr1* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr1, CviJI, Eco52I, SaII, SaiI, Taul	Cfr10I* (G↓CCGGY)	BsaWI* (W↓CCGGA), Kpn2I (T↓CCGGA)	Hpall, Mspl
NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG) Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pd fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGTG) Bcnl, Bme1390I, BssKI, Hpall, Mspl ifrI* (C↓GGCCR) Bsp120I (G↓GGCCC) Bcnl, Bme1390I, BssKI, Cfr9I, Eco52I (C↓GGCCC) BsuR1, Cfr13I, CviJI ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) Bsh1285I, BsuR1, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) Bsh1285I, BsuR1, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI ifrI* (T↓GGCCC) BsuRI, Cfr13I, CviJI BsuRI, Cfr1, CviJI, SatI,		BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), SgrAI* (CR↓CCGGTG)	Cfr10I, Hpall, Mspl
fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGTG) Bcnl, Bme1390I, BssKI, Hpall, Mspl Eco88I* (C↓CCGGG) Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BseDI, BssKI, Cfr9I, Eco88I, Hpall, Mspl, Smal frI* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI, Eco52I (C↓GGCCG) BsuRI, Cfr1, CviJI, Eco52I Notl (GC↓GGCCC) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI frI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul frI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul		Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKl, Hpall, Mspl
fr42I (CCGC↓GG) Bsh1285I* (CGGC↓CG) Ssil fr9I (C↓CCGGG) BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC), Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGTG) Bcnl, Bme1390I, BssKI, Hpall, Mspl Eco88I* (C↓CCGGG) Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BseDI, BssKI, Cfr9I, Eco88I, Hpall, Mspl, Smal frI* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI, Eco52I (C↓GGCCG) BsuRI, Cfr1, CviJI, Eco52I Notl (GC↓GGCCC) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI frI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul frI* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul		NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pdil
Cfr101* (R↓CCGGT), Kpn21 (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG), SgrAI* (CR↓CCGGCG) SgrAI* (CR↓CCGGTG) Eco881* (C↓CCGGG) Bsp1201 (G↓GGCCC) Bsp1201 (G↓GGCCC) Bsp1201 (G↓GGCCC) Bsp1201 (G↓GGCCG) Bsp1201 (G↓GGCCC) Bsp1201 (G↓GGCCC) Bsp1201 (G↓GGCCG) Bsp1201 (G↓GGCCG) Bsp1201 (G↓GGCCGC) Bsp1201 (G↓GGCCC) BsuRI, Cfr131, CviJI Eco521 (C↓GGCCC) BsuRI, Cfr1, CviJI Notl (GC↓GGCCG) BsuRI, Cfr1, CviJI, Satl, Ssil, Taul	Cfr42I (CCGC↓GG)	Bsh1285I* (CGGC↓CG)	Ssil
SgrAl* (CR↓CCGGTG) Eco88I* (C↓CCGGG) Bcnl, Bme1390I, BseDI, BssKI, Cfr9I, Eco88I, Hpall, MspI, Smal frl* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) Bsh1285I, BsuRI, CfrI, CviJI, Eco52I Notl (GC↓GGCCGC) Bsh1285I, BsuRI, CfrI, CviJI, Eco52I, Satl, Ssil, Taul frl* (T↓GGCCR) Bsp120I (G↓GGCCC) Eco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCC) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCG) BsuRI, Cfr1, CviJI, Satl, Ssil, Taul	Cfr9I (C↓CCGGG)	BsaWI* (W \downarrow CCGGA), BsaWI* (W \downarrow CCGGT), BshTI (A \downarrow CCGGT), Cfr10I * (R \downarrow CCGGC),	Bcnl, Bme1390I, BssKI, Hpall, Mspl
Eco88I* (C↓CCCGGG) Bcnl, Bme1390l, BseDl, BssKl, Cfr9l, Eco88I, Hpall, Mspl, Smal fri* (C↓GGCCR) Bsp120l (G↓GGCCC) BsuRl, Cfr13l, CviJl Eco52l (C↓GGCCG) Bsh1285l, BsuRl, Cfrl, CviJl, Eco52l Notl (GC↓GGCCGC) Bsh1285l, BsuRl, Cfrl, CviJl, Eco52l, Satl, Ssil, Taul fri* (T↓GGCCR) Bsp120l (G↓GGCCC) Eco52l (C↓GGCCG) BsuRl, Cfr13l, CviJl fri* (T↓GGCCR) Bsp120l (G↓GGCCC) Bsp120l (G↓GGCCCG) BsuRl, Cfr13l, CviJl Notl (GC↓GGCCCG) BsuRl, Cfr13l, CviJl Notl (GC↓GGCCG) BsuRl, Cfr1, CviJl, Satl, Ssil, Taul		Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG),	
Eco88I, Hpall, Mspl, Smal frl* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I Notl (GC↓GGCCGC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul frl* (T↓GGCCR) Bsp120I (G↓GGCCC) Eco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI frl* (T↓GGCCR) Bsp120I (G↓GGCCC) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) BsuRI, Cfr1, CviJI Notl (GC↓GGCCGC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul		SgrAI* (CR↓CCGGTG)	
fri* (C↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I Notl (GC↓GGCCGC) Bsh1285I, BsuRI, Cfr1, CviJI, Eco52I, SatI, SsiI, Taul frl* (T↓GGCCR) Bsp120I (G↓GGCCC) Eco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI frl* (T↓GGCCR) Bsp120I (G↓GGCCC) Bsp120I (G↓GGCCG) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCG) BsuRI, Cfr1, CviJI Notl (GC↓GGCCGC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul		Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BseDI, BssKI, Cfr9I,
Eco52I (C↓GGCCG) Bsh1285I, BsuRI, CfrI, CviJI, Eco52I Notl (GC↓GGCCGC) Bsh1285I, BsuRI, CfrI, CviJI, Eco52I, SatI, SsiI, Taul frI* (T↓GGCCR) Bsp120I (G↓GGCCC) Bco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) BsuRI, Cfr13I, CviJI Notl (GC↓GGCCG) BsuRI, Cfr1, CviJI Notl (GC↓GGCCG) BsuRI, Cfr1, CviJI Notl (GC↓GGCCGC) BsuRI, Cfr1, CviJI, SatI, SsiI, Taul			Eco88I, Hpall, Mspl, Smal
Noti (GC↓GGCCGC) Bsh12851, BsuRi, Cfrl, CviJi, Eco521, Sati, Ssii, Taul frl* (T↓GGCCR) Bsp1201 (G↓GGCCC) BsuRi, Cfr13i, CviJi Eco521 (C↓GGCCG) BsuRi, Cfrl, CviJi Noti (GC↓GGCCGC) BsuRi, Cfrl, CviJi Noti (GC↓GGCCGC) BsuRi, Cfrl, CviJi	CfrI* (C↓GGCCR)		BsuRI, Cfr13I, CviJI
Noti (GC↓GGCCGC) Bsh12851, BsuRi, Cfrl, CviJi, Eco521, Sati, Ssii, Taul frl* (T↓GGCCR) Bsp1201 (G↓GGCCC) BsuRi, Cfr13i, CviJi Eco521 (C↓GGCCG) BsuRi, Cfrl, CviJi Noti (GC↓GGCCGC) BsuRi, Cfrl, CviJi Noti (GC↓GGCCGC) BsuRi, Cfrl, CviJi			Bsh1285I, BsuRI, CfrI, CviJI, Eco52I
Satl, Ssil, Taul frl* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) BsuRI, CfrI, CviJI Notl (GC↓GGCCGC) BsuRI, CfrI, CviJI, Satl, Ssil, Taul			
frl* (T↓GGCCR) Bsp120I (G↓GGCCC) BsuRI, Cfr13I, CviJI Eco52I (C↓GGCCG) BsuRI, CfrI, CviJI Notl (GC↓GGCCGC) BsuRI, CfrI, CviJI, Sail, Sail, Taul			
Eco52I (C↓GGCCG) BsuRI, CfrI, CviJI Notl (GC↓GGCCGC) BsuRI, CfrI, CviJI, Satl, Ssil, Taul	Cfrl* (T↓GGCCR)	Bsp120I (G↓GGCCC)	
Notl (GC↓GGCCGC) BsuRI, CfrI, CviJI, SatI, SsiI, Taul			
	Cpol* (CG↓GACCG)	Eco47I * ($G \downarrow GACC$), Psp5II * (RG $\downarrow GACCT$)	Cfr13I, Eco47I

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Table 1.26. Newly	y Generated Recognition S	Seauences Resultina	from the Ligation	of Protrudina Co	mpatible DNA Ends.

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme	enzyme	newly generated recognition sequence
	Psp5II* (RG↓GACCC), SanDI* (GG↓GACCC)	BspLI, Cfr13I, Eco47I
Cpol* (CG↓GTCCG)	Eco471* (GJGTCC), Psp5II* (RGJGTCCT)	Cfr13I, Eco47I
	Psp5II* (RG GTCCC), SanDI* (GG GTCCC)	BspLI, Cfr13I, Eco47I CviAll, Fatl, Hin1II
ECOISUL (CVCAIGG)	Afilil* (A↓CATGT), Fatl (↓CATG), <mark>Pagl</mark> (T↓CATGA), <mark>Pscl</mark> (A↓CATGT) Btgl* (C↓CATGG), Ncol (C↓CATGG)	BseDI, Btgl, CviAll, Eco130I, Fatl, Hin1II,
		Ncol
Eco130I* (C↓CTAGG)	Bcul (A↓CTAGT), Nhel (G↓CTAGC), Xbal (T↓CTAGA)	FspBI
	XmaJI (C↓CTAGG)	BseDI, Eco130I, FspBI, XmaJI
Eco24I* (GAGCC↓C)	Sdul* (GAGCC↓C)	CviJI, Eco24I, Sdul
Eco24I* (GAGCT↓C)	Alw211* (GAGCT↓C), SacI (GAGCT↓C), SduI* (GAGCT↓C)	Alul, Alw21I, CviJI, Ecl136II, Eco24I,
		Sacl, Sdul
Eco24I* (GGGCC↓C)	Apal (GGGCC↓C), BseSI* (GGGCC↓C), Sdul* (GGGCC↓C)	Apal, BseSI, Bsp120I, BspLI, BsuRI,
		Cfr13I, CviJI, Eco24I, Sdul
Eco24I* (GGGCT↓C)		CviJI, Eco24I, Sdul
Eco47I* (G↓GACC)	Cpol* (CG↓GACCG), Psp5II* (RG↓GACCT) Psp5II* (RG↓GACCC), SanDI* (GG↓GACCC)	Cfr13I, Eco47I BspLI, Cfr13I, Eco47I
Eco47I* (G↓GTCC)	Cpol* (CG↓GTCCG), Psp5II* (RG↓GTCCT)	Cfr13I, Eco47I
	Psp5II * (RG \downarrow GTCCC), SanDI* (GG \downarrow GTCCC)	BspLI, Cfr13I, Eco47I
Eco52I (C↓GGCCG)	Bsp120I (G↓GGCCC)	BsuRI, Cfr13I, CviJI
	CfrI* (Y↓GGCCA)	BsuRI, CfrI, CviJI
	Cfrl* (Y↓GGCCG)	Bsh1285I, BsuRI, CfrI, CviJI, Eco52I
	Notl (GC↓GGCCGC)	Bsh1285I, BsuRI, CfrI, CviJI, Eco52I,
		Satl, Ssil, Taul
Eco88I* (C↓CCGGG)	BsaWI* (W↓CCGGA), BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGC),	Bcnl, Bme1390I, BssKI, Hpall, Mspl
	Cfr10I* (R↓CCGGT), Kpn2I (T↓CCGGA), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG),	
	SgrAI* (CR+CCGGTG)	Devel Developed Developed VI Official
	Cfr9I (C↓CCGGG)	Bcnl, Bme1390l, BseDl, BssKl, Cfr9l,
Eco88I* (C↓TCGAG)	PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), Smol* (C↓TCGAG),	Eco88I, Hpall, Mspl, Smal Eco88I, Smol, Taql, Xhol
	TSPAT (VC+TCGAGC), TSPAT (VC+TCGAGG), TSPAT (VC+TCGAGT), SHOT (C+TCGAG), Xhol (C+TCGAG)	
	Sall (G↓TCGAC)	Taql
EcoRI (G↓AATTC)	Muni (C \downarrow AATTG), Tasi (\downarrow AATT)	Tasl
	Xapl* (R↓AATTC)	EcoRI, Tasl, Xapl
	Xapl* (RJAATTT)	Tasl, Xapl
EcoRII* (↓CCAGG)	SexAI* (A↓CCAGGT)	Bme1390I, BssKI, EcoRII, Mval
EcoRII* (↓CCTGG)	SexAI* (A CCTGGT)	Bme1390I, BssKI, EcoRII, Mval
Fatl (↓CATG)	Afilil* (A↓CATGT), BtgI* (C↓CATGG), Eco130I* (C↓CATGG), Ncol (C↓CATGG), PagI (T↓CATGA), PscI (A↓CATGT)	CviAll, Fatl, Hin1ll
Hin1I* (GA↓CGYC)	Hin6I (G \downarrow CGC), Ssil* (C \downarrow CGC)	Csel
	Maell (AUCGT), Psp1406I (AAUCGTT)	Maell, Tail
	Narl (GG↓CGCC)	Csel, Hin1I
Hin1I* (GG↓CGYC)	Hin6I (G \downarrow CGC), Ssil* (C \downarrow CGC)	Hhal, Hin6l
	Narl (GG↓CGCC)	Bbel, BshNI, Bsp143II, BspLI, Ehel,
		Hhal, Hin1l, Hin6l, Kasl, Narl
Hin1II (CATG↓)	Pael (GCATG↓C), Xcel* (RCATG↓C), Xcel* (RCATG↓T)	CviAll, Fatl, Hin1ll
Hin6I (G \downarrow CGC) Hpall (C \downarrow CGG)	Hin1I* (GR↓CGCC), Narl (GG↓CGCC), Ssil* (C↓CGC) Hin1I* (GR↓CGCC), Hin6I (G↓CGC), Narl (GG↓CGCC), Ssil* (C↓CGC)	Hhal, Hin6l Ssil
	$Mspl (C\downarrow CGG), Ssil* (G\downarrow CGG)$	Hpall, Mspl
Kasl (G↓GCGCC)	BshNI* (GJGCGCC)	Bbel, BshNI, Bsp143II, BspLI, Ehel,
1431 (0 • 00000)		Hhal, Hin1l, Hin6l, Kasl, Narl
Kpn2I (T↓CCGGA)	BsaWI* (W↓CCGGA)	BsaWI, Hpall, Hpy188III, Kpn2I, Mspl
	BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, Hpall, Mspl
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	Hpall, Mspl
	Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKl, Hpall, Mspl
Maell (AUCGT)	Hin1I* (GR↓CGTC), Psp1406I (AA↓CGTT)	Maell, Tail
MboI (↓GATC)	BamHI (G↓GATCC), BCII (T↓GATCA), BgIII (A↓GATCT), Bsp143I (↓GATC), PsuI* (R↓GATCC), PsuI* (R↓GATCT)	Bsp143I, Dpnl, Mbol
Mlul (A↓CGCGT)	AfIIII* (A↓CGCGT)	Aflill, Bsh1236I, Mlul
	Btgl* (C↓CGCGG)	Bsh1236I
	Paul (G↓CGCGC), Sgsl (GG↓CGCGCC)	Bsh1236I, Hhal, Hin6I
Mph1103I (ATGCA↓T)		HpyCH4V
Mspl (C↓CGG)	Hin11* (GR↓CGCC), Hin6I (G↓CGC), Narl (GG↓CGCC), Ssil* (C↓CGC)	Ssil
		Hpall, Mspl
Muni (C↓AATTG)	EcoRI (G↓AATTC), Tasi (↓AATT), Xapi* (R↓AATTC), Xapi* (R↓AATTT)	Tasl Rhol Bohll Bon14211 Bon14 Ehol
Narl (GG↓CGCC)	Hin1I* (GR↓CGCC)	Bbel, BshNI, Bsp143II, BspLI, Ehel, Hhal, Hin1I, Hin6I, Kasl, Narl
		(continued on next page

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Information on New Cleavage Sites

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PC-CC-

Table 1.26. Newly Generated Recognition Sequences Resulting from the Ligation of Protruding Compatible DNA Ends.

First restriction enzyme	Second restriction enzyme	Restriction enzymes that cleave the newly generated recognition sequence
chzyme		
	Hin1I* (GR↓CGTC) Hin6I (G↓CGC), SsiI* (C↓CGC)	Hin1l Hhal, Hin6l
Ncol (C↓CATGG)	Afilii* (A↓CATGT), Fati (↓CATG), Paqi (T↓CATGA), Psci (A↓CATGT)	CviAll, Fatl, Hin111
	Hill (A↓CATGT), Fall (↓CATG), Pagi (1↓CATGA), PSCI (A↓CATGT) Btgl* (C↓CATGG), Eco1301* (C↓CATGG)	BseDI, Btgl, CviAll, Eco130I, Fatl, Hin1II,
	$Digi \ (C V CAIGG), E COISOI \ (C V CAIGG)$	Ncol
NgoMIV (G↓CCGGC)	BsaWI* (W↓CCGGA), <mark>Kpn2I</mark> (T↓CCGGA)	Hpall, Mspl
	BSaWI (₩↓CCGGA), Kpitzi (1↓CCGGA) BsaWI* (₩↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGT), SgrAI* (CR↓CCGGTG)	Cfr10I, Hpall, Mspl
	Cfr10I* (R↓CCGGC), SgrAI* (CR↓CCGGCG)	Cac8l, Cfr10l, Hpall, Mspl, NgoMIV, Pdil
	Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKl, Hpall, Mspl
Nhel (G↓CTAGC)	Bcul (A↓CTAGT), Ecol30I (C↓CTAGG), Xbal (T↓CTAGA), XmaJI (C↓CTAGG)	
Noti (GC↓GGCCGC)	Bsp120I (G↓GGCCC)	FspBI BsuRI, Cfr13I, CviJI, SatI, Taul
	Cfrl* (Y↓GGCCA)	
	CfrI* (Y↓GGCCG), Eco52I (C↓GGCCG)	BsuRI, CfrI, CviJI, SatI, Taul Bsh1285I, BsuRI, CfrI, CviJI, Eco52I,
		Sati, Taul
Pacl (TTAAT↓TAA)	Bsh1285I* (CGAT↓CG), Pvul (CGAT↓CG), Sqfl (GCGAT↓CGC)	Tru1I
Pael (GCATG↓C)		
		CviAll, Fatl, Hin1ll
	Xcel* (RCATG↓C) Xcel* (RCATG↓T)	Cac8I, CviAll, Fatl, Hin1II, Pael, Xcel
	Afilii* (A↓CATGT), Btgi* (C↓CATGG), Eco130I* (C↓CATGG), Fati (↓CATG), Ncol (C↓CATGG),	CviAll, Fatl, Hin1ll, Xcel
PagI (T↓CATGA)		CviAll, Fatl, Hin1II
	PscI (A↓CATGT)	DeeV/
Pasl* (CC↓CAGGG)		BseYI
Paul (G↓CGCGC)	Afilii* (A↓CGCGT), Bigi* (C↓CGCGG), Miui (A↓CGCGT)	Bsh1236l, Hhal, Hin6l
		Bsh1236l, Cac8l, Hhal, Hin6l, Paul
PfI23II (C↓GTACG)	Acc65I (G↓GTACC), BshNI* (G↓GTACC), Bsp1407I (T↓GTACA), TatI* (W↓GTACA), TatI* (W↓GTACT)	
PscI (A↓CATGT)		AfIIII, CviAII, Fatl, Hin1II, PscI, Xcel
	Btgl* (C↓CATGG), Eco130I* (C↓CATGG), Fatl (↓CATG), Ncol (C↓CATGG), Pagl (T↓CATGA)	CviAll, Fatl, Hin1ll
	Hin1I* (GR↓CGTC), Maell (A↓CGT)	Maell, Tail
Psp5II* (AG↓GACCY)	Cpol* (CGJGACCG), Eco47I* (GJGACC)	Cfr13I, Eco47I
	SanDI* (GG GACCC)	BspLI, Cfr13I, Eco47I, Eco0109I, Psp5I
Psp5II* (AG↓GTCCY)	Cpol* (CG↓GTCCG), Eco47I* (G↓GTCC)	Cfr13I, Eco47I
	SanDI* (GG GTCCC)	BspLI, Cfr13I, Eco47I, Eco0109I, Psp5I
Psp5II* (GG↓GACCY)	Cpol* (CG↓GACCG), Eco47I* (G↓GACC)	BspLI, Cfr13I, Eco47I, Faql
	SanDI* (GG↓GACCC)	BspLI, Cfr13I, Eco47I, Eco0109I, FaqI,
		Psp5II, SanDI
Psp5II^ (GG↓GTCCY)	Cpol* (CGJGTCCG), Eco47I* (GJGTCC)	BspLI, Cfr13I, Eco47I
	SanDI* (GG ¹ GTCCC)	BspLI, Cfr13I, Eco47I, Eco0109I,
		Psp5II, SanDI
PSpXI" (AC↓ICGAGB)	Eco88I* (C↓TCGAG), SmoI* (C↓TCGAG), XhoI (C↓TCGAG)	Eco88I, Smol, Taql, Xhol
		Taql
PSpXI^ (CC↓ICGAGB)	Eco88I* (C↓TCGAG), SmoI* (C↓TCGAG), XhoI (C↓TCGAG)	Eco88I, MnII, Smol, Taql, Xhol
	Sall (GUTCGAC)	MnII, Taql
PspXI* (GC↓TCGAGB)		Eco88I, Smol, Taql, Xhol
	Sall (G↓TCGAC)	Taql
PstI (CTGCA↓G)	Alw21I* (GTGCA↓C), BseSI* (GTGCA↓C), Mph1103I (ATGCA↓T), SduI* (GTGCA↓C)	НруСН4V
	Sdal (CCTGCA GG)	Bfml, HpyCH4V, Pstl
Psul* (A↓GATCY)	BamHI (GJGATCC)	Bsp143I, DpnI, Mbol, Psul
	BCII (T↓GATCA), Bsp143I (↓GATC), MboI (↓GATC)	Bsp143I, DpnI, Mbol
	BgIII (AJGATCT)	BgIII, Bsp143I, DpnI, Mbol, Psul
Psul* (G↓GATCY)	BamHI (G↓GATCC)	BamHI, Bsp143I, BspLI, BspPI, DpnI,
		Mbol, Psul
	BCII (T↓GATCA), Bsp143I (↓GATC), Mbol (↓GATC)	Bsp143I, BspPI, DpnI, Mbol
	BgIII (A↓GATCT)	Bsp143I, BspPI, DpnI, Mbol, Psul
Pvul (CGAT↓CG)	Bsh1285I* (CGAT↓CG), Sgfl (GCGAT↓CGC)	Bsh1285I, Bsp143I, DpnI, Mbol, Pvul
	Pacl (TTAAT J TAA)	Tru1I
<mark>SacI</mark> (GAGCT↓C)	Alw21I* (GAGCT↓C), Eco24I* (GAGCT↓C), SduI* (GAGCT↓C)	Alul, Alw21I, CviJI, Ecl136II, Eco24I,
		Sacl, Sdul
<mark>Sall</mark> (G↓TCGAC)	Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT),	Taql
	Smol* (C↓TCGAG), Xhol (C↓TCGAG)	
SanDI* (GG↓GACCC)	Cpol* (CG↓GACCG), Eco47I* (G↓GACC)	BspLI, Cfr13I, Eco47I, Faql
	Psp5II* (RG↓GACCC)	BspLI, Cfr13I, Eco47I, Eco0109I, FaqI,
		Psp5II, SanDl
	Psp5II* (RG↓GACCT)	BspLI, Cfr13I, Eco47I, Eco0109I, FaqI,
		Psp5II
SanDI* (GG↓GTCCC)	CpoI* (CG↓GTCCG), Eco47I* (G↓GTCC)	BspLI, Cfr13I, Eco47I
	Psp5II* (RG↓GTCCC)	BspLI, Cfr13I, Eco47I, Eco0109I,

(continued on next page) Bulk quantities & custom formulations available on request



Table 1.26. Newl	y Generated Recognition S	Sequences Resulting from	the Ligation of Protruding	Compatible DNA Ends.

First restriction	Second restriction	Restriction enzymes that cleave the
enzyme	enzyme	newly generated recognition sequence
	Psp5II* (RG↓GTCCT)	BspLI, Cfr13I, Eco47I, Eco0109I, Psp5I
Sdal (CCTGCA↓GG)	Alw21I* (GTGCA↓C), BseSI* (GTGCA↓C), Mph1103I (ATGCA↓T), SduI* (GTGCA↓C)	HpyCH4V
	PstI (CTGCAJG)	Bfml, HpyCH4V, Pstl
Sdul* (GAGCA↓C)	Alw21I* (GAGCA↓C)	Alw21I, Sdul
Sdul* (GAGCC↓C)	Eco24I* (GAGCC↓C)	CviJI, Eco24I, Sdul
Sdul* (GAGCT↓C)	Alw21I* (GAGCT↓C), Eco24I* (GAGCT↓C), SacI (GAGCT↓C)	Alul, Alw21I, CviJI, Ecl136II, Eco24I,
		Sacl, Sdul
Sdul* (GGGCA↓C)	BseSI* (GGGCAJC)	BseSI, Sdul
Sdul* (GGGCC↓C)	Apal (GGGCC↓C), BseSI* (GGGCC↓C), Eco24I* (GGGCC↓C)	Apal, BseSI, Bsp120I, BspLI, BsuRI,
		Cfr13I, CviJI, Eco24I, Sdul
Sdul* (GGGCT↓C)	Eco24I* (GGGCTJC)	CviJI, Eco24I, Sdul
Sdul* (GTGCA↓C)	<mark>Alw21I</mark> * (GTGCA↓C), <mark>BseSI</mark> * (GTGCA↓C)	Alw21I, Alw44I, BseSI, Hpy8I, HpyCH4V,
		Sdul
	Mph1103I (ATGCA↓T)	HpyCH4V
	Pstl (CTGCA↓G), Sdal (CCTGCA↓GG)	Bsgl, HpyCH4V
Sdul* (GTGCC↓C)	BseSI* (GTGCC↓C)	BseSI, Sdul
Sdul* (GTGCT↓C)	Alw21I* (GTGCT↓C)	Alw21I, Sdul
SexAI* (A CCAGGT)	EcoRII* (↓CCAGG)	Bme1390I, BssKI, EcoRII, Mval
SexAI* (A↓CCTGGT)	EcoRII* (↓CCTGG)	Bme1390I, BssKI, EcoRII, Mval
Sgfl (GCGAT↓CGC)	Bsh1285I* (CGAT↓CG), Pvul (CGAT↓CG)	Bsh1285I, Bsp143I, DpnI, Mbol, Pvul
	Paci (TTAAT TAA)	Tru1l
SgrAI* (CA↓CCGGYG)	BsaWI* (W↓CCGGA), <mark>Kpn2I</mark> (T↓CCGGA)	BsaWI, Hpall, Mspl
	BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I* (R↓CCGGT)	BsaWI, BshTI, Cfr10I, Hpall, Mspl
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC)	Cfr10I, Hpall, Mspl
	Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
SgrAl* (CG↓CCGGYG)	BsaWI* (W↓CCGGA), <mark>Kpn2I</mark> (T↓CCGGA)	Hpall, Mspl
	BsaWI* (W↓CCGGT), BshTI (A↓CCGGT), Cfr10I * (R↓CCGGT)	Cfr10I, Hpall, Mspl
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC)	Cac8I, Cfr10I, Hpall, Mspl, NgoMIV, Pdil
	Cfr9I (C↓CCGGG), Eco88I* (C↓CCGGG)	Bcnl, Bme1390I, BssKI, Hpall, Mspl
SgsI (GG↓CGCGCC)	Afilli* (A↓CGCGT), BtgI* (C↓CGCGG), MIuI (A↓CGCGT)	Bsh1236I, Hhal, Hin6I
	Paul (G ¹ CGCGC)	Bsh1236I, Cac8I, Hhal, Hin6I, Paul
Smol* (C↓TCGAG)	Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT),	Eco88I, Smol, Taql, Xhol
	Xhol (C↓TCGAG)	
	Sall (G↓TCGAC)	Taql
Smol* (C↓TTAAG)	BspTI (C↓TTAAG)	BspTI, Smol, Tru1I
<mark>Ssil</mark> * (C↓CGC)	Hin1I* (GR↓CGCC), Hin6I (G↓CGC), Narl (GG↓CGCC)	Ssil
	Hpall (C↓CGG), Mspl (C↓CGG)	Hpall, Mspl
<mark>Ssil</mark> * (G↓CGG)	Hin1I* (GR↓CGCC), Hin6I (G↓CGC), Narl (GG↓CGCC)	Hhal, Hin6l
Tail (ACGT↓)	AatII (GACGT↓C)	Maell, Tail
TaqI (T↓CGA)	Bsp119I (TT↓CGAA), Bsu15I (AT↓CGAT), XmiI* (GT↓CGAC)	Taql
Tasl (↓AATT)	EcoRI (G↓AATTC), MunI (C↓AATTG), XapI* (R↓AATTC), XapI* (R↓AATTT)	Tasl
Tatl* (A↓GTACW)	Acc65I (G↓GTACC), BshNI* (G↓GTACC), PfI23II (C↓GTACG)	Csp6l, Rsal
	Bsp1407I (T↓GTACA)	Csp6I, Rsal, Tatl
Tatl* (T↓GTACW)	Acc65I (G↓GTACC), BshNI* (G↓GTACC), PfI23II (C↓GTACG)	Csp6l, Rsal
	Bsp1407I (T↓GTACA)	Bsp1407I, Csp6I, Rsal, Tatl
Tru1I (T↓TAA)	VspI (AT↓TAAT)	Tru1I
VspI (AT↓TAAT)	Tru1I (T↓TAA)	Tru1I
Xapl* (A↓AATTY)	ECORI (G↓AATTC)	Tasl, Xapl
	Muni (C↓AATTG), Tasi (↓AATT)	Tasl
Xapl* (G↓AATTY)	Ecori (GJAATTĆ)	EcoRI, Tasl, Xapl
	Muni (C↓AATTG), Tasi (↓AATT)	Tasl
Xbal (T↓CTAGA)	Bcul (A↓CTAGT), Eco130I* (C↓CTAGG), Nhel (G↓CTAGC), XmaJI (C↓CTAGG)	FspBI
Xcel* (ACATG↓Y)	Hin1II (CATG)	CviAll, Fatl, Hin1II
	Pael (GCATG ¹ C)	CviAll, Fatl, Hin1II, Xcel
		CviAll, Fatl, Hin1ll
Xcel* (GCATG↓Y)	Hin1II (CATG) Pael (GCATG C)	
Xcel* (GCATG↓Y)	Pael (GCATG↓C)	Cac8I, CviAII, FatI, Hin1II, PaeI, XceI
	Pael (GCATG↓C) Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT),	
Xcel* (GCATG↓Y)	Pael (GCATG↓C) Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), SmoI* (C↓TCGAG)	Cac8l, CviAll, Fatl, Hin1ll, Pael, Xcel Eco88l, Smol, Taql, Xhol
Xcel* (GCATG↓Y) Xhol (C↓TCGAG)	Pael (GCATG↓C) Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), SmoI* (C↓TCGAG) Sall (G↓TCGAC)	Cac8l, CviAll, Fatl, Hin1ll, Pael, Xcel Eco88l, Smol, Taql, Xhol Taql
Xcel* (GCATG↓Y)	Pael (GCATG↓C) Eco88I* (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), SmoI* (C↓TCGAG)	Cac8l, CviAll, Fatl, Hin1ll, Pael, Xcel Eco88l, Smol, Taql, Xhol