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 consistency and lot-to-lot reproducibility.
PureExtreme™ Quality will provide the performance you need for your most demanding experiments.

RESTRICTION ENDONUCLEASES

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 approximately 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

Save Time – Digest DNA in Just 5min!

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:

Features

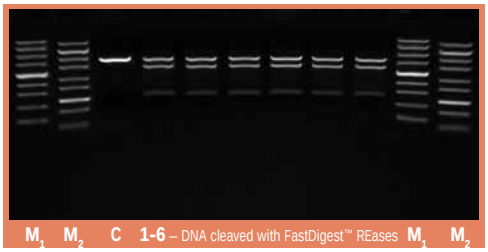
- Single and double digestions of plasmid DNA in just 5 minutes.
- Enhanced performance in one hour DNA cleavage reactions.
- A single reaction buffer for all FastDigest™ enzymes.

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.



M_1, M_2 – ZipRuler™ Express DNA Ladder Set (#SM1373)
C – control pUC19 DNA digested with FastDigest™ EcoRI and HindIII
1-6 – 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).

Table 1.1. FastDigest™ Restriction Endonucleases.

FastDigest™ Enzyme	Specificity 5' → 3'	Catalog #	Page #
ApaI	GGGCC↓C	ER1414	30
BamHI	G↓GATCC	ER0054	31
BcuI (SpeI)	A↓CTAGT	ER1254	33
BglII	A↓GATCT	ER0084	37
BstXI	CCANNNNN↓NTGG	ER1024	52
Bsu15I (ClaI)	AT↓CGAT	ER0144	53
Eco32I (EcoRV)	GAT↓ATC	ER0304	62
EcoRI	G↓AATTC	ER0274	69
HindIII	A↓AGCTT	ER0504	76
KpnI	GGTAC↓C	ER0524	80
NcoI	C↓CATGG	ER0574	88
NdeI	CA↓TATG	ER0584	89
NotI	GC↓GGCCGC	ER0594	90
PaeI (SphI)	GCATG↓C	ER0604	92
PstI	CTGCA↓G	ER0614	99
SacI	GAGCT↓C	ER1134	102
SmaI	CCC↓GGG	ER0664	106
XbaI	T↓CTAGA	ER0684	116
XhoI	C↓TCGAG	ER0694	117

N = G, A, T or C

Analyze Your Clones 3X Faster with Fermentas

Protocol for Fast Clone Analysis

1. Purify DNA from 1.5ml of overnight cultures using GeneJET™ Plasmid Miniprep Kit (#K0501). **13min**
2. Pipette 2µl (~0.2µg) of each miniprep DNA into thin-wall tubes. **2min**
3. Prepare the following reaction master mix: **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

4. Add 18µl of the master mix into each tube with plasmid DNA, mix and spin down. **3min**
5. Incubate at 37°C for 5 minutes to digest DNA. **5min**
6. Add 4µl of an appropriate 6X loading dye solution into each tube and mix. **2min**
7. Load on a 0.8-1% agarose gel and run electrophoresis for 10-20min using the ZipRuler™ Express DNA Ladder Set (#SM1373). **15min**

Total time: **43min**

Note

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

Fermentas Restriction Endonucleases

Table 1.2. Fermentas Restriction Endonucleases.

Fermentas Enzyme	Prototype	Specificity 5' → 3'	Catalog #	Page #
AarI	AarI	CACCTGC(4/8)↓	ER1581/2	24
AasI	DrdI	GACNNNN↓NNGTC	ER1721/2	24
AatII	AatII	GACGT↓C	ER0991/2	25
Acc65I	KpnI (GGTAC↓C)	G↓GTACC	ER0901/2	25
AdeI	DraIII	CACNNN↓GTG	ER1231/2	26
NEW AjiI	BtrI	CAC↓GTC	ER1941	26
NEW AjuI	AjuI	↓(7/12)GAA(N)$_7$TTGG(11/6)↓	ER1951	27
AlfI	AlfI	↓(10/12)GCA(N)$_6$TGC(12/10)↓	ER1801	27
AloI	AloI	↓(7/12-13)GAAC(N)$_6$TCC(12-13/7)↓	ER1491/2	28
AluI	AluI	AG↓CT	ER0011/2	28
Alw21I	HgiAI	GWGCW↓C	ER0021/2	29
Alw26I	BsmAI	GTCTC(1/5)↓	ER0031/2	29
Alw44I	ApaLI	G↓TGCAC	ER0041/2	30
ApaI	ApaI	GGGCC↓C	ER1411/2/4	30
BamHI	BamHI	G↓GATCC	ER0051/2/3/4	31
BauI	BsiI	CACGAG(-5/-1)↓	ER1841	32
BclI	BclI	T↓GATCA	ER0721/2	32
BcnI	CauII	CC↓SGG	ER0061/2	33
BcuI	SpeI	A↓CTAGT	ER1251/2/4	33
NEW BdaI	BdaI	↓(10/12)TGA(N)$_6$TCA(12/10)↓	ER1961	34
BfiI	BfiI	ACTGGG(5/4)↓	ER1591/2	35
BfmI	SfeI	C↓TRYAG	ER1161/2	35
BfuI	BciVI	GTATCC(6/5)↓	ER1501/2	36
BglI	BglI	GCCNNNN↓NGGC	ER0071/2	36
BglII	BglII	A↓GATCT	ER0081/2/4	37
Bme1390I	ScrFI	CC↓NGG	ER1421/2	37
BoxI	PshAI	GACNN↓NNGTC	ER1431	38
BpiI	BbvII	GAAGAC(2/6)↓	ER1011/2	38
BplI	BplI	↓(8/13)GAG(N)$_5$CTC(13/8)↓	ER1311/2	39
Bpu10I	Bpu10I	CCTNAGC(-5/-2)↓	ER1181/2	39
Bpu1102I	EspI	GC↓TNAGC	ER0091/2	40
BseDI	SecI	C↓CNNGG	ER1081/2	40
BseGI	FokI (GGATG(9/13)↓)	GGATG(2/0)↓	ER0871/2	41
BseJI	BsaBI	GATNN↓NNATC	ER1711	41
BseLI	BsiYI	CCNNNNN↓NNGG	ER1201/2	42
BseMI	BsrDI	GCAATG(2/0)↓	ER1261/2	42
BseMII	BseMII	CTCAG(10/8)↓	ER1401/2	43
BseNI	BsrI	ACTGG(1/-1)↓	ER0881/2	43
BseSI	BseSI	GKGCM↓C	ER1441/2	44
BseXI	BbvI	GCAGC(8/12)↓	ER1451/2	44
Bsh1236I	FnuDII	CG↓CG	ER0921/2	45
Bsh1285I	McrI	CGRY↓CG	ER0891	45
BshNI	HgiCI	G↓GYRCC	ER1001	46
BshTI	AgeI	A↓CCGGT	ER1461/2	46
Bsp68I	NruI	TCG↓CGA	ER0111/2	47
Bsp119I	AsuII	TT↓CGAA	ER0121	47
Bsp120I	ApaI (GGGCC↓C)	G↓GGCCC	ER0131/2/3	48
Bsp143I	MboI	↓GATC	ER0781/2	48
Bsp143II	HaeII	RGCGC↓Y	ER0791/2	49
Bsp1407I	Bsp1407I	T↓GTACA	ER0931/2	49
BspLI	NlaIV	GGN↓NCC	ER1151/2	50
BspPI	BinI	GGATC(4/5)↓	ER1321/2	50
BspTI	AflII	C↓TTAAG	ER0831/2	51
Bst1107I	SnaI	GTA↓TAC	ER0701/2	52
BstXI	BstXI	CCANNNNN↓NTGG	ER1021/2/4	52
Bsu15I	ClaI	AT↓CGAT	ER0141/2/4	53
BsuRI	HaeIII	GG↓CC	ER0151/2	53
BveI	BspMI	ACCTGC(4/8)↓	ER1741	54
CaiI	AlwNI	CAGNNN↓CTG	ER1391/2	54
CfrI	CfrI	Y↓GGCCR	ER0161/2	55
Cfr9I	SmaI (CCC↓GGG)	C↓CCGGG	ER0171/2	55
Cfr10I	Cfr10I	R↓CCGGY	ER0181/2	56
Cfr13I	AsuI	G↓GNCC	ER0191/2	56

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Single letter code

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M = A or C; D = A, G or T;
K = G or T; N = G, A, T or C.
S = C or G;

Table 1.2. Fermentas Restriction Endonucleases.

Fermentas Enzyme	Prototype	Specificity 5' → 3'	Catalog #	Page #
Cfr42I	SacII	CCGC↓GG	ER0201/2	57
CpoI	RsrII	CG↓GWCCG	ER0741/2	57
NEW **CseI**	HgaI	GACGC(5/10)↓	ER1901/2	58
Csp6I	RsaI (GT↓AC)	G↓TAC	ER0211	58
DpnI	DpnI	Gm6A↓TC	ER1701/2	59
DraI	AhaIII	TTT↓AAA	ER0221/2/3	59
Eam1104I	Ksp632I	CTCTTC(1/4)↓	ER0231/2	60
Eam1105I	Eam1105I	GACNNN↓NNGTC	ER0241/2	60
Ecl136II	SacI (GAGCT↓C)	GAG↓CTC	ER0251	61
Eco24I	HgiJII	GRGCY↓C	ER0281	61
Eco31I	Eco31I	GGTCTC(1/5)↓	ER0291/2	62
Eco32I	EcoRV	GAT↓ATC	ER0301/2/3/4	62
Eco47I	AvaII	G↓GWCC	ER0311/2	63
Eco47III	Eco47III	AGC↓GCT	ER0321/2	63
Eco52I	XmaIII	C↓GGCCG	ER0331/2	64
Eco57I	Eco57I	CTGAAG(16/14)↓	ER0341/2	64
Eco57MI	Eco57MI	CTGRAG(16/14)↓	ER1671	65
Eco72I	PmaCI	CAC↓GTG	ER0361/2	65
Eco81I	SauI	CC↓TNAGG	ER0371/2	66
Eco88I	AvaI	C↓YCGRG	ER0381	66
Eco91I	BstEII	G↓GTNACC	ER0391/2	67
Eco105I	SnaBI	TAC↓GTA	ER0401/2	67
Eco130I	StyI	C↓CWWGG	ER0411	68
Eco147I	StuI	AGG↓CCT	ER0421/2	68
EcoO109I	DraII	RG↓GNCCY	ER0261	69
EcoRI	EcoRI	G↓AATTC	ER0271/2/3/4	69
NEW **EcoRII**	EcoRII	↓CCWGG	ER1921/2	70
EheI	NarI (GG↓CGCC)	GGC↓GCC	ER0441	70
Esp3I	Esp3I	CGTCTC(1/5)↓	ER0451/2	71
FaqI	FinI	GGGAC(10/14)↓	ER1811	71
FspAI	FspAI	RTGC↓GCAY	ER1661/2	72
FspBI	MaeI	C↓TAG	ER1761/2	72
GsuI	GsuI	CTGGAG(16/14)↓	ER0461/2	73
HhaI	HhaI	GCG↓C	ER1851	73
Hin1I	AcyI	GR↓CGYC	ER0471/2	74
Hin1II	NlaIII	CATG↓	ER1831	74
Hin4I	Hin4I	↓(8/13-14)GAY(N)$_5$VTC(13-14/8)↓	ER1601/2	75
Hin6I	HhaI (GCG↓C)	G↓CGC	ER0481/2	75
HincII	HindII	GTY↓RAC	ER0491/2	76
HindIII	HindIII	A↓AGCTT	ER0501/2/3/4	76
HinfI	HinfI	G↓ANTC	ER0801/2/3	77
HpaII	HpaII	C↓CGG	ER0511/2	77
HphI	HphI	GGTGA(8/7)↓	ER1101/2	78
Hpy8I	MjaIV	GTN↓NAC	ER1571/2	78
NEW **HpyF3I**	DdeI	C↓TNAG	ER1881/2	79
HpyF10VI	MwoI	GCNNNNN↓NNGC	ER1731/2	79
KpnI	KpnI	GGTAC↓C	ER0521/2/3/4	80
Kpn2I	BspMII	T↓CCGGA	ER0531/2	80
KspAI	HpaI	GTT↓AAC	ER1031/2	81
NEW **LguI**	SapI	GCTCTTC(1/4)↓	ER1931/2	81
LweI	SfaNI	GCATC(5/9)↓	ER1621/2	82
MbiI	BsrBI	GAGCGG(-3/-3)↓	ER1271	82
MboI	MboI	↓GATC	ER0811/2	83
MboII	MboII	GAAGA(8/7)↓	ER0821/2	83
MlsI	BalI	TGG↓CCA	ER1211/2	84
MluI	MluI	A↓CGCGT	ER0561/2	84
MnlI	MnlI	CCTC(7/6)↓	ER1071/2	85
Mph1103I	AvaIII	ATGCA↓T	ER0731/2	85
MspI	HpaII	C↓CGG	ER0541/2	86
MssI	PmeI	GTTT↓AAAC	ER1341/2	86
MunI	MfeI	C↓AATTG	ER0751/2	87
MvaI	EcoRII (↓CCWGG)	CC↓WGG	ER0551/2	87
Mva1269I	BsmI	GAATGC(1/-1)↓	ER0961/2	88
NcoI	NcoI	C↓CATGG	ER0571/2/4	88
NdeI	NdeI	CA↓TATG	ER0581/2/4	89
NheI	NheI	G↓CTAGC	ER0971/2	89

(continued on next page)

Table 1.2. Fermentas Restriction Endonucleases.

Fermentas Enzyme	Prototype	Specificity 5' → 3'	Catalog #	Page #
NmuCI	Tsp45I	↓GTSAC	ER1511/2	90
NotI	NotI	GC↓GGCCGC	ER0591/2/3/4	90
NsbI	MstI	TGC↓GCA	ER1221/2	91
OliI	OliI	CACNN↓NNGTG	ER1631/2	91
PaeI	SphI	GCATG↓C	ER0601/2/4	92
PagI	BspHI	T↓CATGA	ER1281/2	92
PasI	PasI	CC↓CWGGG	ER1861	93
PauI	BsePI	G↓CGCGC	ER1091/2	93
PdiI	NaeI	GCC↓GGC	ER1521/2	94
PdmI	XmnI	GAANN↓NNTTC	ER1531/2	94
PfeI	TfiI	G↓AWTC	ER1781	95
Pfl23II	SpII	C↓GTACG	ER0851	95
PfoI	PfoI	T↓CCNGGA	ER1751	96
PpiI	PpiI	↓(7/12)GAAC(N)$_5$CTC(13/8)↓	ER1541/2	96
NEW Ppu21I	BsaAI	YAC↓GTR	ER1971	97
NEW PscI	BspLU11I	A↓CATGT	ER1871/2	97
Psp5II	PpuMI	RG↓GWCCY	ER0761	98
Psp1406I	AclI	AA↓CGTT	ER0941/2	98
PstI	PstI	CTGCA↓G	ER0611/2/3/4	99
PsuI	XhoII	R↓GATCY	ER1551	99
PsyI	Tth111I	GACN↓NNGTC	ER1331	100
PvuI	PvuI	CGAT↓CG	ER0621/2	100
PvuII	PvuII	CAG↓CTG	ER0631/2/3	101
RsaI	RsaI	GT↓AC	ER1121/2	101
SacI	SacI	GAGCT↓C	ER1131/2/3/4	102
SalI	SalI	G↓TCGAC	ER0641/2/3	102
SatI	Fnu4HI	GC↓NGC	ER1641/2	103
ScaI	ScaI	AGT↓ACT	ER0431/2	103
SchI	PleI (GAGTC(4/5)↓)	GAGTC(5/5)↓	ER1371	104
SdaI	Sse8387I	CCTGCA↓GG	ER1191/2	104
SduI	SduI	GDGCH↓C	ER0651	105
SfiI	SfiI	GGCCNNNN↓NGGCC	ER1821	105
NEW SgsI	AscI	GG↓CGCGCC	ER1891/2	106
SmaI	SmaI	CCC↓GGG	ER0661/2/3/4	106
SmiI	SwaI	ATTT↓AAAT	ER1241/2	107
NEW SmoI	SmlI	C↓TYRAG	ER1981	107
SmuI	FauI	CCCGC(4/6)↓	ER1691/2	108
SsiI	AciI	CCGC(-3/-1)↓	ER1791	108
SspI	SspI	AAT↓ATT	ER0771/2	109
TaaI	Tsp4CI	ACN↓GT	ER1361/2	109
TaiI	MaeII (A↓CGT)	ACGT↓	ER1141/2	110
TaqI	TaqI	T↓CGA	ER0671/2/3	110
TasI	TspEI	↓AATT	ER1351/2	111
TatI	TatI	W↓GTACW	ER1291/2	111
TauI	TauI	GCSG↓C	ER1651/2	112
Tru1I	MseI	T↓TAA	ER0981/2/3	112
NEW TsoI	TsoI	TARCCA(11/9)↓	ER1991	113
NEW TstI	TstI	↓(8/13)CAC(N)$_6$TCC(12/7)↓	ER1911	113
Van91I	PflMI	CCANNNN↓NTGG	ER0711/2	114
VspI	VspI	AT↓TAAT	ER0911/2	114
XagI	EcoNI	CCTNN↓NNNAGG	ER1301/2	115
XapI	ApoI	R↓AATTY	ER1381/2	115
XbaI	XbaI	T↓CTAGA	ER0681/2/3/4	116
XceI	NspI	RCATG↓Y	ER1471/2	116
XhoI	XhoI	C↓TCGAG	ER0691/2/3/4	117
XmaJI	AvrII	C↓CTAGG	ER1561/2	117
XmiI	AccI	GT↓MKAC	ER1481/2	118
Nb.Bpu10I	Nb.Bpu10I	GC↓TNAGG CG ANTCC	ER1681	119
I-SceI	I-SceI	TAGGG ATAA↓CAGGGTAAT ATCCC↑TATT GTCCCATTA	ER1771	120

Single letter code

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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 www.fermentas.com/research, **DoubleDigest™** at www.fermentas.com/doubledigest 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".
- DpnI requires the presence of N6-methyladenine within the recognition sequence GATC.
- Enzymes produced by Fermentas are shown in orange.

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
AarI	CACCTGC(4/8)↓	**AarI** (ER1581/2, p.24)	
AasI	GACNNNN↓NNGTC	**AasI** (ER1721/2, p.24)	DrdI, DseDI
AatI	AGG↓CCT	**Eco147I** (ER0421/2, p.68)	**Eco147I**, PceI, StuI
AatII	GACGT↓C	**AatII** (ER0991/2, p.25)	(ZraI)
AccI	GT↓MKAC	**XmiI** (ER1481/2, p.118)	FblI, **XmiI**
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	**NsbI** (ER1221/2, p.91)	AviII, FspI, **NsbI**
Acc36I	ACCTGC(4/8)↓	**BveI** (ER1741, p.54)	BfuAI, BspMI, **BveI**
Acc65I	G↓GTACC	**Acc65I** (ER0901/2/4, p.25), (**KpnI**^{m}) (GGTAC↓C) (ER0521/2/3/4, p.80)	Asp718I, (**KpnI**^{m})
AccB1I	G↓GYRCC	**BshNI** (ER1001, p.46)	BanI, **BshNI**, BspT107I
AccB7I	CCANNNN↓NTGG	**Van91I** (ER0711/2, p.114)	PflMI, **Van91I**
AccBSI	CCGCTC(-3/-3)↓	**MbiI** (ER1271, p.82)	BsrBI, **MbiI**
AciI	CCGC(-3/-1)↓	**SsiI** (ER1791, p.108)	**SsiI**
AclI	AA↓CGTT	**Psp1406I** (ER0941/2, p.98)	**Psp1406I**
AclWI	GGATC(4/5)↓	**BspPI** (ER1321/2, p.50)	AlwI, **BspPI**
AcsI	R↓AATTY	**XapI** (ER1381/2/4, p.115)	ApoI, **XapI**
AcuI	CTGAAG(16/14)↓	**Eco57I** (ER0341/2, p.64)	**Eco57I**
AcyI	GR↓CGYC	**Hin1I** (ER0471/2, p.74)	BsaHI, BstACI, **Hin1I**, Hsp92I
AdeI	CACNNN↓GTG	**AdeI** (ER1231/2, p.26)	DraIII
AfaI	GT↓AC	(**Csp6I**^{m}) (G↓TAC) (ER0211, p.58), **RsaI** (ER1121/2, p.101)	(**Csp6I**^{m}), **RsaI**
AfeI	AGC↓GCT	**Eco47III** (ER0321/2, p.63)	Aor51HI, **Eco47III**
AflII	C↓TTAAG	**BspTI** (ER0831/2, p.51)	BfrI, **BspTI**, Bst98I, Vha464I
AflIII	A↓CRYGT		
AgeI	A↓CCGGT	**BshTI** (ER1461/2, p.46)	AsiGI, **BshTI**, PinAI
AhdI	GACNNN↓NNGTC	**Eam1105I** (ER0241/2, p.60)	AspEI, DriI, **Eam1105I**, EclHKI
AhlI	A↓CTAGT	**BcuI** (ER1251/2, p.33)	**BcuI**, SpeI
AjiI	CACGTC(-3/-3)↓	**AjiI** (ER1941, p.26)	BmgBI, BtrI
AjnI	↓CCWGG	**EcoRII**^{m} (ER1921/2, p.70), (**MvaI**) (CC↓WGG) (ER0551/2, p.87)	(BstNI), (BstOI), (Bst2UI), **EcoRII**^{m}, (**MvaI**), Psp6I, PspGI^{m}
AjuI	↓(7/12)GAANNNNNNNTTGG(11/6)↓	**AjuI** (ER1951, p.27)	
AleI	CACNN↓NNGTG	**OliI** (ER1631/2, p.91)	**OliI**
AlfI	↓(10/12)GCANNNNNNTGC(12/10)↓	**AlfI** (ER1801, p.27)	

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
AloI	↓(7/12-13)GAACNNNNNNTCC(12-13/7)↓	**AloI** (ER1491/2, p.28)	
AluI	AG↓CT	**AluI** (ER0011/2, p.27)	
AlwI	GGATC(4/5)↓	**BspPI** (ER1321/2, p.50)	AclWI, **BspPI**
Alw21I	GWGCW↓C	**Alw21I** (ER0021/2, p.29)	AspHI, Bbv12I, BsiHKAI
Alw26I	GTCTC(1/5)↓	**Alw26I** (ER0031/2, p.29)	BsmAI[m], BstMAI
Alw44I	G↓TGCAC	**Alw44I** (ER0041/2, p.30)	ApaLI[m], VneI
AlwNI	CAGNNN↓CTG	**CaiI** (ER1391/2, p.54)	**CaiI**
Ama87I	C↓YCGRG	**Eco88I** (ER0381/4, p.66)	AvaI, BsoBI, **Eco88I**
Aor13HI	T↓CCGGA	**Kpn2I** (ER0531/2, p.80)	AccIII, BseAI, Bsp13I, BspEI, **Kpn2I**, MroI
Aor51HI	AGC↓GCT	**Eco47III** (ER0321/2, p.63)	AfeI, **Eco47III**
ApaI	GGGCC↓C	**ApaI** (ER1411/2, p.30), (**Bsp120I**) (G↓GGCCC) (ER0131/2/3, p.48)	(**Bsp120I**), (PspOMI)
ApaLI	G↓TGCAC	**Alw44I**[m] (ER0041/2, p.30)	**Alw44I**[m], VneI
ApeKI	G↓CWGC		TseI
ApoI	R↓AATTY	**XapI** (ER1381/2/4, p.115)	AcsI, **XapI**
AscI	GG↓CGCGCC	**SgsI** (ER1891/2, p.106)	PalAI, **SgsI**
AseI	AT↓TAAT	**VspI** (ER0911/2, p.114)	PshBI, **VspI**
AsiGI	A↓CCGGT	**BshTI** (ER1461/2, p.46)	AgeI, **BshTI**, PinAI
AsiSI	GCGAT↓CGC		RgaI, SgfI
AspI	GACN↓NNGTC	**PsyI** (ER1331, p.100)	PflFI, **PsyI**, Tth111I
Asp700I	GAANN↓NNTTC	**PdmI** (ER1531/2, p.94)	MroXI, **PdmI**, XmnI[m]
Asp718I	G↓GTACC	**Acc65I** (ER0901/2/4, p.25), (**KpnI**[m]) (GGTAC↓C) (ER0521/2/3/4, p.80)	**Acc65I**, (**KpnI**[m])
AspA2I	C↓CTAGG	**XmaJI** (ER1561/2, p.117)	AvrII, BlnI, **XmaJI**
AspEI	GACNNN↓NNGTC	**Eam1105I** (ER0241/2, p.60)	AhdI, DriI, **Eam1105I**, EclHKI
AspHI	GWGCW↓C	**Alw21I** (ER0021/2, p.29)	**Alw21I**, Bbv12I, BsiHKAI
AspLEI	GCG↓C	**HhaI** (ER1851, p.73), (**Hin6I**) (G↓CGC) (ER0481/2, p.75)	BstHHI, CfoI, **HhaI**, (**Hin6I**), (HinP1I), (HspAI)
AspS9I	G↓GNCC	**Cfr13I** (ER0191/2, p.56)	**Cfr13I**, Sau96I
AsuC2I	CC↓SGG	**BcnI** (ER0061/2, p.33)	**BcnI**, NciI
AsuHPI	GGTGA(8/7)↓	**HphI** (ER1101/2, p.78)	**HphI**
AsuNHI	G↓CTAGC	**NheI** (ER0971/2, p.89)	(BmtI), **NheI**
AvaI	C↓YCGRG	**Eco88I**[m] (ER0381/4, p.66)	Ama87I, BsoBI[m], **Eco88I**[m]
AvaII	G↓GWCC	**Eco47I** (ER0311/2, p.63)	Bme18I, **Eco47I**, SinI, VpaK11BI
AviII	TGC↓GCA	**NsbI** (ER1221/2, p.91)	Acc16I, FspI, **NsbI**
AvrII	C↓CTAGG	**XmaJI** (ER1561/2, p.117)	AspA2I, BlnI, **XmaJI**
AxyI	CC↓TNAGG	**Eco81I** (ER0371/2, p.66)	Bse21I, Bsu36I, **Eco81I**
BaeI	↓(10/15)ACNNNNGTAYC(12/7)↓		
BalI	TGG↓CCA	**MlsI** (ER1211/2, p.84)	**MlsI**, MluNI, MscI, Msp20I
BamHI	G↓GATCC	**BamHI** (ER0051/2/3/4, p.31)	
BanI	G↓GYRCC	**BshNI** (ER1001, p.46)	AccB1I, **BshNI**, BspT107I
BanII	GRGCY↓C	**Eco24I** (ER0281/4, p.61)	**Eco24I**, EcoT38I, FriOI
BanIII	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	Bsa29I, Bsp106I, BspDI, BspXI, **Bsu15I**, ClaI
BauI	CACGAG(-5/-1)↓	**BauI** (ER1841, p.32)	BssSI, Bst2BI
BbeI	GGCGC↓C	(**EheI**) (GGC↓GCC) (ER0441, p.70)	(EgeI), (**EheI**), (KasI), (Mly113I), (NarI[m]), (SfoI[m])
BbrPI	CAC↓GTG	**Eco72I** (ER0361/2, p.65)	**Eco72I**, PmaCI, PmlI, PspCI
BbsI	GAAGAC(2/6)↓	**BpiI** (ER1011/2, p.38)	**BpiI**, BpuAI, BstV2I
BbuI	GCATG↓C	**PaeI** (ER0601/2/4, p.92)	**PaeI**, SphI
BbvI	GCAGC(8/12)↓	**BseXI** (ER1451/2, p.44)	**BseXI**, BstV1I
Bbv12I	GWGCW↓C	**Alw21I** (ER0021/2, p.29)	**Alw21I**, AspHI, BsiHKAI
BbvCI	CCTCAGC(-5/-2)↓		
BccI	CCATC(4/5)↓		
BceAI	ACGGC(12/14)↓		
BcgI	↓(10/12)CGANNNNNNTGC(12/10)↓		
BciVI	GTATCC(6/5)↓	**BfuI** (ER1501/2, p.36)	**BfuI**
BclI	T↓GATCA	**BclI** (ER0721/2, p.32)	FbaI, Ksp22I
BcnI	CC↓SGG	**BcnI** (ER0061/2, p.33)	AsuC2I, NciI
BcuI	A↓CTAGT	**BcuI** (ER1251/2, p.33)	AhlI, SpeI
BdaI	↓(10/12)TGANNNNNNTCA(12/10)↓	**BdaI** (ER1961, p.34)	
BfaI	C↓TAG	**FspBI** (ER1761/2, p.72)	**FspBI**, MaeI, XspI
BfiI	ACTGGG(5/4)↓	**BfiI** (ER1591/2, p.35)	BmrI
BfmI	C↓TRYAG	**BfmI** (ER1161/2, p.35)	BstSFI, SfcI
BfrI	C↓TTAAG	**BspTI** (ER0831/2, p.51)	AflII, **BspTI**, Bst98I, Vha464I
BfrBI	ATG↓CAT	(**Mph1103I**) (ATGCA↓T) (ER0731/2, p.85)	(EcoT22I), (**Mph1103I**), (NsiI), (Zsp2I)
BfuI	GTATCC(6/5)↓	**BfuI** (ER1501/2, p.36)	BciVI
BfuAI	ACCTGC(4/8)↓	**BveI** (ER1741, p.54)	Acc36I, BspMI, **BveI**

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
BfuCI	↓GATC	**Bsp143I** (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI**[m] (ER0811/2, p.83)	**Bsp143I**, (BstKTI[m]), BstMBI, (**DpnI**[m]), DpnII[m], Kzo9I, (MalI[m]), **MboI**[m], NdeII[m], Sau3AI
BglI	GCCNNNN↓NGGC	**BglI** (ER0071/2, p.36)	
BglII	A↓GATCT	**BglII** (ER0081/2, p.37)	
BisI	GC↓NGC	**SatI**[m] (ER1641/2, p.103)	Fnu4HI[m], Fsp4HI[m], ItaI[m], **SatI**[m]
BlnI	C↓CTAGG	**XmaJI** (ER1561/2, p.117)	AspA2I, AvrII, **XmaJI**
BlpI	GC↓TNAGC	**Bpu1102I** (ER0091/2, p.40)	**Bpu1102I**, Bsp1720I, CelII
Bme18I	G↓GWCC	**Eco47I** (ER0311/2, p.63)	AvaII, **Eco47I**, SinI, VpaK11BI
Bme1390I	CC↓NGG	**Bme1390I** (ER1421/2, p.37)	(BssKI), (BstSCI), MspR9I, ScrFI, (StyD4I)
Bme1580I	GKGCM↓C	**BseSI** (ER1441/2, p.44)	**BseSI**
BmgBI	CACGTC(-3/-3)↓	**AjiI** (ER1941, p.26)	**AjiI**, BtrI
BmrI	ACTGGG(5/4)↓	**BfiI** (ER1591/2, p.35)	**BfiI**
BmtI	GCTAG↓C	(**NheI**[m]) (G↓CTAGC) (ER0971/2, p.89)	(AsuNHI), (**NheI**[m])
BmyI	GDGCH↓C	**SduI** (ER0651, p.105)	Bsp1286I, MhlI, **SduI**
BoxI	GACNN↓NNGTC	**BoxI** (ER1431, p.38)	BstPAI, PshAI
BpiI	GAAGAC(2/6)↓	**BpiI** (ER1011/2, p.38)	BbsI, BpuAI, BstV2I
BplI	↓(8/13)GAGNNNNNCTC(13/8)↓	**BplI** (ER1311/2, p.39)	
BpmI	CTGGAG(16/14)↓	**GsuI**[m] (ER0461/2, p.73)	**GsuI**[m]
Bpu10I	CCTNAGC(-5/-2)↓	**Bpu10I** (ER1181/2, p.39)	
Bpu14I	TT↓CGAA	**Bsp119I** (ER0121, p.47)	**Bsp119I**, BspT104I, BstBI, Csp45I, NspV, SfuI
Bpu1102I	GC↓TNAGC	**Bpu1102I** (ER0091/2, p.40)	BlpI, Bsp1720I, CelII
BpuAI	GAAGAC(2/6)↓	**BpiI** (ER1011/2, p.38)	BbsI, **BpiI**, BstV2I
BpuEI	CTTGAG(16/14)↓		
BsaI	GGTCTC(1/5)↓	**Eco31I** (ER0291/2, p.62)	Bso31I, **Eco31I**
Bsa29I	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsp106I, BspDI, BspXI, **Bsu15I**, ClaI
BsaAI	YAC↓GTR	**Ppu21I** (ER1971, p.97)	BstBAI, **Ppu21I**
BsaBI	GATNN↓NNATC	**BseJI** (ER1711, p.41)	Bse8I, **BseJI**, MamI
BsaHI	GR↓CGYC	**Hin1I**[m] (ER0471/2, p.74)	AcyI, BstACI, **Hin1I**[m], Hsp92I
BsaJI	C↓CNNGG	**BseDI** (ER1081/2, p.40)	**BseDI**, BssECI
BsaMI	GAATGC(1/-1)↓	**Mva1269I** (ER0961/2, p.88)	BsmI, **Mva1269I**, PctI
BsaWI	W↓CCGGW		
BsaXI	↓(9/12)ACNNNNNCTCC(10/7)↓		
Bsc4I	CCNNNNN↓NNGG	**BseLI** (ER1201/2, p.42)	**BseLI**, BsiYI, BslI
Bse1I	ACTGG(1/-1)↓	**BseNI** (ER0881/2, p.43)	**BseNI**, BsrI, BsrSI
Bse8I	GATNN↓NNATC	**BseJI** (ER1711, p.41)	BsaBI, **BseJI**, MamI
Bse21I	CC↓TNAGG	**Eco81I** (ER0371/2, p.66)	AxyI, Bsu36I, **Eco81I**
Bse118I	R↓CCGGY	**Cfr10I** (ER0181/2, p.56)	BsrFI, **Cfr10I**
BseAI	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, (FokI)
BseJI	GATNN↓NNATC	**BseJI** (ER1711, p.41)	BsaBI, Bse8I, MamI
BseLI	CCNNNNN↓NNGG	**BseLI** (ER1201/2, p.42)	Bsc4I, BsiYI, BslI
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
BsePI	G↓CGCGC	**PauI** (ER1091/2, p.93)	BssHII, **PauI**
BseRI	GAGGAG(10/8)↓		
BseSI	GKGCM↓C	**BseSI** (ER1441/2, p.44)	Bme1580I
BseXI	GCAGC(8/12)↓	**BseXI** (ER1451/2, p.52)	BbvI, BstV1I
BseX3I	C↓GGCCG	**Eco52I** (ER0331/2, p.64)	BstZI, EagI, EclXI, **Eco52I**
BseYI	CCCAGC(-5/-1)↓		
BsgI	GTGCAG(16/14)↓		
Bsh1236I	CG↓CG	**Bsh1236I** (ER0921/2, p.45)	AccII, BstFNI, BstUI, MvnI
Bsh1285I	CGRY↓CG	**Bsh1285I** (ER0891, p.45)	BsiEI, BstMCI
BshNI	G↓GYRCC	**BshNI** (ER1001, p.46)	AccB1I, BanI, BspT107I
BshTI	A↓CCGGT	**BshTI** (ER1461/2, p.46)	AgeI, AsiGI, PinAI
BsiEI	CGRY↓CG	**Bsh1285I** (ER0891, p.45)	**Bsh1285I**, BstMCI
BsiHKAI	GWGCW↓C	**Alw21I** (ER0021/2, p.29)	**Alw21I**, AspHI, Bbv12I
BsiWI	C↓GTACG	**Pfl23II** (ER0851, p.95)	**Pfl23II**, PspLI
BsiYI	CCNNNNN↓NNGG	**BseLI** (ER1201/2, p.42)	Bsc4I, **BseLI**, BslI
BslI	CCNNNNN↓NNGG	**BseLI** (ER1201/2, p.42)	Bsc4I, **BseLI**, BsiYI
BslFI	GGGAC(10/14)↓	**FaqI** (ER1811, p.71)	BsmFI, **FaqI**
BsmI	GAATGC(1/-1)↓	**Mva1269I** (ER0961/2, p.88)	BsaMI, **Mva1269I**, PctI
BsmAI	GTCTC(1/5)↓	**Alw26I**[m] (ER0031/2, p.29)	**Alw26I**[m], BstMAI

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
BsmBI	CGTCTC(1/5)↓	**Esp3I** (ER0451/2, p.71)	**Esp3I**
BsmFI	GGGAC(10/14)↓	**FaqI** (ER1811, p.71)	BslFI, **FaqI**
Bso31I	GGTCTC(1/5)↓	**Eco31I** (ER0291/2, p.62)	BsaI, **Eco31I**
BsoBI	C↓YCGRG	**Eco88I**[m] (ER0381/4, p.66)	Ama87I, AvaI[m], **Eco88I**[m]
Bsp13I	T↓CCGGA	**Kpn2I** (ER0531/2, p.80)	AccIII, Aor13HI, BseAI, BspEI, **Kpn2I**, MroI
Bsp19I	C↓CATGG	**NcoI** (ER0571/2, p.88)	**NcoI**
Bsp68I	TCG↓CGA	**Bsp68I** (ER0111/2, p.47)	NruI
Bsp106I	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsa29I, BspDI, BspXI, **Bsu15I**, ClaI
Bsp119I	TT↓CGAA	**Bsp119I** (ER0121, p.47)	Bpu14I, BspT104I, BstBI, Csp45I, NspV, SfuI[m]
Bsp120I	G↓GGCCC	(**ApaI**) (GGGCC↓C) (ER1411/2, p.30), **Bsp120I** (ER0131/2/3, p.48)	(**ApaI**), PspOMI
Bsp143I	↓GATC	**Bsp143I** (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI**[m] (ER0811/2, p.83)	BfuCI, (BstKTI[m]), BstMBI, (**DpnI**[m]), DpnII[m], Kzo9I, (MalI[m]), **MboI**[m], NdeII[m], Sau3AI
Bsp143II	RGCGC↓Y	**Bsp143II** (ER0791/2, p.49)	BstH2I, HaeII[m]
Bsp1286I	GDGCH↓C	**SduI** (ER0651, p.105)	BmyI, MhlI, **SduI**
Bsp1407I	T↓GTACA	**Bsp1407I** (ER0931/2, p.49)	BsrGI, BstAUI, SspBI
Bsp1720I	GC↓TNAGC	**Bpu1102I** (ER0091/2, p.40)	BlpI, **Bpu1102I**, CelII
BspCI	CGAT↓CG	**PvuI** (ER0621/2, p.100)	Ple19I, **PvuI**
BspCNI	CTCAG(9/7)↓	(**BseMII**) (CTCAG(10/8)) (ER1401/2, p.43)	(**BseMII**)
BspDI	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsa29I, Bsp106I, BspXI, **Bsu15I**, ClaI
BspEI	T↓CCGGA	**Kpn2I**[m] (ER0531/2, p.80)	AccIII, Aor13HI, BseAI, Bsp13I, **Kpn2I**[m], MroI[m]
BspHI	T↓CATGA	**PagI**[m] (ER1281/2, p.92)	**PagI**[m], RcaI
BspLI	GGN↓NCC	**BspLI** (ER1151/2, p.50)	NlaIV[m], PspN4I
BspLU11I	A↓CATGT	**PscI** (ER1871/2, p.97)	PciI, **PscI**
BspMI	ACCTGC(4/8)↓	**BveI** (ER1741, p.54)	Acc36I, BfuAI, **BveI**
BspPI	GGATC(4/5)↓	**BspPI** (ER1321/2, p.50)	AclWI, AlwI
BspTI	C↓TTAAG	**BspTI** (ER0831/2, p.51)	AflII, BfrI, Bst98I, Vha464I
BspT104I	TT↓CGAA	**Bsp119I** (ER0121, p.47)	Bpu14I, **Bsp119I**, BstBI, Csp45I, NspV, SfuI[m]
BspT107I	G↓GYRCC	**BshNI** (ER1001, p.46)	AccB1I, BanI, **BshNI**
BspXI	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsa29I, Bsp106I, BspDI, **Bsu15I**, ClaI
BsrI	ACTGG(1/-1)↓	**BseNI** (ER0881/2, p.43)	Bse1I, **BseNI**, BsrSI
BsrBI	CCGCTC(-3/-3)↓	**MbiI**[m] (ER1271, p.82)	AccBSI, **MbiI**[m]
BsrDI	GCAATG(2/0)↓	**BseMI** (ER1261/2, p.42)	Bse3DI, **BseMI**
BsrFI	R↓CCGGY	**Cfr10I** (ER0181/2, p.56)	Bse118I, **Cfr10I**
BsrGI	T↓GTACA	**Bsp1407I** (ER0931/2, p.49)	**Bsp1407I**, BstAUI, SspBI
BsrSI	ACTGG(1/-1)↓	**BseNI** (ER0881/2, p.43)	Bse1I, **BseNI**, BsrI
BssECI	C↓CNNGG	**BseDI** (ER1081/2, p.40)	BsaJI, **BseDI**
BssHII	G↓CGCGC	**PauI** (ER1091/2, p.93)	BsePI, **PauI**
BssKI	↓CCNGG	(**Bme1390I**) (CC↓NGG) (ER1421/2, p.37)	(**Bme1390I**), BstSCI, (MspR9I), (ScrFI), StyD4I
BssNAI	GTA↓TAC	**Bst1107I** (ER0701/2, p.52)	**Bst1107I**, BstZ17I
BssSI	CACGAG(-5/-1)↓	**BauI** (ER1841, p.32)	**BauI**, Bst2BI
BssT1I	C↓CWWGG	**Eco130I** (ER0411, p.65)	**Eco130I**, EcoT14I, ErhI, StyI
Bst6I	CTCTTC(1/4)↓	**Eam1104I** (ER0231/2, p.60)	**Eam1104I**, EarI, Ksp632I
Bst98I	C↓TTAAG	**BspTI** (ER0831/2, p.51)	AflII, BfrI, **BspTI**, Vha464I
Bst1107I	GTA↓TAC	**Bst1107I** (ER0701/2, p.52)	BssNAI, BstZ17I
BstACI	GR↓CGYC	**Hin1I** (ER0471/2, p.74)	AcyI, BsaHI, **Hin1I**, Hsp92I
BstAPI	GCANNNN↓NTGC		
BstAUI	T↓GTACA	**Bsp1407I** (ER0931/2, p.49)	**Bsp1407I**, BsrGI, SspBI
BstBI	TT↓CGAA	**Bsp119I** (ER0121, p.47)	Bpu14I, **Bsp119I**, BspT104I, Csp45I, NspV, SfuI[m]
Bst2BI	CACGAG(-5/-1)↓	**BauI** (ER1841, p.32)	**BauI**, BssSI
BstBAI	YAC↓GTR	**Ppu21I** (ER1971, p.97)	BsaAI, **Ppu21I**
Bst4CI	ACN↓GT	**TaaI** (ER1361/2, p.109)	HpyCH4III, **TaaI**
BstC8I	GCN↓NGC		Cac8I
BstDEI	C↓TNAG	**HpyF3I** (ER1881/2, p.79)	DdeI, **HpyF3I**
BstDSI	C↓CRYGG		BtgI
BstEII	G↓GTNACC	**Eco91I** (ER0391/2, p.67)	BstPI, **Eco91I**, EcoO65I, PspEI
BstENI	CCTNN↓NNNAGG	**XagI** (ER1301/2, p.115)	EcoNI, **XagI**
BstF5I	GGATG(2/0)↓	**BseGI** (ER0871/2, p.41)	**BseGI**, (FokI)
BstFNI	CG↓CG	**Bsh1236I** (ER0921/2, p.45)	AccII, **Bsh1236I**, BstUI, MvnI
BstH2I	RGCGC↓Y	**Bsp143II** (ER0791/2, p.48)	**Bsp143II**, HaeII
BstHHI	GCG↓C	**HhaI** (ER1851, p.73), (**Hin6I**) (G↓CGC) (ER0481/2, p.75)	AspLEI, CfoI, **HhaI**, (**Hin6I**), (HinP1I), (HspAI)
BstKTI	GAT↓C	(**Bsp143I**[m]) (↓GATC) (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), (**MboI**) (↓GATC) (ER0811/2, p.83)	(BfuCI[m]), (**Bsp143I**[m]), (BstMBI), (**DpnI**[m]), (DpnII), (Kzo9I[m]), (MalI[m]), (**MboI**), (NdeII), (Sau3AI[m])

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
BstMAI	GTCTC(1/5)↓	**AIw26I** (ER0031/2, p.29)	**AIw26I**, BsmAI
BstMBI	↓GATC	**Bsp143I** (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI** (ER0811/2, p.83)	BfuCI, **Bsp143I**, (BstKTI), (**DpnI**[m]), DpnII, Kzo9I, (MalI), **MboI**, NdeII, Sau3AI
BstMCI	CGRY↓CG	**Bsh1285I** (ER0891, p.45)	**Bsh1285I**, BsiEI
BstMWI	GCNNNNN↓NNGC	**HpyF10VI** (ER1731/2, p.79)	**HpyF10VI**, MwoI
BstNI	CC↓WGG	(**EcoRII**[m]) (↓CCWGG) (ER1921/2, p.70), **MvaI** (ER0551/2, p.87)	(AjnI), BstOI, Bst2UI, (**EcoRII**[m]), **MvaI**, (Psp6I), (PspGI[m])
BstNSI	RCATG↓Y	**XceI** (ER1471/2, p.116)	NspI, **XceI**
BstOI	CC↓WGG	(**EcoRII**[m]) (↓CCWGG) (ER1921/2, p.70), **MvaI** (ER0551/2, p.87)	(AjnI), BstNI, Bst2UI, (**EcoRII**[m]), **MvaI**, (Psp6I), (PspGI[m])
BstPI	G↓GTNACC	**Eco91I** (ER0391/2, p.67)	BstEII, **Eco91I**, EcoO65I, PspEI
BstPAI	GACNN↓NNGTC	**BoxI** (ER1431, p.38)	**BoxI**, PshAI
BstSCI	↓CCNGG	(**Bme1390I**) (CC↓NGG) (ER1421/2, p.37)	(**Bme1390I**), BssKI, (MspR9I), (ScrFI), StyD4I
BstSFI	C↓TRYAG	**BfmI** (ER1161/2, p.35)	**BfmI**, SfcI
BstSNI	TAC↓GTA	**Eco105I** (ER0401/2, p.67)	**Eco105I**, SnaBI
BstUI	CG↓CG	**Bsh1236I** (ER0921/2, p.45)	AccII, **Bsh1236I**, BstFNI, MvnI
Bst2UI	CC↓WGG	(**EcoRII**[m]) (↓CCWGG) (ER1921/2, p.70), **MvaI** (ER0551/2, p.87)	(AjnI), BstNI, BstOI, (**EcoRII**[m]), **MvaI**, (Psp6I), (PspGI[m])
BstV1I	GCAGC(8/12)↓	**BseXI** (ER1451/2, p.44)	BbvI, **BseXI**
BstV2I	GAAGAC(2/6)↓	**BpiI** (ER1011/2, p.38)	BbsI, **BpiI**, BpuAI
BstXI	CCANNNNN↓NTGG	**BstXI** (ER1021/2, p.44)	
BstX2I	R↓GATCY	**PsuI** (ER1551, p.99)	BstYI, MflI, **PsuI**, XhoII
BstYI	R↓GATCY	**PsuI** (ER1551, p.99)	BstX2I, MflI[m], **PsuI**, XhoII
BstZI	C↓GGCCG	**Eco52I** (ER0331/2, p.64)	BseX3I, EagI, EclXI, **Eco52I**
BstZ17I	GTA↓TAC	**Bst1107I** (ER0701/2, p.52)	BssNAI, **Bst1107I**
Bsu15I	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsa29I, Bsp106I, BspDI, BspXI, ClaI
Bsu36I	CC↓TNAGG	**Eco81I** (ER0371/2, p.66)	AxyI, Bse21I, **Eco81I**
BsuRI	GG↓CC	**BsuRI** (ER0151/2, p.53)	HaeIII, PaII, PhoI
BtgI	C↓CRYGG		BstDSI
BtgZI	GCGATG(10/14)↓		
BtrI	CACGTC(-3/-3)↓	**AjiI** (ER1941, p.26)	**AjiI**, BmgBI
BtsI	GCAGTG(2/0)↓		
BveI	ACCTGC(4/8)↓	**BveI** (ER1741, p.54)	Acc36I, BfuAI, BspMI
Cac8I	GCN↓NGC		BstC8I
CaiI	CAGNNN↓CTG	**CaiI** (ER1391/2, p.54)	AlwNI
CciNI	GC↓GGCCGC	**NotI** (ER0591/2/3, p.90)	**NotI**
CelII	GC↓TNAGC	**Bpu1102I** (ER0091/2, p.40)	BlpI, **Bpu1102I**, Bsp1720I
CfoI	GCG↓C	**HhaI** (ER1851, p.73), (**Hin6I**) (G↓CGC) (ER0481/2, p.75)	AspLEI, BstHHI, **HhaI**, (**Hin6I**), (HinP1I), (HspAI)
CfrI	Y↓GGCCR	**CfrI** (ER0161/2, p.55)	EaeI
Cfr9I	C↓CCGGG	**Cfr9I** (ER0171/2/4, p.55), (**SmaI**[m]) (CCC↓GGG) (ER0661/2/3/4, p.106)	PspAI, (**SmaI**[m]), XmaI, XmaCI
Cfr10I	R↓CCGGY	**Cfr10I** (ER0181/2, p.56)	Bse118I, BsrFI
Cfr13I	G↓GNCC	**Cfr13I** (ER0191/2, p.56)	AspS9I, Sau96I
Cfr42I	CCGC↓GG	**Cfr42I** (ER0201/2, p.57)	KspI, SacII, Sfr303I
ClaI	AT↓CGAT	**Bsu15I** (ER0141/2, p.53)	BanIII, Bsa29I, Bsp106I, BspDI, BspXI, **Bsu15I**
CpoI	CG↓GWCCG	**CpoI** (ER0741/2, p.57)	CspI, RsrII, Rsr2I
CseI	GACGC(5/10)↓	**CseI** (ER1901/2, p.58)	HgaI
CspI	CG↓GWCCG	**CpoI** (ER0741/2, p.57)	**CpoI**, RsrII, Rsr2I
Csp6I	G↓TAC	**Csp6I** (ER0211, p.58), (**RsaI**[m]) (GT↓AC) (ER1121/2, p.101)	(AfaI[m]), (**RsaI**[m])
Csp45I	TT↓CGAA	**Bsp119I** (ER0121, p.47)	Bpu14I, **Bsp119I**, BspT104I, BstBI, NspV, SfuI
CspCI	↓(11/13)CAANNNNNGTGG(12/10)↓		
CviAII	C↓ATG	(**Hin1II**) (CATG↓) (ER1831, p.74)	(FatI), (**Hin1II**), (Hsp92II), (NlaIII[m])
CviJI	RG↓CY		CviTI
DdeI	C↓TNAG	**HpyF3I** (ER1881/2, p.79)	BstDEI, **HpyF3I**
DpnI	GA↓TC	(**Bsp143I**[m]) (↓GATC) (ER0781/2, p.48), **DpnI** (ER1701/2, p.59), (**MboI**[m]) (↓GATC) (ER0811/2, p.83)	(BfuCI[m]), (**Bsp143I**[m]), (BstKTI[m]), (BstMBI[m]), (DpnII[m]), (Kzo9I[m]), MalI, (**MboI**[m]), (NdeII[m]), (Sau3AI[m])
DpnII	↓GATC	**Bsp143I**[m] (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI** (ER0811/2, p.83)	BfuCI[m], **Bsp143I**[m], (BstKTI), BstMBI, (**DpnI**[m]), Kzo9I[m], (MalI), **MboI**, NdeII, Sau3AI[m]
DraI	TTT↓AAA	**DraI** (ER0221/2/3, p.59)	
DraII	RG↓GNCCY	**EcoO109I** (ER0261, p.69)	**EcoO109I**
DraIII	CACNNN↓GTG	**AdeI** (ER1231/2, p.26)	**AdeI**

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
DrdI	GACNNNN↓NNGTC	**AasI** (ER1721/2, p.24)	**AasI**, DseDI
DriI	GACNNN↓NNGTC	**Eam1105I** (ER0241/2, p.60)	AhdI, AspEI, **Eam1105I**, EclHKI
DseDI	GACNNNN↓NNGTC	**AasI** (ER1721/2, p.24)	**AasI**, DrdI
EaeI	Y↓GGCCR	**CfrI** (ER0161/2, p.55)	**CfrI**
EagI	C↓GGCCG	**Eco52I** (ER0331/2, p.64)	BseX3I, BstZI, EclXI, **Eco52I**
Eam1104I	CTCTTC(1/4)↓	**Eam1104I** (ER0231/2, p.60)	Bst6I, EarI, Ksp632I
Eam1105I	GACNNN↓NNGTC	**Eam1105I** (ER0241/2, p.60)	AhdI, AspEI, DriI, EclHKI
EarI	CTCTTC(1/4)↓	**Eam1104I** (ER0231/2, p.60)	Bst6I, **Eam1104I**, Ksp632I
EciI	GGCGGA(11/9)↓		
Ecl136II	GAG↓CTC	**Ecl136II** (ER0251/4, p.61), (**SacI**[m]) (GAGCT↓C) (ER1131/2/3, p.102)	EcoICRI, (Psp124BI), (**SacI**[m])
EclHKI	GACNNN↓NNGTC	**Eam1105I** (ER0241/2, p.60)	AhdI, AspEI, DriI, **Eam1105I**
EclXI	C↓GGCCG	**Eco52I** (ER0331/2, p.64)	BseX3I, BstZI, EagI, **Eco52I**
Eco24I	GRGCY↓C	**Eco24I** (ER0281/4, p.61)	BanII, EcoT38I, FriOI
Eco31I	GGTCTC(1/5)↓	**Eco31I** (ER0291/2, p.62)	BsaI, Bso31I
Eco32I	GAT↓ATC	**Eco32I** (ER0301/2/3, p.62)	EcoRV
Eco47I	G↓GWCC	**Eco47I** (ER0311/2, p.63)	AvaII, Bme18I, SinI, VpaK11BI
Eco47III	AGC↓GCT	**Eco47III** (ER0321/2, p.63)	AfeI, Aor51HI
Eco52I	C↓GGCCG	**Eco52I** (ER0331/2, p.64)	BseX3I, BstZI, EagI, EclXI
Eco57I	CTGAAG(16/14)↓	**Eco57I** (ER0341/2, p.64)	AcuI
Eco72I	CAC↓GTG	**Eco72I** (ER0361/2, p.65)	BbrPI, PmaCI, PmlI, PspCI
Eco81I	CC↓TNAGG	**Eco81I** (ER0371/2, p.66)	AxyI, Bse21I, Bsu36I
Eco88I	C↓YCGRG	**Eco88I** (ER0381/4, p.66)	Ama87I, AvaI[m], BsoBI[m]
Eco91I	G↓GTNACC	**Eco91I** (ER0391/2, p.67)	BstEII, BstPI, EcoO65I, PspEI
Eco105I	TAC↓GTA	**Eco105I** (ER0401/2, p.67)	BstSNI, SnaBI
Eco130I	C↓CWWGG	**Eco130I** (ER0411, p.68)	BssT1I, EcoT14I, ErhI, StyI
Eco147I	AGG↓CCT	**Eco147I** (ER0421/2, p.68)	AatI, PceI, StuI
EcoICRI	GAG↓CTC	**Ecl136II** (ER0251/4, p.61), (**SacI**[m]) (GAGCT↓C) (ER1131/2/3/4, p.102)	**Ecl136II**, (Psp124BI), (**SacI**[m])
Eco57MI	CTGRAG(16/14)↓	**Eco57MI** (ER1671, p.65)	
EcoNI	CCTNN↓NNNAGG	**XagI** (ER1301/2, p.115)	BstENI, **XagI**
EcoO65I	G↓GTNACC	**Eco91I** (ER0391/2, p.67)	BstEII, BstPI, **Eco91I**, PspEI
EcoO109I	RG↓GNCCY	**EcoO109I** (ER0261, p.69)	DraII
EcoP15I	CAGCAG(25/27)↓		
EcoRI	G↓AATTC	**EcoRI** (ER0271/2/3/4, p.69)	
EcoRII	↓CCWGG	**EcoRII** (ER1921/2, p.70), (**MvaI**[m]) (CC↓WGG) (ER0551/2, p.87)	AjnI[m], (BstNI[m]), (BstOI[m]), (Bst2UI[m]), (**MvaI**[m]), Psp6I, PspGI
EcoRV	GAT↓ATC	**Eco32I** (ER0301/2/3, p.62)	**Eco32I**
EcoT14I	C↓CWWGG	**Eco130I** (ER0411, p.68)	BssT1I, **Eco130I**, ErhI, StyI
EcoT22I	ATGCA↓T	**Mph1103I** (ER0731/2, p.85)	(BfrBI), **Mph1103I**, NsiI[m], Zsp2I
EcoT38I	GRGCY↓C	**Eco24I** (ER0281/4, p.61)	BanII, **Eco24I**, FriOI
EgeI	GGC↓GCC	**EheI** (ER0441, p.70)	(BbeI), **EheI**, (KasI), (Mly113I), (NarI), SfoI
EheI	GGC↓GCC	**EheI** (ER0441, p.70)	(BbeI), EgeI, (KasI[m]), (Mly113I), (NarI), SfoI[m]
ErhI	C↓CWWGG	**Eco130I** (ER0411, p.68)	BssT1I, **Eco130I**, EcoT14I, StyI
Esp3I	CGTCTC(1/5)↓	**Esp3I** (ER0451/2, p.71)	BsmBI
FalI	↓(8/13)AAGNNNNNCTT(13/8)↓		
FaqI	GGGAC(10/14)↓	**FaqI** (ER1811, p.71)	BslFI, BsmFI
FatI	↓CATG	(**Hin1II**) (CATG↓) (ER1831, p.74)	(CviAII[m]), (**Hin1II**), (Hsp92II), (NlaIII[m])
FauI	CCCGC(4/6)↓	**SmuI** (ER1691/2, p.108)	**SmuI**
FauNDI	CA↓TATG	**NdeI** (ER0581/2, p.89)	**NdeI**
FbaI	T↓GATCA	**BclI** (ER0721/2, p.32)	**BclI**, Ksp22I
FblI	GT↓MKAC	**XmiI** (ER1481/2, p.118)	AccI, **XmiI**
Fnu4HI	GC↓NGC	**SatI** (ER1641/2, p.103)	BisI[m], Fsp4HI, ItaI[m], **SatI**
FokI	GGATG(9/13)↓	(**BseGI**) (GGATG(2/0)) (ER0871/2, p.41)	(**BseGI**), (BstF5I)
FriOI	GRGCY↓C	**Eco24I** (ER0281/4, p.61)	BanII, **Eco24I**, EcoT38I
FseI	GGCCGG↓CC		
FspI	TGC↓GCA	**NsbI** (ER1221/2, p.91)	Acc16I, AviII, **NsbI**
FspAI	RTGC↓GCAY	**FspAI** (ER1661/2, p.72)	
FspBI	C↓TAG	**FspBI** (ER1761/2, p.72)	BfaI, MaeI, XspI
Fsp4HI	GC↓NGC	**SatI** (ER1641/2, p.103)	BisI[m], Fnu4HI, ItaI, **SatI**
GsuI	CTGGAG(16/14)↓	**GsuI** (ER0461/2, p.73)	BpmI[m]
HaeII	RGCGC↓Y	**Bsp143II**[m] (ER0791/2, p.49)	**Bsp143II**[m], BstH2I
HaeIII	GG↓CC	**BsuRI** (ER0151/2, p.54)	**BsuRI**, PalI, PhoI
HapII	C↓CGG	**HpaII** (ER0511/2, p.77), **MspI**[m] (ER0541/2, p.86)	**HpaII**, **MspI**[m]
HgaI	GACGC(5/10)↓	**CseI** (ER1901/2, p.58)	**CseI**

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
HhaI	GCG↓C	**HhaI** (ER1851, p.73), (**Hin6I**) (G↓CGC) (ER0481/2, p.75)	AspLEI, BstHHI, CfoI, (**Hin6I**), (HinP1I), (HspAI)
Hin1I	GR↓CGYC	**Hin1I** (ER0471/2, p.74)	AcyI, BsaHI[m], BstACI, Hsp92I
Hin1II	CATG↓	**Hin1II** (ER1831, p.74)	(CviAII), (FatI), Hsp92II, NlaIII
Hin4I	↓(8/13)GAYNNNNNVTC(13/8)↓	**Hin4I** (ER1601/2, p.75)	
Hin6I	G↓CGC	(**HhaI**) (GCG↓C) (ER1851, p.73), **Hin6I** (ER0481/2, p.75)	(AspLEI), (BstHHI), (CfoI), (**HhaI**), HinP1I, HspAI
HinP1I	G↓CGC	(**HhaI**) (GCG↓C) (ER1851, p.73), **Hin6I** (ER0481/2, p.75)	(AspLEI), (BstHHI), (CfoI), (**HhaI**), **Hin6I**, HspAI
HincII	GTY↓RAC	**HincII** (ER0491/2/4, p.76)	HindII
HindII	GTY↓RAC	**HincII** (ER0491/2/4, p.76)	**HincII**
HindIII	A↓AGCTT	**HindIII** (ER0501/2/3/4, p.76)	
HinfI	G↓ANTC	**HinfI** (ER0801/2/3, p.77)	
HpaI	GTT↓AAC	**KspAI** (ER1031/2, p.81)	**KspAI**
HpaII	C↓CGG	**HpaII** (ER0511/2, p.77), **MspI**[m] (ER0541/2, p.86)	HapII, **MspI**[m]
HphI	GGTGA(8/7)↓	**HphI** (ER1101/2, p.78)	AsuHPI
Hpy8I	GTN↓NAC	**Hpy8I** (ER1571/2, p.78)	
Hpy99I	CGWCG↓		
Hpy188I	TCN↓GA		
Hpy188III	TC↓NNGA		
HpyCH4III	ACN↓GT	**TaaI** (ER1361/2, p.109)	Bst4CI, **TaaI**
HpyCH4IV	A↓CGT	(**TaiI**[m]) (ACGT↓) (ER1141/2, p.110)	MaeII, (**TaiI**[m]), (TscI)
HpyCH4V	TG↓CA		
HpyF3I	C↓TNAG	**HpyF3I** (ER1881/2, p.79)	BstDEI, DdeI
HpyF10VI	GCNNNNN↓NNGC	**HpyF10VI** (ER1731/2, p.79)	BstMWI, MwoI
Hsp92I	GR↓CGYC	**Hin1I** (ER0471/2, p.74)	AcyI, BsaHI, BstACI, **Hin1I**
Hsp92II	CATG↓	**Hin1II** (ER1831, p.74)	(CviAII), (FatI), **Hin1II**, NlaIII
HspAI	G↓CGC	(**HhaI**) (GCG↓C) (ER1851, p.73), **Hin6I** (ER0481/2, p.75)	(AspLEI), (BstHHI), (CfoI), (**HhaI**), **Hin6I**, HinP1I
ItaI	GC↓NGC	**SatI** (ER1641/2, p.103)	BisI[m], Fnu4HI[m], Fsp4HI[m], **SatI**
KasI	G↓CGCGC	(**EheI**[m]) (GGC↓GCC) (ER0441, p.70)	(BbeI), (EgeI), (**EheI**[m]), (MlyI13I), (NarI[m]), (SfoI[m])
KpnI	GGTAC↓C	(**Acc65I**[m]) (G↓GTACC) (ER0901/2/4, p.25), **KpnI** (ER0521/2/3/4, p.80)	(**Acc65I**[m]), (Asp718I[m])
Kpn2I	T↓CCGGA	**Kpn2I** (ER0531/2, p.80)	AccIII[m], Aor13HI, BseAI[m], Bsp13I, BspEI[m], MroI
KspI	CCGC↓GG	**Cfr42I** (ER0201/2, p.57)	**Cfr42I**, SacII, Sfr303I
Ksp22I	T↓GATCA	**BclI** (ER0721/2, p.32)	**BclI**, FbaI
Ksp632I	CTCTTC(1/4)↓	**Eam1104I** (ER0231/2, p.60)	Bst6I, **Eam1104I**, EarI
KspAI	GTT↓AAC	**KspAI** (ER1031/2, p.81)	HpaI
Kzo9I	↓GATC	**Bsp143I** (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI**[m] (ER0811/2, p.83)	BfuCI, **Bsp143I**, (BstKTI[m]), BstMBI, (**DpnI**[m]), DpnII[m], (MalI[m]), **MboI**[m], NdeII[m], Sau3AI
LguI	GCTCTTC(1/4)↓	**LguI** (ER1931/2, p.81)	SapI
LweI	GCATC(5/9)↓	**LweI** (ER1621/2, p.82)	SfaNI
MabI	A↓CCWGGT		SexAI[m]
MaeI	C↓TAG	**FspBI** (ER1761/2, p.72)	BfaI, **FspBI**, XspI
MaeII	A↓CGT	(**TaiI**) (ACGT↓) (ER1141/2, p.110)	HpyCH4IV, (**TaiI**), (TscI)
MaeIII	↓GTNAC		
MalI	GA↓TC	(**Bsp143I**[m]) (↓GATC) (ER0781/2, p.48), **DpnI** (ER1701/2, p.59), (**MboI**[m]) (↓GATC) (ER0811/2, p.83)	(BfuCI[m]), (**Bsp143I**[m]), (BstKTI[m]), (BstMBI[m]), **DpnI**, (DpnII[m]), (Kzo9I[m]), (**MboI**[m]), (NdeII[m]), (Sau3AI[m])
MamI	GATNN↓NNATC	**BseJI** (ER1711, p.41)	BsaBI, Bse8I, **BseJI**
MbiI	CCGCTC(-3/-3)↓	**MbiI** (ER1271, p.82)	AccBSI, BsrBI[m]
MboI	↓GATC	**Bsp143I**[m] (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI** (ER0811/2, p.83)	BfuCI[m], **Bsp143I**[m], (BstKTI), BstMBI, (**DpnI**[m]), DpnII, Kzo9I[m], (MalI[m]), NdeII, Sau3AI[m]
MboII	GAAGA(8/7)↓	**MboII** (ER0821/2, p.83)	
MfeI	C↓AATTG	**MunI** (ER0751/2, p.87)	**MunI**
MflI	R↓GATCY	**PsuI**[m] (ER1551, p.99)	BstX2I, BstYI[m], **PsuI**[m], XhoII[m]
MhlI	GDGCH↓C	**SduI** (ER0651, p.105)	BmyI, Bsp1286I, **SduI**
MlsI	TGG↓CCA	**MlsI** (ER1211/2, p.84)	BalI, MluNI, MscI, Msp20I
MluI	A↓CGCGT	**MluI** (ER0561/2, p.84)	
MluNI	TGG↓CCA	**MlsI** (ER1211/2, p.84)	BalI, **MlsI**, MscI, Msp20I
MlyI	GAGTC(5/5)↓	**SchI** (ER1371, p.104)	(PleI[m]), (PpsI), **SchI**
MlyI13I	GG↓CGCC	(**EheI**) (GGC↓GCC) (ER0441, p.70)	(BbeI), (EgeI), (**EheI**), (KasI), NarI, (SfoI)
MmeI	TCCRAC(20/18)↓		

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
MnlI	CCTC(7/6)↓	**MnlI** (ER1071/2, p.85)	
Mph1103I	ATGCA↓T	**Mph1103I** (ER0731/2, p.85)	(BfrBI), EcoT22I, NsiI, Zsp2I
MroI	T↓CCGGA	**KpnI2** (ER0531/2, p.80)	AccIII[m], Aor13HI, BseAI[m], Bsp13I, BspEI[m], **KpnI2**
MroNI	G↓CCGGC	(**PdiI**) (GCC↓GGC) (ER1521/2, p.94)	(NaeI), NgoMIV, (**PdiI**)
MroXI	GAANN↓NNTTC	**PdmI** (ER1531/2, p.94)	Asp700I, **PdmI**, XmnI
MscI	TGG↓CCA	**MlsI** (ER1211/2, p.84)	BalI, **MlsI**, MluNI, Msp20I
MseI	T↓TAA	**Tru1I** (ER0981/2/3, p.112)	**Tru1I**, Tru9I
MslI	CAYNN↓NNRTG		SmiMI
MspI	C↓CGG	**HpaII**[m] (ER0511/2, p.77), **MspI** (ER0541/2, p.86)	HapII[m], **HpaII**[m]
Msp20I	TGG↓CCA	**MlsI** (ER1211/2, p.84)	BalI, **MlsI**, MluNI, MscI
MspA1I	CMG↓CKG		
MspR9I	CC↓NGG	**Bme1390I** (ER1421/2, p.37)	**Bme1390I**, (BssKI), (BstSCI), ScrFI, (StyD4I)
MssI	GTTT↓AAAC	**MssI** (ER1341/2, p.86)	PmeI[m]
MunI	C↓AATTG	**MunI** (ER0751/2, p.87)	MfeI
MvaI	CC↓WGG	(**EcoRII**[m]) (↓CCWGG) (ER1921/2, p.70), **MvaI** (ER0551/2, p.87)	(AjnI), BstNI, BstOI, Bst2UI, (**EcoRII**[m]), (Psp6I), (PspGI[m])
Mva1269I	GAATGC(1/-1)↓	**Mva1269I** (ER0961/2, p.88)	BsaMI, BsmI, PctI
MvnI	CG↓CG	**Bsh1236I** (ER0921/2, p.45)	AccII, **Bsh1236I**, BstFNI, BstUI
MwoI	GCNNNNN↓NNGC	**HpyF10VI** (ER1731/2, p.79)	BstMWI, **HpyF10VI**
NaeI	GCC↓GGC	**PdiI** (ER1521/2, p.94)	(MroNI), (NgoMIV), **PdiI**
NarI	GG↓CGCC	(**EheI**) (GGC↓GCC) (ER0441, p.70)	(BbeI[m]), (EgeI), (**EheI**), (KasI[m]), Mly113I, (SfoI[m])
NciI	CC↓SGG	**BcnI** (ER0061/2, p.33)	AsuC2I, **BcnI**
NcoI	C↓CATGG	**NcoI** (ER0571/2, p.88)	Bsp19I
NdeI	CA↓TATG	**NdeI** (ER0581/2, p.89)	FauNDI
NdeII	↓GATC	**Bsp143I**[m] (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI** (ER0811/2, p.83)	BfuCI[m], **Bsp143I**[m], (BstKTI), BstMBI, (**DpnI**[m]), DpnII, Kzo9I[m], (MalI[m]), **MboI**, Sau3AI[m]
NgoMIV	G↓CCGGC	(**PdiI**) (GCC↓GGC) (ER1521/2, p.94)	MroNI, (NaeI), (**PdiI**)
NheI	G↓CTAGC	**NheI** (ER0971/2, p.89)	AsuNHI, (BmtI[m])
NlaIII	CATG↓	**Hin1II** (ER1831, p.74)	(CviAII[m]), (FatI[m]), **Hin1II**, Hsp92II
NlaIV	GGN↓NCC	**BspLI**[m] (ER1151/2, p.50)	**BspLI**[m], PspN4I
NmuCI	↓GTSAC	**NmuCI** (ER1511/2, p.90)	Tsp45I
NotI	GC↓GGCCGC	**NotI** (ER0591/2/3, p.90)	CciNI
NruI	TCG↓CGA	**Bsp68I** (ER0111/2, p.47)	**Bsp68I**
NsbI	TGC↓GCA	**NsbI** (ER1221/2, p.91)	Acc16I, AviII, FspI
NsiI	ATGCA↓T	**Mph1103I** (ER0731/2, p.85)	(BfrBI), EcoT22I[m], **Mph1103I**, Zsp2I
NspI	RCATG↓Y	**XceI** (ER1471/2, p.116)	BstNSI, **XceI**
NspV	TT↓CGAA	**Bsp119I** (ER0121, p.47)	Bpu14I, **Bsp119I**, BspT104I, BstBI, Csp45I, SfuI
OliI	CACNN↓NNGTG	**OliI** (ER1631/2, p.91)	AleI
PacI	TTAAT↓TAA		
PaeI	GCATG↓C	**PaeI** (ER0601/2/4, p.92)	BbuI, SphI
PaeR7I	C↓TCGAG	**XhoI** (ER0691/2/3, p.117)	Sfr274I, TliI, **XhoI**
PagI	T↓CATGA	**PagI** (ER1281/2, p.92)	BspHI[m], RcaI
PalI	GG↓CC	**BsuRI** (ER0151/2, p.53)	**BsuRI**, HaeIII, PhoI
PalAI	GG↓CGCGCC	**SgsI** (ER1891/2, p.106)	AscI, **SgsI**
PasI	CC↓CWGGG	**PasI** (ER1861, p.93)	
PauI	G↓CGCGC	**PauI** (ER1091/2, p.93)	BsePI, BssHII
PceI	AGG↓CCT	**Eco147I** (ER0421/2, p.68)	AatI, **Eco147I**, StuI
PciI	A↓CATGT	**PscI** (ER1871/2, p.97)	BspLU11I, **PscI**
PctI	GAATGC(1/-1)↓	**Mva1269I** (ER0961/2, p.88)	BsaMI, BsmI, **Mva1269I**
PdiI	GCC↓GGC	**PdiI** (ER1521/2, p.94)	(MroNI), NaeI, (NgoMIV)
PdmI	GAANN↓NNTTC	**PdmI** (ER1531/2, p.94)	Asp700I, MroXI, XmnI[m]
PfeI	G↓AWTC	**PfeI** (ER1781, p.95)	TfiI
Pfl23II	C↓GTACG	**Pfl23II** (ER0851, p.95)	BsiWI, PspLI
PflFI	GACN↓NNGTC	**PsyI** (ER1331, p.100)	AspI, **PsyI**, Tth111I
PflMI	CCANNNN↓NTGG	**Van91I** (ER0711/2, p.114)	AccB7I, **Van91I**
PfoI	T↓CCNGGA	**PfoI** (ER1751, p.96)	
PhoI	GG↓CC	**BsuRI** (ER0151/2, p.53)	**BsuRI**, HaeIII, PalI
PinAI	A↓CCGGT	**BshTI** (ER1461/2, p.46)	AgeI, AsiGI, **BshTI**
PleI	GAGTC(4/5)↓	(**SchI**[m]) (GAGTC(5/5)) (ER1371, p.104)	(MlyI[m]), PpsI, (**SchI**[m])
Ple19I	CGAT↓CG	**PvuI** (ER0621/2, p.100)	BspCI, **PvuI**
PmaCI	CAC↓GTG	**Eco72I** (ER0361/2, p.65)	BbrPI, **Eco72I**, PmlI, PspCI
PmeI	GTTT↓AAAC	**MssI**[m] (ER1341/2, p.86)	**MssI**[m]
PmlI	CAC↓GTG	**Eco72I** (ER0361/2, p.65)	BbrPI, **Eco72I**, PmaCI, PspCI
PpiI	↓(7/12)GAACNNNNNCTC(13/8)↓	**PpiI** (ER1541/2, p.96)	

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
PpsI	GAGTC(4/5)↓	(**SchI**) (GAGTC(5/5)) (ER1371, p.104)	(MlyI), PleI, (**SchI**)
Ppu21I	YAC↓GTR	**Ppu21I** (ER1971, p.97)	BsaAI, BstBAI
PpuMI	RG↓GWCCY	**Psp5II** (ER0761, p.98)	**Psp5II**, PspPPI
PscI	A↓CATGT	**PscI** (ER1871/2, p.97)	BspLU11I, PciI
PshAI	GACNN↓NNGTC	**BoxI** (ER1431, p.38)	**BoxI**, BstPAI
PshBI	AT↓TAAT	**VspI** (ER0911/2, p.114)	AseI, **VspI**
PsiI	TTA↓TAA		
Psp5II	RG↓GWCCY	**Psp5II** (ER0761, p.98)	PpuMI, PspPPI
Psp6I	↓CCWGG	**EcoRII** (ER1921/2, p.70), (**MvaI**) (CC↓WGG) (ER0551/2, p.87)	AjnI[m], (BstNI), (BstOI), (Bst2UI[m]), **EcoRII**, (**MvaI**), PspGI
Psp1406I	AA↓CGTT	**Psp1406I** (ER0941/2, p.98)	AclI
PspAI	C↓CCGGG	**Cfr9I** (ER0171/2/4, p.55), (**SmaI**) (CCC↓GGG) (ER0661/2/3/4, p.106)	**Cfr9I**, (**SmaI**), XmaI, XmaCI
Psp124BI	GAGCT↓C	(**Ecl136II**) (GAG↓CTC) (ER0251/4, p.61), **SacI** (ER1131/2/3/4, p.102)	(**Ecl136II**), (EcoICRI), **SacI**
PspCI	CAC↓GTG	**Eco72I** (ER0361/2, p.65)	BbrPI, **Eco72I**, PmaCI, PmlI
PspEI	G↓GTNACC	**Eco91I** (ER0391/2, p.67)	BstEII, BstPI, **Eco91I**, EcoO65I
PspGI	↓CCWGG	**EcoRII** (ER1921/2, p.70), (**MvaI**[m]) (CC↓WGG) (ER0551/2, p.87)	AjnI, (BstNI[m]), (BstOI[m]), (Bst2UI), **EcoRII**, (**MvaI**[m]), Psp6I
PspLI	C↓GTACG	**Pfl23II** (ER0851, p.95)	BsiWI, **Pfl23II**
PspN4I	GGN↓NCC	**BspLI** (ER1151/2, p.50)	**BspLI**, NlaIV
PspOMI	G↓GGCCC	(**ApaI**) (GGGCC↓C) (ER1411/2, p.30), **Bsp120I** (ER0131/2/3, p.48)	(**ApaI**), **Bsp120I**
PspPPI	RG↓GWCCY	**Psp5II** (ER0761, p.98)	PpuMI, **Psp5II**
PspXI	VC↓TCGAGB		
PsrI	↓(7/12)GAACNNNNNNTAC(12/7)↓		
PstI	CTGCA↓G	**PstI** (ER0611/2/3/4, p.99)	
PsuI	R↓GATCY	**PsuI** (ER1551, p.99)	BstX2I, BstYI, MflI[m], XhoII
PsyI	GACN↓NNGTC	**PsyI** (ER1331, p.100)	AspI, PflFI, Tth111I
PvuI	CGAT↓CG	**PvuI** (ER0621/2, p.100)	BspCI, Ple19I
PvuII	CAG↓CTG	**PvuII** (ER0631/2/3, p.101)	
RcaI	T↓CATGA	**PagI** (ER1281/2, p.92)	BspHI, **PagI**
RgaI	GCGAT↓CGC		AsiSI, SgfI
RsaI	GT↓AC	(**Csp6I**[m]) (G↓TAC) (ER0211, p.58), **RsaI** (ER1121/2, p.101)	AfaI, (**Csp6I**[m])
RsrII	CG↓GWCCG	**CpoI** (ER0741/2, p.58)	**CpoI**, CspI, Rsr2I
Rsr2I	CG↓GWCCG	**CpoI** (ER0741/2, p.58)	**CpoI**, CspI, RsrII
SacI	GAGCT↓C	(**Ecl136II**[m]) (GAG↓CTC) (ER0251/4, p.61), **SacI** (ER1131/2/3/4, p.102)	(**Ecl136II**[m]), (EcoICRI), Psp124BI
SacII	CCGC↓GG	**Cfr42I** (ER0201/2, p.57)	**Cfr42I**, KspI, Sfr303I
SalI	G↓TCGAC	**SalI** (ER0641/2/3/4, p.102)	
SanDI	GG↓GWCCC		
SapI	GCTCTTC(1/4)↓	**LguI** (ER1931/2, p.81)	**LguI**
SatI	GC↓NGC	**SatI** (ER1641/2, p.103)	BisI[m], Fnu4HI, Fsp4HI, ItaI[m]
Sau96I	G↓GNCC	**Cfr13I** (ER0191/2, p.56)	AspS9I, **Cfr13I**
Sau3AI	↓GATC	**Bsp143I** (ER0781/2, p.48), (**DpnI**[m]) (GA↓TC) (ER1701/2, p.59), **MboI**[m] (ER0811/2, p.83)	BfuCI, **Bsp143I**[m], (BstKTI[m]), BstMBI, (**DpnI**[m]), DpnII[m], Kzo9I, (MalI[m]), **MboI**[m], NdeII[m]
SbfI	CCTGCA↓GG	**SdaI** (ER1191/2/4, p.104)	**SdaI**, Sse8387I
ScaI	AGT↓ACT	**ScaI** (ER0431/2, p.103)	ZrmI
SchI	GAGTC(5/5)↓	**SchI** (ER1371, p.104)	MlyI, (PleI), (PpsI)
ScrFI	CC↓NGG	**Bme1390I** (ER1421/2, p.37)	**Bme1390I**, (BssKI), (BstSCI), MspR9I, (StyD4I)
SdaI	CCTGCA↓GG	**SdaI** (ER1191/2/4, p.104)	SbfI, Sse8387I
SduI	GDGCH↓C	**SduI** (ER0651, p.105)	BmyI, Bsp1286I, MhlI
SexAI	A↓CCWGGT		MabI
SfaNI	GCATC(5/9)↓	**LweI** (ER1621/2, p.82)	**LweI**
SfcI	C↓TRYAG	**BfmI** (ER1161/2, p.35)	**BfmI**, BstSFI
SfiI	GGCCNNNN↓NGGCC	**SfiI** (ER1821, p.105)	
SfoI	GGC↓GCC	**EheI**[m] (ER0441, p.70)	(BbeI[m]), EgeI, **EheI**[m], (KasI[m]), (Mly113I), (NarI[m])
Sfr274I	C↓TCGAG	**XhoI** (ER0691/2/3, p.117)	PaeR7I, TliI, **XhoI**
Sfr303I	CCGC↓GG	**Cfr42I** (ER0201/2, p.57)	**Cfr42I**, KspI, SacII
SfuI	TT↓CGAA	**Bsp119I**[m] (ER0121, p.47)	Bpu14I, **Bsp119I**[m], BspT104I[m], BstBI[m], Csp45I, NspV[m]
SgfI	GCGAT↓CGC		AsiSI, RgaI
SgrAI	CR↓CCGGYG		
SgsI	GG↓CGCGCC	**SgsI** (ER1891/2, p.106)	AscI, PalAI
SinI	G↓GWCC	**Eco47I** (ER0311/2, p.63)	AvaII, Bme18I, **Eco47I**, VpaK11BI

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Table 1.3. Alphabetic List of Commercially Available Restriction Endonucleases.

Enzyme	Specificity 5' → 3'	Fermentas enzyme (cat. #, page #)	Commercially available isoschizomers
SmaI	CCC↓GGG	(**Cfr9I**[m]) (C↓CCGGG) (ER0171/2, p.55), **SmaI** (ER0661/2/3/4, p.106)	(**Cfr9I**[m]), (PspAI), (XmaI[m]), (XmaCI)
SmiI	ATTT↓AAAT	**SmiI** (ER1241/2, p.107)	SwaI
SmiMI	CAYNN↓NNRTG		MslI
SmlI	C↓TYRAG	**SmoI** (ER1981, p.107)	**SmoI**
SmoI	C↓TYRAG	**SmoI** (ER1981, p.107)	SmlI
SmuI	CCCGC(4/6)↓	**SmuI** (ER1691/2, p.108)	FauI
SnaBI	TAC↓GTA	**Eco105I** (ER0401/2, p.67)	BstSNI, **Eco105I**
SpeI	A↓CTAGT	**BcuI** (ER1251/2, p.33)	AhlI, **BcuI**
SphI	GCATG↓C	**PaeI** (ER0601/2/4, p.92)	BbuI, **PaeI**
SrfI	GCCC↓GGGC		
Sse9I	↓AATT	**TasI** (ER1351/2, p.111)	**TasI**, Tsp509I, TspEI
Sse8387I	CCTGCA↓GG	**SdaI** (ER1191/2/4, p.104)	SbfI, **SdaI**
SsiI	CCGC(-3/-1)↓	**SsiI** (ER1791, p.108)	AciI
SspI	AAT↓ATT	**SspI** (ER0771/2, p.109)	
SspBI	T↓GTACA	**Bsp1407I** (ER0931/2, p.49)	**Bsp1407I**, BsrGI, BstAUI
StuI	AGG↓CCT	**Eco147I** (ER0421/2, p.68)	AatI, **Eco147I**, PceI
StyI	C↓CWWGG	**Eco130I** (ER0411, p.68)	BssT1I, **Eco130I**, EcoT14I, ErhI
StyD4I	↓CCNGG	(**Bme1390I**) (CC↓NGG) (ER1421/2, p.37)	(**Bme1390I**), BssKI, BstSCI, (MspR9I), (ScrFI)
SwaI	ATTT↓AAAT	**SmiI** (ER1241/2, p.107)	**SmiI**
TaaI	ACN↓GT	**TaaI** (ER1361/2, p.109)	Bst4CI, HpyCH4III
TaiI	ACGT↓	**TaiI** (ER1141/2, p.110)	(HpyCH4IV), (MaeII), TscI
TaqI	T↓CGA	**TaqI** (ER0671/2/3, p.110)	
TaqII	GACCGA(11/9)↓, CACCCA(11/9)↓		
TasI	↓AATT	**TasI** (ER1351/2, p.111)	Sse9I, Tsp509I, TspEI
TatI	W↓GTACW	**TatI** (ER1291/2, p.111)	
TauI	GCSG↓C	**TauI** (ER1651/2, p.112)	
TfiI	G↓AWTC	**PfeI** (ER1781, p.95)	**PfeI**
TliI	C↓TCGAG	**XhoI** (ER0691/2/3, p.117)	PaeR7I, Sfr274I, **XhoI**
Tru1I	T↓TAA	**Tru1I** (ER0981/2/3, p.112)	MseI, Tru9I
Tru9I	T↓TAA	**Tru1I** (ER0981/2/3, p.112)	MseI, **Tru1I**
TscI	ACGT↓	**TaiI** (ER1141/2, p.110)	(HpyCH4IV[m]), (MaeII), **TaiI**
TseI	G↓CWGC		ApeKI
TsoI	TARCCA(9/11)↓	**TsoI** (ER1991, p.113)	
Tsp45I	↓GTSAC	**NmuCI** (ER1511/2, p.90)	**NmuCI**
Tsp509I	↓AATT	**TasI** (ER1351/2, p.111)	Sse9I, **TasI**, TspEI
TspEI	↓AATT	**TasI** (ER1351/2, p.111)	Sse9I, **TasI**, Tsp509I
TspGWI	ACGGA(11/9)↓		
TspRI	CASTGNN(2/-7)↓		
TstI	↓(8/13)CACNNNNNNTCC(12/7)↓	**TstI** (ER1911, p.113)	
Tth111I	GACN↓NNGTC	**PsyI** (ER1331, p.100)	AspI, PflFI, **PsyI**
Van91I	CCANNNN↓NTGG	**Van91I** (ER0711/2, p.114)	AccB7I, PflMI
Vha464I	C↓TTAAG	**BspTI** (ER0831/2, p.51)	AflII, BfrI, **BspTI**, Bst98I
VneI	G↓TGCAC	**Alw44I** (ER0041/2, p.30)	**Alw44I**, ApaLI
VpaK11BI	G↓GWCC	**Eco47I** (ER0311/2, p.63)	AvaII, Bme18I, **Eco47I**, SinI
VspI	AT↓TAAT	**VspI** (ER0911/2, p.114)	AseI, PshBI
XagI	CCTNN↓NNNAGG	**XagI** (ER1301/2, p.115)	BstENI, EcoNI
XapI	R↓AATTY	**XapI** (ER1381/2/4, p.115)	AcsI, ApoI
XbaI	T↓CTAGA	**XbaI** (ER0681/2/3/4, p.116)	
XceI	RCATG↓Y	**XceI** (ER1471/2, p.116)	BstNSI, NspI
XcmI	CCANNNNN↓NNNNTGG		
XhoI	C↓TCGAG	**XhoI** (ER0691/2/3, p.117)	PaeR7I, Sfr274I, TliI
XhoII	R↓GATCY	**PsuI** (ER1551, p.99)	BstX2I, BstYI, MflI[m], **PsuI**
XmaI	C↓CCGGG	**Cfr9I** (ER0171/2/4, p.55), (**SmaI**[m]) (CCC↓GGG) (ER0661/2/3/4, p.106)	**Cfr9I**, PspAI, (**SmaI**[m]), XmaCI
XmaCI	C↓CCGGG	**Cfr9I** (ER0171/2/4, p.55), (**SmaI**) (CCC↓GGG) (ER0661/2/3/4, p.106)	**Cfr9I**, PspAI, (**SmaI**), XmaI
XmaJI	C↓CTAGG	**XmaJI** (ER1561/2, p.117)	AspA2I, AvrII, BlnI
XmiI	GT↓MKAC	**XmiI** (ER1481/2, p.118)	AccI, FbII
XmnI	GAANN↓NNTTC	**PdmI**[m] (ER1531/2, p.94)	Asp700I, MroXI, **PdmI**[m]
XspI	C↓TAG	**FspBI** (ER1761/2, p.72)	BfaI, **FspBI**, MaeI
ZraI	GAC↓GTC	(**AatII**[m])(GACGT↓C) (ER0991/2, p.25)	(**AatII**[m])
ZrmI	AGT↓ACT	**ScaI** (ER0431/2, p.103)	**ScaI**
Zsp2I	ATGCA↓T	**Mph1103I** (ER0731/2, p.85)	(BfrBI), EcoT22I, **Mph1103I**, NsiI

Recognition Specificities

Table 1.4. Recognition Specificities.

Specificity 5'→ 3'	Enzyme
A	
AA↓CGTT	**Psp1406I**
A↓AGCTT	**HindIII**
AAGGAG(20/18)↓	CstMI
↓(8/13)AAG(N)$_5$CTT(13/8)↓	FalI
AAT↓ATT	**SspI**
↓AATT	**TasI**
ACAYNNNNNGTA	UbaF9I
A↓CATGT	**PscI**
A↓CCGGT	**BshTI**
ACCNNNNNNGGT	HgiEII
ACCTGC(4/8)↓	**BveI**
A↓CCWGGT	SexAI
A↓CGCGT	**MluI**
ACGGA(11/9)↓	TspGWI
ACGGC(12/14)↓	BceAI
ACGGG	BscGI
A↓CGT	MaeII
ACGT↓	**TaiI**
↓(10/15)AC(N)$_4$GTAYC(12/7)↓	BaeI
ACN↓GT	**TaaI**
ACNNNNNCTC	CjeNII
↓(9/12)AC(N)$_5$CTCC(10/7)↓	BsaXI
A↓CRYGT	AflIII
A↓CTAGT	**BcuI**
ACTGGG(5/4)↓	**BfiI**
ACTGG(1/-1)↓	**BseNI**
↓(9/15)AC(N)$_6$TGG(14/8)↓	CjeI
A↓GATCT	**BglII**
AGC↓GCT	**Eco47III**
AG↓CT	**AluI**
AGG↓CCT	**Eco147I**
AG↓GWCCT	Sse8647I
AGT↓ACT	**ScaI**
AT↓CGAT	**Bsu15I**
ATGAA(11/9)↓	TspDTI
ATG↓CAT	BfrBI
ATGCA↓T	**Mph1103I**
AT↓TAAT	**VspI**
ATTT↓AAAT	**SmiI**
C	
CAACAC	BsbI
↓(11/13)CAA(N)$_5$GTGG(12/10)↓	CspCI
CAARCA(11/9)↓	Tth111II
C↓AATTG	**MunI**
CACCCA(11/9)↓	TaqII
CACCTGC(4/8)↓	**AarI**
CACGAG(-5/-1)↓	**BaeI**
CAC↓GTC	**AjiI**
CAC↓GTG	**Eco72I**
CACNNN↓GTG	**AdeI**
CACNN↓NNGTG	**OliI**
↓(8/13)CAC(N)$_6$TCC(12/7)↓	**TstI**
↓(0/2)CACTGC	BtsI
CAGCTC(7/11)↓	AceIII
CAG↓CTG	**PvuII**
CAGNNN↓CTG	**CaiI**
CASTG(2/-7)↓	TspRI
CAYNNNNNRTG	UbaF10I
CAYNN↓NNRTG	MslI
CA↓TATG	**NdeI**
CATCAG	SsmI
↓(0/2)CATCC	**BseGI**
↓(13/9)CATCC	FokI
(14/10)CATCC	StsI
CATC↓G	CdiI
↓(14/10)CATCGC	BtgZI
↓CATG	FatI
CATG↓	**Hin1II**
C↓ATG	CviAII
↓(0/2)CATTGC	**BseMI**
↓(6/11)CCAA(N)$_7$TTC(12/7)↓	**AjuI**
↓(10/12)CCAC(N)$_5$TTG(13/11)↓	CspCI
CCAGA	BspNCI
↓(-1/1)CCAGT	**BseNI**
↓(8/14)CCA(N)$_6$GT(15/9)↓	CjeI
↓(7/12)CCA(N)$_6$GTC(13/8)↓	Bsp24I
↓(7/13)CCA(N)$_7$TC(14/8)↓	CjePI
CCANNNN↓NTGG	**Van91I**
CCANNNNN↓NNNNTGG	XcmI
CCANNNNN↓NTGG	**BstXI**
CCATC	BccI
C↓CATGG	**NcoI**
C↓CRYGG	DsaI
CCCACA(12/9)↓	RleAI
↓(4/5)CCCAGT	**BfiI**
C↓CCAGC	BseYI
CCCGC(4/6)↓	**SmuI**
C↓CCGGG	**Cfr9I**
CCC↓GGG	**SmaI**
CCCG(4/8)↓	Sth132I
CCCGT	BscGI
CC↓CWGGG	**PasI**
CCGC(-3/-1)↓	**SsiI**
CCGC↓GG	**Cfr42I**
CCGCTC(-3/-3)↓	**MbiI**
C↓CGG	**HpaII, MspI**
↓CCNGG	BssKI
CC↓NGG	**Bme1390I**
C↓CNNGG	**BseDI**
CCNNNNN↓NNGG	**BseLI**
C↓CRYGG	DsaI
↓CCSGG	EcoHI
CC↓SGG	**BcnI**
C↓CTAGG	**XmaJI**
CCTCAGC(-5/-2)↓	BbvCI
CCTC(7/6)↓	**MnlI**
CCTGCA↓GG	**SdaI**
CC↓TNAGC	**Bpu10I**
CC↓TNAGG	**Eco81I**
CCTNN↓NNNAGG	**XagI**
CCTTC	Hin4II
↓CCWGG	**EcoRII**
CC↓WGG	**MvaI**
C↓CWWGG	**Eco130I**
CGAACG	UbaPI
CGAT↓CG	**PvuI**
↓(10/12)CGA(N)$_6$TGC(12/10)↓	BcgI
CG↓CCGGCG	Sse232I
↓CGCG	SelI
CG↓CG	**Bsh1236I**
C↓GGCCG	**Eco52I**
↓(-1/-5)CGGCCR	GdiII
↓(8/4)CGGG	Sth132I
CG↓GWCCG	**CpoI**
CGRY↓CG	**Bsh1285I**
C↓GTACG	**Pfl23II**
CGTCGACG	SgrDI
CGTCTC(1/5)↓	**Esp3I**
CGTTCG	UbaPI
CGWCG↓	Hpy99I
CMG↓CKG	MspA1I
CR↓CCGGYG	SgrAI
CTACGA	Pfl1108I
C↓TAG	**FspBI**
CTCAG(10/8)↓	**BseMII**
↓(14/16)CTCCAG	**GsuI**
↓(8/10)CTCCTC	BseRI
↓(18/20)CTCCTT	CstMI
C↓TCGAG	**XhoI**
CTC↓GAG	SciI
↓(14/16)CTCAAG	BpuEI
CTCGTG(-5/-1)↓	**BauI**
CTCTTC(1/4)↓	**Eam1104I**
CTGAAG(16/14)↓	**Eco57I**
CTGATG	SsmI
↓(14/16)CTGCAC	BsgI
CTGCA↓G	**PstI**
CTGGAC	BspGI
↓(8/10)CTGAG	**BseMII**
CTGGAG(16/14)↓	**GsuI**
CTGRAG(16/14)↓	**Eco57MI**
C↓TNAG	**HpyF3I**
C↓TRYAG	**BfmI**
C↓TTAAG	**BspTI**
↓(14/16)CTTCAG	**Eco57I**
CTTGAG(16/14)↓	BpuEI
C↓TYRAG	**SmoI**
↓(14/16)CTYCAG	**Eco57MI**
C↓YCGRG	**Eco88I**
CYCGR↓G	Nli3877I
G	
GAACCA	DrdII
↓(7/12)GAA(N)$_7$TTGG(11/6)↓	**AjuI**
↓(7/12)GAAC(N)$_5$CTC(13/8)↓	**PpiI**
↓(7/12)GAAC(N)$_6$TAC(12/7)↓	PsrI
↓(7/12-13)GAAC(N)$_6$TCC(12-13/7)↓	**AloI**
GAAGAC(2/6)↓	**BpiI**
GAAGAC(7/11)↓	Bbr7I
↓(4/1)GAAGAG	**Eam1104I**
↓(4/1)GAAGAGC	**LguI**
GAAGA(8/7)↓	**MboII**
GAANN↓NNTTC	**PdmI**
GAATGC(1/-1)↓	**Mva1269I**
G↓AATTC	**EcoRI**
↓(8/13-14)GAY(N)$_5$VTC(13-14/8)↓	**Hin4I**
↓(0/-3)GACCC	SimI
GACCGA(11/9)↓	TaqII
GACGC(5/10)↓	**CseI**
GACGT↓C	**AatII**
GAC↓GTG	**AjiI**
↓(5/5)GACTC	**SchI**
↓(5/4)GACTC	PleI
GACN↓NNGTC	**PsyI**
GACNN↓NNGTC	**BoxI**
GACNNN↓NNGTC	**Eam1105I**
GACNNNN↓NNGTC	**AasI**
↓(8/13) GAC(N)$_6$TGG(12/7)↓	Bsp24I
↓(5/1)GAGAC	**Alw26I**
↓(5/1)GAGACC	**Eco31I**
↓(5/1)GAGACG	**Esp3I**
↓(-3/-3)GAGCGG	**MbiI**

(continued on next page)

Table 1.4. Recognition Specificities.

Specificity 5'→ 3'	Enzyme
GAG↓CTC	**Ecl136II**
GAGCT↓C	**SacI**
↓(11/7)GAGCTG	AceIII
↓(6/7)GAGG	**MnlI**
GAGNNNCTC	TssI
GAGNNNNNGT	CjeNII
GAGTC(5/5)↓	**SchI**
GAGTC(4/5)↓	PleI
↓(8/13)GAG(N)$_5$CTC(13/8)↓	**BplI**
GAGGAG(10/8)↓	BseRI
↓(8/13)GAG(N)$_5$GTTC(12/7)↓	**PpiI**
G↓ANTC	**HinfI**
↓(7/13) GAY(N)$_5$RTC(14/9)↓	HaeIV
GAT↓ATC	**Eco32I**
↓GATC	**Bsp143I**, **MboI**
GAT↓C	BstKTI
GATC↓	ChaI
Gm^{6}A↓TC	**DpnI**
↓(5/4)GATCC	**BspPI**
↓(9/5)GATGC	**LweI**
↓(6/4)GATGC	BscAI
GATGG	BccI
↓(8/14)GA(N)$_7$TGG(13/7)↓	CjePI
GATNN↓NNATC	**BseJI**
G↓AWTC	**PfeI**
GCAATG(2/0)↓	**BseMI**
GCAGC(8/12)↓	**BseXI**
GCAGGTG	**AarI**
↓(8/4)GCAGGT	**BveI**
GCAGTG(2/0)↓	BtsI
GCANNNN↓NTGC	BstAPI
GCANNNNN↓TGC	ApaBI
↓(10/12)GCA(N)$_6$TGC(12/10)↓	**AlfI**
GCATC(5/9)↓	**LweI**
GCATC(4/6)↓	BscAI
↓(10/12)GCA(N)$_6$TCG(12/10)↓	BcgI
GCATG↓C	**PaeI**
↓(-1/1)GCATTC	**Mva1269I**
GCCC↓GGGC	SrfI
GCCGC	AspCNI
G↓CCGGC	NgoMIV
GCC↓GGC	**PdiI**
↓(14/12)GCCGT	BceAI
GCCNNNN↓NGGC	**BglI**
GCGAC	TsuI
GCGAT↓CGC	SgfI
GCGATG(10/14)↓	BtgZI
G↓CGC	**Hin6I**
GCG↓C	**HhaI**
G↓CGCGC	**PauI**
GCGG(-3/-1)↓	**SsiI**
GC↓GGCCGC	**NotI**
↓(6/4)GCGGG	**SmuI**
GC↓NGC	**SatI**
GCN↓NGC	Cac8I
GCNNNNN↓NNGC	**HpyF10VI**
↓(10/5)GCGTC	**CseI**
GCSG↓C	**TauI**
G↓CTAGC	**NheI**
GCTAG↓C	BmtI
GCTCTTC(1/4)↓	**LguI**
GCTGAGG(-5/-2)↓	BbvCI
↓(12/8)GCTGC	**BseXI**
G↓CTGGG	BseYI
GC↓TNAGC	**Bpu1102I**
GC↓TNAGG	**Bpu10I**

Specificity 5'→ 3'	Enzyme
G↓CWGC	TseI
GDGCH↓C	**SduI**
↓(7/10)GGAG(N)$_5$GT(12/9)↓	BsaXI
G↓GATCC	**BamHI**
GGATC(4/5)↓	**BspPI**
↓(7/12)GGA(N)$_6$GTG(13/8)↓	**TstI**
↓(7/12-13)GGA(N)$_6$GTTC(12-13/7)↓	**AloI**
↓(5/6)GGATAC	**BfuI**
GGATG(2/0)↓	**BseGI**
GGATG(9/13)↓	FokI
GGATG(10/14)	StsI
GG↓CC	**BsuRI**
GGCCGG↓CC	FseI
GGCCNNNN↓NGGCC	**SfiI**
G↓GCGCC	KasI
GG↓CGCC	NarI
GGC↓GCC	**EheI**
GGCGC↓C	BbeI
GG↓CGCGCC	**SgsI**
GGCGGA(11/9)↓	EciI
GGGAC(10/14)↓	**FaqI**
G↓GGCCC	**Bsp120I**
GGGCC↓C	**ApaI**
GGGCMC	BmgI
GGGTC(-3/0)↓	SimI
GG↓GWCCC	SanDI
↓GGNCC	UnbI
G↓GNCC	**Cfr13I**
GGNC↓C	FmuI
GGN↓NCC	**BspLI**
G↓GYRCC	**BshNI**
G↓GTACC	**Acc65I**
GGTAC↓C	**KpnI**
GGTCTC(1/5)↓	**Eco31I**
GGTGA(8/7)↓	**HphI**
GGTGA(8/8)↓	SspD5I
G↓GTNACC	**Eco91I**
↓GGWCC	VpaK11AI
G↓GWCC	**Eco47I**
GKGCCC	BmgI
GKGCM↓C	**BseSI**
GR↓CGYC	**Hin1I**
GRGCY↓C	**Eco24I**
↓(7/12)GRTAC(N)$_4$GT(15/10)↓	BaeI
G↓TAC	**Csp6I**
GT↓AC	**RsaI**
↓(7/12)GTA(N)$_6$GTTC(12/7)↓	PsrI
GTA↓TAC	**Bst1107I**
GTATCC(6/5)↓	**BfuI**
GTCCAG	BspGI
↓(14/10)GTCCC	**FaqI**
G↓TCGAC	**SalI**
GTCGC	TsuI
GTCTC(1/5)↓	**Alw26I**
↓(6/2)GTCTTC	**BpiI**
↓(11/7)GTCTTC	Bbr7I
G↓TGCAC	**Alw44I**
GTGCAG(16/14)↓	BsgI
GTGTTG	BsbI
GT↓MKAC	**XmiI**
↓GTNAC	MaeIII
GTN↓NAC	**Hpy8I**
↓GTSAC	**NmuCI**
↓(18/20)GTYGGA	MmeI
GTY↓RAC	**HincII**
GTT↓AAC	**KspAI**

Specificity 5'→ 3'	Enzyme
GTTT↓AAAC	**MssI**
GWGCW↓C	**Alw21I**
R	
R↓AATTY	**XapI**
RCATG↓Y	**XceI**
R↓CCGGY	**Cfr10I**
R↓GATCY	**PsuI**
RGC↓GCY	LpnI
RGCGC↓Y	**Bsp143II**
RG↓CY	CviJI
RG↓GWCCY	**Psp5II**
RG↓GNCCY	**Eco0109I**
RGGNC↓CY	PssI
RTGC↓GCAY	**FspAI**
Y	
YAC↓GTR	**Ppu21I**
YGGCCG(-5/-1)↓	GdiII
Y↓GGCCR	**CfrI**
T	
TAC↓GTA	**Eco105I**
TACNNNNNRTGT	UbaF9I
TARCCA(9/11)↓	**TsoI**
↓(7/8)TCACC	**HphI**
↓(8/8)TCACC	SspD5I
T↓CATGA	**PagI**
↓(9/11)TCCGCC	EciI
T↓CCGGA	**Kpn2I**
T↓CCNGGA	**PfoI**
TCCRAC(20/18)↓	MmeI
T↓CGA	**TaqI**
TCG↓CGA	**Bsp68I**
TCGTAG	Pfl1108I
TCN↓GA	Hpy188I
TC↓NNGA	Hpy188III
T↓CTAGA	**XbaI**
TCTGG	BspNCI
↓(7/8)TCTTC	**MboII**
↓(10/12)TGA(N)$_6$TCA(12/10)↓	**BdaI**
T↓GATCA	**BclI**
TG↓CA	CviRI
TGC↓GCA	**NsbI**
TGG↓CCA	**MlsI**
↓(9/11)TTCGGTC	TaqII
↓(9/11)TTGGGTG	TaqII
TGGTTC	DrdII
↓(9/11)TGYTTG	Tth111II
T↓GTACA	**Bsp1407I**
↓(9/12)TGTGGG	RleAI
T↓TAA	**Tru1I**
TTAAT↓TAA	PacI
TTA↓TAA	PsiI
↓(9/11)TTCAT	TspDTI
TT↓CGAA	**Bsp119I**
TTT↓AAA	**DraI**
V	
VC↓TCGAGB	PspXI
W	
W↓CCGGW	BsaWI
WGG↓CCW	HaeI
W↓GTACW	**TatI**

Note

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

Commercial Restriction Enzymes Sorted by the Type of DNA Ends Generated

Enzymes Generating 5'-protruding Ends

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

Recognition sequence	Enzyme	5'-protruding end (5'→ 3') 1nt	2nt	3nt	4nt	5nt
AA↓CGTT	**Psp1406I**		CG			
A↓AGCTT	**HindIII**				AGCT	
↓AATT	**TasI**				AATT	
A↓CATGT	**PscI**				CATG	
A↓CCGGT	**BshTI**				CCGG	
ACCTGC(4/8)↓*	**BveI**				NNNN	
A↓CCWGGT	SexAI					CCWGG
A↓CGCGT	**MluI**				CGCG	
A↓CRYGT	AflIII				CRYG	
A↓CTAGT	**BcuI**				CTAG	
A↓GATCT	**BglII**				GATC	
AT↓CGAT	**Bsu15I**		CG			
AT↓TAAT	**VspI**		TA			
C↓AATTG	**MunI**				AATT	
CACCTGC(4/8)↓*	**AarI**				NNNN	
CACGAG(-5/-1)↓*	**BauI**				ACGA	
CA↓TATG	**NdeI**		TA			
↓(13/9)CATCC*	FokI				NNNN	
↓(14/10)CATCGC	BtgZI				NNNN	
C↓CATGG	**NcoI**				CATG	
C↓CCAGC	BseYI				CCAG	
CCCGC(4/6)↓*	**SmuI**		NN			
C↓CCGGG	**CfrI**				CCGG	
CC↓CWGGG	**PasI**			CWG		
CCGC(-3/-1)↓*	**SsiI**		CG			
C↓CGG	**HpaII**		CG			
C↓CGG	**MspI**		CG			
↓CCNGG	BssKI					CCNGG
CC↓NGG	**Bme1390I**	N				
C↓CNNGG	**BseDI**				CNNG	
C↓CRYGG	DsaI				CRYG	
CC↓SGG	**BcnI**	S				
C↓CTAGG	**XmaJI**				CTAG	
CCTCAGC(-5/-2)↓*	BbvCI			TCA		
CCTNAGC(-5/-2)↓*	**Bpu10I**			TNA		
CC↓TNAGG	**Eco81I**			TNA		
CCTNN↓NNNAGG	**XagI**	N				
↓CCWGG	**EcoRII**					CCWGG
CC↓WGG	**MvaI**	W				
C↓CWWGG	**Eco130I**				CWWG	
C↓GGCCG	**Eco52I**				GGCC	
CG↓GWCCG	**CpoI**			GWC		
C↓GTACG	**Pfl23II**				GTAC	
CGTCTC(1/5)↓*	**Esp3I**				NNNN	
C↓YCGRG	**Eco88I**				YCGR	
CR↓CCGGYG	SgrAI				CCGG	
C↓TAG	**FspBI**		TA			
C↓TCGAG	**XhoI**				TCGA	
CTCGTG(-5/-1)↓*	**BauI**				TCGT	
CTCTTC(1/4)↓*	**Eam1104I**			NNN		
C↓TYRAG	**SmoI**				TYRA	
C↓TNAG	**HpyF3I**			TNA		
C↓TRYAG	**BfmI**				TRYA	
C↓TTAAG	**BspTI**				TTAA	
GAAGAC(2/6)↓*	**BpiI**				NNNN	
↓(4/1)GAAGAG*	**Eam1104I**			NNN		
↓(4/1)GAAGAGC*	**LguI**			NNN		
G↓AATTC	**EcoRI**				AATT	
GACGC(5/10)↓*	**CseI**					NNNNN
GACN↓NNGTC	**PsyI**	N				
↓(5/4)GACTC*	PleI	N				
↓(5/1)GAGAC*	**Alw26I**				NNNN	
↓(5/1)GAGACC*	**Eco31I**				NNNN	
↓(5/1)GAGACG*	**Esp3I**				NNNN	
GAGTC(4/5)↓*	PleI	N				
G↓ANTC	**HinfI**			ANT		
↓GATC	**Bsp143I**				GATC	
↓GATC	**MboI**				GATC	
↓(5/4)GATCC*	**BspPI**	N				
↓(9/5)GATGC*	**LweI**				NNNN	

Recognition sequence	Enzyme	5'-protruding end (5'→ 3') 1nt	2nt	3nt	4nt	5nt
G↓AWTC	**PfeI**			AWT		
GCAGC(8/12)↓*	**BseXI**				NNNN	
↓(8/4)GCAGGT*	**BveI**				NNNN	
GCATC(5/9)↓*	**LweI**				NNNN	
GCGATG(10/14)↓	BtgZI				NNNN	
G↓CGC	**Hin6I**		CG			
GCG↓C	**HhaI**		CG			
G↓CGCGC	**PauI**				CGCG	
GCGG(-3/-1)↓*	**SsiI**		CG			
GC↓GGCCGC	**NotI**				GGCC	
↓(6/4)GCGGG*	**SmuI**		NN			
↓(10/5)GCGTC*	**CseI**					NNNNN
GC↓NGC	**SatI**	N				
G↓CTAGC	**NheI**				CTAG	
GCTCTTC(1/4)↓*	**LguI**			NNN		
GCTGAGG(-5/-2)↓*	BbvCI			TGA		
↓(12/8)GCTGC*	**BseXI**				NNNN	
G↓CTGGG	BseYI				CTGG	
GC↓TNAGC	**Bpu1102I**			TNA		
GCTNAGG(-5/-2)↓*	**Bpu10I**			TNA		
G↓CWGC	TseI			CWG		
GGATC(4/5)↓*	**BspPI**	N				
G↓GATCC	**BamHI**				GATC	
GGATG(9/13)↓*	FokI				NNNN	
GG↓CGCGCC	**SgsI**				CGCG	
GGGAC(10/14)↓*	**FaqI**				NNNN	
G↓GGCCC	**Bsp120I**				GGCC	
GG↓GWCCC	SanDI			GWC		
G↓GYRCC	**BshNI**				GYRC	
G↓GNCC	**Cfr13I**			GNC		
G↓GTACC	**Acc65I**				GTAC	
GGTCTC(1/5)↓*	**Eco31I**				NNNN	
G↓GTNACC	**Eco91I**					GTNAC
G↓GWCC	**Eco47I**			GWC		
GR↓CGYC	**Hin1I**		CG			
G↓TAC	**Csp6I**		TA			
↓(14/10)GTCCC*	**FaqI**				NNNN	
G↓TCGAC	**SalI**				TCGA	
GTCTC(1/5)↓*	**Alw26I**				NNNN	
↓(6/2)GTCTTC*	**BpiI**				NNNN	
G↓TGCAC	**Alw44I**				TGCA	
GT↓MKAC	**XmiI**		MK			
↓GTNAC	MaeIII					GTNAC
↓GTSAC	**NmuCI**					GTSAC
Y↓GGCCR	**CfrI**				GGCC	
R↓AATTY	**XapI**				AATT	
R↓CCGGY	**Cfr10I**				CCGG	
R↓GATCY	**PsuI**				GATC	
RG↓GNCCY	**Eco0109I**			GNC		
RG↓GWCCY	**Psp5II**			GWC		
T↓CATGA	**PagI**				CATG	
T↓CCGGA	**Kpn2I**				CCGG	
T↓CCNGGA	**PfoI**					CCNGG
T↓CGA	**TaqI**		CG			
T↓CTAGA	**XbaI**				CTAG	
T↓GATCA	**BclI**				GATC	
T↓GTACA	**Bsp1407I**				GTAC	
T↓TAA	**Tru1I**		TA			
TT↓CGAA	**Bsp119I**		CG			
VC↓TCGAGB	PspXI				TCGA	
W↓CCGGW	BsaWI				CCGG	
W↓GTACW	**TatI**				GTAC	

Single letter code

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

Note

- Asymmetric sequences are indicated by "*".
- Enzymes produced by Fermentas are shown in orange.

(continued on next page)

Enzymes Generating 3'-protruding Ends

Recognition sequence	Enzyme	3'-protruding end (5'→ 3') 1nt	2nt	3nt	4nt	9nt
ACGGA(11/9)↓*	TspGWI		NN			
ACGT↓	**TaiI**				ACGT	
ACN↓GT	**TaaI**	N				
ACTGG(1/-1)↓*	**BseNI**		GN			
ACTGGG(5/4)↓*	**BfiI**	N				
ATGAA(11/9)↓*	TspDTI		NN			
ATGCA↓T	**Mph1103I**				TGCA	
CACNNN↓GTG	**AdeI**			NNN		
↓(0/2)CACTGC*	BtsI		NN			
CAGNNN↓CTG	**CaiI**			NNN		
CASTG(2/-7)↓*	TspRI					NNCASTGNN
↓(0/2)CATCC*	**BseGI**		NN			
CATG↓	**Hin1II**				CATG	
↓(0/2)CATTGC*	**BseMI**		NN			
↓(-1/1)CCAGT*	**BseNI**		NC			
CCA$(N)_5$↓$(N)_4$TGG	XcmI	N				
CCANNNNN↓NTGG	**BstXI**				NNNN	
CCANNNN↓NTGG	**Van91I**			NNN		
↓(4/5)CCCAGT*	**BfiI**	N				
CCGC↓GG	**Cfr42I**		GC			
CCNNNNN↓NNGG	**BseLI**			NNN		
CCTC(7/6)↓*	**MnlI**	N				
CCTGCA↓GG	**SdaI**				TGCA	
CGAT↓CG	**PvuI**		AT			
CGRY↓CG	**Bsh1285I**		RY			
CTCAG(10/8)↓*	**BseMII**		NN			
↓(14/16)CTCCAG*	**GsuI**		NN			
↓(8/10)CTCCTC*	BseRI		NN			
CTGAAG(16/14)↓*	**Eco57I**		NN			
CTGRAG(16/14)↓*	**Eco57MI**		NN			
↓(8/10)CTGAG*	**BseMII**		NN			
↓(14/16)CTGCAC*	BsgI		NN			
CTGCA↓G	**PstI**				TGCA	
CTGGAG(16/14)↓*	**GsuI**		NN			
↓(14/16)CTTCAG*	**Eco57I**		NN			
GAAGA(8/7)↓*	**MboII**	N				
GAATGC(1/-1)↓*	**Mva1269I**		CN			
GACGT↓C	**AatII**				ACGT	
GACNNN↓NNGTC	**Eam1105I**	N				
GACNNNN↓NNGTC	**AasI**		NN			
GAGCT↓C	**SacI**				AGCT	
↓(6/7)GAGG*	**MnlI**	N				
GAGGAG(10/8)↓*	BseRI		NN			
GCAATG(2/0)↓*	**BseMI**		NN			
GCAGTG(2/0)↓*	BtsI		NN			
GCANNNN↓NTGC	BstAPI			NNN		
GCATG↓C	**PaeI**				CATG	
↓(-1/1)GCATTC*	**Mva1269I**		NG			
GCCNNNN↓NGGC	**BglI**			NNN		
GCGAT↓CGC	SgfI		AT			
GCNNNNN↓NNGC	**HpyF10VI**			NNN		
GDGCH↓C	**SduI**				DGCH	
↓(5/6)GGATAC*	**BfuI**	N				
GGATG(2/0)↓*	**BseGI**		NN			
GGCCGG↓CC	FseI				CCGG	
GGCC$(N)_4$↓NGGCC	**SfiI**			NNN		
GGCGGA(11/9)↓*	EciI		NN			
GGGCC↓C	**ApaI**				GGCC	
GCSG↓C	**TauI**			CSG		
GGTAC↓C	**KpnI**				GTAC	
GGTGA(8/7)↓*	**HphI**	N				
GKGCM↓C	**BseSI**				KGCM	
GRGCY↓C	**Eco24I**				RGCY	
GTATCC(6/5)↓*	**BfuI**	N				
GTGCAG(16/14)↓*	BsgI		NN			
GWGCW↓C	**Alw21I**				WGCW	
RCATG↓Y	**XceI**				CATG	
RGCGC↓Y	**Bsp143II**				GCGC	
TARCCA(11/9)↓*	**TsoI**		NN			
↓(7/8)TCACC*	**HphI**	N				
↓(9/11)TCCGCC*	EciI		NN			
↓(9/11)TCCGT*	TspGWI		NN			
↓(7/8)TCTTC*	**MboII**	N				
↓(9/11)TGGYTA*	**TsoI**		NN			
TTAAT↓TAA	PacI		AT			
↓(9/11)TTCAT*	TspDTI		NN			

Enzymes Generating Blunt Ends

Recognition sequence	Enzyme
AAT↓ATT	**SspI**
AGC↓GCT	**Eco47III**
AG↓CT	**AluI**
AGG↓CCT	**Eco147I**
AGT↓ACT	**ScaI**
ATTT↓AAAT	**SmiI**
CAC↓GTC	**AjiI**
CAC↓GTG	**Eco72I**
CACNN↓NNGTG	**OliI**
CAG↓CTG	**PvuII**
CAYNN↓NNRTG	MslI
CCC↓GGG	**SmaI**
CCGCTC(-3/-3)↓*	**MbiI**
CG↓CG	**Bsh1236I**
CMG↓CKG	NspBII
GAANN↓NNTTC	**PdmI**
GACGTG(-3/-3)↓*	**AjiI**
GACNN↓NNGTC	**BoxI**
↓(5/5)GACTC*	**SchI**
GAGCGG(-3/-3)↓*	**MbiI**
GAG↓CTC	**Ecl136II**
GAGTC(5/5)↓*	**SchI**
GAT↓ATC	**Eco32I**
GATNN↓NNATC	**BseJI**
Gm^6A↓TC	**DpnI**
GCCC↓GGGC	SrfI
GCC↓GGC	**PdiI**
GCN↓NGC	Cac8I
GG↓CC	**BsuRI**
GGC↓GCC	**EheI**
GGN↓NCC	**BspLI**
GT↓AC	**RsaI**
GTA↓TAC	**Bst1107I**
GTN↓NAC	**Hpy8I**
GTY↓RAC	**HincII**
GTT↓AAC	**KspAI**
GTTT↓AAAC	**MssI**
YAC↓GTR	**Ppu21I**
RG↓CY	CviJI
RTGC↓GCAY	**FspAI**
TAC↓GTA	**Eco105I**
TCG↓CGA	**Bsp68I**
TG↓CA	CviRI
TGC↓GCA	**NsbI**
TGG↓CCA	**MlsI**
TTA↓TAA	PsiI
TTT↓AAA	**DraI**

Enzymes Cleaving DNA on Both Sides of Their Recognition Sequence

Recognition sequence	Enzyme
↓(8/13)AAG$(N)_5$CTT(13/8)↓	FalI
↓(10/15)AC$(N)_4$GTAYC(12/7)↓	BaeI
↓(11/13)CAA$(N)_5$GTGG(12/10)↓	CspCI
↓(8/13)CAC$(N)_6$TCC(12/7)↓	**TstI**
↓(6/11)CCAA$(N)_7$TTC(12/7)↓	**AjuI**
↓(10/12)CCAC$(N)_5$TTG(13/11)↓	CspCI
↓(10/12)CGA$(N)_6$TGC(12/10)↓	BcgI
↓(7/12)GAA$(N)_7$TTGG(11/6)↓	**AjuI**
↓(7/12)GAAC$(N)_6$CTC(13/8)↓	**PpiI**
↓(7/12)GAAC$(N)_6$TAC(12/7)↓	PsrI
↓(7/12-13)GAAC$(N)_6$TCC(12-13/7)↓	**AloI**
↓(8/13)GAG$(N)_5$CTC(13/8)↓	**BpiI**
↓(8/13)GAG$(N)_5$GTTC(12/7)↓	**PpiI**
↓(8/13-14)GAY$(N)_5$VTC(13-14/8)↓	**Hin4I**
↓(10/12)GCA$(N)_6$TCG(12/10)↓	BcgI
↓(10/12)GCA$(N)_6$TGC(12/10)↓	**AlfI**
↓(7/12)GGA$(N)_6$GTG(13/8)↓	**TstI**
↓(7/12-13)GGA$(N)_6$GTTC(12-13/7)↓	**AloI**
↓(7/12)GRTAC$(N)_4$GT(15/10)↓	BaeI
↓(7/12)GTA$(N)_6$GTTC(12/7)↓	PsrI
↓(10/12)TGA$(N)_6$TCA(12/10)↓	**BdaI**

Note

- Asymmetric sequences are indicated by "*".
- Enzymes produced by Fermentas are shown 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 KCl, 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 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/µl; a number of enzymes are also available at high concentration (HC) – 50u/µl.

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'-[^{32}P]-labeled synthetic oligonucleotides (single-stranded and double-stranded) 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.

(continued on next page)

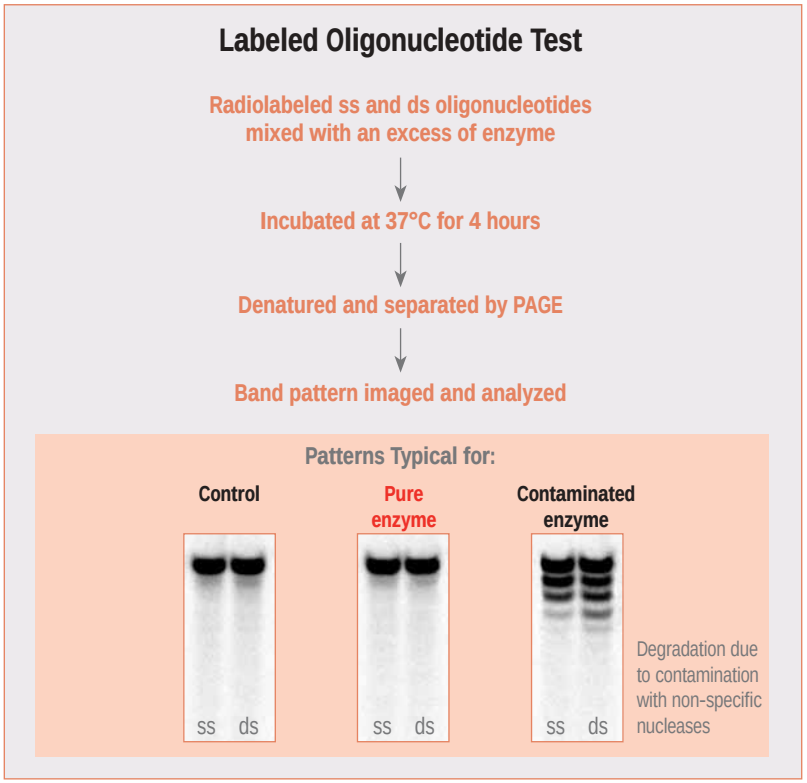


Figure 1.1. Labeled Oligonucleotide (LO) Test.
ss – single-stranded radiolabeled oligonucleotide **ds** – double-stranded radiolabeled oligonucleotide
Pure enzyme – Fermentas NotI
Contaminated enzyme – competitor's NotI

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 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/PstI and pUC57/Eco32I DNA fragments representing three different types of termini (3'-overhang, 5'-overhang and blunt ends).

Storage and Shipping

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°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.

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 (*see* p.122).

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.

Incubation Temperature. Indicates the optimal incubation temperature in °Celsius.

Ligation Efficiency. Indicates the ligation efficiency of DNA fragments generated by digestion with the restriction endonuclease (*see* conditions on p.21).

Star Activity. Restriction enzyme may exhibit star activity under the conditions described (*see* p.131).

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 (*see* pp.133-135).

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 (*see* pp.133-138).

Thermal Inactivation. Indicates thermal inactivation of the enzyme at 65°C or 80°C in 20min (*see* p.123).

Indicates that only small amounts of restriction endonuclease (up to 10 units) can be inactivated at 80°C in 20min.

High Concentration. Indicates that the enzyme is available at a high concentration (50u/µl).

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 *E.coli* designed to overexpress this enzyme.

Blue/White Certified. Indicates that the restriction enzyme was tested by the Blue/White cloning assay (*see* p.21).

FastDigest™ Enzyme. Indicates the availability of a special formulation of the enzyme for fast digestion (*see* p.2).

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

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

1 2 **BcuI** (SpeI)

3 Tango 37° 80% NO

4

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

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

7 **#ER1251** 8 400u
Supplied with:
10X Buffer Tango™ 1ml

#ER1252 2000u
Supplied with:
10X Buffer Tango™ 1ml

9 **FastDigest™ BcuI** (*see* p.2)
#ER1254 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

6 

10 Ad2 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
BcuI is supplied in storage buffer:
10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% glycerol.

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

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
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 Adenovirus-2 DNA in 16 hours.

11 ***Note***
Assayed using Adenovirus-2 DNA.

12

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	Ad2
0	0	0	0	0	0	0	1	1	0	0	3

1 Restriction endonuclease.
2 Its prototype.
3 Icons. For more information *see* p.22.
4 Relative enzyme activity (%) in Five Buffer System.
* – 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.
6 Electrophoretic pattern of cleavage products.
7 Catalog number.
8 Number of enzyme activity units in package.
9 FastDigest™ restriction endonuclease, *see* p.2.
10 DNA substrate and concentration of gel.
11 Other important information about specific enzyme features.
12 Number of recognition sites in the phage or plasmid DNA.

Bulk quantities & custom formulations available on request

AarI

Unique | Oligo | 37° | 95% | ★ | CG | YES 65° | ✂ | DNA

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
NR	NR	0-20	0-20	NR	50-100

5'...C A C C T G C (N)$_4$↓...3'
3'...G T G G A C G (N)$_8$↑...5'

#ER1581 25u
Supplied with:
10X Buffer AarI 1ml
10X Buffer Tango™ 1ml
50X oligonucleotide 25µl

#ER1582 125u
Supplied with:
10X Buffer AarI 1ml
10X Buffer Tango™ 1ml
50X oligonucleotide 2x25µl

λ DNA
1.0% agarose

Concentration
1-3u/µl

Conditions for 100% Activity
[1X Buffer AarI] + oligonucleotide:
[10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$, 100mM KCl, 0.1mg/ml BSA] + 1µM of oligonucleotide (*see* Note).
Incubate at 37°C.

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

Ligation and Recleavage
After 10-fold overdigestion with AarI, 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 AarI at least two copies of its recognition sequence are required.
- Inclusion of 1µM oligonucleotide with the AarI recognition sequence in the reaction mixture significantly improves cleavage of DNAs, especially of those with a single AarI site. Still, a complete cleavage of some substrates with AarI is difficult to achieve.
- Greater than 10-fold overdigestion with AarI may result in star activity.
- AarI 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
12	0	0	0	0	0	0	0	0	0	0

AasI (DrdI)

B | 37° | 95% | ★ | CG | YES 80° | ✂

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	20-50	0-20	0-20	50-100*	0-20

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

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

#ER1721 300u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1722 1500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
AasI 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.

Ligation and Recleavage
After 10-fold overdigestion with AasI, 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: 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	1	1	2	2	2	2	2	2	1	1

AatII

Tango 37° 95% CG YES 65° ✗

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↓C...3'
3'...C↑T G C A G...5'

#ER0991 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER0992 1500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
AatII 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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
10	1	0	1	1	1	0	0	0	1	0

AccI Fermentas enzyme **XmiI**, p.118

AccII Fermentas enzyme **Bsh1236I**, p.45

AccIII Fermentas enzyme **Kpn2I** (different sensitivity to methylation), p.80

Acc65I (KpnI*)

O 37° 95% Dcm CG YES 65° ✗

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

** Unlike KpnI, Acc65I produces DNA fragments with a 4-base 5'-extension*

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

#ER0901 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0902 5000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 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
Acc65I 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 Acc65I, approximately 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.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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	1	1	1	1	1	0	0

AccB7I Fermentas enzyme **Van91I**, p.114

AciI Fermentas enzyme **SsiI**, p.108

AclI Fermentas enzyme **Psp1406I**, p.98

AcsI Fermentas enzyme **XapI**, p.115

AcuI Fermentas enzyme **Eco57I**, p.64

AcyI Fermentas enzyme **Hin1I**, p.74

AdeI (DraIII)

G | 37° | 80% | ★ | CG | NO* | ✂ | 🧫

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

#ER1231 500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER1232 2500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
AdeI 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.

Ligation and Recleavage
After 10-fold overdigestion with AdeI, more than 80% of the DNA fragments can be ligated and more than 90% of these can be recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	100	20-50	100	100*	20-50

* 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.136).
EcoKI: may overlap – effect not determined.
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
Greater than 20-fold overdigestion with AdeI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
10	1	1	0	0	0	1	1	1	1	0

AfaI Fermentas enzyme **Csp6I** (different cleavage position) p.58 and **RsaI**, p.101

AfeI Fermentas enzyme **Eco47III**, p.63

AflII Fermentas enzyme **BspTI**, p.51

AgeI Fermentas enzyme **BshTI**, p.46

AhaIII Fermentas enzyme **DraI**, p.59

AhdI Fermentas enzyme **Eam1105I**, p.60

NEW

AjiI (BtrI)

Unique | 37° | 80% | CG | YES 65° | ✂ | 🧫

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

#ER1941 200u
Supplied with:
10X Buffer AjiI 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
5u/µl

Conditions for 100% Activity
1X Buffer AjiI:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
AjiI 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 AjiI, 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 AjiI. The remaining uncleaved ligation products may be cut by AatII and Eco72I (PmaCI).

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
NR	NR	20-50*	NR	NR	20-50*

* 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: completely overlaps – cleavage 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 is 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
17	0	0	0	0	0	0	0	0	0	0

NEW

AjuI

R SAM 37° 90% YES 65° HC

Activity in Five Buffer System, %

B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	Tango$_{+SAM}$	2X Tango$_{+SAM}$
0-20	50-100	20-50	**100**	50-100	50-100

5'...↓$_{7}$ (N) GAA (N)$_{7}$TTGG (N)$_{11}$ ↓...3'
3'...↑$_{12}$ (N) CTT (N)$_{7}$AACC (N)$_{6}$ ↑...5'

#ER1951 100u

Supplied with:

10X Buffer R	1ml
50X SAM	0.1ml
10X BufferTango™	1ml

λ DNA
0.7% agarose

Concentration
5u/µl

Conditions for 100% Activity
[1X Buffer R] + SAM:
[10mM Tris-HCl (pH 8.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA] + 0.01mM S-adenosylmethionine.
Incubate at 37°C.

Storage Buffer
AjuI 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 AjuI, 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.

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 AjuI is difficult to achieve.
- AjuI concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.
- AjuI 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	1	0	0	0	0	0	0	0	1	0

AleI

Fermentas enzyme **OliI**, p.91

AlfI

R SAM 37° 70% YES 65° HC

Activity in Five Buffer System, %

B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	Tango$_{+SAM}$	2X Tango$_{+SAM}$
0-20	0-20	0-20	**100**	0-20	20-50

5'...↓$_{10}$ (N) GCA (N)$_{6}$TGC (N)$_{12}$ ↓...3'
3'...↑$_{12}$ (N) CGT (N)$_{6}$ACG (N)$_{10}$ ↑...5'

#ER1801 50u

Supplied with:

10X Buffer R	1ml
50X SAM	0.1ml
10X BufferTango™	1ml

λ DNA
1.0% agarose

Concentration
1-3u/µl

Conditions for 100% Activity
[1X Buffer R] + SAM:
[10mM Tris-HCl (pH 8.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA] + 0.01mM S-adenosylmethionine.
Incubate at 37°C.

Storage Buffer
AlfI 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.

Ligation and Recleavage
After 20-fold overdigestion with AlfI, 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: 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 AlfI is difficult to achieve.
- AlfI concentration is determined by a maximal cleavage level achieved when no change in the fragmentation pattern is observed with the further enzyme increase.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
22	0	1	3	0	1	0	0	0	1	3

AloI

R | 30° | 70% | ★ | CG | YES 65° | ○ | ▭

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	0-20	0-20	100	20-50	100

5'...↓ 7 (N)GAAC(N)6TCC(N)12-13 ↓...3'
3'...↑ 12-13 (N)CTTG(N)6AGG(N)7 ↑...5'

#ER1491 100u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1492 500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
1-3u/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 30°C.

Storage Buffer
AloI 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 AloI, approximately 70% of the DNA fragments can be ligated and more than 80% of these can be recut.

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

Note
- Incubation at 37°C results in 20% activity.
- AloI 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 (12 or 13 nt away).
- The presence of SAM in the reaction mixture results in incomplete cleavage with AloI.
- Greater than 10-fold overdigestion with AloI may result in star activity.
- AloI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	0	1	0	0	0	1	1	1	0	0

AluI

Tango | 37° | 95% | YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	0-20	0-20	0-20	100	20-50

5'...A G↓C T...3'
3'...T C↑G A...5'

#ER0011 600u
Supplied with:
10X Buffer Tango™ 1ml

#ER0012 3000u
Supplied with:
10X Buffer Tango™ 2x1ml

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.
Incubate at 37°C.

Storage Buffer
AluI 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 AluI, approximately 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 – blocked (p.138).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
143	24	27	17	16	16	17	17	17	12	13

AlwI

Fermentas enzyme **BspPI**, p.50

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

AlwI (HgiAI)

O | 37° | 95% | YES 65°

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

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

#ER0021 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0022 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
AlwI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 300mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with AlwI, 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 – no effect (p.136).
EcoKI: may overlap – no effect (p.138).
EcoBI: may overlap – effect not determined.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
28	3	3	8	5	5	4	4	4	2	5

AlwI (BsmAI)

Tango | 37° | 95% | CG | YES 65°

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

5'...G T C T C(N)$_1$↓...3'
3'...C A G A G(N)$_5$↑...5'

#ER0031 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0032 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

λ DNA
1.0% 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
AlwI 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 AlwI, 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: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
37	4	5	3	4	4	2	2	2	5	3

AlwI 44I (ApaLI)

Tango | 37° | 90% | CG | YES 65° | ✂

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

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

#ER0041 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0042 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

λ 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
AlwI 44I 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 AlwI 44I, 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 – blocked (p.136).
EcoKI: may overlap – no effect (p.138).
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
4	1	1*	3	3	3	2	2	2	1	0

* According to our experimental data, AlwI 44I does not cut M13mp18/19 DNA.

AlwNI Fermentas enzyme **CaiI**, p.54

Aor13HI Fermentas enzyme **Kpn2I**, p.80

Aor51HI Fermentas enzyme **Eco47III**, p.63

ApaI

B | 37° | 95% | Dcm | CG | YES 65° | ✂ | ◎ | ⏱

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

5'...G G G C C↓C...3'
3'...C↑C C G G G...5'

#ER1411 3000u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

#ER1412 5x3000u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ ApaI** (*see* p.2)
#ER1414 100 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with ApaI, 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.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
Incubation at 30°C results in a 2-fold increase in activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	0	0	0	1	0	1	1	0	0

ApaLI Fermentas enzyme **AIw44I** (different sensitivity to methylation), p.30

ApoI Fermentas enzyme **XapI**, p.115

AscI Fermentas enzyme **SgsI**, p.106

AseI Fermentas enzyme **VspI**, p.114

AspI Fermentas enzyme **PsyI**, p.100

Asp700I Fermentas enzyme **PdmI**, p.94

Asp718I Fermentas enzymes **Acc65I**, p.25 and **KpnI** (different cleavage position and different sensitivity to methylation), p.80

AspEI Fermentas enzyme **Eam1105I**, p.60

AspHI Fermentas enzyme **AIw21I**, p.29

AsuI Fermentas enzyme **Cfr13I**, p.56

AsuII Fermentas enzyme **Bsp119I**, p.47

AvaI Fermentas enzyme **Eco88I**, p.66

AvaII Fermentas enzyme **Eco47I**, p.63

AvaIII Fermentas enzyme **Mph1103I**, p.85

AviII Fermentas enzyme **NsbI**, p.91

AvrII Fermentas enzyme **XmaJI**, p.117

BalI Fermentas enzyme **MlsI**, p.84

BamHI

Unique | 37° | 95% | 80° | HC | Genome Qualified | Blue/White Certified | FastDigest™ Enzyme

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

#ER0051 4000u
Supplied with:
10X Buffer BamHI 2x1ml
10X Buffer Tango™ 1ml

#ER0052 5x4000u
Supplied with:
10X Buffer BamHI 4x1ml
10X Buffer Tango™ 1ml

#ER0053 HC, 20000u
Supplied with:
10X Buffer BamHI 4x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ BamHI** (*see* p.2)

#ER0054 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
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.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50*	100	20-50	50-100*	100*	50-100

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

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

Methylation Effects
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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	1	1	1	1	1	1	1	1	1

BanI Fermentas enzyme **BshNI**, p.46

BanII Fermentas enzyme **Eco24I**, p.61

Bulk quantities & custom formulations available on request

BauI (BsiI)

Tango | 37° | 95% | YES 65° | ✂

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

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

#ER1841 200u
Supplied with:
10X Buffer Tango™ 1ml


λ 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
BauI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with BauI, 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 – no effect.
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
8	2	0	3	3	3	2	2	2	5	1

BbeI Fermentas enzyme **EheI** (different cleavage position), p.70

BbrPI Fermentas enzyme **Eco72I**, p.65

BbsI Fermentas enzyme **BpiI**, p.38

BbuI Fermentas enzyme **PaeI**, p.92

BbvI Fermentas enzyme **BseXI**, p.44

BbvII Fermentas enzyme **BpiI**, p.38

BciVI Fermentas enzyme **BfuI**, p.36

BclI

G | 55° | 95% | ★ | Dam | 80° | ⬭

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	100	20-50	20-50	100*	100

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

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

#ER0721 1000u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0722 5000u
Supplied with:
10X Buffer G 2x1ml
10X Buffer Tango™ 1ml


λ DNA (*dam*⁻)
0.7% agarose

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 55°C.

Storage Buffer
BclI 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 BclI, 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: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked (p.138).

Note
- Incubation at 37°C results in 50% activity.
- Greater than 15-fold overdigestion with BclI may result in star activity.
- Assayed using λ DNA (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
8	0	0	0	0	0	0	0	0	0	0

BcnI (CauII)

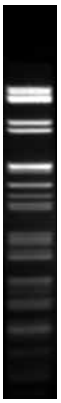
Tango | 37° | 80% | CG | YES 65°

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

5'...C C↓C G G...3'
3'...G G G↑C C...5'

#ER0061 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0062 5000u
Supplied with:
10X Buffer Tango™ 2x1ml



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.
Incubate at 37°C.

Storage Buffer
BcnI is supplied in:
10mM potassium phosphate (pH 7.4 at 25°C),
200mM NaCl, 1mM EDTA,
7mM 2-mercaptoethanol, 0.2mg/ml BSA and
50% (v/v) glycerol.

Ligation and Recleavage
After 10-fold overdigestion with BcnI, 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.

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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
114	1	4	10	7	7	5	6	6	6	10

BcuI (SpeI)

Tango | 37° | 80% | NO | Genome Qualified | Blue/White Certified | FastDigest™ Enzyme

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

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

#ER1251 400u
Supplied with:
10X Buffer Tango™ 1ml

#ER1252 2000u
Supplied with:
10X Buffer Tango™ 1ml

NEW **FastDigest™ BcuI** (*see* p.2)
#ER1254 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

Ad2 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
BcuI 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.

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

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: may overlap – effect not determined.
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 Adenovirus-2 DNA in 16 hours.

Note
Assayed using Adenovirus-2 DNA.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	Ad2
0	0	0	0	0	0	0	1	1	0	0	3

NEW

BdaI

G | SAM | 30° | 70% | ★ | Dam | YES 65°

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

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

5'...↓10 (N) TGA (N)6 TCA (N) 12↓...3'
3'...↑12 (N) ACT (N)6 AGT (N) 10↑...5'

#ER1961 50u

Supplied with:

10X Buffer G	1ml
50X SAM	0.1ml
10X BufferTango™	1ml

λ DNA (*dam*⁻)
0.7% agarose

Concentration
1-3u/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] + 0.05mM S-adenosylmethionine.
Incubate at 30°C.

Storage Buffer
BdaI 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 BdaI, 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: 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.

Note
- Incubation at 37°C results in 30% activity.
- Requires S-adenosylmethionine for activity. A complete cleavage of some substrates with BdaI is difficult to achieve.
- BdaI concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.
- BdaI 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 BdaI may result in star activity.
- BdaI 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 (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
28	4	7	4	2	2	2	2	2	3	6

BfaI

Fermentas enzyme FspBI, p.72

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

BfiI

Tango 37° 40% YES 65°

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

5'...A C T G G G(N)$_5$↓...3'
3'...T G A C C C(N)$_4$↑...5'

#ER1591 50u
Supplied with:
10X Buffer Tango™ 1ml
3X BfiI Stop Solution 0.5ml

#ER1592 250u
Supplied with:
10X Buffer Tango™ 1ml
3X BfiI Stop Solution 0.5ml

λ DNA
0.7% agarose

Concentration
1-3u/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.

Termination of Digestion Reaction
Do not attempt to stop a digestion reaction by adding EDTA (*see* Note). If no further enzymatic manipulations with the digested DNA are required, use Stop Solution containing **SDS** or BfiI Stop Solution to terminate the reaction by incubating at 65°C for 10min. If the digested DNA is to be used in down-stream manipulations, inactivate BfiI by incubating at 65°C for 20min.

3X BfiI Stop Solution *(supplied with)*
(Warm up, to ensure the solution is homogeneous before use.)
0.6% SDS, 0.05% bromophenol blue and
50% glycerol.

Storage Buffer
BfiI 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.

Ligation and Recleavage
After 10-fold overdigestion with BfiI, more than 40% of the pBR322 DNA fragments can be ligated and more than 90% of these can be recut.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

Note
- BfiI is the only known restriction endonuclease that cleaves DNA specifically both in the presence and absence of Mg^{2+} ions in the reaction mixture (Sapranauskas, R., et al., J.Biol.Chem., 275, 30878-30885, 2000).
- Chelating of Mg^{2+} ions by EDTA does not inhibit BfiI activity and may cause non-specific products. The non-specific cleavage increases at temperatures over 37°C.
- Greater than 40-fold overdigestion with BfiI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
4	0	1	5	2	2	2	2	2	1	3

BfmI (SfeI)

Tango 37° 95% YES 65°

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

5'...C↓T Pu Py A G...3'
3'...G A Py Pu T↑C...5'

#ER1161 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1162 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
BfmI 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.

Ligation and Recleavage
After 50-fold overdigestion with BfmI, 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.

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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
38	6	7	4	4	4	6	6	6	1	1

Bulk quantities & custom formulations available on request

CG Sensitivity to CpG Methylation | YES 65° Thermal Inactivation | HC High Concentration | Genome Qualified | Recombinant Enzyme | Blue/White Certified | FastDigest™ Enzyme

BfrI Fermentas enzyme **BspTI**, p.51

BfrBI Fermentas enzyme **Mph1103I** (different cleavage position), p.85

BfuI (BciVI)

Unique | 37° | 80% | ★ | YES 80° | ✂

5'...G T A T C C(N)$_6$↓...3'
3'...C A T A G G(N)$_5$↑...5'

#ER1501 100u
Supplied with:
10X Buffer BfuI 1ml
10X Buffer Tango™ 1ml

#ER1502 500u
Supplied with:
10X Buffer BfuI 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
5u/µl

Conditions for 100% Activity
1X Buffer BfuI:
50mM Tris-acetate (pH 7.9 at 37°C), 15mM Mg-acetate, 100mM K-acetate and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
BfuI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 300mM NaCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
NR	NR	0-20	0-20	NR	50-100*

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

Ligation and Recleavage
After 5-fold overdigestion with BfuI, 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 than 90% of these can be 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: 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 5-fold overdigestion with BfuI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
26	4	0	2	2	2	2	2	2	1	0

BfuAI Fermentas enzyme **BveI**, p.54

BfuCI Fermentas enzymes **Bsp143I**, p.48, **DpnI** (different cleavage position and different sensitivity to methylation), p.59 and **MboI** (different sensitivity to methylation), p.83

BglI

O | 37° | 95% | CG | YES 65° | ✂

5'...G C C N N N N↓N G G C...3'
3'...C G G N↑N N N N C C G...5'

#ER0071 2000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0072 5x2000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 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
BglI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 300mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	50-100	**100**	100	0-20	100

Ligation and Recleavage
After 50-fold overdigestion with BglI, 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 – 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
29	0	1	3	2	2	2	2	2	1	2

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

BglII

O 37° 95% NO* Genome Qualified Blue/White Certified FastDigest™ Enzyme

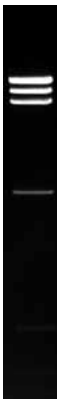
Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
0-20	20-50	**100**	50-100	0-20	100

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

#ER0081 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0082 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ BglII** (*see* p.2)
#ER0084 50 reactions
Supplied with:
10X FastDigest™ Buffer 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
BglII 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.

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

Methylation Effects
Dam: completely overlaps – no effect (p.133).
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – cleavage impaired (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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
6	0	1	0	0	0	0	0	0	0	0

BinI Fermentas enzyme **BspPI**, p.50

BlnI Fermentas enzyme **XmaJI**, p.117

BlpI Fermentas enzyme **Bpu1102I**, p.40

Bme1390I (ScrFI)

O 37° 80% Dcm CG YES 80°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	50-100	**100**	50-100	50-100	50-100

5'...C C↓N G G...3'
3'...G G N↑C C...5'

#ER1421 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1422 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA (*dcm*⁻)
1.4% 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
Bme1390I 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 Bme1390I, 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 than 90% of these can be 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.

Note
Assayed using λ DNA (*dcm*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
184	3	11	16	12	12	10	11	11	14	22

Bme1508I Fermentas enzyme **BseSI**, p.44

BmgBI Fermentas enzyme **AjiI**, p.26

BmrI Fermentas enzyme **BfiI**, p.35

BmtI Fermentas enzyme **NheI** (different cleavage position), p.89

BmyI Fermentas enzyme **SduI**, p.105

Bulk quantities & custom formulations available on request

BoxI (PshAI)

Tango 37° 90% CG YES 80°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	0-20	0-20	20-50	100	20-50

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

#ER1431 500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
BoxI 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	1	0	1	0	0	0	0	0	0	2

BpiI (BbvII)

G 37° 95% YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	100	50-100	50-100	50-100	50-100

5'...G A A G A C(N)$_2$↓...3'
3'...C T T C T G(N)$_6$↑...5'

#ER1011 200u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER1012 1000u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
BpiI 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 BpiI, 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 – 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
BpiI cleaves downstream of its recognition site and can generate any desired 4 base 5'-over-hangs. This feature is useful for direct PCR product cloning (p.140).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	3	0	3	0	0	0	0	0	1	3

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

BpII

Tango | SAM | 37° | 70% | YES 65° | CG | Blue/White Certified

Activity in Five Buffer System, %					
B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	2X $Tango_{+SAM}$
0-20	20-50	0-20	0-20	100	20-50

5'...↓ 8 (N)G A G(N)5C T C(N) 13↓...3'
3'...↑ 13 (N)C T C(N)5G A G(N) 8↑...5'

#ER1311 100u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 0.1ml

#ER1312 500u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 2x0.1ml

λ DNA
0.7% agarose

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
BpII 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.

Ligation and Recleavage
After 10-fold overdigestion with BpII, 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
- BpII requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.05mM S-adenosylmethionine gives more than a 100-fold increase in BpII activity. Still, a complete cleavage of some substrates with BpII is difficult to achieve.
- BpII concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	0	0	0	0	0	0	0	0	0

BpmI

Fermentas enzyme **GsuI**, p.73

Bpu10I

Unique | 37° | 90% | YES 80° | Recombinant Enzyme

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

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

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

#ER1181 200u
Supplied with:
10X Buffer Bpu10I 1ml
10X Buffer Tango™ 1ml

#ER1182 1000u
Supplied with:
10X Buffer Bpu10I 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
5u/µl

Conditions for 100% Activity
1X Buffer Bpu10I:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Bpu10I 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 Bpu10I, more than 90% 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. No more than 50% of these can be recut due to asymmetric recognition sequence of Bpu10I. The remaining uncleaved ligation products may be cut by Eco81I (SauI) and Bpu1102I (EspI).

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 – no effect (p.138).

Note
- A large excess of enzyme (>4u/1µg DNA) may result in incomplete DNA cleavage. Therefore, we recommend increasing the incubation time instead of using an excess of Bpu10I.
- Low salt, high glycerol (>5%) concentrations, pH >7.0 or a large excess of enzyme may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
19	7	4	1	0	0	0	0	0	5	3

Bulk quantities & custom formulations available on request

Bpu1102I (EspI)

Tango | 37° | 80% | YES 80° | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	20-50	20-50	100	20-50

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

#ER0091 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER0092 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
Bpu1102I 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 Bpu1102I, 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 than 95% of these can be 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
6	0	0	0	0	0	0	0	0	0	0

BpuAI Fermentas enzyme **BpiI**, p.38

BsaI Fermentas enzyme **Eco31I**, p.62

BsaAI Fermentas enzyme **Ppu21I**, p.97

BsaBI Fermentas enzyme **BseJI**, p.41

BsaHI Fermentas enzyme **Hin1I**, p.74

BsaJI Fermentas enzyme **BseDI**, p.40

BsaMI Fermentas enzyme **Mva1269I**, p.88

BseAI Fermentas enzyme **Kpn2I**, p.80

BseDI (SecI)

Tango | 55° | 95% | YES 80°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	20-50	0-20	0-20	100	50-100

5'...C↓C N N G G...3'
3'...G G N N C↑C...5'

#ER1081 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER1082 1500u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 55°C.

Storage Buffer
BseDI 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 BseDI, 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: never overlaps – no effect.

Note
Incubation at 37°C results in 10% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
105	6	9	8	5	4	5	6	6	12	18

BseGI (FokI*)

Tango | 55° | 95% | YES 80°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	50-100	20-50	20-50	**100**	20-50

** Unlike FokI, BseGI cleaves closer to the recognition sequence and produces DNA fragments with a 2-base 3'-extension*

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

#ER0871 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0872 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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 55°C.

Storage Buffer
BseGI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

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.

Note
Incubation at 37°C results in 25% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
150	8	4	12	5	5	4	4	4	10	7

BseJI (BsaBI)

O | 65° | 95% | Dam | NO

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	100*	**100**	NR	NR	100*

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

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

#ER1711 2000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA (*dam*⁻)
1.0% 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 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

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

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

Note
- Incubation at 37°C results in less than 10% activity.
- Greater than 20-fold overdigestion with BseJI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
21	2	2	1	0	0	0	0	0	0	1

BseLI (BsiYI)

Tango 55° 95% Dcm CG NO

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

5'...C C N N N N N↓N N G G...3'
3'...G G N N↑N N N N N C C...5'

#ER1201 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1202 2500u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 55°C.

Storage Buffer
BseLI 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 BseLI, 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.136).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Note
Incubation at 37°C results in 40% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
176	19	17	20	6	6	7	8	8	8	21

BseMI (BsrDI)

R 55° 95% YES 80°

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

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

#ER1261 100u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1262 500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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:
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 BseMI, more than 95% of the DNA fragments can be ligated and approximately 80% of these can be 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.

Note
Incubation at 37°C results in 20% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
44	4	3	2	2	2	2	2	2	3	1

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

BseMII

Tango | SAM | 55° | 90% | YES 80°

Activity in Five Buffer System, %					
B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
50-100	50-100	50-100	50-100	100	50-100

5'...C T C A G(N)$_{10}$↓...3'
3'...G A G T C(N)$_{8}$↑...5'

#ER1401 100u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 0.1ml

#ER1402 500u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 2x0.1ml

λ DNA
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] + 0.01mM S-adenosylmethionine.
Incubate at 55°C.

Storage Buffer
BseMII 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 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.

Note
- 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
79	10	23	7	5	5	4	4	4	13	8

BseNI (BsrI)

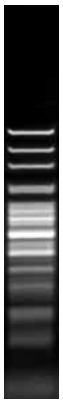
B | 65° | 95% | YES 80°

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:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0882 5000u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml



λ DNA
1.0% agarose

Concentration
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.
Incubate at 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

Storage Buffer
BseNI 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 BseNI, 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: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

Note
Incubation at 37°C results in less than 10% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
110	9	18	19	11	11	12	12	12	14	15

BsePI

Fermentas enzyme **Paul**, p.93

BseSI

G | 55° | 95% | 80°

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

```
    G     A
5'...G T G C C↓C...3'
3'...C↑A C G G G...5'
    C     T
```

#ER1441 500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER1442 2500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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 55°C.

Storage Buffer
BseSI is supplied in:
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.

Ligation and Recleavage
After 50-fold overdigestion with BseSI, 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: may overlap – cleavage impaired (p.138).
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
- Incubation at 37°C results in 20% activity.
- 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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
10	1	2*	3	3	4	2	3	3	2	1

* According to our experimental data, BseSI has only one site at position 2088.

BseXI (BbvI)

Unique | 65° | 95% | ★ | YES 80°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	NR	NR	NR	NR	NR

```
5'...G C A G C(N)8↓...3'
3'...C G T C G(N)12↑...5'
```

#ER1451 100u
Supplied with:
10X Buffer BseXI 1ml

#ER1452 500u
Supplied with:
10X Buffer BseXI 1ml

λ DNA
1.4% agarose

Concentration
1-3u/µl

Conditions for 100% Activity
1X Buffer BseXI:
50mM Tris-HCl (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-HCl (pH 7.4 at 25°C), 200mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

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

Note
- Assayed using pBR322 DNA (#SD0041).
- Incubation at 37°C results in 10% activity.
- Greater than 40-fold overdigestion with BseXI may result in the star activity.
- BseXI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
199	14	10	21	12	12	12	13	13	7	15

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution p.336

Fermentas Restriction Endonucleases:
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- 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

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

Bsh1236I (FnuDII)

R | 37° | 95% | CG | YES 65°

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

5'...C G↓C G...3'
3'...G C↑G C...5'

#ER0921 500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0922 2500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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
Bsh1236I 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
157	14	18	23	10	11	11	15	15	10	18

Bsh1285I (McrI)

G | 37° | 95% | CG | YES 80° | ✗

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

5'...C G Pu Py↓C G...3'
3'...G C↑Py Pu G C...5'

#ER0891 600u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

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
Bsh1285I is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 150mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Bsh1285I, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: may overlap – no effect (p.133).
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
22	1	4	7	5	5	5	6	6	6	6

BshNI (HgiCI)

O | 37° | 95% | Dcm | CG | YES 65° | ✂

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 C...3'
3'...C C Pu Py G↑G...5'

#ER1001 2000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
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 ligated and recut.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: may overlap – cleavage impaired (p.134).
CpG: may overlap – cleavage impaired (p.136).
EcoKI: may overlap – effect not determined.
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
25	3	7	9	4	4	4	4	4	1	10

BshTI (AgeI)

O | 37° | 95% | CG | YES 65° | ✂ | ◯

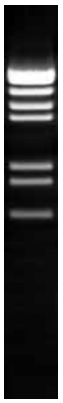
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	20-50	**100**	50-100	20-50	20-50

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

#ER1461 200u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1462 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 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
BshTI 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 BshTI, 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 20 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
13	0	0	0	0	0	0	0	0	2	4

BsiI Fermentas enzyme **BauI**, p.32

BsiEI Fermentas enzyme **Bsh1285I**, p.45

BsiHKAI Fermentas enzyme **Alw21I**, p.29

BsiWI Fermentas enzyme **Pfl23II**, p.95

BsiYI Fermentas enzyme **BseLI**, p.42

BslI Fermentas enzyme **BseLI**, p.42

BsmI Fermentas enzyme **Mva1269I**, p.88

BsmAI Fermentas enzyme **Alw26I**, p.29

BsmBI Fermentas enzyme **Esp3I**, p.71

BsmFI Fermentas enzyme **FaqI**, p.71

BsoBI Fermentas enzyme **Eco88I** (different sensitivity to methylation), p.66

Bsp68I (NruI)

O | 37° | 80% | ★ | CG | YES 65° | ✗ | ⬭

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

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

#ER0111 800u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0112 4000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 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
Bsp68I is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 200mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 10-fold overdigestion with Bsp68I, 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 than 90% of these can be recut.

Methylation Effects
Dam: may overlap – no effect (p.133).
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 Bsp68I may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	2	0	1	0	1	0	0	0	1	1

Bsp106I

BsoBI Fermentas enzyme **Bsu15I**, p.53

Bsp119I (AsuII)

Tango | 37° | 95% | CG | YES 80° | ✗ | ⬭

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

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

#ER0121 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
Bsp119I 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 Bsp119I, 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: 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	0	0	0	0	0	0	0	0	1	2

Bulk quantities & custom formulations available on request

CG Sensitivity to CpG Methylation | YES 65° Thermal Inactivation | HC High Concentration | ✗ Genome Qualified | Recombinant Enzyme | Blue/White Certified | FastDigest™ Enzyme

Bsp120I (ApaI*)

B | 37° | 95% | Dam | CG | YES 80° | HC

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	20-50	0-20	20-50	50-100	0-20

** Unlike ApaI, Bsp120I produces DNA fragments with a 4-base 5'-extension*

5'...G↓G G C C C...3'
3'...C C C G G↑G...5'

#ER0131 1500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0132 5x1500u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

#ER0133 HC, 7500u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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.

Storage Buffer
Bsp120I 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 Bsp120I, 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.

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

Bsp143I (MboI*)

Unique | 37° | 95% | CG | YES 65°

Activity in Five Buffer System, %

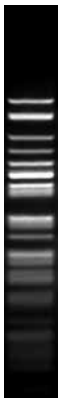
B	G	O	R	Tango	2X Tango
20-50	20-50	0-20	0-20	50-100	20-50

** Unlike MboI, Bsp143I is not blocked by Dam methylation*

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

#ER0781 300u
Supplied with:
10X Buffer Bsp143I 1ml
10X Buffer Tango™ 1ml

#ER0782 1500u
Supplied with:
10X Buffer Bsp143I 1ml
10X Buffer Tango™ 1ml



λ DNA
1.4% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Bsp143I:
33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate, 0.02% Triton X-100 and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Bsp143I is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Bsp143I more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: completely overlaps – no effect (p.133).
Dcm: never overlaps – no effect.
CpG: may overlap – blocked (p.136).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – no effect (p.138).

Lambda	ΦX174	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
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Bsp143II (HaeII)

Tango | 37° | 95% | CG | YES 65° | Genome Qualified | Recombinant Enzyme

5'...Pu G C G C↓Py...3'
3'...Py↑C G C G Pu...5'

#ER0791 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0792 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

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

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100*	20-50	0-20	20-50	100*	20-50

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

Ligation and Recleavage
After 5-fold overdigestion with Bsp143II 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: 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
Greater than 5-fold overdigestion with Bsp143II may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
48	8	6	11	3	3	4	4	4	2	11

Bsp1286I

Fermentas enzyme **SduI**, p.105

Bsp1407I

Tango | 37° | 95% | YES 65° | Genome Qualified | Blue/White Certified

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

#ER0931 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER0932 1500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
Bsp1407I 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.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	20-50	0-20	20-50	100	50-100

Ligation and Recleavage
After 50-fold overdigestion with Bsp1407I 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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	1	0	0	0	0	0	0	0	0

BspCI

Fermentas enzyme **PvuI**, p.100

BspCNI

Fermentas enzyme **BseMII**, p.43

BspDI

Fermentas enzyme **Bsu15I**, p.53

BspEI

Fermentas enzyme **Kpn2I**, p.80

BspHI

Fermentas enzyme **PagI**, p.92

Bulk quantities & custom formulations available on request

BspLI (NlaIV)

Tango | 37° | 90% | Dcm | CG | YES 65°

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

5'...G G N↓N C C...3'
3'...C C N↑N G G...5'

#ER1151 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1152 1000u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
BspLI is supplied in:
10mM Tris-HCl (pH 7.4 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 BspLI, approximately 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: may overlap – cleavage impaired (p.134).
CpG: may overlap – cleavage impaired (p.136).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
82	6	18	24	11	12	13	15	15	10	23

BspLU11I Fermentas enzyme **PscI**, p.97

BspMI Fermentas enzyme **BveI**, p.54

BspMII Fermentas enzyme **Kpn2I**, p.80

BspPI (BinI)

Tango | 55° | 70% | Dam | YES 80°

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

5'...G G A T C(N)$_4$↓...3'
3'...C C T A G(N)$_5$↑...5'

#ER1321 100u
Supplied with:
10X Buffer Tango™ 1ml

#ER1322 500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA (*dam*⁻)
1.4% agarose

Concentration
1-3u/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 55°C.

Storage Buffer
BspPI 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 BspPI, approximately 70% 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 50% of these can be recut.

Methylation Effects
Dam: completely overlaps – blocked (p.133).
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.

Note
- Incubation at 37°C results in 30% activity.
- Assayed using λ DNA (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
58	0	4	12	10	10	10	10	10	13	4

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

BspTI (AflII)

O | 37° | 95% | YES 65° | ✗ | Blue/White

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

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

#ER0831 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0832 5000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 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
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.

Ligation and Recleavage
After 50-fold overdigestion with BspTI, more than 95% of the ΦX174 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: 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	2	0	0	0	0	0	0	0	0	0

BspT104I Fermentas enzyme **Bsp119I**, p.47

BspT107I Fermentas enzyme **BshNI**, p.46

BsrI Fermentas enzyme **BseNI**, p.43

BsrBI Fermentas enzyme **MbiI**, p.82

BsrDI Fermentas enzyme **BseMI**, p.42

BsrFI Fermentas enzyme **Cfr10I**, p.56

BsrGI Fermentas enzyme **Bsp1407I**, p.49

BsrSI Fermentas enzyme **BseNI**, p.43

BssHII Fermentas enzyme **PauI**, p.93

BssKI Fermentas enzyme **Bme1309I** (different cleavage position), p.37

BssSI Fermentas enzyme **BauI**, p.32

Bst98I Fermentas enzyme **BspTI**, p.51

Bst1107I (SnaI)

O | 37° | 90% | CG | YES 65° | | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	50-100	**100**	100	20-50	100

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

#ER0701 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0702 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 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
Bst1107I 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 Bst1107I, more than 90% of the pBR322 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: 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	0	1	0	0	0	0	0	1	1

BstBI Fermentas enzyme **Bsp119I**, p.47

BstEII Fermentas enzyme **Eco91I**, p.67

BstF5I Fermentas enzyme **BseGI**, p.41

BstNI Fermentas enzymes **EcoRII** (different cleavage position and different sensitivity to methylation), p.70 and **MvaI**, p.87

BstOI Fermentas enzymes **EcoRII** (different cleavage position and different sensitivity to methylation), p.70 and **MvaI**, p.87

BstPI Fermentas enzyme **Eco91I**, p.67

BstUI Fermentas enzyme **Bsh1236I**, p.45

BstXI

O | 55° | 95% | | Dcm | YES 65° | | | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	100	**100**	50-100	50-100	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
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1022 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ BstXI** (*see* p.2)
#ER1024 50 reactions
Supplied with:
10X FastDigest™ Buffer 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 55°C.

Storage Buffer
BstXI 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 BstXI, approximately 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: 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
- Incubation at 37°C results in 50% activity.
- Greater than 15-fold overdigestion with BstXI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
13	3	0	0	0	0	0	1	1	0	0

BstYI Fermentas enzyme **PsuI**, p.99

BstZI Fermentas enzyme **Eco52I**, p.64

BstZ17I Fermentas enzyme **Bst1107I**, p.52

Bsu15I (ClaI)

Tango | 37° | 95% | Dam | CG | YES 65° | Genome Qualified | Blue/White Certified | FastDigest™

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	20-50	20-50	20-50	**100**	20-50

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

#ER0141 600u
Supplied with:
10X Buffer Tango™ 1ml

#ER0142 3000u
Supplied with:
10X Buffer Tango™ 2x1ml

NEW **FastDigest™ Bsu15I** (*see* p.2)

#ER0144 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ 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
Bsu15I is supplied in:
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.

Ligation and Recleavage
After 50-fold overdigestion with Bsu15I, 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.
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
15	0	2	1	0	0	0	1	1	1	1

Bsu36I Fermentas enzyme **Eco81I**, p.66

BsuRI (HaeIII)

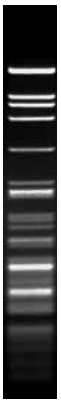
R | 37° | 95% | YES 80°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	20-50	50-100	**100**	50-100	100

5'...G G↓C C...3'
3'...C C↑G G...5'

#ER0151 3000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

#ER0152 5x3000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml



λ DNA
1.4% agarose

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
BsuRI 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 BsuRI, 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: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
149	11	15	22	11	13	12	14	14	13	24

BtrI Fermentas enzyme **AjiI**, p.26

Bulk quantities & custom formulations available on request

BveI (BspMI)

O | Oligo | 37° | 90% | CG | YES 65° | plasmid

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	20-50	**100**	20-50	50-100	100

5'...A C C T G C (N)$_4$↓...3'
3'...T G G A C G (N)$_8$↑...5'

#ER1741 250u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml
50X oligonucleotide 25µl

λ DNA
0.7% agarose

Concentration
5u/µl

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 Buffer
BveI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 150mM KCl,
1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and
50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with BveI, more than 90% 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: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

Note
- At least two copies of BveI recognition site are required for efficient cleavage.
- Inclusion of 1µM oligonucleotide with the BveI recognition sequence in the reaction mixture significantly improves cleavage of plasmid DNAs, especially of those with a single BveI site. Still, a complete cleavage of some substrates with BveI is difficult to achieve.
- BveI 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.
- BveI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
41	3	3	1	1	0	1	0	0	0	1

CaiI (AlwNI)

Tango | 37° | 95% | Dcm | YES 65° | star

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	20-50	20-50	50-100	**100**	50-100

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

#ER1391 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1392 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
CaiI 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 CaiI, 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 complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
41	0	1	1	1	1	1	1	1	1	3

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

CauII Fermentas enzyme **BcnI**, p.33

CelII Fermentas enzyme **Bpu1102I**, p.40

CfoI Fermentas enzymes **Hin6I** (different cleavage position), p.75 and **HhaI**, p.73

CfrI

Tango 37° 95% Dcm CG YES 65° ✗

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

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

5'...Py↓G G C C Pu...3'
3'...Pu C C G G↑Py...5'

#ER0161 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER0162 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA (*dcm*⁻)
1.0% 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
CfrI is supplied in:
10mM Tris-HCl (pH 8.5 at 25°C), 500mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with CfrI, 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.

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).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
39	2	3	6	3	3	3	4	4	4	8

Cfr9I (SmaI*)

Unique 37° 95% CG YES 65° ✗

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

** Unlike SmaI, Cfr9I produces DNA fragments with a 4-base 5'-extension*

5'...C↓C C G G G...3'
3'...G G G C C↑C...5'

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

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

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Cfr9I:
10mM Tris-HCl (pH 7.2 at 37°C), 5mM $MgCl_2$, 200mM sodium glutamate and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Cfr9I is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 250mM KCl, 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	1	0	1	1	1	1	1	1	0

Cfr10I

Unique · 37° · 95% · ★ · CG · NO* · ✂ · ◎

Activity in Five Buffer System, %

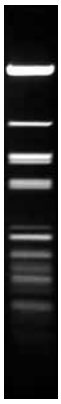
B	G	O	R	Tango	2X Tango
0-20	20-50	20-50	50-100*	20-50	50-100

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

5'...Pu↓C C G G Py...3'
3'...Py G G C C↑Pu...5'

#ER0181 200u
Supplied with:
10X Buffer Cfr10I 1ml
10X Buffer Tango™ 1ml

#ER0182 1000u
Supplied with:
10X Buffer Cfr10I 1ml
10X Buffer Tango™ 1ml



λ DNA
1.0% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Cfr10I:
10mM Tris-HCl (pH 8.0 at 37°C), 5mM $MgCl_2$, 100mM NaCl, 0.02% Triton X-100 and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Cfr10I is supplied in:
10mM potassium phosphate (pH 7.4 at 25°C), 100mM KCl, 2mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
61	0	1	7	1	1	2	2	2	5	12

Cfr13I (AsuI)

Tango · 37° · 95% · Dcm · CG · YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	20-50	20-50	100	20-50

5'...G↓G N C C...3'
3'...C C N G↑G...5'

#ER0191 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0192 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

λ DNA
1.0% agarose

Concentration
10u/µl

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

Storage Buffer
Cfr13I is supplied in:
10mM potassium phosphate (pH 7.5 at 25°C), 100mM KCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Cfr13I, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
74	2	4	15	6	8	6	8	8	9	11

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Cfr42I (SacII)

B | 37° | 95% | CG | YES 65° | Genome Qualified | Recombinant Enzyme | Blue/White Certified

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'

#ER0201 1200u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0202 5x1200u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
Cfr42I 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 50-fold overdigestion with Cfr42I, 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.

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.

Note
- Certain sites in λ and ΦX174 DNAs are difficult to cleave with Cfr42I, the same as with its prototype SacII.
- Cfr42I activity is affected by high salt concentration. Trace amounts of sodium chloride remaining in the substrate DNA after completion of upstream applications may inhibit enzyme activity and result in impaired DNA cleavage.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
4	1	0	0	0	0	0	1	1	1	1

ClaI

Fermentas enzyme **Bsu15I**, p.53

CpoI (RsrII)

Tango | 37° | 95% | CG | YES 65° | Blue/White Certified

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 G...3'
3'...G C C T G↑G C...5'
A

#ER0741 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER0742 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
CpoI 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 CpoI, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	0	0	0	0	0	0	0	0	0

NEW

CseI (HgaI)

R | 37° | 95% | CG | 80°

5'...G A C G C (N)5↓...3'
3'...C T G C G (N)10↑...5'

#ER1901 100u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1902 500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

pBR322 DNA
1.4% agarose

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 37°C.

Storage Buffer
CseI 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.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
NR	50-100*	50-100	100	100*	50-100

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

Ligation and Recleavage
After 50-fold overdigestion with CseI, 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: completely overlaps – blocked (p.135).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Note
- Assayed using pBR322 DNA (#SD0041).
- Low salt, high glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.
- CseI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
102	14	7	11	4	4	4	4	4	5	9

CspI
Fermentas enzyme CpoI, p.57

Csp6I (RsaI*)

B | 37° | 95% | YES 65°

** Unlike RsaI, Csp6I produces DNA fragments with a 2-base 5'-extension*

5'...G↓T A C...3'
3'...C A T↑G...5'

#ER0211 1500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
Csp6I 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.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	50-100	0-20	0-20	50-100	0-20

Ligation and Recleavage
After 50-fold overdigestion with Csp6I, 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 – no effect (p.136).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
113	11	19	3	3	3	2	2	2	4	3

Csp45I
Fermentas enzyme Bsp119I, p.47

CviAII
Fermentas enzyme Hin1II (different cleavage position), p.74

DdeI
Fermentas enzyme HpyF3I, p.79

DpnI

Tango | 37° | 70% | YES 80° | ◯

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
100	100	50-100	50-100	**100**	50-100

5'...G A↓T C...3'
3'...C T↑A G...5'
CH3 CH3

#ER1701 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1702 2500u
Supplied with:
10X Buffer Tango™ 1ml

pBR322 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.
Incubate at 37°C.

Storage Buffer
DpnI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 400mM KCl,
1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and
50% (v/v) glycerol.

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

Note
- DpnI requires the presence of N6-methyl-adenine within the recognition sequence to cleave DNA.
- Assayed using pBR322 DNA (#SD0041).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
116	0	7	22	15	15	15	15	15	22	15

DpnII

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

DraI (AhaIII)

Tango | 37° | 95% | YES 65° | HC | ✗

Activity in Five Buffer System, %					
B	G	O	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'

#ER0221 1500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0222 5x1500u
Supplied with:
10X Buffer Tango™ 2x1ml

#ER0223 HC, 7500u
Supplied with:
10X Buffer Tango™ 2x1ml

λ DNA
0.7% agarose

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 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with DraI, 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: may overlap – blocked (p.138).
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
13	2	5	3	3	3	3	3	3	3	2

DraII

Fermentas enzyme **Eco0109I**, p.69

DraIII

Fermentas enzyme **AdeI**, p.26

DrdI

Fermentas enzyme **AasI**, p.24

EaeI

Fermentas enzyme **CfrI**, p.55

EagI

Fermentas enzyme **Eco52I**, p.64

Bulk quantities & custom formulations available on request

Eam1104I (Ksp632I)

Tango | 37° | 95% | YES 65° | ✂ | ◯

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	0-20	0-20	100	0-20

5'...C T C T T C(N)$_1$↓...3'
3'...G A G A A G(N)$_4$↑...5'

#ER0231 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER0232 1500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
Eam1104I 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 Eam1104I, 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 – 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
34	2	2	2	3	3	3	3	3	2	1

Eam1105I

Unique | 37° | 80% | YES 65° | ✂

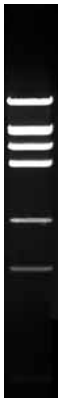
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	50-100	0-20	0-20	50-100	20-50

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

#ER0241 1000u
Supplied with:
10X Buffer Eam1105I 1ml
10X Buffer Tango™ 1ml

#ER0242 5000u
Supplied with:
10X Buffer Eam1105I 2x1ml
10X Buffer Tango™ 1ml



λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Eam1105I:
10mM Tris-HCl (pH 7.5 at 37°C), 5mM $MgCl_2$, 100mM NaCl and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Eam1105I 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 Eam1105I, 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 than 90% of these can be 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: 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
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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
9	1	0	1	1	1	1	1	1	1	1

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

EarI

Fermentas enzyme **Eam1104I**, p.60

Ecl136II (SacI*)

Unique 37° 90% CG YES 65° Genome Qualified Blue/White Certified

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

** Unlike SacI, Ecl136II produces DNA fragments with blunt ends*

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

#ER0251 1500u

Supplied with:
10X Buffer Ecl136II 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Ecl136II:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$ and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Ecl136II 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 Ecl136II, more than 90% 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 complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	1	1	1	1	1	0	0

EclHKI

Fermentas enzyme **Eam1105I**, p.60

EclXI

Fermentas enzyme **Eco52I**, p.64

Eco24I (HgiJII)

Tango 37° 95% YES 65° Genome Qualified Blue/White Certified

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↓C...3'
3'...C↑Py C G Pu G...5'

#ER0281 1500u

Supplied with:
10X Buffer Tango™ 1ml

λ 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
Eco24I 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 Eco24I, 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.

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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	0	2	2	1	2	2	3	3	1	2

Eco31I

G | 37° | 95% | Dcm | CG | YES 65° | ✂ | ◯

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	**100**	0-20	0-20	50-100	20-50

5'...G G T C T C(N)$_1$↓...3'
3'...C C A G A G(N)$_5$↑...5'

#ER0291 1000u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0292 5000u
Supplied with:
10X Buffer G 2x1ml
10X Buffer Tango™ 1ml

λ DNA (*dcm*⁻)
0.7% agarose

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
Eco31I 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 Eco31I, more than 95% of 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.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).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	1	1	1	1	1	1	1	0

Eco32I (EcoRV)

R | 37° | 95% | YES 65° | HC | ✂ | ▭ | ◷

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	50-100	50-100	**100**	20-50	100

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

#ER0301 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0302 5x2000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

#ER0303 HC, 10000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ Eco32I** (*see* p.2)
#ER0304 100 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
1.0% agarose

Concentration
10u/µl
50u/µl, HC

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
Eco32I is supplied in:
25mM Tris-HCl (pH 7.5 at 25°C), 200mM NaCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Eco32I, 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 – 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
21	0	0	1	0	1	0	1	1	0	1

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Eco47I (AvaII)

R | 37° | 95% | Dam | CG | YES 65° | ◯

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

5'...G↓GACC...3'
3'...CCTG↑G...5'
T A

#ER0311 800u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0312 4000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

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
Eco47I 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
35	1	1	8	2	2	2	2	2	4	5

Eco47III

O | 37° | 80% | CG | YES 65° | ✂ | ◯

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

5'...AGC↓GCT...3'
3'...TCG↑CGA...5'

#ER0321 200u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0322 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 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
Eco47III 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 Eco47III, more than 80% of pBR322 DNA fragments can be ligated and more than 90% of these can be 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 – 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	2*	4	0	0	0	0	0	2	5

* According to our experimental data, Eco47III has only one recognition site at position 3039.

Eco52I (XmaIII)

Unique | 37° | 95% | CG | YES 65° | ✗ | ◯

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

5'...C↓G G C C G...3'
3'...G C C G G↑C...5'

#ER0331 500u
Supplied with:
10X Buffer Eco52I 1ml
10X Buffer Tango™ 1ml

#ER0332 2500u
Supplied with:
10X Buffer Eco52I 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
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.

Storage Buffer
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.

Ligation and Recleavage
After 50-fold overdigestion with Eco52I, 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.

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

Eco57I

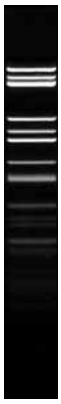
G | SAM | 37° | 70% | YES 65° | ◯

Activity in Five Buffer System, %					
B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
100	100	20-50	20-50	50-100	50-100

5'...C T G A A G(N)$_{16}$↓...3'
3'...G A C T T C(N)$_{14}$↑...5'

#ER0341 200u
Supplied with:
10X Buffer G 1ml
50X SAM 0.1ml
10X Buffer Tango™ 1ml

#ER0342 1000u
Supplied with:
10X Buffer G 1ml
50X SAM 2x0.1ml
10X Buffer Tango™ 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
Eco57I 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 Eco57I, 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.
EcoBI: may overlap – effect not determined.

Note
- Eco57I requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.01mM S-adenosylmethionine gives a 100-fold increase in Eco57I activity. Still, a complete cleavage of some substrates with Eco57I is difficult to achieve.
- Eco57I 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.
- Eco57I 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
40	0	0	2	2	2	2	2	2	1	2

Eco57MI*

B | SAM | 37° | 90% | Dcm | YES 65° | ◯

Activity in Five Buffer System, %					
B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
100	50-100	0-20	20-50	50-100	0-20

5'...C T G Pu A G(N)$_{16}$↓...3'
3'...G A C Py T C(N)$_{14}$↑...5'

#ER1671 50u
Supplied with:
10X Buffer B 1ml
50X SAM 0.1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
1-3u/µl

Conditions for 100% Activity
[1X Buffer B] + SAM:
[10mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$ and 0.1mg/ml BSA] +
1µM S-adenosylmethionine.
Incubate at 37°C.

Storage Buffer
Eco57MI 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 Eco57MI, approximately 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.

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.

Note
- Eco57MI requires only Mg^{2+} 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	ΦX174	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 | 37° | 80% | ✶ | CG | YES 65° | ✗ | ◯ | ▭

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

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

#ER0361 2000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0362 5x2000u
Supplied with:
10X Buffer Tango™ 2x1ml

λ 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
Eco72I 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 Eco72I, 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 than 90% of these can be 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 10-fold overdigestion with Eco72I may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	0	0	0	0	0	0	0	0	0

Bulk quantities & custom formulations available on request

Eco81I (SauI)

Tango | 37° | 80% | YES 80° | | |

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

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

#ER0371 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0372 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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 and 66mM K-acetate and
0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Eco81I 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 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	0	0	0	0	0	0	1

Eco88I (AvaI)

Tango | 37° | 95% | CG | YES 65° | | | |

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

5'...C↓Py C G Pu G...3'
3'...G Pu G C Py↑C...5'

#ER0381 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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 and 66mM K-acetate and
0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Eco88I 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 Eco88I, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
8	1	2	1	1	1	1	2	2	2	1

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

Eco91I (BstEII)

O | 37° | 95% | YES 65° | Genome Qualified | Blue/White Certified

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	20-50	**100**	50-100	50-100	100

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

#ER0391 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0392 5000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 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
Eco91I is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 150mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
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).
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
13	0	0	0	0	0	0	0	0	1	0

Eco105I (SnaBI)

Tango | 37° | 80% | Star Activity | CG | YES 65° | Genome Qualified | Recombinant Enzyme

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

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

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

#ER0401 600u
Supplied with:
10X Buffer Tango™ 1ml

#ER0402 3000u
Supplied with:
10X Buffer Tango™ 2x1ml

λ 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 and 66mM K-acetate and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
Eco105I is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 1mM phenylmethylsulfonylfluoride, 0.2mg/ml BSA and 50% (v/v) glycerol.

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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	1	0	0	0	0	0	0	0	0

Eco130I (StyI)

O | 37° | 95% | YES 65° | | |

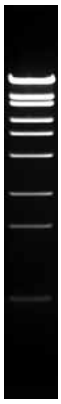
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	20-50	**100**	50-100	50-100	100

```
    A A
5'...C↓C T T G G...3'
3'...G G A A C↑C...5'
      T T
```

#ER0411 2500u

Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml



λ DNA
1.0% 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
Eco130I 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 Eco130I, 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.

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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
10	0	0	1	0	0	0	0	0	0	2

Eco147I (StuI)

B | 37° | 90% | Dcm | YES 80° | | | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	50-100	20-50	20-50	50-100	0-20

```
5'...A G G↓C C T...3'
3'...T C C↑G G A...5'
```

#ER0421 1000u

Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0422 5000u

Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
Eco147I 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 Eco147I, more than 90% 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 complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
6	1	0	0	0	1	0	0	0	2	0

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

EcoICRI Fermentas enzymes **Ecl136II**, p.61 and **SacI** (different cleavage position), p.102

EcoNI Fermentas enzyme **XagI**, p.115

EcoO65I Fermentas enzyme **Eco91I**, p.67

EcoO109I (DraI)

Tango · 37° · 95% · Dcm · YES 65° · Genome Qualified

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
50-100	20-50	20-50	20-50	**100**	100

5'...Pu G↓G N C C Py...3'
3'...Py C C N G↑G Pu...5'

#ER0261 2000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
EcoO109I is supplied in:
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.

Ligation and Recleavage
After 50-fold overdigestion with EcoO109I, 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 – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

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

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	0	4	1	1	0	1	1	1	4

EcoRI

Unique · 37° · 95% · YES 65° · HC · Genome Qualified · Recombinant Enzyme · Blue/White Certified · FastDigest™ Enzyme

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
0-20	NR	100	100*	NR	100

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

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

#ER0271 5000u
Supplied with:
10X Buffer EcoRI 2ml
10X Buffer Tango™ 1ml

#ER0272 5x5000u
Supplied with:
10X Buffer EcoRI 5x1ml
10X Buffer Tango™ 1ml

#ER0273 HC, 25000u
Supplied with:
10X Buffer EcoRI 5x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ EcoRI** (*see* p.2)

#ER0274 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

Conditions for 100% Activity
1X Buffer EcoRI:
50mM Tris-HCl (pH 7.5 at 37°C),
10mM $MgCl_2$, 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.

Ligation and Recleavage
After 50-fold overdigestion with EcoRI, 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 – 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
- Low salt concentration, large excess of the enzyme, pH >8.0, or the replacement of Mg^{2+} by Mn^{2+} 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	1	1	1	1	1	1	1	0	1

Bulk quantities & custom formulations available on request

NEW

EcoRII

O | 37° | 90% | Dcm | YES 80° | ◯

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	50-100	**100**	50-100	20-50	50-100

```
      T
5'...↓C C A G G ...3'
3'...  G G T C C↑...5'
       A
```

#ER1921 200u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1922 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA (*dcm*⁻)
1.4% 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
EcoRII 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 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 MvaI, neoschizomer (#ER0551) of EcoRII, is recommended.
- MvaI, 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
70	2	7	6	5	5	5	5	5	8	12

EcoRV Fermentas enzyme **Eco32I**, p.62

EcoT14I Fermentas enzyme **Eco130I**, p.68

EcoT22I Fermentas enzyme **Mph1103I**, p.85

EheI (NarI*)

Tango | 37° | 80% | CG | YES 65° | ✂

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

** Unlike NarI, EheI produces DNA fragments with blunt ends*

```
5'...G G C↓G C C...3'
3'...C C G↑C G G...5'
```

#ER0441 500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
EheI is supplied in:
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.

Ligation and Recleavage
After 50-fold overdigestion with EheI, more 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.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: may overlap – no effect (p.134).
CpG: completely overlaps – blocked (p.135).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

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.

Note
- Unlike NarI, EheI completely digests λ and pBR322 DNAs.
- 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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	2	1	4	1	1	0	0	0	0	4

EspI Fermentas enzyme **Bpu1102I**, p.40

Esp3I

Tango DTT 37° 95% CG YES 65° Genome Qualified Recombinant Enzyme

Activity in Five Buffer System, %					
B_{+DTT}	G_{+DTT}	O_{+DTT}	R_{+DTT}	$Tango_{+DTT}$	2X $Tango_{+DTT}$
100	20-50	0-20	0-20	**100**	0-20

5'...C G T C T C(N)$_1$↓...3'
3'...G C A G A G(N)$_5$↑...5'

#ER0451 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER0452 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
10u/µl

Conditions 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
Esp3I is supplied in:
10mM potassium phosphate (pH 7.4 at 25°C), 100mM KCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Esp3I, 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 – 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.
- Esp3I 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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	0	1	1	2	2	0	0	0	1	2

FaqI (FinI)

Tango SAM 37° 90% CG YES 80° Blue/White Certified

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

5'...G G G A C(N)$_{10}$↓...3'
3'...C C C T G(N)$_{14}$↑...5'

#ER1811 100u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 0.1ml

λ DNA
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
FaqI 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 10-fold overdigestion with FaqI, 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.

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.

Note
- FaqI 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 FaqI activity. Still, a complete cleavage of some substrates is difficult to achieve.
- FaqI concentration is determined by a maximal cleavage level achieved when no change in the fragmentation patterns is observed with the further enzyme increase.
- FaqI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
38	2	2	4	0	0	0/1	0/1	0/1	1	7

FatI Fermentas enzyme **Hin1II** (different cleavage position), p.74

FauI Fermentas enzyme **SmuI**, p.108

FbaI Fermentas enzyme **BclI**, p.32

Bulk quantities & custom formulations available on request

CG Sensitivity to CpG Methylation | YES 65° Thermal Inactivation | HC High Concentration | Genome Qualified | Recombinant Enzyme | Blue/White Certified | FastDigest™ Enzyme

FinI Fermentas enzyme **Faq**, p.71

FnuDII Fermentas enzyme **Bsh1236**, p.45

Fnu4HI Fermentas enzyme **SatI**, p.103

FokI Fermentas enzyme **BseGI** (different cleavage position), p.41

FspI Fermentas enzyme **NsbI**, p.91

FspAI

O | 37° | 80% | CG | YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	0-20	100	50-100	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
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1662 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA
0.5% agarose

Concentration
5u/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
FspAI 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 FspAI, more than 80% of the DNA fragments can be ligated and more than 90% of these can be 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.

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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	2	0	0	0	0	0	0	2

FspBI (Mael)

Tango | 37° | 80% | YES 65° | ✂

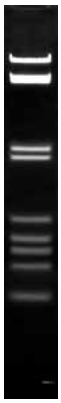
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	20-50	0-20	0-20	100	0-20

5'...C↓T A G...3'
3'...G A T↑C...5'

#ER1761 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1762 2500u
Supplied with:
10X Buffer Tango™ 1ml



λ 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
FspBI 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 FspBI, 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 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: 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 4 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	3	5	5	4	4	5	6	6	4	4

GsuI

B | 30° | 90% | Dcm | YES 65° | ◯

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 G(N)$_{16}$↓...3'
3'...G A C C T C(N)$_{14}$↑...5'

#ER0461 100u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0462 500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
5u/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.
Incubate at 30°C.

Storage Buffer
GsuI is supplied in:
10mM potassium phosphate (pH 7.5 at 25°C), 1mM EDTA, 7mM 2-mercaptoethanol, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 10-fold overdigestion with GsuI, approximately 90% 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: never overlaps – no effect.

Note
- Incubation at 37°C results in 70% activity.
- GsuI requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.01mM S-adenosylmethionine gives a 2-fold increase in activity.
- GsuI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
25	3	2	4	1	1	1	1	2	1	4

HaeII Fermentas enzyme **Bsp143II**, p.49

HaeIII Fermentas enzyme **BsuRI**, p.53

HapII Fermentas enzymes **HpaII**, p.77 and **MspI** (different sensitivity to methylation), p.86

HgaI Fermentas enzyme **CseI**, p.58

HgiAI Fermentas enzyme **Alw21I**, p.29

HgiCI Fermentas enzyme **BshNI**, p.46

HgiJII Fermentas enzyme **Eco24I**, p.61

HhaI

Tango | 37° | 95% | CG | YES 80°

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

5'...G C G↓C...3'
3'...C↑G C G...5'

#ER1851 2000u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with HhaI, 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.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
215	18	26	31	17	17	20	24	24	16	26

Bulk quantities & custom formulations available on request

Hin1I (AcyI)

G | 37° | 95% | Dcm | CG | YES 65°

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

5'...G Pu↓C G Py C...3'
3'...C Py G C↑Pu G...5'

#ER0471 300u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0472 1500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

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
Hin1I 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 50-fold overdigestion with Hin1I, 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: 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
40	7	1	6	3	3	1	1	1	2	4

Hin1II (NlaIII)

G | 37° | 95% | YES 65°

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

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

#ER1831 300u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

Concentration
5u/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
Hin1II is supplied in:
10mM potassium phosphate (pH 7.4 at 25°C), 500mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.15% Triton X-100, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Hin1II, 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.

Note
More stable when stored at -70°C.
At -20°C the half-life of Hin1II is 6 months.

Lambda	ΦX174	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
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

Hin4I

Tango | SAM | 37° | 95% | Dam | CG | YES 65°

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	0-20

5'...↓8 (N)GAPy(N)5(A/C/G)TC(N)13-14↓...3'
3'...↑13-14 (N)CTPu(N)5(T/G/C)AG(N)8↑...5'

#ER1601 50u
Supplied with:
10X Buffer Tango™ 1ml
50X SAM 0.1ml

#ER1602 250u
Supplied with:
10X Buffer Tango™ 1ml
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.

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 Mg^{2+} 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
58	9	5	5	2	3	2	2	2	8	6

Hin6I (HhaI*)

Tango | 37° | 95% | CG | YES 65°

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

** Unlike HhaI, Hin6I produces DNA fragments with a 2-base 5'-extension*

5'...G↓C G C...3'
3'...C G C↑G...5'

#ER0481 2000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0482 5x2000u
Supplied with:
10X Buffer Tango™ 2x1ml

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.
Incubate at 37°C.

Storage Buffer
Hin6I 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 50-fold overdigestion with Hin6I, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
215	18	26	31	17	17	20	24	24	16	26

HinP1I

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

HincII (HindII)

Tango | 37° | 95% | CG | YES 65°

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

5'...G T Py↓Pu A C...3'
3'...C A Pu↑Py T G...5'

#ER0491 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0492 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
HincII is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 200mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with HincII, 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: may overlap – blocked (p.138).
EcoBI: may overlap – blocked (p.138).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
35	13	1	2	1	1	1	1	1	1	2

HindII

Fermentas enzyme **HincII**, *see above*

HindIII

R | 37° | 95% | YES 65° | HC

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

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

#ER0501 5000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0502 5x5000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

#ER0503 HC, 25000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ HindIII** *(see p.2)*

#ER0504 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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

Ligation and Recleavage
After 50-fold overdigestion with HindIII, 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 – cleavage impaired (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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	0	1	1	1	1	1	1	1	1	1

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

HinfI

R | 37° | 95% | CG | YES 65° | HC

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

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

#ER0801 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0802 5x2000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

#ER0803 HC, 10000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

Concentration
10u/µl
50u/µl, HC

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
HinfI 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.

Ligation and Recleavage
After 50-fold overdigestion with HinfI, 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).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked (p.138).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
148	21	27	10	6	5	9	8	8	13	9

HpaI

Fermentas enzyme **KspAI**, p.81

HpaII

Tango | 37° | 95% | CG | YES 65°

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

5'...C↓C G G...3'
3'...G G C↑C...5'

#ER0511 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0512 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

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.
Incubate at 37°C.

Storage Buffer
HpaII is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with HpaII, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
328	5	18	26	13	13	12	13	13	16	34

HphI

B | 37° | 70% | Dam | YES 65° | ◯

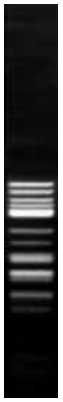
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	0-20	0-20	0-20	20-50	0-20

5'...G G T G A(N)$_8$↓...3'
3'...C C A C T(N)$_7$↑...5'

#ER1101 300u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1102 1500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml



λ DNA (*dam*⁻)
1.4% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
HphI 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 HphI, more than 70% of the DNA fragments can be ligated and more than 90% of these can be recut.

Methylation Effects
Dam: may overlap – blocked (p.133).
Dcm: may overlap – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked (p.138).

Note
- High glycerol (>5%) concentrations, pH >8.0 or a large excess of enzyme may result in star activity.
- Assayed using λ DNA (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
168	9	18	12	7	7	6	6	6	17	16

Hpy8I (MjaIV)

Tango | 37° | 95% | CG | YES 80° | ◯

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	0-20	20-50	100	50-100

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

#ER1571 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1572 1000u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
Hpy8I 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 Hpy8I, 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).
EcoKI: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
125	23	11	8	5	5	7	7	7	8	6

HpyCH4III
Fermentas enzyme **TaaI**, p.109

HpyCH4IV
Fermentas enzyme **TaiI** (different cleavage position), p.110

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

NEW HpyF3I (DdeI)

Tango 37° 95% YES 65° ◯

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

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

#ER1881 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1882 2500u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
HpyF3I 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 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
104	14	29	8	6	6	4	4	4	14	9

HpyF10VI (MwoI)

Tango 37° 95% CG YES 80° ◯

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

5'...G C N N N N N↓N N G C...3'
3'...C G N N↑N N N N N C G...5'

#ER1731 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER1732 1500u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
HpyF10VI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM NaCl,
1mM DTT, 1mM EDTA, 0.2mg/ml BSA and
50% (v/v) glycerol.

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).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
346	21	20	34	13	13	14	16	16	11	36

Hsp92I Fermentas enzyme **Hin1I**, p.74

Hsp92II Fermentas enzyme **Hin1II**, p.74

ItaI Fermentas enzyme **SatI**, p.103

KasI Fermentas enzyme **EheI** (different cleavage position), p.70

KpnI

Unique | 37° | 95% | YES 80° | HC

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
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
Supplied with:
10X Buffer KpnI 2x1ml
10X Buffer Tango™ 1ml

#ER0522 5x4000u
Supplied with:
10X Buffer KpnI 4x1ml
10X Buffer Tango™ 1ml

#ER0523 HC, 20000u
Supplied with:
10X Buffer KpnI 4x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ KpnI** (see p.2)

#ER0524 100 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

Conditions for 100% Activity
1X Buffer KpnI:
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
KpnI 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 KpnI, 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.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 λ DNA in 16 hours.

Note
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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	1	1	1	1	1	0	0

Kpn2I (BspMII)

Tango | 55° | 95% | CG | YES 80°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	0-20	20-50	100	50-100

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

#ER0531 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0532 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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 55°C.

Storage Buffer
Kpn2I 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 Kpn2I, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: may overlap – no effect (p.133).
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
Incubation at 37°C results in 50% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	0	0	1	0	0	0	0	0	0	2

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

KspI Fermentas enzyme **Cfr42I**, p.57

Ksp632I Fermentas enzyme **Eam1104I**, p.60

KspAI (HpaI)

B | 37° | 90% | CG | YES 65° | ✗

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

#ER1031 500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1032 2500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
KspAI 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 KspAI, more than 90% of the DNA fragments can be ligated and recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	50-100*	20-50	20-50	100*	50-100

* 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.

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 >8.0 or a large excess of enzyme may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	3	0	0	0	0	0	0	0	0	0

NEW LguI (SapI)

Tango | 37° | 90% | YES 65° | ✗

5'...G C T C T T C(N)$_1$↓...3'
3'...C G A G A A G(N)$_4$↑...5'

#ER1931 100u
Supplied with:
10X Buffer Tango™ 1ml

#ER1932 500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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 37°C.

Storage Buffer
LguI 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 LguI, more than 90% of the DNA fragments can be ligated and recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	50-100	20-50	20-50	100	20-50

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – 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.

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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
10	1	0	1	1	1	1	1	1	0	0

Bulk quantities & custom formulations available on request

CG Sensitivity to CpG Methylation | YES 65° Thermal Inactivation | HC High Concentration | ✗ Genome Qualified | Recombinant Enzyme | Blue/White Certified | FastDigest™ Enzyme

LweI (SfaNI)

Tango 37° 95% CG YES 65°

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

5'...G C A T C(N)$_5$↓...3'
3'...C G T A G(N)$_9$↑...5'

#ER1621 100u
Supplied with:
10X Buffer Tango™ 1ml

#ER1622 500u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
LweI 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.

Ligation and Recleavage
After 50-fold overdigestion with LweI, 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).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Note
LweI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
169	12	7	22	8	9	4	4	4	17	16

MaeI Fermentas enzyme **FspBI**, p.72

MaeII Fermentas enzyme **TaiI** (different cleavage position), p.110

MamI Fermentas enzyme **BseJI**, p.41

MbiI (BsrBI)

Tango 37° 80% CG YES 65°

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

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

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

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.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with MbiI, 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 MbiI. The remaining uncleaved ligation products may be cut by Cfr42I (SacII) and Ecl136II (SacI).

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
17	1	4	2	3	3	4	5	5	3	2

MboI

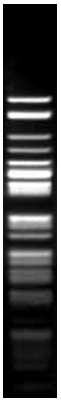
R | 37° | 95% | Dam | YES 65°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
50-100	50-100	50-100	**100**	50-100	100

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

#ER0811 300u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0812 1500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml



λ DNA (*dam*⁻)
1.4% agarose

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
MboI 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 MboI, 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).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
116	0	7	22	15	15	15	15	15	22	15

MboII

B | 37° | 80% | ★ | Dam | YES 65° | ◯

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

5'...G A A G A(N)$_8$↓...3'
3'...C T T C T(N)$_7$↑...5'

#ER0821 300u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0822 1500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA (*dam*⁻)
1.4% agarose

Concentration
5u/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.
Incubate at 37°C.

Storage Buffer
MboII 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 5-fold overdigestion with MboII, 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 than 80% of these can be recut.

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.
EcoBI: may overlap – blocked (p.138).

Note
- Greater than 15-fold overdigestion with MboII may result in star activity.
- MboII 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 (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
130	11	11	11	7/8	8	9	8	8	15	11

McrI Fermentas enzyme **Bsh1285I**, p.45

MfeI Fermentas enzyme **MunI**, p.87

MflI Fermentas enzyme **PsuI**, p.99

MjaIV Fermentas enzyme **Hpy8I**, p.78

MlsI (Ball)

R | 37° | 90% | Dcm | YES 65° | ✂ | plasmid

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

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

#ER1211 200u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1212 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA (*dcm*⁻)
0.7% agarose

Concentration
5u/µ

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
MlsI 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 MlsI, 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: may overlap – blocked (p.134).
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

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.

Note
Assayed using λ DNA (*dcm*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
18	0	1	1	0	0	0	0	0	0	2

MluI

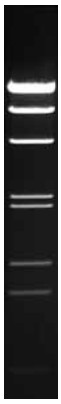
R | 37° | 95% | CG | YES 80° | ✂ | plasmid

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

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

#ER0561 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0562 5000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml



λ DNA
0.7% agarose

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
MluI 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 MluI, 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 – no effect (p.138).
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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	2	0	0	0	0	0	0	0	0	0

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

MluNI Fermentas enzyme **MlsI**, p.84

MlyI Fermentas enzyme **SchI**, p.104

MnlI

G | 37° | 80% | YES 65° | ◯

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	100	20-50	20-50	20-50	20-50

5'...C C T C(N)$_7$↓...3'
3'...G G A G(N)$_6$↑...5'

#ER1071 300u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER1072 1500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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
MnlI 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 MnlI, approximately 80% of the DNA fragments can be ligated and more than 90% of these can be 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: may overlap – blocked (p.138).

Note
MnlI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
262	34	61	26	13	14	12	14	14	26	27

Mph1103I (AvaIII)

R | 37° | 95% | YES 65° | ▭

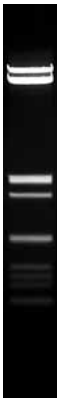
Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	50-100	20-50	100	50-100	50-100

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

#ER0731 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0732 5000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml



λ DNA
0.7% agarose

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
Mph1103I is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 200mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Mph1103I, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	0	0	0	0	1	0	0	0	2	0

MroI Fermentas enzyme **Kpn2I**, p.80

MscI Fermentas enzyme **MlsI**, p.84

MseI Fermentas enzyme **Tru1I**, p.112

Bulk quantities & custom formulations available on request

MspI (HpaII)

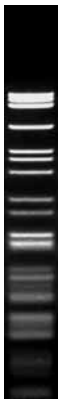
Tango | 37° | 95% | YES 65'

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

5'...C↓C G G...3'
3'...G G C↑C...5'

#ER0541 3000u
Supplied with:
10X Buffer Tango™ 1ml

#ER0542 5x3000u
Supplied with:
10X Buffer Tango™ 2x1ml



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.
Incubate at 37°C.

Storage Buffer
MspI is supplied in:
10mM potassium phosphate (pH 7.5 at 25°C), 200mM NaCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with MspI, 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 – no effect (p.135).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
328	5	18	26	13	13	12	13	13	16	34

MssI (PmeI)

B | 37° | 90% | YES 65' | ✂ | ⊂⊃

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

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

#ER1341 250u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1342 1250u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
5u/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.
Incubate at 37°C.

Storage Buffer
MssI 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 10-fold overdigestion with MssI, 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: never overlaps – no effect.
CpG: may overlap – no effect (p.137).
EcoKI: may overlap – blocked (p.138).
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	0	0	0	0	0	0	0	0

MstI

Fermentas enzyme NsbI, p.91

Supporting Products

- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

MunI (MfeI)

G | 37° | 95% | YES 65° | Genome Qualified | Recombinant Enzyme | Blue/White Certified

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

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

#ER0751 300u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0752 1500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
MunI 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 MunI, 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.

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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
8	1	0	0	0	0	0	0	0	0	0

MvaI (EcoRII*)

R | 37° | 90% | 80° | Recombinant Enzyme

Activity in Five Buffer System, %					
B	G	O	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).

** Unlike EcoRII, MvaI produces DNA fragments with a 1-base 5'-extension and is not blocked by Dcm methylation*

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

#ER0551 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0552 5x2000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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

Ligation and Recleavage
After 10-fold overdigestion with MvaI, 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, MvaI does not require multiple copies of recognition site for efficient cleavage.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
70	2	7	6	5	5	5	5	5	8	12

Mva1269I (BsmI)

R | 37° | 95% | YES 65' | | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	20-50	50-100	100	0-20	50-100

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

#ER0961 200u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0962 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

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
Mva1269I 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 Mva1269I, 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 – no effect (p.137).
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
46	4	1	1	0	1	0	0	0	2	3

MvnI Fermentas enzyme **Bsh1236I**, p.45

MwoI Fermentas enzyme **HpyF10VI**, p.79

NaeI Fermentas enzyme **PdiI**, p.94

NarI Fermentas enzyme **EheI** (different cleavage position), p.70

NciI Fermentas enzyme **BcnI**, p.33

NcoI

Tango | 37° | 95% | YES 65' | | | |

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	20-50	20-50	50-100	100	100

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

#ER0571 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0572 2500u
Supplied with:
10X Buffer Tango™ 1ml

NEW **FastDigest™ NcoI** (see p.2)
#ER0574 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ 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
NcoI 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 NcoI, 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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
4	0	0	0	0	0	0	0	0	0	1

NdeI

O | 37° | 95% | YES 65° | Genome Qualified | FastDigest™ Enzyme

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

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

#ER0581 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0582 2500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ NdeI** (*see* p.2)
#ER0584 50 reactions
Supplied with:
10X FastDigest™ Buffer 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
NdeI 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 NdeI, 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 – 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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
7	0	3	1	1	1	0	0	0	0	0

NdeII

Fermentas enzymes **Bsp143I** (different sensitivity to methylation), p.48, **DpnI** (different cleavage position and different sensitivity to methylation), p.59 and **MboI**, p.83

NgoMIV

Fermentas enzyme **PdiI** (different cleavage position), p.94

NheI

Tango | 37° | 95% | CG | YES 65° | Genome Qualified | Blue/White Certified

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

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

#ER0971 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0972 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
NheI is supplied in:
10mM Tris-HCl (pH 8.0 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 NheI, more than 95% of 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).
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
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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	0	1	0	0	0	0	0	1	2

NlaIII

Fermentas enzyme **Hin1II**, p.74

NlaIV

Fermentas enzyme **BspLI**, p.50

NmuCI (Tsp45I)

R | 37° | 95% | CG | YES 65°

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

5'...G T G A C ...3'
3'... C A C T G ...5'
(C above the first G; G below the last C)

#ER1511 200u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1512 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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
NmuCI 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.

Ligation and Recleavage
After 50-fold overdigestion with NmuCI, 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).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
81	8	8	9	4	4	4	4	4	5	7

NotI

O | 37° | 95% | CG | YES 80° | HC

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

5'...G C G G C C G C...3'
3'...C G C C G G C G...5'
(↓ after first C; ↑ before last G)

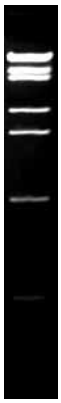
#ER0591 300u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0592 1500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0593 HC, 1500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ NotI** (*see* p.2)

#ER0594 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml



Ad2 DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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
NotI is supplied in:
20mM Tris-HCl (pH 7.8 at 25°C), 100mM NaCl, 0.1mM EDTA, 10mM 2-mercaptoethanol, 0.02% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with NotI, more than 95% of 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.

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
Assayed using Adenovirus-2 DNA.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	Ad2
0	0	0	0	0	0	0	1	1	0	0	7

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

NruI

Fermentas enzyme **Bsp68I**, p.47

NsbI (MstI)

Tango | 37° | 80% | CG | YES 65° | ✗

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

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

#ER1221 400u
Supplied with:
10X Buffer Tango™ 1ml

#ER1222 2000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
NsbI 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 NsbI, more than 80% of the DNA fragments can be ligated and more tha 95% of these can be recut.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked (p.135).
EcoKI: may overlap – no effect (p.138).
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
15	1	1	4	2	2	2	2	2	1	3

NsiI

Fermentas enzyme **Mph1103I**, p.85

NspI

Fermentas enzyme **XceI**, p.116

NspV

Fermentas enzyme **Bsp119I**, p.47

OliI

R | 37° | 80% | CG | YES 65° | Blue/White

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

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

#ER1631 200u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1632 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
OliI 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 OliI, more than 80% of the DNA fragments can be ligated and more than 90% of these can be recut.

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: may overlap – blocked (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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
20	1	0*	0	0	0	0	1	1	0	0

* According to our experimental data, OliI has one recognition site in M13mp18/19 DNA at a position 6573.

PaeI (SphI)

B | 37° | 95% | YES 65° | | | |

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

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

#ER0601 500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER0602 2500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ PaeI** (*see* p.2)
#ER0604 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml


λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
PaeI is supplied in:
10mM potassium-phosphate (pH 7.0 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.15% Triton X-100, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with PaeI, 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 – no effect (p.137).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked (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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
6	0	1	1	1	1	1	0	0	0	1

PaeR7I

Fermentas enzyme XhoI, p.117

PagI (BspHI)

O | 37° | 95% | Dam | YES 80° |

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

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

#ER1281 400u
Supplied with:
10X Buffer O 1ml

#ER1282 2000u
Supplied with:
10X Buffer O 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
PagI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

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

Methylation Effects
Dam: may overlap – cleavage impaired (p.133).
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 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
8	3	1	4	3	3	2	2	2	4	1

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

PasI

Unique | 55° | 90% | YES 80° | Blue/White Certified

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	NR	NR	NR	NR	NR

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

#ER1861 200u
Supplied with:
10X Buffer PasI 1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer PasI:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA.
Incubate at 55°C.

Storage Buffer
PasI 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 PasI, more than 90% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: completely overlaps – no effect.
CpG: never overlaps – no effect.
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
- Incubation at 37°C results in 30% activity.
- Greater than 10-fold overdigestion with PasI results in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	0	0	0	0	0	0	0	2

PalI

Fermentas enzyme **BsuRI**, p.53

PauI (BsePI)

R | 37° | 95% | CG | YES 80° | Blue/White Certified

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

5'...G↓C G C G C...3'
3'...C G C G C↑G...5'

#ER1091 200u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1092 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
PauI 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 PauI, 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.

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

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
6	1	0	0	0	0	0	2	2	0	0

PciI

Fermentas enzyme **PscI**, p.97

PdiI (NaeI)

Tango 37° 90% CG YES 65°

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

5'...G C C↓G G C...3'
3'...C G G↑C C G...5'

#ER1521 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1522 1000u
Supplied with:
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
PdiI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 500mM KCl,
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 PdiI, more than 90% 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.

Digestion of Agarose-embedded DNA
Minimum 20 units of the enzyme are required for complete digestion of 1µg of agarose-embedded pBR322 DNA in 16 hours.

Note
- Certain sites in pBR322 are difficult to cleave with PdiI, the same as with its prototype NaeI.
- Assayed using pBR322 DNA (#SD0041).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	1	4	0	0	1	1	1	0	5

PdmI (XmnI)

Tango 37° 95% CG YES 65°

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

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

#ER1531 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1532 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
PdmI 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.

Ligation and Recleavage
After 50-fold overdigestion with PdmI, 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).
EcoKI: may overlap – effect not determined.
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	3	2	2	1	1	1	1	1	2	1

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

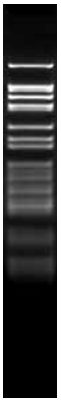
PfeI (TfiI)

O | 37° | 95% | CG | YES 65°

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

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

#ER1781 500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml



λ DNA
1.4% 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
PfeI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 250mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with PfeI, 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 – blocked (p.137).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
87	11	19	6	2	2	2	2	2	7	5

PfI23II (SpII)

Tango | 37° | 95% | CG | YES 65° | | |

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

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

#ER0851 300u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
1-3u/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
PfI23II is supplied in:
10mM potassium phosphate (pH7.4 at 25°C), 200mM NaCl, 1mM EDTA, 7mM 2-mercaptoethanol, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 10-fold overdigestion with PfI23II, 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.

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

PflFI
Fermentas enzyme **PsyI**, p.100

PflMI
Fermentas enzyme **Van91I**, p.114

PfoI

Tango | 37° | 95% | Dam | Dcm | CG | YES 65° | ✂

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

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

#ER1751 200u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
PfoI 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 PfoI, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: may overlap – cleavage impaired (p.133).
Dcm: may overlap – blocked (p.134).
CpG: may overlap – blocked (p.137).
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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
15	0	0	1	1	1	0	0	0	1	1

PhoI
Fermentas enzyme **BsuRI**, p.53

PinAI
Fermentas enzyme **BshTI**, p.46

PleI
Fermentas enzyme **SchI** (different cleavage position), p.104

PmaCI
Fermentas enzyme **Eco72I**, p.65

PmeI
Fermentas enzyme **MssI**, p.86

PmlI
Fermentas enzyme **Eco72I**, p.65

PpiI

R | 30° | 90% | ★ | CG | YES 65°

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

5'...↓$_{7}$(N)G A A C(N)$_{5}$C T C(N)$_{13}$↓...3'
3'...↑$_{12}$(N)C T T G(N)$_{5}$G A G(N)$_{8}$↑...5'

#ER1541 50u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1542 250u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
1-3u/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 30°C.

Storage Buffer
PpiI 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 PpiI, more than 90% 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).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Note
- Incubation at 37°C results in 60% activity.
- PpiI cleaves certain DNA sequences at random 7 or 8 nt away on the top strand of the recognition sequence:
  5'...↓$_{7\text{-}8}$(N) G A A C (N)$_{5}$ C T C (N)$_{13}$↓...3'
  3'...↑$_{12}$(N) C T T G (N)$_{5}$ G A G (N)$_{8}$ ↑...5'
- The presence of SAM in a reaction mixture results in incomplete cleavage with PpiI.
- Greater than 10-fold overdigestion with PpiI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	1	2	2	2	3	3	3	2	0

NEW Ppu21I (BsaAI)

Unique | 30° | 90% | CG | YES 65° | Genome Qualified | Blue/White Certified

5'...Py A C↓G T Pu...3'
3'...Pu T G↑C A Py...5'

#ER1971 500u
Supplied with:
10X Buffer Ppu21I 1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer Ppu21I:
10mM Tris-HCl (pH 7.2 at 37°C), 3mM $MgCl_2$, 150mM NaCl and 0.1mg/ml BSA.
Incubate at 30°C.

Storage Buffer
Ppu21I 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 50-fold overdigestion with Ppu21I, more than 90% of the DNA fragments can be ligated and recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100*	100*	20-50	NR	NR	NR

* 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: 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
- Incubation at 37°C results in less than 30% activity.
- 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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	2	5	1	0	0	1	1	1	0	2

PpuMI

Fermentas enzyme **Psp5II**, p.98

NEW PscI (BspLU11I)

Tango | 37° | 95% | YES 65° | Genome Qualified

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

#ER1871 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1872 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
PscI 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 PscI, more than 95% of the DNA fragments can be ligated and recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
20-50	20-50	0-20	0-20	100	0-20

Methylation Effects
Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
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
PscI, like its isoschizomers BspLU11I and PciI, is inhibited by nonionic detergents Triton X-100 (>0.002%) and Nonidet P40 (>0.001%).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	3	1	1	1	1	1	1	0	0

PshAI

Fermentas enzyme **BoxI**, p.38

PshBI

Fermentas enzyme **VspI**, p.114

Psp5II (PpuMI)

G | 37° | 95% | Dcm | YES 80° | ✂ | ⊂⊃

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

```
      T
5'...Pu G↓G A C C Py...3'
3'...Py C C T G↑G Pu...5'
      A
```

#ER0761 500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
Psp5II is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.1% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with Psp5II, 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 complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	0	2	0	0	0	0	0	0	2

Psp1406I (AclI)

Tango | 37° | 95% | CG | YES 65° | ✂

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

```
5'...A A↓C G T T...3'
3'...T T G C↑A A...5'
```

#ER0941 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER0942 1500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
Psp1406I 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 Psp1406I, 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 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded λ DNA in 16 hours.

Lambda	ΦX174	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
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

Tango Recommended Buffer | DTT Requires DTT | SAM Requires SAM | 37° Incubation Temperature | 95% Ligation Efficiency | ★ Star Activity | Dam Sensitivity to Dam Methylation

PspAI Fermentas enzymes **Cfr9I**, p.55 and **SmaI** (different cleavage position), p.106

PspGI Fermentas enzymes **EcoRII**, p.70 and **MvaI** (different cleavage position and different sensitivity to methylation), p.87

PspOMI Fermentas enzymes **ApaI** (different cleavage position), p.30 and **Bsp120I**, p.48

PstI

O | 37° | 95% | 80° | HC | Genome Qualified | Blue/White Certified | FastDigest™ Enzyme

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	**100**	100	50-100	50-100

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

#ER0611 3000u
Supplied with:
10X Buffer O 2x1ml
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
10X Buffer Tango™ 1ml

NEW **FastDigest™ PstI** (*see* p.2)

#ER0614 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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
PstI 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.

Ligation and Recleavage
After 50-fold overdigestion with PstI, 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.

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

PsuI (XhoII)

B | 37° | 95% | YES 80°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	20-50	0-20	0-20	50-100	0-20

5'...Pu↓G A T C Py...3'
3'...Py C T A G↑Pu...5'

#ER1551 500u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
5u/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.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with PsuI, more than 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).
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Note
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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
21	0	3	8	7	7	7	7	7	6	2

PsyI (Tth111I)

B | 37° | 60% | YES 80' | | |

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

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

#ER1331 1000u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml


λ DNA
0.7% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
PsyI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 50mM NaCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with PsyI, more than 60% 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 95% of these can be 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: 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	1	0	0	0	0	0	0	1

PvuI

R | 37° | 90% | CG | 80' | |

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

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

#ER0621 300u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0622 1500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml


λ DNA
0.7% agarose

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
PvuI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 0.1mM EDTA, 1mM DTT, 0.2mg/ml BSA and 50% (v/v) glycerol.

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

Methylation Effects
Dam: completely overlaps – no effect (p.133).
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.

Lambda	ΦX174	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 Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

PvuII

G 37° 90% YES 80° HC

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

#ER0631 2500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0632 5x2500u
Supplied with:
10X Buffer G 2x1ml
10X Buffer Tango™ 1ml

#ER0633 HC, 12500u
Supplied with:
10X Buffer G 2x1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
PvuII 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 PvuII, more than 90% of the DNA fragments can be ligated and more than 95% of these can be recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100*	100	20-50	50-100	20-50*	20-50*

* 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: 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.

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

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
15	0	3	1	2	2	2	2	2	0	2

RcaI

Fermentas enzyme **PagI**, p.92

RsaI

Tango 37° 95% CG YES 65°

5'...G T↓A C...3'
3'...C A↑T G...5'

#ER1121 1000u
Supplied with:
10X Buffer Tango™ 1ml

#ER1122 5000u
Supplied with:
10X Buffer Tango™ 2x1ml

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.
Incubate at 37°C.

Storage Buffer
RsaI 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 RsaI, more than 95% of the DNA fragments can be ligated and recut.

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	20-50	0-20	0-20	100	0-20

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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
113	11	19	3	3	3	2	2	2	4	3

RsrII

Fermentas enzyme **CpoI**, p.57

SacI

Unique | 37° | 95% | YES 65° | HC

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	20-50	0-20	0-20	50-100	20-50

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

#ER1131 1200u
Supplied with:
10X Buffer SacI 1ml
10X Buffer Tango™ 1ml

#ER1132 5x1200u
Supplied with:
10X Buffer SacI 2x1ml
10X Buffer Tango™ 1ml

#ER1133 HC, 6000u
Supplied with:
10X Buffer SacI 2x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ SacI** (*see* p.2)

#ER1134 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml


λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

Conditions for 100% Activity
1X Buffer SacI:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$ and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
SacI 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 SacI, 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 – no effect (p.137).
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	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	0	1	1	1	1	1	0	0

SacII

Fermentas enzyme **CfrI**, p.57

SalI

O | 37° | 95% | CG | YES 65° | HC

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
0-20	0-20	**100**	20-50	0-20	50-100

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

#ER0641 1500u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0642 5x1500u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 1ml

#ER0643 HC, 7500u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 1ml


λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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

Ligation and Recleavage
After 50-fold overdigestion with SalI, 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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	1	1	1	1	1	1	1	0	1

SapI

Fermentas enzyme **LguI**, p.81

SatI (Fnu4HI)

G | 37° | 60% | CG | YES 65°

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

5'...G C↓N G C...3'
3'...C G N↑C G...5'

#ER1641 200u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER1642 1000u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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
SatI 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 SatI, more than 60% of the DNA fragments can be ligated in a reaction mixture containing 10-30u 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: never overlaps – no effect.
CpG: may overlap – blocked (p.137).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
380	31	17	42	19	19	20	23	23	19	37

SauI
Fermentas enzyme **Eco81I**, p.66

Sau96I
Fermentas enzyme **Cfr13I**, p.56

Sau3AI
Fermentas enzymes **Bsp143I**, p.48, **DpnI** (different cleavage position and different sensitivity to methylation), p.59 and **MboI** (different sensitivity to methylation), p.83

SbfI
Fermentas enzyme **SdaI**, p.104

ScaI

Unique | 37° | 80% | 80° |

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 T...3'
3'...T C A↑T G A...5'

#ER0431 1000u
Supplied with:
10X Buffer ScaI 1ml

#ER0432 5000u
Supplied with:
10X Buffer ScaI 2x1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer ScaI:
10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
ScaI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with ScaI, more than 80% of the DNA fragments can be ligated and more than 90% 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 – blocked (p.138).

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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	0	1	1	1	1	1	1	1	1

SchI (PleI*)

Tango 37° 90% ★ YES 65°

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

** Unlike PleI, SchI produces DNA fragments with blunt ends*

5'...G A G T C(N)$_5$↓...3'
3'...C T C A G(N)$_5$↑...5'

#ER1371 1000u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 37°C.

Storage Buffer
SchI 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.

Ligation and Recleavage
After 10-fold overdigestion with SchI, approximately 90% of DNA fragments can be ligated and more than 90% of these can be 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: may overlap – effect not determined.

Note
- Greater than 10-fold overdigestion with SchI may result in star activity.
- SchI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
61	10	8	4	4	3	7	6	6	6	4

ScrFI

Fermentas enzyme **Bme1390I**, p.37

SdaI (Sse8387I)

Unique 37° 95% ★ YES 65°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	NR	0-20	0-20	NR	20-50

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

#ER1191 300u
Supplied with:
10X Buffer SdaI 1ml
10X Buffer Tango™ 1ml

#ER1192 1500u
Supplied with:
10X Buffer SdaI 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer SdaI:
37mM Tris-acetate (pH 7.0 at 37°C), 15mM Mg-acetate, 150mM K-acetate and 0.1mg/ml BSA.
Incubate at 37°C.

Storage Buffer
SdaI 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.

Ligation and Recleavage
After 5-fold overdigestion with SdaI, 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.

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 SdaI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
5	0	1	0	1	0	1	0	0	0	0

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

SduI

Unique | 37° | 95% | YES 65°

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	50-100*	50-100	0-20	NR	NR

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

```
    T     T
    A     A
5'...G G G C C↓C...3'
3'...C↑C C G G G...5'
    T     T
    A     A
```

#ER0651 500u

Supplied with:
10X Buffer SduI 1ml

λ DNA
1.0% agarose

Concentration
10u/µl

Conditions for 100% Activity
1X Buffer SduI:
10mM Tris-HCl (pH 7.2 at 37°C), 3mM $MgCl_2$, 150mM NaCl and 0.1mg/ml BSA.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with SduI, 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.137).
EcoKI: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
38	3	5	10	5	6	5	6	6	4	8

SecI
Fermentas enzyme **BseDI**, p.40

SfaNI
Fermentas enzyme **LweI**, p.82

SfcI
Fermentas enzyme **BfmI**, p.35

SfeI
Fermentas enzyme **BfmI**, p.35

SfiI

G | 50° | 95% | Dcm | CG | YES 80° | | |

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

```
5'...G G C C N N N N↓N G G C C...3'
3'...C C G G N↑N N N N C C G G...5'
```

#ER1821 1000u

Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

Ad2 DNA
0.7% agarose

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 50°C.

Storage Buffer
SfiI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 300mM NaCl, 10mM $MgCl_2$, 1mM DTT, 0.15% Triton X-100, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 50-fold overdigestion with SfiI, 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 SfiI recognition site are required for efficient cleavage.
- Incubation at 37°C results in 10% activity.
- Assayed using Adenovirus-2 DNA.

Lambda	ΦX174	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

SfoI
Fermentas enzyme **EheI**, p.70

SfuI
Fermentas enzyme **Bsp119I**, p.47

NEW

SgsI (AscI)

Tango 37° 95% CG YES 65° HC

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 G C C...3'
3'...C C G C G C↑G G...5'

#ER1891 300u
Supplied with:
10X Buffer Tango™ 1ml

#ER1892 1500u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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 37°C.

Storage Buffer
SgsI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

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

Methylation Effects
Dam: never overlaps – no effect.
Dcm: may overlap – 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	0	0	0	0	0	0	0	0

SinI

Fermentas enzyme Eco47I, p.63

SmaI

Tango 30° 90% CG YES 65° 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...3'
3'...G G G↑C C C...5'

#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

NEW

FastDigest™ SmaI (*see* p.2)
#ER0664 50 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

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
SmaI 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 SmaI, more than 90% of the pUC19 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.

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
- Incubation at 37°C results in 50% activity.
- Incubation at 25°C results in 100% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
3	0	1	0	1	1	1	1	1	1	0

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

SmiI (SwaI)

O | 30° | 80% | YES 65° | Genome Qualified | Blue/White Certified

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

5'...A T T T↓A A A T...3'
3'...T A A A↑T T T A...5'

#ER1241 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER1242 5000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 1ml

Ad2 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 30°C.

Storage Buffer
SmiI 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 SmiI, 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 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 10 units of the enzyme are required for complete digestion of 1µg of agarose-embedded Adenovirus-2 DNA in 16 hours.

Note
- Incubation at 37°C results in 70% activity.
- Assayed using Adenovirus-2 DNA.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184	Ad2
0	0	1	0	0	0	0	0	0	0	0	1

SmiII

Fermentas enzyme **SmoI**, *see* below

NEW

SmoI (SmiI)

Tango | 55° | 95% | Dcm | YES 80° | Genome Qualified

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

5'...C↓T Py Pu A G...3'
3'...G A Pu Py T↑C...5'

#ER1981 200u
Supplied with:
10X Buffer Tango™ 1ml

λ 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 55°C.

Storage Buffer
SmoI 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 SmoI, more than 95% of the DNA fragments can be ligated and recut.

Methylation Effects
Dam: may overlap – cleavage impaired (p.133).
Dcm: never overlap – no effect.
CpG: may overlap – no effect (p.137).
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
- Incubation at 37°C results in 10% activity.
- 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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
17	5	5	6	4	4	4	5	5	4	4

SmuI (FauI)

Tango | 37° | 95% | ★ | CG | YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	0-20	20-50	**100**	20-50

5'...C C C G C(N)$_4$↓...3'
3'...G G G C G(N)$_6$↑...5'

#ER1691 50u
Supplied with:
10X Buffer Tango™ 1ml

#ER1692 250u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
1-3u/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
SmuI is supplied in:
10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.2mg/ml BSA and 50% (v/v) glycerol.

Ligation and Recleavage
After 10-fold overdigestion with SmuI, 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.

Note
Greater than 40-fold overdigestion with SmuI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
90	0	10	10	5	5	5	5	5	1	10

SnaI Fermentas enzyme **Bst1107I**, p.52

SnaBI Fermentas enzyme **Eco105I**, p.67

SpeI Fermentas enzyme **BcuI**, p.33

SphI Fermentas enzyme **PaeI**, p.92

SplI Fermentas enzyme **Pfl23II**, p.95

Sse8387I Fermentas enzyme **SdaI**, p.104

SsiI (AciI)

O | 37° | 95% | CG | YES 65°

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
NR	20-50	**100**	50-100	NR	100

5'...C↓C G C...3'
3'...G G C↑G...5'

#ER1791 200u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

λ DNA
2.0% 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
SsiI 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 SsiI, approximately 95% of the DNA fragments can be ligated. No more than 50% of these can be recut due to asymmetric recognition sequence of SsiI. The remaining uncleaved ligation products may be cut by HpaII and HhaI.

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.

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(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
516	36	42	67	34	34	32	36	36	32	56

SspI

G | 37° | 90% | YES 65° | CG | NO

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

5'...A A T↓A T T...3'
3'...T T A↑T A A...5'

#ER0771 500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

#ER0772 2500u
Supplied with:
10X Buffer G 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
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
SspI 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 SspI, more than 90% 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 – 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
Greater than 10-fold overdigestion with SspI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
20	1	6	1	1	1	2	2	2	2	2

SspBI Fermentas enzyme **Bsp1407I**, p.49

StuI Fermentas enzyme **Eco147I**, p.68

StyI Fermentas enzyme **Eco130I**, p.68

StyD4I Fermentas enzyme **Bme1390I** (different cleavage position), p.37

SwaI Fermentas enzyme **SmiI**, p.107

TaaI (Tsp4CI)

Tango | 65° | 90% | CG | NO

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

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

#ER1361 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1362 1000u
Supplied with:
10X Buffer Tango™ 1ml

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.
Incubate at 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

Storage Buffer
TaaI 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.

Ligation and Recleavage
After 50-fold overdigestion with TaaI, approximately 90% of the DNA fragments can be ligated and more than 90% of these can be recut.

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

Note
Incubation at 37°C results in 10% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
187	15	31	14	8	8	7	8	8	14	20

TaqI (MaeII*)

R | 65° | 95% | CG | NO*

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	20-50	100	100	50-100

** Unlike MaeII, TaqI produces DNA fragments with a 4-base 3'-extension*

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

#ER1141 400u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1142 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

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 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

Storage Buffer
TaqI 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 TaqI, more than 95% of 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.

Note
Incubation at 37°C results in less than 10% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
143	19	22	10	5	5	8	8	8	7	9

TaqI

Unique | 65° | 95% | Dam | NO* | HC

Activity in Five Buffer System, %

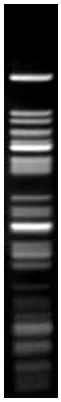
B	G	O	R	Tango	2X Tango
0-20	20-50	20-50	20-50	20-50	20-50

5'...T↓C G A...3'
3'...A G C↑T...5'

#ER0671 3000u
Supplied with:
10X Buffer TaqI 2x1ml
10X Buffer Tango™ 1ml

#ER0672 5x3000u
Supplied with:
10X Buffer TaqI 4x1ml
10X Buffer Tango™ 1ml

#ER0673 HC, 15000u
Supplied with:
10X Buffer TaqI 4x1ml
10X Buffer Tango™ 1ml



λ DNA (*dam*⁻)
1.4% agarose

Concentration
10u/µl,
50u/µl, HC

Conditions for 100% Activity
1X Buffer TaqI:
10mM Tris-HCl (pH 8.0 at 37°C), 5mM $MgCl_2$, 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
TaqI is supplied in:
10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% (v/v) glycerol.

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

Methylation Effects
Dam: may overlap – blocked (p.133).
Dcm: never overlaps – no effect.
CpG: completely overlaps – no effect (p.135).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Note
- Incubation at 37°C results in 10% activity. We recommend using TaqI, HC (#ER0673) at 37°C.
- Assayed using λ DNA (*dam*⁻) (#SD0021).

Lambda	ΦX174	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 p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

TasI (TspEI)

B | 65° | 95% | NO

Activity in Five Buffer System, %					
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
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1352 5000u
Supplied with:
10X Buffer B 2x1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
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.
Incubate at 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

Storage Buffer
TasI 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 TasI, 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.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked (p.138).

Note
Incubation at 37°C results in less than 10% activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
189	25	64	8	7	7	10	12	12	14	13

TatI

Tango | 65° | 95% | NO

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
NR	50-100*	20-50	20-50	100*	0-20

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

5'... (A/T)↓G T A C (A/T) ...3'
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™ 1ml

λ DNA
1.0% agarose

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.

Storage Buffer
TatI 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.

Ligation and Recleavage
After 5-fold overdigestion with TatI, 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.

Note
- Incubation at 37°C results in 20% activity.
- Greater than 5-fold overdigestion with TatI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	0	5	2	2	2	1	1	1	1	1

TaqI

B | 55° | 90% | CG | NO* | ○

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
100	50-100	0-20	0-20	20-50	0-20

5'...G C C G↓C...3'
3'...C↑G G C G...5'
C

#ER1651 50u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1652 250u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

Concentration
1-3u/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.
Incubate at 55°C.

Storage Buffer
TaqI 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 TaqI, more than 90% of 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.

Note
- Incubation at 37°C results in 30% activity.
- TaqI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
181	17	7	21	7	7	8	10	10	12	22

TfiI
Fermentas enzyme **PfeI**, p.95

TliI
Fermentas enzyme **XhoI**, p.117

Tru1I (MseI)

R | 65° | 90% | NO* | HC

Activity in Five Buffer System, %

B	G	O	R	Tango	2X Tango
50-100	50-100	20-50	100	50-100	100

5'...T↓T A A...3'
3'...A A T↑T...5'

#ER0981 300u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0982 1500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0983 HC, 1500u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
1.4% agarose

Concentration
10u/µl
50u/µl, HC

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 65°C.
To ensure higher efficiency of digestion, perform the cleavage reaction under paraffin oil.

Storage Buffer
Tru1I 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 Tru1I, more than 90% 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.
EcoKI: may overlap – blocked (p.138).
EcoBI: never overlaps – no effect.

Note
Incubation at 37°C results in 10% activity. We recommend using Tru1I, HC (#ER0983) at 37°C.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
195	35	63	15	13	13	18	19	19	16	12

Tru9I
Fermentas enzyme **Tru1I**, p.112

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

NEW TsoI

G | SAM | 55° | 80% | YES 80°

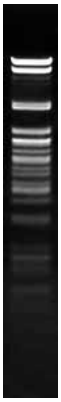
Activity in Five Buffer System, %					
B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
NR	100	50-100	0-20	50-100	20-50

5'...T A G C C A (N)$_{11}$↓...3'
3'...A T C G G T (N)$_{9}$↑...5'
T A

#ER1991 50u

Supplied with:

10X Buffer G	1ml
50X SAM	0.1ml
10X Buffer Tango™	1ml



λ DNA
1.0% agarose

Concentration
1-3u/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] + 0.05mM S-adenosylmethionine.
Incubate at 55°C.

Storage Buffer
TsoI 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 TsoI, approximately 80% 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: may overlap – no effect.
CpG: never overlaps – no effect.
EcoKI: may overlap – effect not determined.
EcoBI: never overlaps – no effect.

Note
- Incubation at 37° results in 10% activity.
- TsoI requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.05mM S-adenosylmethionine gives a 2-fold increase in TsoI activity. Still, a complete cleavage of some substrates with TsoI is difficult to achieve.
- TsoI 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.
- TsoI 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	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
43	6	4	4	1	1	3	3	3	3	4

Tsp4CI
Fermentas enzyme **TaaI**, p.109

Tsp45I
Fermentas enzyme **NmuCI**, p.90

Tsp509I
Fermentas enzyme **TasI**, p.111

NEW TstI

R | 37° | 80% | Dam | NO | ★ | ✂ | ◎

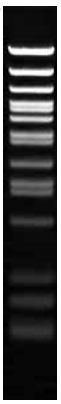
Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
0-20	0-20	0-20	100	0-20	100

5'...↓$_{8}$(N)CAC(N)$_{6}$TCC(N)$_{12}$↓...3'
3'...↑$_{13}$(N)GTG(N)$_{6}$AGG(N)$_{7}$↑...5'

#ER1911 100u

Supplied with:

10X Buffer R	1ml
10X Buffer Tango™	1ml



λ DNA (*dam*⁻)
0.8% agarose

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 37°C.

Storage Buffer
TstI 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 10-fold overdigestion with TstI, more than 80% of DNA fragments can be ligated and recut.

Methylation Effects
Dam: may overlap – blocked (p.133).
Dcm: may overlap – no effect.
CpG: may overlap – 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.

Note
- The presence of SAM in a reaction mixture results in incomplete cleavage with TstI.
- Greater than 10-fold overdigestion with TstI may result in star activity.
- TstI 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 (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
24	0	1	1	0	0	0	0	0	2	2

Bulk quantities & custom formulations available on request

TthIIII

Fermentas enzyme PsyI, p.100

Van91I (PflMI)

R | 37° | 90% | Dcm | YES 65° | ✂ | ⊂⊃

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

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

#ER0711 400u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0712 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
Van91I 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 Van91I, more than 90% of 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: 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
14	2	0	2	0	0	0	0	0	1	4

VpaK11BI

Fermentas enzyme Eco47I, p.63

VspI

O | 37° | 95% | YES 65° | ✂

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

5'...A T↓T A A T...3'
3'...T A A T↑T A...5'

#ER0911 1000u
Supplied with:
10X Buffer O 1ml
10X Buffer Tango™ 1ml

#ER0912 5000u
Supplied with:
10X Buffer O 2x1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
VspI 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 VspI, 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.
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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
17	2	7	1	3	3	3	3	3	2	1

Supporting Products
- Dilution Buffer p.121
- 0.5M EDTA, pH 8.0 p.369
- 6X Loading Dye & SDS Solution 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

XagI (EcoNI)

R | 37° | 80% | YES 65° | Genome Qualified | Blue/White Certified

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X 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'
3'...G G A N N N↑N N T C C...5'

#ER1301 1000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER1302 5000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

λ DNA
0.7% agarose

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
XagI 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 XagI, approximately 80% of DNA fragments can be ligated in the reaction mixture containing 20-40u of T4 DNA Ligase/1µg of fragments and 10% of PEG. More than 95% of these can be recut.

Methylation Effects
Dam: never overlaps – no effect.
Dcm: may overlap – 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
9	0	0	1	0	0	0	0	0	1	1

XapI (ApoI)

Tango | 37° | 95% | Star Activity | YES 80° | Genome Qualified | Blue/White Certified

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

5'...Pu↓A A T T Py...3'
3'...Py T T A A↑Pu...5'

#ER1381 500u
Supplied with:
10X Buffer Tango™ 1ml

#ER1382 2500u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
XapI 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 XapI, 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 – no effect (p.137).
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
Greater than 15-fold overdigestion with XapI may result in star activity.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
58	7	11	1	1	1	3	3	3	4	3

XbaI

Tango 37° 95% Dam YES 65° HC

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

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

#ER0681 1500u
Supplied with:
10X Buffer Tango™ 1ml

#ER0682 5x1500u
Supplied with:
10X Buffer Tango™ 2x1ml

#ER0683 HC, 7500u
Supplied with:
10X Buffer Tango™ 2x1ml

NEW **FastDigest™ XbaI** (*see* p.2)

#ER0684 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA (*dam*⁻)
0.7% agarose

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 37°C.

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

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

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 (*dam*⁻) (#SD0021).

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	0	1	0	1	1	1	1	1	0	1

XceI (NspI)

Tango 37° 95% YES 65°

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

5'...Pu C A T G↓Py...3'
3'...Py↑G T A C Pu...5'

#ER1471 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1472 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ 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
XceI 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.

Ligation and Recleavage
After 50-fold overdigestion with XceI, 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 – no effect (p.137).
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
32	0	6	4	3	3	2	1	1	1	2

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

XhoI

R | 37° | 95% | CG | YES 80° | HC | Genome Qualified | Blue/White Certified | FastDigest™ Enzyme

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 G...3'
3'...G A G C T↑C...5'

#ER0691 2000u
Supplied with:
10X Buffer R 1ml
10X Buffer Tango™ 1ml

#ER0692 5x2000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

#ER0693 HC, 10000u
Supplied with:
10X Buffer R 2x1ml
10X Buffer Tango™ 1ml

NEW **FastDigest™ XhoI** (*see* p.2)

#ER0694 200 reactions
Supplied with:
10X FastDigest™ Buffer 1ml

λ DNA
0.7% agarose

Concentration
10u/µl
50u/µl, HC

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
XhoI 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 XhoI, 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.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
1	1	0	0	0	0	0	1	1	1	0

XhoII
Fermentas enzyme **PsuI**, p.99

XmaI
Fermentas enzymes **Cfr9I**, p.55 and **SmaI** (different cleavage position), p.106

XmaIII
Fermentas enzyme **Eco52**, p.64

XmaCI
Fermentas enzymes **Cfr9I**, p.55 and **SmaI** (different cleavage position), p.106

XmaJI (AvrII)

Tango | 37° | 95% | YES 80° | Genome Qualified | Blue/White Certified

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
20-50	50-100	50-100	50-100	**100**	50-100

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

#ER1561 200u
Supplied with:
10X Buffer Tango™ 1ml

#ER1562 1000u
Supplied with:
10X Buffer Tango™ 1ml

λ DNA
1.0% 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
XmaJI 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 XmaJI, 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.

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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
2	0	0	0	0	0	0	0	0	0	0

XmiI (AccI)

B | 37° | 95% | CG | YES 65° | |

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

```
      A T
5'...G T↓C G A C...3'
3'...C A G C↑T G...5'
      T A
```

#ER1481 400u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

#ER1482 2000u
Supplied with:
10X Buffer B 1ml
10X Buffer Tango™ 1ml

λ DNA
1.0% agarose

Concentration
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.
Incubate at 37°C.

Storage Buffer
XmiI is supplied in:
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.

Ligation and Recleavage
After 50-fold overdigestion with XmiI, 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 – blocked (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 λ DNA in 16 hours.

Lambda	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
9	2	1	2	1	1	1	1	1	1	2

XmnI Fermentas enzyme **PdmI**, p.94

XspI Fermentas enzyme **FspBI**, p.72

ZraI Fermentas enzyme **AatII** (different cleavage position), p.25

Supporting Products
- **Dilution Buffer** p.121
- **0.5M EDTA, pH 8.0** p.369
- **6X Loading Dye & SDS Solution** 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 www.fermentas.com/doubledigest for optimal double digest buffer
– REsearch™ at www.fermentas.com/research for complete information on restriction enzymes

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

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.Bpu10I*

R | 37° | YES 80° | Recombinant Enzyme | Blue/White Certified

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

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

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 37°C.

Storage Buffer
Nb.Bpu10I 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% 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.

Applications
- 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, double-stranded 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	ΦX174	M13mp18/19	pBR322	pUC18/19	pUC57	pTZ19R/U	pBluescriptIIKS(-/+)	pBluescriptIISK(-/+)	pACYC177	pACYC184
19	7	4	1	0	0	0	0	0	5	3

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

Homing Enzyme

I-SceI 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 intron-plus (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 high-resolution physical mapping, genome organization analysis, gene cloning and site-directed induced-recombination and for studying double-strand-break repair in diverse biological systems (4, 6).

I-SceI

Tango | 37° | 90% | YES 65° | | | |

Activity in Five Buffer System, %					
B	G	O	R	Tango	2X Tango
50-100	50-100	50-100	50-100	100	50-100

5'...TAGGG ATAA↓CAGGGTAAT...3'
3'...ATCCC↑TATT GTCCCATTA...5'

#ER1771 250u

Supplied with:

10X Buffer Tango™	1ml
10X Buffer Tango™ (without Mg-acetate)	1ml
100mM Mg-acetate	1ml

pUC-I-SceI 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.
Incubate at 37°C.

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

Ligation and Recleavage
After 50-fold overdigestion with I-SceI, 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.

Digestion of Agarose-embedded DNA
Minimum 20 units of the enzyme are required for complete digestion of 1µg of agarose-embedded pUC-I-SceI 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-SceI 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-SceI is unstable in the presence of Mg^{2+} ions.
- Assayed using pUC-I-SceI DNA.

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

1. 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.
2. Add 20u of the enzyme.
3. Incubate 2 hours on ice.
4. Add 1/10 volume of the 100mM Mg-acetate solution (supplied with the enzyme).
5. Incubate at 37°C for 1 hour.

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-SceI, 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.

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

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.

❶ Add components in the following order:

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

❷ Mix gently, spin down briefly.

❸ 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, AjuI, AlfI, BdaI, BplI, BseMII, FaqI, Eco57I, Eco57MI, Hin4I, TsoI require S-adenosylmethionine, which is supplied with the enzyme, while AarI and BveI require oligonucleotide (also supplied with the enzyme), and Esp3I requires DTT*.

The following enzymes require unique buffers for optimal digestions: AarI, AjiI, BamHI, BfuI, Bpu10I, BseXI, Bsp143I, Cfr9I, Cfr10I, Eam1105I, Ecl136II, Eco52I, EcoRI, KpnI, PasI, Ppu21I, SacI, ScaI, SdaI, SduI 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.

Fermentas buffer	Cat. #	Quantity	1X buffer composition
Buffer B	BB5	5x1ml 10X buffer	10mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$ and 0.1mg/ml BSA.
Buffer G	BG5	5x1ml 10X buffer	10mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$, 50mM NaCl and 0.1mg/ml BSA.
Buffer O	BO5	5x1ml 10X buffer	50mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$, 100mM NaCl and 0.1mg/ml BSA.
Buffer R	BR5	5x1ml 10X buffer	10mM Tris-HCl (pH 8.5 at 37°C), 10mM $MgCl_2$, 100mM KCl 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-HCl (pH 7.4 at 25°C), 100mM KCl, 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 Buffers for Restriction Endonucleases.

Buffer	Cat. #	Quantity 10X buffer, ml	1X buffer composition												pH at 37°C
			Tris-HCl, mM	Tris-acetate, mM	Bis-Tris Propane-HCl, mM	$MgCl_2$, mM	NaCl, mM	KCl, mM	Mg-acetate, mM	K-acetate, mM	Sodium glutamate, mM	Triton X-100, %	BME, mM	BSA, mg/ml	
FIVE BUFFER SYSTEM															
B	BB5	5x1	10			10								0.1	7.5
G	BG5	5x1	10			10	50							0.1	7.5
O	BO5	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 BUFFERS															
AarI, AjiI, Bpu10I, ScaI, PasI	B27	1			10	10		100						0.1	6.5
BamHI	B57	5x1	10			5		100				0.02	1	0.1	8.0
BfuI	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
Cfr9I	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, SacI	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
KpnI	B29	1	10			10						0.02		0.1	7.5
SdaI	B24	1		37					15	150				0.1	7.0
SduI, Ppu21I	B23	1	10			3	150							0.1	7.2
TaqI	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.

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 Mg^{2+}, 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. BfiI is the only known restriction endonuclease that does not require Mg^{2+} 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.

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

1. Mix your sample with 0.5 volume of TE-saturated phenol and 0.5 volume of chloroform. Then, centrifuge (10,000rpm, 5min, room temperature).
2. Transfer the upper phase to a fresh tube. Add an equal volume of chloroform and mix. Then, centrifuge (10,000rpm, 5min, room temperature).
3. 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.
5. Incubate the mixture for 30-60min at -20°C.
6. Centrifuge for 10min at 10,000rpm. Then discard the supernatant and rinse the pellet twice with 70% cold ethanol.
7. 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.

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.

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

Table 1.8. Reaction Conditions for Restriction Endonucleases.

Enzyme	Recommended buffer	Units for overnight incubation, u/µg DNA	Thermal inactivation in 20min	Enzyme activity, %					
				B (blue) 1X	G (green) 1X	O (orange) 1X	R (red) 1X	Tango™ (yellow) 1X	Tango™ (yellow) 2X
AarI	AarI +oligo	1.0	65°C	NR (+oligo)	NR (+oligo)	0-20 (+oligo)	0-20 (+oligo)	NR (+oligo)	50-100 (+oligo)
AasI	B	0.1	80°C	100	20-50	0-20	0-20	50-100*	0-20
AatII	Tango™	0.3	65°C	50-100	20-50	0-20	0-20	100	20-50
Acc65I	O	0.3	65°C	0-20	20-50	100	20-50	20-50	50-100
AdeI	G	0.5	No	0-20	100	20-50	100	100*	20-50
AjiI	AjiI	0.5	65°C	NR	NR	20-50*	NR	NR	20-50*
AjuI	R +SAM	0.5	65°C	0-20 (+SAM)	50-100 (+SAM)	20-50 (+SAM)	100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)
AlfI	R +SAM	1.0	65°C	0-20 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)
AloI (30°C)	R	0.1	65°C	0-20	0-20	0-20	100	20-50	100
AluI	Tango™	0.1	65°C	50-100	0-20	0-20	0-20	100	20-50
Alw21I	O	0.1	65°C	0-20	20-50	100	50-100	20-50	50-100
Alw26I	Tango™	0.2	65°C	50-100	100	0-20	0-20	100	100
Alw44I	Tango™	0.1	65°C	50-100	100	0-20	50-100	100	50-100
ApaI	B	0.2	65°C	100	20-50	0-20	0-20	20-50	0-20
BamHI	BamHI	0.5	80°C (10u)	20-50*	100	20-50	50-100*	100*	50-100
BauI	Tango™	0.2	65°C	0-20	50-100	0-20	50-100	100	50-100
BclI (55°C)	G	0.1	80°C (10u)	20-50	100	20-50	20-50	100*	100
BcnI	Tango™	0.2	65°C	20-50	50-100	50-100	50-100	100	50-100
BcuI	Tango™	0.5	No	50-100	50-100	0-20	20-50	100	0-20
BdaI (30°C)	G +SAM	1.0	65°C	NR (+SAM)	100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)	50-100* (+SAM)	50-100 (+SAM)
BfiI	Tango™	1.0	65°C	20-50	20-50	0-20	0-20	100	0-20
BfmI	Tango™	1.0	65°C	0-20	50-100	0-20	0-20	100	20-50
BfuI	BfuI	1.0	80°C	NR	NR	0-20	0-20	NR	50-100*
BglI	O	0.1	65°C	0-20	50-100	100	100	0-20	100
BglII	O	0.1	No	0-20	20-50	100	50-100	0-20	100
Bme1390I	O	0.2	80°C	20-50	50-100	100	50-100	50-100	50-100
BoxI	Tango™	0.5	80°C	0-20	0-20	0-20	20-50	100	20-50
BpiI	G	0.3	65°C	20-50	100	50-100	50-100	50-100	50-100
BplI	Tango™ +SAM	0.3	65°C	0-20 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)
Bpu10I	Bpu10I	0.2	80°C	0-20	20-50*	50-100*	100*	50-100*	100*
Bpu1102I	Tango™	0.1	80°C	50-100	50-100	20-50	20-50	100	20-50
BseDI (55°C)	Tango™	0.2	80°C	50-100	20-50	0-20	0-20	100	50-100
BseGI (55°C)	Tango™	0.2	80°C	20-50	50-100	20-50	20-50	100	20-50
BseJI (65°C)	O	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	80°C	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	100 (+SAM)	50-100 (+SAM)
BseNI (65°C)	B	0.1	80°C	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
Bsh1236I	R	0.1	65°C	0-20	0-20	50-100	100	20-50	50-100
Bsh1285I	G	0.2	80°C	20-50	100	20-50	0-20	0-20	20-50
BshNI	O	0.3	65°C	0-20	20-50	100	50-100	0-20	100
BshTI	O	0.1	65°C	0-20	20-50	100	50-100	20-50	20-50
Bsp68I	O	0.2	65°C	0-20	20-50	100	50-100	20-50	50-100
Bsp119I	Tango™	0.1	80°C	20-50	0-20	0-20	0-20	100	100
Bsp120I	B	0.1	80°C	100	20-50	0-20	20-50	50-100	0-20
Bsp143I	Bsp143I	0.1	65°C	20-50	20-50	0-20	0-20	50-100	20-50
Bsp143II	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
BspLI	Tango™	0.3	65°C	50-100	50-100	0-20	20-50	100	20-50
BspPI (55°C)	Tango™	0.5	80°C	20-50	20-50	0-20	0-20	100	0-20
BspTI	O	0.1	65°C	0-20	0-20	100	20-50	0-20	50-100
Bst1107I	O	0.1	65°C	20-50	50-100	100	100	20-50	100
BstXI (55°C)	O	0.1	65°C	20-50	100	100	50-100	50-100	100
Bsu15I	Tango™	0.1	65°C	20-50	20-50	20-50	20-50	100	20-50
BsuRI	R	0.1	80°C	20-50	20-50	50-100	100	50-100	100
BveI	O +oligo	0.2	65°C	0-20 (+oligo)	20-50 (+oligo)	100 (+oligo)	20-50 (+oligo)	50-100 (+oligo)	100 (+oligo)
CaiI	Tango™	0.2	65°C	20-50	20-50	20-50	50-100	100	50-100
CfrI	Tango™	1.0	65°C	50-100*	50-100	0-20	0-20	100	0-20
Cfr9I	Cfr9I	0.2	65°C	0-20	0-20	0-20	0-20	20-50	0-20
Cfr10I	Cfr10I	0.1	No	0-20	20-50	20-50	50-100*	20-50	50-100

(continued on next page)

Table 1.8. Reaction Conditions for Restriction Endonucleases.

Enzyme	Recommended buffer	Units for overnight incubation, u/µg DNA	Thermal inactivation in 20min	Enzyme activity, %					
				B (blue) 1X	G (green) 1X	O (orange) 1X	R (red) 1X	Tango™ (yellow) 1X	Tango™ (yellow) 2X
Cfr13I	Tango™	0.3	65°C	50-100	50-100	20-50	20-50	**100**	20-50
Cfr42I	B	0.1	65°C	**100**	50-100	0-20	0-20	50-100	0-20
CpoI	Tango™	0.5	65°C	20-50	50-100	50-100	20-50	**100**	50-100
CseI	R	0.5	80°C (10u)	NR	50-100*	50-100	**100**	100*	50-100
Csp6I	B	0.1	65°C	**100**	50-100	0-20	0-20	50-100	0-20
DpnI	Tango™	0.1	80°C	100	100	50-100	50-100	**100**	50-100
DraI	Tango™	0.1	65°C	50-100	50-100	20-50	20-50	**100**	50-100
Eam1104I	Tango™	0.5	65°C	50-100	50-100	0-20	0-20	**100**	0-20
Eam1105I	Eam1105I	0.1	65°C	20-50	50-100	0-20	0-20	50-100	20-50
Ecl136II	Ecl136II	0.2	65°C	50-100	20-50	0-20	0-20	50-100	0-20
Eco24I	Tango™	0.2	65°C	50-100	50-100	0-20	20-50	**100**	0-20
Eco31I	G	0.3	65°C	50-100	**100**	0-20	0-20	50-100	20-50
Eco32I	R	0.1	65°C	0-20	50-100	50-100	**100**	20-50	100
Eco47I	R	0.3	65°C	0-20	50-100	50-100	**100**	50-100	50-100
Eco47III	O	0.1	65°C	0-20	20-50	**100**	100	50-100	100
Eco52I	Eco52I	0.2	65°C	0-20	0-20	0-20	20-50	0-20	20-50
Eco57I	G +SAM	1.0	65°C	100 (+SAM)	**100** (+SAM)	20-50 (+SAM)	20-50 (+SAM)	50-100 (+SAM)	50-100 (+SAM)
Eco57MI	B +SAM	1.0	65°C	**100** (+SAM)	50-100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)	50-100 (+SAM)	0-20 (+SAM)
Eco72I	Tango™	0.5	65°C	NR	NR	0-20	0-20	**100**	20-50
Eco81I	Tango™	0.1	80°C	50-100	100	0-20	0-20	**100**	0-20
Eco88I	Tango™	0.2	65°C	100	50-100	0-20	0-20	**100**	20-50
Eco91I	O	0.1	65°C	20-50	20-50	**100**	50-100	50-100	100
Eco105I	Tango™	0.5	65°C	100*	50-100	0-20	0-20	**100**	0-20
Eco130I	O	0.2	65°C	0-20	20-50	**100**	50-100	50-100	100
Eco147I	B	0.1	80°C	**100**	50-100	20-50	20-50	50-100	0-20
EcoO109I	Tango™	0.2	65°C	50-100	20-50	20-50	20-50	**100**	100
EcoRI	EcoRI	0.2	65°C	0-20	NR	100	100*	NR	100
EcoRII	O	0.1	80°C	20-50	50-100	**100**	50-100	20-50	50-100
EheI	Tango™	1.0	65°C	20-50	50-100	0-20	0-20	**100**	20-50
Esp3I	Tango™ +DTT	0.2	65°C	100 (+DTT)	20-50 (+DTT)	0-20 (+DTT)	0-20 (+DTT)	**100** (+DTT)	0-20 (+DTT)
FaqI	Tango™ +SAM	1.0	80°C	20-50 (+SAM)	20-50 (+SAM)	0-20(+SAM)	0-20 (+SAM)	**100** (+SAM)	20-50 (+SAM)
FspAI	O	0.2	65°C	0-20	0-20	**100**	50-100	0-20	50-100
FspBI	Tango™	0.3	65°C	50-100	20-50	0-20	0-20	**100**	0-20
GsuI (30°C)	B	1.0	65°C	**100**	50-100	20-50	20-50	100	50-100
HhaI	Tango™	0.1	80°C	50-100	50-100	20-50	20-50	**100**	20-50
Hin1I	G	0.1	65°C	20-50	**100**	20-50	20-50	20-50	20-50
Hin1II	G	0.3	65°C	50-100	**100**	20-50	50-100	50-100	50-100
Hin4I	Tango™ +SAM	0.2	65°C	20-50 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	**100** (+SAM)	0-20 (+SAM)
Hin6I	Tango™	0.1	65°C	50-100	50-100	20-50	20-50	**100**	50-100
HincII	Tango™	0.1	65°C	50-100	50-100	20-50	50-100	**100**	50-100
HindIII	R	0.1	65°C	0-20	20-50	0-20	**100**	50-100	50-100
HinfI	R	0.1	65°C	0-20	20-50	50-100	**100**	50-100	50-100
HpaII	Tango™	0.1	65°C	50-100	50-100	0-20	20-50	**100**	20-50
HphI	B	0.1	65°C	**100**	0-20	0-20	0-20	20-50	0-20
Hpy8I	Tango™	0.1	80°C	50-100	50-100	0-20	20-50	**100**	50-100
HpyF3I	Tango™	0.2	65°C	20-50	20-50	20-50	20-50	**100**	50-100
HpyF10VI	Tango™	0.1	80°C	0-20	0-20	0-20	0-20	**100**	50-100
KpnI	KpnI	0.2	80°C	20-50	0-20	0-20	0-20	20-50	0-20
Kpn2I (55°C)	Tango™	0.1	80°C	50-100	50-100	0-20	20-50	**100**	50-100
KspAI	B	0.5	65°C	**100**	50-100*	20-50	20-50	100*	50-100
LguI	Tango™	0.5	65°C	20-50	50-100	20-50	20-50	**100**	20-50
LweI	Tango™	0.2	65°C	0-20	0-20	0-20	20-50	**100**	20-50
MbiI	Tango™	0.2	65°C	20-50	100	20-50	20-50	**100**	20-50
MboI	R	0.1	65°C	50-100	50-100	50-100	**100**	50-100	100
MboII	B	1.0	65°C	**100**	50-100	20-50	0-20	50-100	20-50
MlsI	R	0.5	65°C	0-20	20-50	0-20	**100**	20-50	50-100
MluI	R	0.1	80°C	0-20	20-50	50-100	**100**	20-50	50-100
MnlI	G	0.5	65°C	50-100	**100**	20-50	20-50	20-50	20-50
Mph1103I	R	0.3	65°C	0-20	50-100	20-50	**100**	50-100	50-100
MspI	Tango™	0.3	65°C	50-100	50-100	0-20	0-20	**100**	50-100
MssI	B	0.5	65°C	**100**	0-20	0-20	0-20	20-50	0-20
MunI	G	0.1	65°C	100	**100**	0-20	0-20	100	0-20
MvaI	R	0.1	80°C (10u)	20-50	20-50	50-100	**100**	20-50*	100
Mva1269I	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.

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Bulk quantities & custom formulations available on request

Table 1.8. Reaction Conditions for Restriction Endonucleases.

Enzyme	Recommended buffer	Units for overnight incubation, u/µg DNA	Thermal inactivation in 20min	Enzyme activity, %					
				B (blue) 1X	G (green) 1X	O (orange) 1X	R (red) 1X	Tango™ (yellow) 1X	Tango™ (yellow) 2X
NcoI	Tango™	0.1	65°C	20-50	20-50	20-50	50-100	**100**	100
NdeI	O	0.2	65°C	0-20	0-20	**100**	50-100	0-20	50-100
NheI	Tango™	0.2	65°C	100	20-50	0-20	0-20	**100**	0-20
NmuCI	R	0.1	65°C	0-20	20-50	50-100	**100**	20-50	50-100
NotI	O	0.1	80°C	0-20	0-20	**100**	20-50	0-20	20-50
NsbI	Tango™	0.1	65°C	20-50	50-100	0-20	20-50	**100**	20-50
OliI	R	0.2	65°C	0-20	0-20	0-20	**100**	0-20	50-100
PaeI	B	0.1	65°C	**100**	50-100	0-20	0-20	50-100	0-20
PagI	O	0.2	80°C	0-20	50-100	**100**	NR	NR	NR
PasI (55°C)	PasI	0.2	80°C	NR	NR	NR	NR	NR	NR
PauI	R	0.2	80°C	0-20	0-20	100	**100**	0-20	100
PdiI	Tango™	0.5	65°C	50-100	20-50	0-20	0-20	**100**	50-100
PdmI	Tango™	0.5	65°C	20-50	50-100	0-20	0-20	**100**	0-20
PfeI	O	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
PfoI	Tango™	0.1	65°C	0-20	20-50	50-100	0-20	**100**	50-100
PpiI (30°C)	R	0.1	65°C	0-20	0-20	0-20	**100**	50-100	50-100
Ppu21I (30°C)	Ppu21I	0.5	65°C	50-100*	100*	20-50	NR	NR	NR
PscI	Tango™	0.2	65°C	20-50	20-50	0-20	0-20	**100**	0-20
Psp5II	G	0.2	80°C	0-20	**100**	20-50	20-50	50-100	100
Psp1406I	Tango™	0.5	65°C	100	50-100	0-20	20-50	**100**	0-20
PstI	O	0.2	80°C (10u)	50-100	50-100	**100**	100	50-100	50-100
PsuI	B	0.5	80°C	**100**	20-50	0-20	0-20	50-100	0-20
PsyI	B	0.1	80°C	**100**	50-100	0-20	0-20	50-100	0-20
PvuI	R	0.2	80°C (10u)	0-20	20-50	50-100	**100**	50-100	100
PvuII	G	0.2	80°C	50-100*	**100**	20-50	50-100	20-50*	20-50*
RsaI	Tango™	0.2	65°C	50-100	20-50	0-20	0-20	**100**	0-20
SacI	SacI	0.2	65°C	50-100	20-50	0-20	0-20	50-100	20-50
SalI	O	0.1	65°C	0-20	0-20	**100**	20-50	0-20	50-100
SatI	G	0.1	65°C	20-50	**100**	20-50	20-50	50-100	20-50
ScaI	ScaI	0.5	80°C (10u)	0-20	0-20	0-20	0-20	0-20	0-20
SchI	Tango™	0.2	65°C	20-50	50-100	0-20	0-20	**100**	0-20
SdaI	SdaI	0.3	65°C	NR	NR	0-20	0-20	NR	20-50
SduI	SduI	0.3	65°C	NR	50-100*	50-100	0-20	NR	NR
SfiI (50°C)	G	0.2	80°C	50-100	**100**	20-50	0-20	100	0-20
SgsI	Tango™	0.1	65°C	0-20	0-20	0-20	50-100	**100**	50-100
SmaI (30°C)	Tango™	0.2	65°C	50-100	0-20	0-20	0-20	**100**	0-20
SmiI (30°C)	O	0.1	65°C	0-20	0-20	**100**	20-50	0-20	20-50
SmoI (55°C)	Tango™	0.2	80°C	50-100	20-50	0-20	20-50	**100**	20-50
SmuI	Tango™	0.2	65°C	50-100	50-100	0-20	20-50	**100**	20-50
SsiI	O	0.5	65°C	NR	20-50	**100**	50-100	NR	100
SspI	G	0.1	65°C	20-50	**100**	0-20	50-100	100	20-50
TaaI (65°C)	Tango™	0.2	No	0-20	0-20	0-20	50-100	**100**	100
TaiI (65°C)	R	0.3	No	50-100	50-100	20-50	**100**	100	50-100
TaqI (65°C)	TaqI	0.3	No	0-20	20-50	20-50	20-50	20-50	20-50
TasI (65°C)	B	0.3	No	**100**	50-100	20-50	0-20	20-50	0-20
TauI (55°C)	B	1.0	No	**100**	50-100	0-20	0-20	20-50	0-20
TatI (65°C)	Tango™	0.2	No	NR	50-100*	20-50	20-50	**100***	0-20
Tru1I (65°C)	R	0.2	No	50-100	50-100	20-50	**100**	50-100	100
TsoI (55°C)	G +SAM	1.0	80°C	NR (+SAM)	**100 (+SAM)**	50-100 (+SAM)	0-20 (+SAM)	50-100 (+SAM)	20-50 (+SAM)
TstI	R	0.1	No	0-20	0-20	0-20	**100**	0-20	100
Van91I	R	0.1	65°C	0-20	50-100	50-100	**100**	20-50	50-100
VspI	O	0.1	65°C	0-20	50-100	**100**	20-50	100	100
XagI	R	0.1	65°C	0-20	20-50	50-100	**100**	20-50	50-100
XapI	Tango™	0.1	80°C	50-100	100	0-20	0-20	**100**	20-50
XbaI	Tango™	0.1	65°C	50-100	50-100	20-50	0-20	**100**	50-100
XceI	Tango™	0.2	65°C	50-100	0-20	0-20	0-20	**100**	0-20
XhoI	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
XmiI	B	0.1	65°C	**100**	0-20	0-20	0-20	50-100	20-50
I-SceI	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.

How do I Perform a Double Digest?

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

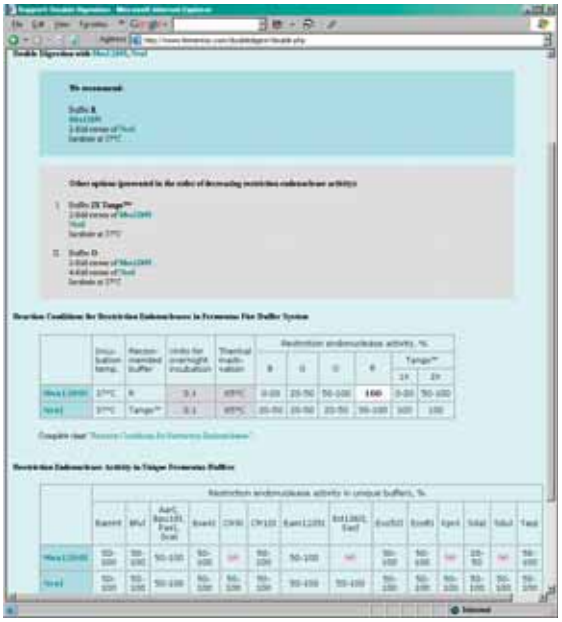
1. The Fermentas DoubleDigest™ Engine

Use www.fermentas.com/doubledigest 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

2. Submit

3. Read the recommendations



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 Tango™ buffer.

1. 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.
3. 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.

1. 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.
3. 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., SmaI, TaaI), 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.

1. 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

Table 1.9. Double Digestions using Universal Tango™ Buffer.

Buffers for double digestions			
Fermentas enzyme	Recommended buffer	Tango™ 1X	Tango™ 2X
AarI	AarI +oligo	NR	+oligo
AasI	B	*	NR
AatII	Tango™		
Acc65I	O		
AdeI	G	*	
AjiI	AjiI	NR	*
AjuI	R +SAM	+SAM	+SAM
AloI (30°C)	R		
AlfI	R +SAM	NR	+SAM
AluI	Tango™		
Alw21I	O		
Alw26I	Tango™		
Alw44I	Tango™		
ApaI	B		NR
BamHI	BamHI	*	
BauI	Tango™		
BclI (55°C)	G	*	
BcnI	Tango™		
BcuI	Tango™		NR
BdaI (30°C)	G +SAM	+SAM *	+SAM
BfiI	Tango™		NR
BfmI	Tango™		
BfuI	BfuI	NR	*
BglI	O	NR	
BglII	O	NR	
Bme1390I	O		
BoxI	Tango™		
BpiI	G		
BplI	Tango™ +SAM	+SAM	NR
Bpu10I	Bpu10I	*	*
Bpu1102I	Tango™		
BseDI (55°C)	Tango™		
BseGI (55°C)	Tango™		
BseJI (65°C)	O	NR	*
BseLI (55°C)	Tango™		
BseMI (55°C)	R		
BseMII (55°C)	Tango™ +SAM	+SAM	+SAM
BseNI (65°C)	B		
BseSI (55°C)	G		NR
BseXI (65°C)	BseXI	NR	NR
Bsh1236I	R		
Bsh1285I	G	NR	
BshNI	O	NR	
BshTI	O		
Bsp68I	O		
Bsp119I	Tango™		
Bsp120I	B		NR
Bsp143I	Bsp143I		
Bsp143II	Tango™	*	
Bsp1407I	Tango™		
BspLI	Tango™		
BspPI (55°C)	Tango™		NR
BspTI	O	NR	
Bst1107I	O		
BstXI (55°C)	O		
Bsu15I	Tango™		
BsuRI	R		
BveI	O +oligo	+oligo	+oligo
CaiI	Tango™		
CfrI	Tango™		NR
Cfr9I	Cfr9I		NR
Cfr10I	Cfr10I		
Cfr13I	Tango™		

Buffers for double digestions			
Fermentas enzyme	Recommended buffer	Tango™ 1X	Tango™ 2X
Cfr42I	B		NR
CpoI	Tango™		
CseI	R	*	
Csp6I	B		NR
DpnI	Tango™		
DraI	Tango™		
Eam1104I	Tango™		NR
Eam1105I	Eam1105I		
Ecl136II	Ecl136II		NR
Eco24I	Tango™		NR
Eco31I	G		
Eco32I	R		
Eco47I	R		
Eco47III	O		
Eco52I	Eco52I	NR	
Eco57I	G +SAM	+SAM	+SAM
Eco57MI	B +SAM	+SAM	NR
Eco72I	Tango™		
Eco81I	Tango™		NR
Eco88I	Tango™		
Eco91I	O		
Eco105I	Tango™		NR
Eco130I	O		
Eco147I	B		NR
EcoO109I	Tango™		
EcoRI	EcoRI	NR	
EcoRII	O		
EheI	Tango™		
Esp3I	Tango™ +DTT	+DTT	NR
FaqI	Tango™ +SAM	+SAM	+SAM
FspAI	O	NR	
FspBI	Tango™		NR
GsuI (30°C)	B		
HhaI	Tango™		
Hin1I	G		
Hin1II	G		
Hin4I	Tango™ +SAM	+SAM	NR
Hin6I	Tango™		
HincII	Tango™		
HindIII	R		
HinfI	R		
HpaII	Tango™		
HphI	B		NR
Hpy8I	Tango™		
HpyF3I	Tango™		
HpyF10VI	Tango™		
KpnI	KpnI		NR
Kpn2I (55°C)	Tango™		
KspAI	B	*	
LguI	Tango™		
LweI	Tango™		
MbiI	Tango™		
MboI	R		
MboII	B		
MlsI	R		
MluI	R		
MnlI	G		
Mph1103I	R		
MspI	Tango™		
MssI	B		NR
MunI	G		NR
MvaI	R	*	
Mva1269I	R	NR	

(continued on next page)

Table 1.9. Double Digestions using Universal Tango™ Buffer.

Fermentas enzyme	Buffers for double digestions		
	Recommended buffer	Tango™ 1X	Tango™ 2X
NcoI	Tango™		
NdeI	O	NR	
NheI	Tango™		NR
NmuCI	R		
NotI	O	NR	
NsbI	Tango™		
OliI	R	NR	
PaeI	B		NR
PagI	O	NR	NR
PasI (55°C)	PasI	NR	NR
PauI	R	NR	
PdiI	Tango™		
PdmI	Tango™		NR
PfeI	O		
Pfl23II	Tango™		NR
PfoI	Tango™		
PpiI (30°C)	R		
Ppu21I (30°C)	Ppu21I	NR	NR
PscI	Tango™		NR
Psp5II	G		
Psp1406I	Tango™		NR
PstI	O		
PsuI	B		NR
PsyI	B		NR
PvuI	R		
PvuII	G	*	*
RsaI	Tango™		NR
SacI	SacI		
SalI	O	NR	
SatI	G		
ScaI	ScaI	NR	NR
SchI	Tango™		NR
SdaI	SdaI	NR	
SduI	SduI	NR	NR
SfiI (50°C)	G		NR
SgsI	Tango™		
SmaI (30°C)	Tango™		NR
SmiI (30°C)	O	NR	
SmoI (55°C)	Tango™		
SmuI	Tango™		
SsiI	O	NR	
SspI	G		
TaaI (65°C)	Tango™		
TaiI (65°C)	R		
TaqI (65°C)	TaqI		
TasI (65°C)	B		NR
TauI (55°C)	B		NR
TatI (65°C)	Tango™	*	NR
Tru1I (65°C)	R		
TsoI (55°C)	G +SAM	+SAM	+SAM
TstI	R	NR	
Van91I	R		
VspI	O		
XagI	R		
XapI	Tango™		
XbaI	Tango™		
XceI	Tango™		NR
XhoI	R		
XmaJI	Tango™		
XmiI	B		
I-SceI	Tango™		
Nb.Bpu10I	R		

Note

Cleavage efficiency:

yellow shaded buffers 50-100%

grey shaded buffers 20-50%

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 37°C unless otherwise indicated in parenthesis.

Activity of Mesophilic and Thermophilic Enzymes at 37°C

Table 1.10. Activity of Mesophilic and Thermophilic Enzymes at 37°C.

Enzyme	Optimal temperature	Activity at 37°C, %
AloI	30	20
BclI	55	50
BdaI	30	30
BseDI	55	10
BseGI	55	25
BseJI	65	<10
BseLI	55	40
BseMI	55	20
BseMII	55	30
BseNI	65	<10
BseSI	55	20
BseXI	65	10
BspPI	55	30
BstXI	55	50
GsuI	30	70
Kpn2I	55	50
PasI	55	30
PpiI	30	60
Ppu21I	30	<30
SfiI	50	10
SmaI (HC)*	30	50
SmiI	30	70
SmoI	55	10
TaaI	65	10
TaiI	65	<10
TasI	65	<10
TauI	55	30
TaqI (HC)*	65	10
TatI	65	20
Tru1I (HC)*	65	10
TsoI	55	10

* – 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, NaeI, NarI, Ksp632I, BspMI, Eco57I, 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, NaeI), 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 (SfiI, Cfr10I, NgoMIV, BspMI) cleave both recognition sequences in a concerted reaction (11-14). Type IIS enzymes, such as FokI, BpmI, BsgI, MboII, 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 BveI, Cfr42I, Eam1104I and PdiI and their prototypes BspMI, SacII, Ksp632I and NaeI). However, some isoschizomers cleave "resistant" sites at the same rate as other normal sites. For example, EheI cleaves the target DNA more efficiently than its prototype NarI. Thus, one recognition site of NarI on λ DNA and two sites on pBR322 are not cleaved to completion, even after incubation with 50 units of NarI for 16 hours. Unlike NarI, Fermentas' neoschizomer EheI cleaves λ DNA and pBR322 DNA completely under standard conditions. Site preferences are a characteristic feature of the following Fermentas prototype enzymes: AarI, AjuI, AlfI, AloI, BdaI, BplI, BseMII, Eco57I, Eco57MI, PpiI, TsoI and TstI. The properties of these enzymes differ significantly from other type II enzymes.

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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 Mg^{2+} for other divalent cations, such as Mn^{2+} or Co^{2+}.

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.

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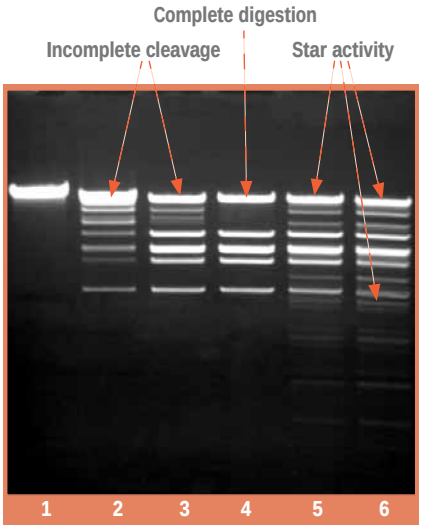

Figure 1.2. Enzyme star activity.

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)

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 C5-position 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: $AAC(N)_6GTGC$ 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 tissue-specific 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, MboI (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 MvaI 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.

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

Enzyme couple	Recognition and cleavage sites	Sensitivity to methylation
Acc65I	G↓GTACC	Overlapping Dcm or CpG methylation may influence DNA cleavage.
KpnI	GGTAC↓C	Not influenced by Dcm or CpG methylation.
ApaI	GGGCC↓C	Overlapping Dcm or CpG methylation may influence DNA cleavage.
Bsp120II	G↓GGCCC	Blocked by overlapping Dcm or CpG methylation.
Bsp143I	↓GATC	Not influenced by Dam, blocked by CpG methylation.
MboI	↓GATC	Blocked by Dam methylated DNA.
DpnI	GA↓TC	Cleaves only Dam methylated DNA.
Cfr9I	C↓CCGGG	CpG methylation may influence DNA cleavage.
SmaI	CCC↓GGG	Blocked by CpG methylation.
Csp6I	G↓TAC	Not influenced by CpG methylation.
RsaI	GT↓AC	Overlapping CpG methylation may influence DNA cleavage.
Ecl136II	GAG↓CTC	Overlapping CpG methylation may influence DNA cleavage.
SacI	GAGCT↓C	Not influenced by CpG methylation.
EcoRII	↓CCWGG	Blocked by Dcm methylation.
MvaI	CC↓WGG	Not influenced by Dcm methylation.
HpaII	C↓CGG	Blocked by CpG methylation.
MspI	C↓CGG	Not influenced by CpG methylation.

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Single letter code

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

Effect of Dam Methylation on DNA Cleavage by Restriction Enzymes

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. Completely Overlapping Dam Methylation and Recognition Sites.

Enzyme	Sequence	Effect
BamHI	GGm6ATCC	↓
BclI	TGm6ATCA	⇊
BglII	AGm6ATCT	↓
Bsp143I	Gm6ATC	↓
BspPI	GGm6ATC	⇊
MboI	Gm6ATC	⇊
PsuI	RGm6ATCY	↓
PvuI	CGm6ATCG	↓

Note

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

Table 1.13. Partially Overlapping Dam Methylation and Recognition Sites.

Enzyme	Sequence*	Effect
AloI	5'...GAAC(N)$_4$Gm6A TCC...3' 3'...CTTG(N)$_4$C Tm6AGG...5'	?
BdaI	5'...TGm6A TC(N)$_4$TCA...3' 3'...AC Tm6AG(N)$_4$AGT...5'	⇊
BseJI	5'...Gm6A TC(N)$_3$ATC...3' 3'...C Tm6AG(N)$_3$TAG...5'	⇊
Bsh1285I	5'...CGm6A TCG...3' 3'...GC Tm6AGC...5'	↓
Bsp68I	5'...Gm6A TCGCGm6A TC...3' 3'...C Tm6AGCGC Tm6AG...5'	↓
Bsu15I	5'...ATCGm6A TC...3' 3'...TAGC Tm6AG...5'	⇊
Hin4I	5'...Gm6A TC(N)$_4$VTC...3' 3'...C Tm6AG(N)$_4$BAG...5'	⇊
	5'...GAY(N)$_4$Gm6A TC...3' 3'...CTR(N)$_4$C Tm6AG...5'	⇊
HphI	5'...GGTGm6A TC...3' 3'...CCAC Tm6AG...5'	⇊
Kpn2I	5'...TCCGGm6A TC...3' 3'...AGGCC Tm6AG...5'	↓
	5'...Gm6A TCCGGm6A TC...3' 3'...C Tm6AGGCC Tm6AG...5'	?
MboII	5'...GAAGm6A TC...3' 3'...CTTC Tm6AG...5'	⇊
PagI	5'...TCATGm6A TC...3' 3'...AGTAC Tm6AG...5'	⇓
	5'...Gm6A TCATGm6A TC...3' 3'...C Tm6AGTAC Tm6AG...5'	?
PfoI	5'...TCCNGGm6A TC...3' 3'...AGGNCC Tm6AG...5'	⇓
SmoI	5'...CTYRAGm6A TC...3' 3'...GARYTC Tm6AG...5'	⇓
TaqI	5'...TCGm6A TC...3' 3'...AGC Tm6AG...5'	⇊
XbaI	5'...TCTAGm6A TC...3' 3'...AGATC Tm6AG...5'	⇊

Note

* 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;

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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**	↓
MvaI	**Cm6CWGG**	↓
PasI	**CCm5CWGGG**	↓

Table 1.15. Partially Overlapping Dcm Methylation and Recognition Sites.

Enzyme	Sequence*	Effect
Acc65I	5'...**GGTACm5CW** GG...3' 3'...**CCATG GWm5CC**...5'	↓
	5'...Cm5CW **GGTACm5CW** GG...3' 3'...G GW**m5CCATG GWm5CC**...5'	↓
AloI	5'...**GAAC**(N)$_6$**TCm5CW** GG...3' 3'...**CTTG**(N)$_6$**AG GWm5CC**...5'	↓
ApaI	5'...**GGGCCm5CW** GG...3' 3'...**CCCGG GWm5CC**...5'	↓
BamHI	5'...Cm5CW **GGATCm5CW** GG...3' 3'...G GW**m5CCTAG GWm5CC**...5'	↓
BfuI	5'...**GTATCm5CW** GG...3' 3'...**CATAG GWm5CC**...5'	↓
BglI	5'...**GCm5CW** GGNNGGC...3' 3'...**CG GWm5CC**NNCCG...5'	↓
Bme1390I	5'...**Cm5CW GG**...3' 3'...**G GWm5CC**...5'	↓
BseDI	5'...**Cm5CW** GGG...3' 3'...**G GW**m5CCC...5'	↓
BseGI	5'...Cm5CW **GGATG**...3' 3'...G GW**m5CCTAC**...5'	↓
BseLI	5'...**Cm5CW** GG(N)$_4$**GG**...3' 3'...**G GW**m5CC(N)$_4$**CC**...5'	↓
	5'...**Cm5CW** GGNCm5CW **GG**...3' 3'...**G GW**m5CCNG GW**m5CC**...5'	↓
BseSI	5'...**GKGCCm5CW** GG...3' 3'...**CMCGG GWm5CC**...5'	↓
BshNI	5'...**GGYRCm5CW** GG...3' 3'...**CCRYG GWm5CC**...5'	↓
	5'...Cm5CW **GGYRCm5CW** GG...3' 3'...G GW**m5CCRYG GWm5CC**...5'	?
Bsp120I	5'...**GGGCCm5CW** GG...3' 3'...**CCCGG GWm5CC**...5'	↓
Bsp143II	5'...Cm5CW **GGCGCm5CW** GG...3' 3'...G GW**m5CCGCG GWm5CC**...5'	↓
BspLI	5'...**GGNNCm5CW** GG...3' 3'...**CCNNG GWm5CC**...5'	↓
	5'...Cm5CW **GGNNCm5CW** GG...3' 3'...G GW**m5CCNNG GWm5CC**...5'	↓
BspPI	5'...Cm5CW **GGATC**...3' 3'...G GW**m5CCTAG**...5'	↓
BstXI	5'...**Cm5CA** GG(N)$_4$**TGG**...3' 3'...**G GT**m5CC(N)$_4$**ACC**...5'	↓
	5'...**Cm5CA** GGNNCm5C**T GG**...3' 3'...**G GT**m5CCNNG G**Am5CC**...5'	↓
BsuRI	5'...**GGCm5CW** GG...3' 3'...**CCG GWm5CC**...5'	↓
	5'...Cm5CW **GGCm5CW** GG...3' 3'...G GW**m5CCG GWm5CC**...5'	↓
CaiI	5'...**CAG**NNCm5CT **GG**...3' 3'...**GTC**NNG **GTm5CC**...5'	↓
CfrI	5'...**YGGCm5CW** GG...3' 3'...**RCCG GWm5CC**...5'	↓
Cfr13I	5'...**GGNCm5CW** GG...3' 3'...**CCNG GWm5CC**...5'	↓
Eco24I	5'...Cm5CW **GGGCCm5CW** GG...3' 3'...G GW**m5CCCGG GWm5CC**...5'	↓
Eco31I	5'...Cm5CW **GGTCTC**...3' 3'...G GW**m5CCAGAG**...5'	↓
Eco47I	5'...**GGWCm5CW** GG...3' 3'...**CCWG GWm5CC**...5'	↓
Eco91I	5'...Cm5CW **GGTNACm5CW** GG...3' 3'...G GW**m5CCANTG GWm5CC**...5'	↓
Eco57MI	5'...Cm5CT **GGAG**...3' 3'...G **GAm5CCTC**...5'	↓
Eco147I	5'...**AGGCm5CT** GG...3' 3'...**TCCG GA**m5CC...5'	↓
Eco0109I	5'...**RGGNCm5CT** GG...3' 3'...**YCCNG GA**m5CC...5'	↓
EheI	5'...Cm5CW **GGCGCm5CW** GG...3' 3'...G GW**m5CCGCG GWm5CC**...5'	↓
FaqI	5'...Cm5CW **GGGAC**...3' 3'...G GW**m5CCCTG**...5'	↓
GsuI	5'...**CTCm5CA GG**...3' 3'...**GAG GTm5CC**...5'	↓
Hin1I	5'...**GRCGCm5CW** GG...3' 3'...**CYGCG GWm5CC**...5'	↓
HphI	5'...Cm5CW **GGTGA**...3' 3'...G GW**m5CCACT**...5'	↓
KpnI	5'...**GGTACm5CW** GG...3' 3'...**CCATG GWm5CC**...5'	↓
	5'...Cm5CW **GGTACm5CW** GG...3' 3'...G GW**m5CCATG GWm5CC**...5'	↓
MlsI	5'...**TGGCm5CA** GG...3' 3'...**ACCG GT**m5CC...5'	↓
PfoI	5'...**TCm5CA GGA**...3' 3'...**AG GTm5CCT**...5'	↓
Psp5II	5'...**RGGWCm5CT** GG...3' 3'...**YCCWG GA**m5CC...5'	↓
SduI	5'...Cm5CW **GGGCCm5CW** GG...3' 3'...G GW**m5CCCGG GWm5CC**...5'	↓
SfiI	5'...**GGCm5CW** GGNNGGCC...3' 3'...**CCG GWm5CC**NNCCGG...5'	↓
	5'...Cm5CW **GGCC**(N)$_5$**GGCm5CW** GG...3' 3'...G GW**m5CCGG**(N)$_5$**CCG GWm5CC**...5'	↓
SgsI	5'...Cm5CW **GGCGCGCm5CW** GG...3' 3'...G GW**m5CCGCGCG GWm5CC**...5'	↓
TsoI	5'...**TARCm5CA** GG...3' 3'...**ATYG GT**m5CC...5'	↓
TstI	5'...**CAC**(N)$_6$**TCm5CW** GG...3' 3'...**GTG**(N)$_6$**AG GWm5CC**...5'	↓
Van91I	5'...**Cm5CA** GG(N)$_3$**TGG**...3' 3'...**G GT**m5CC(N)$_3$**ACC**...5'	↓
XagI	5'...**Cm5CT** GG(N)$_3$**AGG**...3' 3'...**G GA**m5CC(N)$_3$**TCC**...5'	↓
	5'...**Cm5CT** GGNCm5C**A GG**...3' 3'...**G GA**m5CCNG G**Tm5CC**...5'	↓

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

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M = A or C;	D = A, G or T;
K = G or T;	N = G, A, T or C.
S = C or G;	

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Effect of CpG Methylation on DNA Cleavage by Restriction Enzymes

Methylated DNA substrates were prepared with SssI methyltransferase.

Table 1.16. CpG is Located Inside the Recognition Site.

Enzyme	Sequence	Effect
AatII	GACGTC	⇊
AjiI	CACGTC	⇊
BauI	CACGAG	↓
BcnI	CCSGG	↓
Bsh1236I	CGCG	⇊
Bsh1285I	CGRYCG	⇊
BshTI	ACCGGT	⇊
Bsp68I	TCGCGA	⇊
Bsp119I	TTCGAA	⇊
Bsp143II	RGCGCY	⇊
Bsu15I	ATCGAT	⇊
Cfr9I	CCCGGG	↓
Cfr10I	RCCGGY	⇊
Cfr42I	CCGCGG	⇊
CpoI	CGGWCCG	⇊
CseI	GACGC	⇊
Eco47III	AGCGCT	⇊
Eco52I	CGGCCG	⇊
Eco72I	CACGTG	⇊
Eco88I	CYCGRG	↓
Eco105I	TACGTA	⇊
EheI	GGCGCC	⇊
Esp3I	CGTCTC	⇊
FspAI	RTGCGCAY	⇊
HhaI	GCGC	⇊

Enzyme	Sequence	Effect
Hin1I	GRCGYC	⇊
Hin6I	GCGC	⇊
HpaII	CCGG	⇊
Kpn2I	TCCGGA	⇊
MbiI	GAGCGG	↓
MluI	ACGCGT	⇊
MspI	CCGG	↓
NotI	GCGGCCGC	⇊
NsbI	TGCGCA	⇊
PauI	GCGCGC	⇊
PdiI	GCCGGC	⇊
Pfl23II	CGTACG	⇊
Ppu21I	YACGTR	⇊
Psp1406I	AACGTT	⇊
PvuI	CGATCG	⇊
SalI	GTCGAC	⇊
SgsI	GGCGCGCC	⇊
SmaI	CCCGGG	⇊
SmuI	CCCGC	⇊
SsiI	CCGC	⇊
TaiI	ACGT	⇊
TaqI	TCGA	↓
TauI	GCSGC	⇊
XhoI	CTCGAG	↓

Note

↓ Cleavage not blocked.

⇊ Cleavage blocked.

↓ Cleavage rate is slowed significantly by methylation.

Single letter code

R = G or A; H = A, C or T;
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K = G or T; N = G, A, T or C.
S = C or G;

(continued on next page)

Table 1.17. CpG Partially Overlaps the Recognition Site.

Enzyme	Sequence*	Effect
AarI	5'...CACCTGm5C G...3' 3'...GTGGAC Gm5C...5'	↓
AasI	5'...m5C GAC (N)$_6$GTC...3' 3'... Gm5CTG (N)$_6$CA Gm5C...5'	↓
	5'...m5C GAC (N)$_6$GTm5C G...3' 3'... Gm5CTG (N)$_6$CA Gm5C...5'	?
	5'...GAm5C G (N)$_5$GTC...3' 3'...CT Gm5C (N)$_5$CAG...5'	↓
	5'...GAm5C G (N)$_4$m5C GTC...3' 3'...CT Gm5C (N)$_4$ Gm5CAG...5'	?
Acc65I	5'...GGTACm5C G...3' 3'...CCATG Gm5C...5'	↓
	5'...m5C GGTACm5C G...3' 3'... Gm5CCATG Gm5C...5'	↓↓
AdeI	5'...CAm5C GNNGTG...3' 3'...GT Gm5CNNCAC...5'	↓
	5'...CAm5C GNm5C GTG...3' 3'...GT Gm5CN Gm5CAC...5'	↓↓
AjuI	5'...m5C GAA (N)$_7$TTGG...3' 3'... Gm5CTT (N)$_7$AACC...5'	↓
AlfI	5'...GCA (N)$_6$TGm5C G...3' 3'...CGT (N)$_6$AC Gm5C...5'	↓
AloI	5'...m5C GAAC (N)$_6$TCC...3' 3'... Gm5CTTG (N)$_6$AGG...5'	↓↓
	5'...GAAC (N)$_5$TCm5C G...3' 3'...CTTG (N)$_6$AG Gm5C...5'	↓
	5'...GAAm5C G (N)$_5$TCC...3' 3'...CTT Gm5C (N)$_5$AGG...5'	↓
Alw21I	5'...m5C GWGCWm5C G...3' 3'... Gm5CWCGW Gm5C...5'	↓
Alw26I	5'...GTCTm5C G...3' 3'...CAGA Gm5C...5'	↓
	5'...m5C GTCTC...3' 3'... Gm5CAGAG...5'	↓↓
Alw44I	5'...GTGCAm5C G...3' 3'...CACGT Gm5C...5'	↓↓
ApaI	5'...GGGCCm5C G...3' 3'...CCCGG Gm5C...5'	↓
	5'...m5C GGGCCm5C G...3' 3'... Gm5CCCGG Gm5C...5'	↓
BamHI	5'...m5C GGATCm5C G...3' 3'... Gm5CCTAG Gm5C...5'	↓
BfuI	5'...m5C GTATCC...3' 3'... Gm5CATAGG...5'	↓
	5'...GTATCm5C G...3' 3'...CATAG Gm5C...5'	↓
BglI	5'...GCm5C G (N)$_3$m5C GGC...3' 3'...CG Gm5C (N)$_3$ Gm5CCG...5'	↓
	5'...GCC (N)$_5$GGm5C G...3' 3'...CGG (N)$_5$CC Gm5C...5'	↓
	5'...m5C GCC (N)$_5$GGm5C G...3' 3'... Gm5CGG (N)$_5$CC Gm5C...5'	↓↓
Bme1390I	5'...Cm5C GGG...3' 3'...G Gm5CCC...5'	↓↓
BoxI	5'...GAm5C G (N)$_3$GTC...3' 3'...CT Gm5C (N)$_3$CAG...5'	↓
	5'...GAm5C GNNm5C GTC...3' 3'...CT Gm5CNN Gm5CAG...5'	↓↓
	5'...GAC (N)$_4$GTm5C G...3' 3'...CTG (N)$_4$CA Gm5C...5'	↓
BpiI	5'...GAAGAm5C G...3' 3'...CTTCT Gm5C...5'	↓
	5'...m5C GAAGAC...3' 3'... Gm5CTTCTG...5'	↓
BplI	5'...m5C GAG (N)$_5$CTm5C G...3' 3'... Gm5CTC (N)$_5$GA Gm5C...5'	↓
Bpu10I	5'...CCTNAGm5C G...3' 3'...GGANTC Gm5C...5'	↓
Bpu1102I	5'...m5C GCTNAGm5C G...3' 3'... Gm5CGANTC Gm5C...5'	↓
BseDI	5'...Cm5C Gm5C GG...3' 3'...G Gm5C Gm5CC...5'	↓
BseGI	5'...m5C GGATG...3' 3'... Gm5CCTAC...5'	↓
BseJI	5'...GAT (N)$_4$ATm5C G...3' 3'...CTA (N)$_4$TA Gm5C...5'	↓
	5'...m5C GAT (N)$_4$ATm5C G...3' 3'... Gm5CTA (N)$_4$TA Gm5C...5'	?
BseLI	5'...Cm5C G (N)$_6$GG...3' 3'...G Gm5C (N)$_6$CC...5'	↓
	5'...Cm5C G (N)$_5$m5C GG...3' 3'...G Gm5C (N)$_5$ Gm5CC...5'	↓↓
BseMI	5'...m5C GCAATG...3' 3'... Gm5CGTTAC...5'	↓
BseSI	5'...m5C GKGCMm5C G...3' 3'... Gm5CMCGK Gm5C...5'	↓
BseXI	5'...m5C GCAGm5C G...3' 3'... Gm5CGTC Gm5C...5'	↓
BshNI	5'...GGYRCm5C G...3' 3'...CCRYG Gm5C...5'	↓
	5'...m5C GGYRCm5C G...3' 3'... Gm5CCRYG Gm5C...5'	↓↓
	5'...GGm5C GCC...3' 3'...CC Gm5CGG...5'	↓
Bsp120I	5'...GGGCCm5C G...3' 3'...CCCGG Gm5C...5'	↓↓
Bsp143I	5'...GATm5C G...3' 3'...CTA Gm5C...5'	↓↓
BspLI	5'...GGNNCm5C G...3' 3'...CCNNG Gm5C...5'	↓
	5'...m5C GGNNCm5C G...3' 3'... Gm5CCNNG Gm5C...5'	↓↓
BspPI	5'...GGATm5C G...3' 3'...CCTA Gm5C...5'	↓
	5'...m5C GGATC...3' 3'... Gm5CCTAG...5'	↓
Bst1107I	5'...GTATAm5C G...3' 3'...CATAT Gm5C...5'	↓
	5'...m5C GTATAm5C G...3' 3'... Gm5CATAT Gm5C...5'	↓↓
BsuRI	5'...m5C GGCm5C G...3' 3'... Gm5CCG Gm5C...5'	↓
BveI	5'...ACCTGm5C G...3' 3'...TGGAC Gm5C...5'	↓
CfrI	5'...YGGCm5C G...3' 3'...RCCG Gm5C...5'	↓↓
Cfr13I	5'...GGNCm5C G...3' 3'...CCNG Gm5C...5'	↓↓
Csp6I	5'...m5C GTAm5C G...3' 3'... Gm5CAT Gm5C...5'	↓
DpnI	5'...Gm6A Tm5C G...3' 3'...C Tm6A Gm5C...5'	↓
Eam1104I	5'...CTCTTm5C G...3' 3'...GAGAA Gm5C...5'	↓
Eam1105I	5'...GAm5C G (N)$_3$m5C GTC...3' 3'...CT Gm5C (N)$_3$ Gm5CAG...5'	↓
	5'...GAC (N)$_5$GTm5C G...3' 3'...CTG (N)$_5$CA Gm5C...5'	↓
	5'...m5C GAC (N)$_5$GTm5C G...3' 3'... Gm5CTG (N)$_5$CA Gm5C...5'	?
EcI136II	5'...GAGCTm5C G...3' 3'...CTCGA Gm5C...5'	↓
	5'...m5C GAGCTm5C G...3' 3'... Gm5CTCGA Gm5C...5'	↓↓
Eco24I	5'...m5C GRGCYm5C G...3' 3'... Gm5CYCGR Gm5C...5'	↓
Eco31I	5'...GGTCTm5C G...3' 3'...CCAGA Gm5C...5'	↓
	5'...m5C GGTCTC...3' 3'... Gm5CCAGAG...5'	↓
Eco32I	5'...m5C GATATm5C G...3' 3'... Gm5CTATA Gm5C...5'	↓
Eco47I	5'...GGWCm5C G...3' 3'...CCWG Gm5C...5'	↓↓
Eco91I	5'...m5C GGTNACm5C G...3' 3'... Gm5CCANTG Gm5C...5'	↓
EcoRI	5'...m5C GAATTm5C G...3' 3'... Gm5CTTAA Gm5C...5'	↓
FaqI	5'...GGGAm5C G...3' 3'...CCCT Gm5C...5'	↓↓
	5'...m5C GGGAC...3' 3'... Gm5CCCTG...5'	↓↓
Hin4I	5'...m5C GAY (N)$_5$VTC...3' 3'... Gm5CTR (N)$_5$BAG...5'	↓
	5'...GAY (N)$_5$VTm5C G...3' 3'...CTR (N)$_5$BA Gm5C...5'	↓
	5'...m5C GAY (N)$_5$VTm5C G...3' 3'... Gm5CTR (N)$_5$BA Gm5C...5'	?
HincII	5'...GTYRAm5C G...3' 3'...CARYT Gm5C...5'	↓
	5'...m5C GTYRAm5C G...3' 3'... Gm5CARYT Gm5C...5'	↓↓
	5'...GTm5C GAC...3' 3'...CA Gm5CTG...5'	↓

(continued on next page)

Table 1.17. CpG Partially Overlaps the Recognition Site.

Enzyme	Sequence*	Effect
HinfI	5'...**GA**N**Tm5C** G...3' 3'...**CTNA** **Gm5C**...5'	↓
	5'...m5C **GANTm5C** G...3' 3'... **Gm5CTNA** **Gm5C**...5'	↓
HphI	5'...m5C **GGTGA**...3' 3'... **Gm5CCACT**...5'	↓
Hpy8I	5'...**GTNNAm5C** G...3' 3'...**CANNT** **Gm5C**...5'	↓
	5'...m5C **GTNNAm5C** G...3' 3'... **Gm5CANNT** **Gm5C**...5'	?
HpyF10VI	5'...**GC** (N)$_7$**Gm5C** G...3' 3'...**CG** (N)$_7$**C** **Gm5C**...5'	↓
	5'...m5C **GC** (N)$_7$**Gm5C** G...3' 3'... **Gm5CG** (N)$_7$**C** **Gm5C**...5'	↓
	5'...**Gm5C** G (N)$_6$**GC**...3' 3'...**C** **Gm5C** (N)$_6$**CG**...5'	↓
	5'...**Gm5C** G (N)$_5$m5C **GC**...3' 3'...**C** **Gm5C** (N)$_5$ **Gm5CG**...5'	?
KpnI	5'...m5C **GGTAC**m5C G...3' 3'... **Gm5CCATG** **Gm5C**...5'	↓
KspAI	5'...**GTTAAm5C** G...3' 3'...**CAATT** **Gm5C**...5'	↓
	5'...m5C **GTTAAm5C** G...3' 3'... **Gm5CAATT** **Gm5C**...5'	↓
LguI	5'...m5C **GCTCTTC**...3' 3'... **Gm5CGAGAAG**...5'	↓
	5'...**GCTCTTm5C** G...3' 3'...**CGAGAA** **Gm5C**...5'	↓
LweI	5'...**GCATm5C** G...3' 3'...**CGTA** **Gm5C**...5'	↓
MbiI	5'...m5C **GAGCGG**...3' 3'... **Gm5CTCGCC**...5'	↓
MboI	5'...m5C **GATm5C** G...3' 3'... **Gm5CTA** **Gm5C**...5'	↓
MboII	5'...m5C **GAAGA**...3' 3'... **Gm5CTTCT**...5'	↓
MnlI	5'...**CCTm5C** G...3' 3'...**GGA** **Gm5C**...5'	↓
MssI	5'...**GTTTAAAm5C** G...3' 3'...**CAAATTT** **Gm5C**...5'	↓
	5'...m5C **GTTTAAAm5C** G...3' 3'... **Gm5CAAATTT** **Gm5C**...5'	↓
Mva1269I	5'...**GAATGm5C** G...3' 3'...**CTTAC** **Gm5C**...5'	↓
	5'...m5C **GAATGC**...3' 3'... **Gm5CTTACG**...5'	↓
NheI	5'...**GCTAGm5C** G...3' 3'...**CGATC** **Gm5C**...5'	↓
	5'...m5C **GCTAGm5C** G...3' 3'... **Gm5CGATC** **Gm5C**...5'	↓
NmuCI	5'...**GTCAm5C** G...3' 3'...**CAGT** **Gm5C**...5'	↓
	5'...m5C **GTCAm5C** G...3' 3'... **Gm5CAGT** **Gm5C**...5'	↓
OliI	5'...**CAm5C** GNNm5C **GTG**...3' 3'...**GT** **Gm5C**NN **Gm5CAC**...5'	↓
PaeI	5'...m5C **GCATGm5C** G...3' 3'... **Gm5CGTAC** **Gm5C**...5'	↓

Enzyme	Sequence*	Effect
PdmI	5'...**GAA** (N)$_4$**TTm5C** G...3' 3'...**CTT** (N)$_4$**AA** **Gm5C**...5'	↓
	5'...m5C **GAA** (N)$_4$**TTm5C** G...3' 3'... **Gm5CTT** (N)$_4$**AA** **Gm5C**...5'	↓
PfeI	5'...**GAWTm5C** G...3' 3'...**CTWA** **Gm5C**...5'	↓
PfoI	5'...**TCm5C** **GGGA**...3' 3'...**AG** **Gm5CCCT**...5'	↓
PpiI	5'...m5C **GAAC** (N)$_5$**CTC**...3' 3'... **Gm5CTTG** (N)$_5$**GAG**...5'	↓
	5'...**GAAC** (N)$_5$**CTm5C** G...3' 3'...**CTTG** (N)$_5$**GA** **Gm5C**...5'	↓
	5'...**GAAm5C** G (N)$_4$**CTC**...3' 3'...**CTT** **Gm5C** (N)$_4$**GAG**...5'	↓
PsuI	5'...m5C **GGATCm5C** G...3' 3'... **Gm5CCTAG** **Gm5C**...5'	↓
PsyI	5'...**GAm5C** GNN**GTm5C** G...3' 3'...**CT** **Gm5C**NN**CA** **Gm5C**...5'	↓
RsaI	5'...**GTAm5C** G...3' 3'...**CAT** **Gm5C**...5'	↓
	5'...m5C **GTAm5C** G...3' 3'... **Gm5CAT** **Gm5C**...5'	↓
SacI	5'...m5C **GAGCTm5C** G...3' 3'... **Gm5CTCGA** **Gm5C**...5'	↓
SatI	5'...**Gm5C** **GC**...3' 3'...**C** **Gm5CG**...5'	↓
	5'...**GCNGm5C** G...3' 3'...**CGNC** **Gm5C**...5'	↓
SchI	5'...**GAGTm5C** G...3' 3'...**CTCA** **Gm5C**...5'	↓
	5'...m5C **GAGTC**...3' 3'... **Gm5CTCAG**...5'	↓
SduI	5'...m5C **GDGCHm5C** G...3' 3'... **Gm5CHCGD** **Gm5C**...5'	↓
SfiI	5'...m5C **GGCC** (N)$_5$**GGCm5C** G...3' 3'... **Gm5CCGG** (N)$_5$**CCG** **Gm5C**...5'	↓
	5'...**GGCm5C** G (N)$_4$**GGCC**...3' 3'...**CCG** **Gm5C** (N)$_4$**CCGG**...5'	↓
	5'...**GGCm5C** G (N)$_3$m5C **GGCC**...3' 3'...**CCG** **Gm5C** (N)$_3$ **Gm5CCGG**...5'	↓
SmoI	5'...**CTm5C** **GGT**...3' 3'...**GA** **Gm5CCA**...5'	↓
TaaI	5'...**Am5C** **GAG**...3' 3'...**T** **Gm5CTC**...5'	↓
Tst	5'...**CAm5C** G (N)$_5$**TCC**...3' 3'...**GT** **Gm5C** (N)$_5$**AGG**...5'	↓
	5'...**CAC** (N)$_5$**TCm5C** G...3' 3'...**GTG** (N)$_5$**AG** **Gm5C**...5'	↓
XapI	5'...m5C **GAATTm5C** G...3' 3'... **Gm5CTTAA** **Gm5C**...5'	↓
XceI	5'...**RCATGm5C** G...3' 3'...**YGTAC** **Gm5C**...5'	↓
	5'...m5C **GCATGm5C** G...3' 3'... **Gm5CGTAC** **Gm5C**...5'	?
XmiI	5'...**GTm5C** **GAC**...3' 3'...**CA** **Gm5CTG**...5'	↓
	5'...**GTMKAm5C** G...3' 3'...**CAKMT** **Gm5C**...5'	↓

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;	

Effect of EcoKI and EcoBI Methylation on DNA Cleavage by Restriction Enzymes

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

Table 1.18. EcoBI Overlapping Methylation.

Enzyme	Sequence*	Effect
AatII	5'...TGm6ACGTC $(N)_4$ TGCT...3' 3'...AC TGCAG $(N)_4$m6ACGA...5'	↓
AluI	5'...TGm6AGCT $(N)_5$ TGCT...3' 3'...AC TCGA $(N)_5$m6ACGA...5'	⤫
BclI	5'...TGm6A TCA $(N)_5$ TGCT...3' 3'...AC Tm6AGT $(N)_5$m6ACGA...5'	⤫
BglII	5'...TGm6AGATCT $(N)_3$ TGCT...3' 3'...AC TCTAGA $(N)_3$m6ACGA...5'	↓
Bpu10I	5'...CCTGm6AGC $(N)_6$ TGCT...3' 3'...GGAC TCG $(N)_6$m6ACGA...5'	↓
Bsp143I	5'...TGm6ATC $(N)_6$ TGCT...3' 3'...AC TAG $(N)_6$m6ACGA...5'	↓
EcoRI	5'...TGm6AATTC $(N)_4$ TGCT...3' 3'...AC TTAAG $(N)_4$m6ACGA...5'	↓
HincII	5'...GTTGm6AC $(N)_7$ TGCT...3' 3'...CAAC TG $(N)_7$m6ACGA...5'	⤫
HindIII	5'...TGm6AAGCTT $(N)_3$ TGCT...3' 3'...AC TTCGAA $(N)_3$m6ACGA...5'	↓
HinfI	5'...TGm6ANTC $(N)_5$ TGCT...3' 3'...AC TNAG $(N)_5$m6ACGA...5'	⤫
HphI	5'...GGTGm6A $(N)_8$ TGCT...3' 3'...CCAC T $(N)_8$m6ACGA...5'	⤫
MboI	5'...TGm6ATC $(N)_6$ TGCT...3' 3'...AC TAG $(N)_6$m6ACGA...5'	⤫
MboII	5'...TGm6AAGA $(N)_5$ TGCT...3' 3'...AC TTCT $(N)_5$m6ACGA...5'	↓
MluI	5'...TGm6ACGCGT $(N)_3$ TGCT...3' 3'...AC TGCGCA $(N)_3$m6ACGA...5'	↓
MnlI	5'...TGm6AGG $(N)_6$ TGCT...3' 3'...AC TCC $(N)_6$m6ACGA...5'	⤫
NdeI	5'...TGm6A $(N)_4$CATA TGCT...3' 3'...AC T $(N)_4$GTATm6ACGA...5'	↓
OliI	5'...TGm6A $(N)_4$CACG TGCT...3' 3'...AC T $(N)_4$GTGCm6ACGA...5'	⤫
PaeI	5'...TGm6A $(N)_5$GCA TGCT...3' 3'...AC T $(N)_5$CGTm6ACGA...5'	⤫
PagI	5'...TCATGm6A $(N)_8$ TGCT...3' 3'...AGTAC T $(N)_8$m6ACGA...5'	↓
SacI	5'...TGm6AGCTC $(N)_4$ TGCT...3' 3'...AC TCGAG $(N)_4$m6ACGA...5'	↓
ScaI	5'...TGm6AGTACT $(N)_3$ TGCT...3' 3'...AC TCATGA $(N)_3$m6ACGA...5'	⤫
SspI	5'...TGm6AATATT $(N)_3$ TGCT...3' 3'...AC TTATAA $(N)_3$m6ACGA...5'	↓
TasI	5'...TGm6AATT $(N)_5$ TGCT...3' 3'...AC TTAA $(N)_5$m6ACGA...5'	⤫

Table 1.19. EcoKI Overlapping Methylation.

Enzyme	Sequence*	Effect
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'	↓
DraI	5'...TTTAAm6AC $(N)_6$G TGC...3' 3'...AAATT TG $(N)_6$Cm6ACG...5'	⤫
HincII	5'...GTYAm6AC $(N)_6$G TGC...3' 3'...CART TG $(N)_6$Cm6ACG...5'	⤫
KspAI	5'...GTTAm6AC $(N)_6$G TGC...3' 3'...CAAT TG $(N)_6$Cm6ACG...5'	⤫
MluI	5'...Am6ACGCGTNNG TGC...3' 3'...T TGCGCANNCm6ACG...5'	↓
MssI	5'...GTTTAAm6AC $(N)_6$G TGC...3' 3'...CAAATT TG $(N)_6$Cm6ACG...5'	⤫
NsbI	5'...Am6AC $(N)_6$G TGCGCA...3' 3'...T TG $(N)_6$Cm6ACGCGT...5'	↓
OliI	5'...Am6ACAC $(N)_4$G TGC...3' 3'...T TGTG $(N)_4$Cm6ACG...5'	⤫
Tru1I	5'...TTAm6AC $(N)_6$G TGC...3' 3'...AAT TG $(N)_6$Cm6ACG...5'	⤫

Note

* Recognition sequence is indicated in bold.
m6A = N6-methyladenine.

↓ Cleavage not blocked.

⤫ Cleavage blocked.

↓ Cleavage rate is slowed significantly by methylation.

The recognition sequences of the following endonucleases may also overlap with DNA sequences methylated by EcoBI and EcoKI.

The following enzymes have not been tested for sensitivity to EcoBI methylation: **AarI**, **AdeI**, **AjiI**, **Alw21I**, **BauI**, **BcuI**, **BfiI**, **BglII**, **BoxI**, **BpiI**, **Bpu1102I**, **BseJI**, **BseGI**, **BseMI**, **BseNI**, **BseXI**, **BshTI**, **Bsp143II**, **BspPI**, **Bsu15I**, **BveI**, **CaiI**, **Cfr10I**, **CseI**, **DpnI**, **Eam1104I**, **Ecl136II**, **Eco24I**, **Eco31I**, **Eco32I**, **Eco47III**, **Eco57I**, **Eco57MI**, **Eco72I**, **Eco81I**, **Eco91I**, **Eco147I**, **EcoO109I**, **Esp3I**, **FspAI**, **HindIII**, **Hin1I**, **Hin1II**, **Hin4I**, **Hpy8I**, **HpyF3I**, **LguI**, **LweI**, **MbiI**, **Mph1103I**, **MunI**, **Mva1269I**, **NdeI**, **NmuCI**, **PdmI**, **PfeI**, **Ppu21I**, **PscI**, **Psp5II**, **Psp1406I**, **PsuI**, **PsyI**, **PvuII**, **SchI**, **SduI**, **SmiI**, **SmoI**, **SspI**, **TaaI**, **TaiI**, **TatI**, **TstI**, **VspI**, **XagI**, **XapI** and **XceI**.

The following enzymes have not been tested for sensitivity to EcoKI methylation: **AdeI**, **AjiI**, **BauI**, **BcuI**, **BfiI**, **BseNI**, **BshNI**, **BshTI**, **Bsp119I**, **BveI**, **Cfr10I**, **Eco72I**, **Eco91I**, **FspAI**, **Hpy8I**, **PdmI**, **Ppu21I**, **PscI**, **Psp1406I**, **SduI**, **TaaI**, **TaiI**, **TsoI** and **XceI**.

Single letter code

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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;

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 [^{32}P] 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

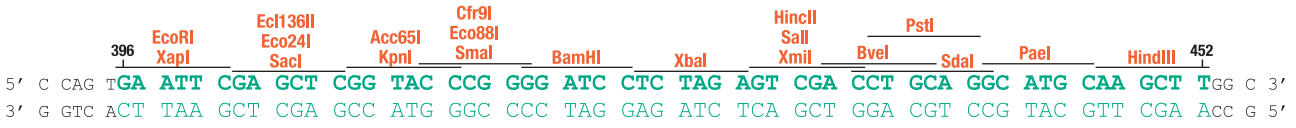

Table 1.20. Cleavage of Restriction Targets Located in Close Vicinity within pUC19 Multiple Cloning Site.

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/XapI	Acc65I	XapI	50-100	7
	XapI	Acc65I	50-100	7
Acc65I/BamHI	Acc65I	BamHI	100	4
	BamHI	Acc65I	100	4
Acc65I/Ecl136II	Acc65I	Ecl136II	50-100	3
	Ecl136II	Acc65I	100	1
Acc65I/Eco24I	Acc65I	Eco24I	0	1
	Eco24I	Acc65I	100	1
Acc65I/SacI	Acc65I	SacI	0-20	1
	SacI	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	HincII	100	9
	HincII	BamHI	50-100	7
BamHI/KpnI	BamHI	KpnI	100	4
	KpnI	BamHI	100	4
BamHI/SalI	BamHI	SalI	100	7
	SalI	BamHI	50-100	7
BamHI/XbaI	BamHI	XbaI	100	1
	XbaI	BamHI	20-50	1
Cfr9I/Ecl136I	Cfr9I	Ecl136II	100	7
	Ecl136II	Cfr9I	50-100	5
Cfr9I/Eco24I	Cfr9I	Eco24I	100	5
	Eco24I	Cfr9I	95	5
Cfr9I/SacI	Cfr9I	SacI	50-100	5
	SacI	Cfr9I	50-100	5
Cfr9I/XbaI	Cfr9I	XbaI	50-100	6
	XbaI	Cfr9I	50-100	6
Ecl136I/Eco88I	Ecl136II	Eco88I	50-100	5
	Eco88I	Ecl136II	100	7
Ecl136I/EcoRI	Ecl136II	EcoRI	100	1
	EcoRI	Ecl136II	100	3
Ecl136I/XapI	Ecl136II	XapI	50-100	1
	XapI	Ecl136II	50-100	3
Eco24I/Eco88I	Eco24I	Eco88I	50-100	5
	Eco88I	Eco24I	100	5
Eco24I/EcoRI	Eco24I	EcoRI	100	1
	EcoRI	Eco24I	100	1
Eco24I/KpnI	Eco24I	KpnI	50-100	1
	KpnI	Eco24I	0	1
Eco24I/XapI	Eco24I	XapI	50-100	1
	XapI	Eco24I	20-50	1
Eco88I/SacI	Eco88I	SacI	100	5
	SacI	Eco88I	50-100	5
Eco88I/XbaI	Eco88I	XbaI	100	6
	XbaI	Eco88I	100	6
EcoRI/Cfr9I	EcoRI	Cfr9I	50-100	11
	Cfr9I	EcoRI	100	11
EcoRI/Eco88I	EcoRI	Eco88I	50-100	11
	Eco88I	EcoRI	100	11
EcoRI/KpnI	EcoRI	KpnI	100	7
	KpnI	EcoRI	100	7
EcoRI/SacI	EcoRI	SacI	30-50	1
	SacI	EcoRI	50-100	1
HincII/PaeI	HincII	PaeI	100	7
	PaeI	HincII	100	9
HincII/PstI	HincII	PstI	20-50	1
	PstI	HincII	100	3
HincII/SdaI	HincII	SdaI	50	1
	SdaI	HincII	0-20	2
HincII/XbaI	HincII	XbaI	50-100	1
	XbaI	HincII	50-100	3
HindIII/PaeI	HindIII	PaeI	0	1
	PaeI	HindIII	50-100	1
HindIII/PstI	HindIII	PstI	100	7
	PstI	HindIII	100	7
HindIII/SdaI	HindIII	SdaI	50-100	7
	SdaI	HindIII	50-100	6
KpnI/Ecl136II	KpnI	Ecl136II	50-100	1
	Ecl136II	KpnI	100	3
KpnI/SacI	KpnI	SacI	100	1
	SacI	KpnI	50-100	1
KpnI/XapI	KpnI	XapI	50-100	7
	XapI	KpnI	100	7
PstI/BamHI	PstI	BamHI	50-100	13
	BamHI	PstI	50-100	13
PaeI/PstI	PaeI	PstI	20-50	1
	PstI	PaeI	0	1
PaeI/SalI	PaeI	SalI	100	7
	SalI	PaeI	50-100	7
PaeI/SdaI	PaeI	SdaI	20-50	1
	SdaI	PaeI	0	0
PstI/SalI	PstI	SalI	50-100	1
	SalI	PstI	0	1
PstI/XbaI	PstI	XbaI	50-100	7
	XbaI	PstI	100	7
SacI/SmaI	SacI	SmaI	50-100	7
	SmaI	SacI	100	5
SacI/XapI	SacI	XapI	50-100	1
	XapI	SacI	0-20	1
SalI/SdaI	SalI	SdaI	20-50	1
	SdaI	SalI	0	0
SalI/XbaI	SalI	XbaI	0-20	1
	XbaI	SalI	50-100	6
SdaI/XbaI	SdaI	XbaI	50-100	6
	XbaI	SdaI	100	7
SmaI/Acc65I	SmaI	Acc65I	0-20	1
	Acc65I	SmaI	0	-1
SmaI/BamHI	SmaI	BamHI	50-100	2
	BamHI	SmaI	0-20	0
SmaI/Ecl136II	SmaI	Ecl136II	50-100	7
	Ecl136II	SmaI	100	7
SmaI/Eco24I	SmaI	Eco24I	50-100	7
	Eco24I	SmaI	100	5
SmaI/KpnI	SmaI	KpnI	0	1
	KpnI	SmaI	0	-1
SmaI/XbaI	SmaI	XbaI	50-100	8
	XbaI	SmaI	100	6

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

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

❶ Reaction Contents:

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

❷ Mix gently.

❸ 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 [^{32}P] 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 (0.5µg) 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)

Table 1.21. Cleavage Efficiency Close to the Termini of PCR Fragments.

Enzyme	bp from the recognition site to fragment end				
	1	2	3	4	5
AarI	20-50		50-100		
AasI	50-100				
AatII	0	0-20	20-50	50-100	
Acc65I	0-20	50-100			
AdeI	50-100				
AjiI	50-100				
AluI	0-20	20-50	50-100		
Alw21I	50-100				
Alw26I	50-100				
Alw44I	0	20-50	50-100		
ApaI	50-100				
BamHI	50-100				
BauI	0-20	20-50	50-100		
BcnI	20-50		50-100		
BclI	0	50-100			
BcuI	50-100				
BfiI	50-100				
BfmI	50-100				
BfuI	50-100				
BglI	20-50	50-100			
BglII	0	50-100			
Bme1390I	20-50		50-100		
BoxI	0	50-100			
BpiI	50-100				
Bpu10I	20-50	50-100			
Bpu1102I	50-100				
BseDI	0	50-100			
BseGI	50-100				
BseJI	0	50-100			
BseLI	0	50-100			
BseMI	0-20	50-100			
BseMII	50-100				
BseNI	0	50-100			
BseSI	50-100				
BseXI	20-50	50-100			
Bsh1236I	50-100				
Bsh1285I	0-20	50-100			
BshNI	50-100				
BshTI	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		
Bst1107I	0-20	50-100			
BstXI	0		50-100		
Bsu15I	50-100				
BsuRI	0-20	20-50	50-100		
BveI	0-20		50-100		
CaiI	0	0-20	50-100		
CfrI	0	50-100			
Cfr9I	20-50	50-100			
Cfr10I	20-50	50-100			
Cfr13I	50-100				
Cfr42I	50-100				
CpoI	50-100				

Enzyme	bp from the recognition site to fragment end				
	1	2	3	4	5
CseI	50-100				
Csp6I	50-100				
DpnI	50-100				
DraI	0	0-20	50-100		
Eam1104I	0	50-100			
Eam1105I	0	50-100			
Ecl136II	50-100				
Eco24I	50-100				
Eco31I	20-50		50-100		
Eco32I	20-50	50-100			
Eco47I	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			
EcoO109I	50-100				
EcoRI	50-100				
EcoRII	0	0-20	20-50	50-100	
EheI	20-50		50-100		
Esp3I	50-100				
FaqI	0-20	50-100			
FspAI	0-20	20-50	50-100		
FspBI	20-50	50-100			
GsuI	50-100				
HhaI	50-100				
Hin1I	50-100				
Hin1II	0	0-20	50-100		
Hin6I	0		0-20	50-100	
HincII	50-100				
HindIII	0	0-20	50-100		
HinfI	50-100				
HpaII	0-20	50-100			
HphI	0	50-100			
Hpy8I	0-20	50-100			
HpyF3I	20-50	50-100			
HpyF10VI	50-100				
KpnI	50-100				
Kpn2I	0	50-100			
KspAI	20-50	50-100			
LguI	50-100				
LweI	20-50	50-100			
MbiI	0	0-20	50-100		
MboI	50-100				
MboII	50-100				
MlsI	0-20	50-100			
MluI	20-50		50-100		
MnlI	0	50-100			
Mph1103I	20-50	50-100			
MspI	0-20	50-100			
MssI	20-50	50-100			
MunI	20-50	50-100			
MvaI	0	20-50	50-100		
Mva1269I	0	50-100			

Enzyme	bp from the recognition site to fragment end				
	1	2	3	4	5
NcoI	0	50-100			
NdeI*	0-20	20-50	50-100		
NheI	0	20-50		50-100	
NmuCI	20-50	50-100			
NotI	20-50	50-100			
NsbI	0	0-20	50-100		
OliI	0-20	20-50	50-100		
PaeI	0	0-20		20-50	50-100
PagI	20-50	50-100			
PasI	50-100				
PauI	0	50-100			
PdiI	0-20	50-100			
PdmI	50-100				
PfeI	50-100				
Pfl23II	0-20	20-50	50-100		
PfoI	0	20-50	50-100		
Ppu21I	50-100				
PscI	0	50-100			
Psp5II	0	50-100			
Psp1406I	0-20	50-100			
PsuI	0-20	50-100			
PstI	0-20	50-100			
PsuI	0-20	50-100			
PsyI	0	50-100			
PvuI	20-50		50-100		
PvuII	50-100				
RsaI	50-100				
SacI	50-100				
SalI	20-50		50-100		
SatI	0	50-100			
ScaI	0-20	50-100			
SchI	50-100				
SdaI	0-20	50-100			
SduI	50-100				
SfiI	50-100				
SgsI	50-100				
SmaI	50-100				
SmiI	0-20	50-100			
SmoI	0	50-100			
SmuI	50-100				
SsiI	50-100				
SspI	0-20	50-100			
TaaI	20-50	50-100			
TaiI	50-100				
TaqI	0	20-50		50-100	
TasI	0	20-50	50-100		
TatI	0-20		20-50	50-100	
TauI	20-50	50-100			
Tru1I	0	0-20		50-100	
TsoI	20-50	50-100			
Van91I	0	50-100			
VspI	50-100				
XagI	20-50	50-100			
XapI	20-50		50-100		
XbaI	20-50	50-100			
XceI	0	50-100			
XhoI	0-20	50-100			
XmaJI	50-100				
XmiI	50-100				
I-SceI	50-100				

Cleavage efficiency

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

Note

***** Incubation was performed for 16 hours.

Fermentas Guide for Successful Digestions

Table 1.22. Fermentas Guide for Successful Digestions.

Problem	Possible cause	Recommended solution
No digestion or incomplete digestion	The DNA sample contains impurities that inhibit restriction enzymes.	*To check if these contaminants inhibit restriction enzymes, digest the control DNA. In parallel, digest your sample supplemented with the control DNA.* • If PCR products are used directly after amplification, dilute the sample 3-fold with the recommended buffer prior digestion (*see* p.140 for more details). • If DNA is purified using silica or resin suspensions, re-centrifuge your 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 1µg 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 *see* Site Preferences by Restriction Endonucleases on p.130).	• Add 5-10u of restriction enzymes per 1µg of DNA. • Try another isoschizomer (*see* Table 1.3 on pp.6-15).
	Some restriction enzymes, like AarI, BveI, Cfr10I, Eam1104, Eco57I, EcoRII, SfiI, require at least two target sites per DNA molecule for efficient cleavage (for more details *see* 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 AarI (#ER1581) and BveI (#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. ***Note*** PCR products are NOT methylated when the PCR is carried out with standard dNTPs and non-methylated primers.	1. Identify which type of DNA methylation can occur (*see* Digestion of Methylated DNA on p.132). 2. Use the Tables 1.12-1.19 on pp.133-138 to check if methylation could influence DNA digestion. 3. If methylation is the reason for impaired DNA cleavage, we suggest the following: – propagate your plasmid in *E.coli dam*$^-$, *dcm*$^-$ strain. (*E.coli* GM2163 *dam*$^-$, *dcm*$^-$, strain is available for free upon request under #M0099), – use the REsearch™ engine on www.fermentas.com/research or check the Fermentas catalog for availability of a restriction endonuclease isoschizomer which is not sensitive to DNA methylation.
	Restriction enzyme DpnI was used to digest DNA containing unmethylated targets.	• If the cleavage site is not important for your experiment use other neoschizomer: Bsp143I or MboI. • If the cleavage site must be retained, propagate your plasmid in *E.coli dam*$^+$ 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.

(continued on next page)

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 *see* p.123).
	The restriction enzyme was added to a reaction buffer with low ionic strength in the absence of stabilizing agents.	The restriction endonuclease should always be the last component added to the reaction mixture.
	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. • Alw21I, BpiI, 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 0.02-0.1µg/µl.
	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 (*see* 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 (*see* 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 Certificate 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 electrophoretic mobility of digestion products (gel shift). Restriction endonucleases AarI, AloI, BdaI, BspPI, EcoRII, Eco57I, GsuI, TauI, TsoI 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 fragment ligation	Restriction enzyme is still active in the ligation mixture.	• 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 difficult 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 neccessary information regarding the newly discovered restriction enzymes.
Use **REsearch™** at www.fermentas.com/research, **DoubleDigest™** at www.fermentas.com/doubledigest 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 = G or A; Y = C or T; W = A or T; M = A or C; K = G or T; S = C or G;
H = A, C or T; V = A, C or G; B = C, G or T; D = A, G or T; N = G, A, T or C.

Table 1.23. Newly Generated Recognition Sequences Resulting from the Removal of a 3'-overhang and Self-ligation.

Restriction enzyme	Recognition sequence	Newly generated sequence after reaction	Restriction enzymes that cleave the newly generated recognition sequence
AasI	GACNNNN↓NNGTC	GACNNNNGTC	**BoxI**
AdeI	CACNNN↓GTG	CACGTG	**Eco72I**, MaeII, **Ppu21I**, **TaiI**
BglI	GCCNNNN↓NGGC	GCCNNGGC	**BseDI**
Bsh1285I	CGRY↓CG	CGCG	**Bsh1236I**
CaiI	CAGNNN↓CTG	CAGCTG	**AluI**, CviJI, MspA1I, **PvuII**
Cfr42I	CCGC↓GG	CCGG	**HpaII**, **MspI**
Eam1105I	GACNNN↓NNGTC	GACNNNNGTC	**BoxI**
FseI	GGCCGG↓CC	GGCC	[**BsuRI**], [CviJI]
Hpy188I	TCN↓GA	TCGA	**TaqI**
PacI	TTAAT↓TAA	TTATAA	PsiI
PvuI	CGAT↓CG	CGCG	**Bsh1236I**
SdaI	CCTGCA↓GG	CCGG	**HpaII**, **MspI**
SfiI	GGCCNNNN↓NGGCC	GGCCNNGGCC	**BseDI**, [**BsuRI**], [CviJI]
SgfI	GCGAT↓CGC	GCGCGC	**Bsh1236I**, Cac8I, **HhaI**, **Hin6I**, **PauI**
TaaI	ACN↓GT	ACGT	MaeII, **TaiI**

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 www.fermentas.com/doubledigest 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 C.
S = C or G;

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

Restriction enzyme	Recognition sequence	Newly generated sequence after reaction	Restriction enzymes that cleave the newly generated recognition sequence
Acc65I	G↓GTACC	GGTACGTACC	[**Csp6I**], **Eco105I**, MaeII, **Ppu21I**, [**RsaI**], **TaiI**
AflIII*	A↓CACGT	ACACGCACGT	[MaeII], [**TaiI**]
AflIII*	A↓CATGT	ACATGCATGT	BfrBI, [CviAII], [FatI], [**Hin1II**], HpyCH4V, **Mph1103I**, [**XceI**]
AflIII*	A↓CGCGT	ACGCGCGCGT	[**Bsh1236I**], Cac8I, **HhaI**, **Hin6I**, **PauI**
AflIII*	A↓CGTGT	ACGTGCGTGT	[MaeII], [**TaiI**]
Alw44I	G↓TGCAC	GTGCATGCAC	Cac8I, CviAII, FatI, **Hin1II**, [HpyCH4V], **PaeI**, **XceI**
BamHI	G↓GATCC	GGATCGATCC	[**Bsp143I**], [**BspPI**], **Bsu15I**, [**DpnI**], [**MboI**], **TaqI**
BbvCI	CC↓TCAGC	CCTCATCAGC	[**MnlI**]
BclI	T↓GATCA	TGATCGATCA	[**Bsp143I**], **Bsu15I**, [**DpnI**], [**MboI**], **TaqI**
BcnI	CC↓SGG	CCSSGG	**BseDI**
BcnI*	CC↓CGG	CCCCGG	[**BcnI**], [**Bme1390I**], **BseDI**, [BssKI], [**HpaII**], [**MspI**]
BcnI*	CC↓GGG	CCGGGG	[**BcnI**], [**Bme1390I**], **BseDI**, [BssKI], [**HpaII**], [**MspI**]
BcuI	A↓CTAGT	ACTAGCTAGT	**AluI**, CviJI, [**FspBI**]
BfmI*	C↓TGCAG	CTGCATGCAG	Cac8I, CviAII, FatI, **Hin1II**, [HpyCH4V], **PaeI**, **XceI**
BglII	A↓GATCT	AGATCGATCT	[**Bsp143I**], **Bsu15I**, [**DpnI**], [**MboI**], **TaqI**
Bme1390I	CC↓NGG	CCNNGG	**BseDI**
Bme1390I*	CC↓AGG	CCAAGG	**BseDI**, **Eco130I**
Bme1390I*	CC↓CGG	CCCCGG	[**BcnI**], [**Bme1390I**], **BseDI**, [BssKI], [**HpaII**], [**MspI**]
Bme1390I*	CC↓GGG	CCGGGG	[**BcnI**], [**Bme1390I**], **BseDI**, [BssKI], [**HpaII**], [**MspI**]
Bme1390I*	CC↓TGG	CCTTGG	**BseDI**, **Eco130I**
Bpu10I*	CC↓TCAGC	CCTCATCAGC	[**MnlI**]
BsaWI	W↓CCGGW	WCCGGCCGGW	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**, [**HpaII**], [**MspI**]
BseYI	C↓CCAGC	CCCAGCCAGC	[BseYI], Cac8I, CviJI
BshNI*	G↓GCACC	GGCACGCACC	Cac8I
BshNI*	G↓GCGCC	GGCGCGCGCC	**Bsh1236I**, Cac8I, [**HhaI**], [**Hin6I**], **PauI**
BshNI*	G↓GTACC	GGTACGTACC	[**Csp6I**], **Eco105I**, MaeII, **Ppu21I**, [**RsaI**], **TaiI**
BshNI*	G↓GTGCC	GGTGCGTGCC	Cac8I
BshTI	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], FseI, **HpaII**, **MspI**, NgoMIV, **PdiI**
Bsp1407I	T↓GTACA	TGTACGTACA	[**Csp6I**], **Eco105I**, MaeII, **Ppu21I**, [**RsaI**], **TaiI**
Bsp143I	↓GATC	GATCGATC	[**Bsp143I**], **Bsu15I**, [**DpnI**], [**MboI**], **TaqI**
BspTI	C↓TTAAG	CTTAATTAAG	PacI, **TasI**, [**Tru1I**]
BssKI	↓CCNGG	CCNGGCCNGG	[**Bme1390I**], [BssKI], **BsuRI**, CviJI
BssKI*	↓CCAGG	CCAGGCCAGG	[**Bme1390I**], [BssKI], **BsuRI**, CviJI, [**EcoRII**], [**MvaI**]

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

Restriction enzyme	Recognition sequence	Newly generated sequence after reaction	Restriction enzymes that cleave the newly generated recognition sequence
BssKI*	↓CCCGG	CCCGGCCCGG	[BcnI], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpaII], [MspI]
BssKI*	↓CCGGG	CCGGGCCGGG	[BcnI], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpaII], [MspI]
BssKI*	↓CCTGG	CCTGGCCTGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
Bsu15I	AT↓CGAT	ATCGCGAT	Bsh1236I, Bsp68I, Hpy188III
BtgI*	C↓CATGG	CCATGCATGG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
BtgI*	C↓CGCGG	CCGCGCGCGG	[Bsh1236I], Cac8I, HhaI, Hin6I, PauI, [SsiI]
Cfr10I	R↓CCGGY	RCCGGCCGGY	Bsh1285I, BsuRI, [Cfr10I], CfrI, CviJI, Eco52I, [HpaII], [MspI]
Cfr13I*	G↓GCCC	GGCCGCCC	[BsuRI], [CviJI], SatI, SsiI, TauI
Cfr13I*	G↓GGCC	GGGCGGCC	[BsuRI], [CviJI], SatI, TauI
Cfr9I	C↓CCGGG	CCCGGCCGGG	[BcnI], [Bme1390I], Bsh1285I, [BssKI], BsuRI, CfrI, CviJI, Eco52I, [HpaII], [MspI]
CfrI	Y↓GGCCR	YGGCCGGCCR	[BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], FseI, HpaII, MspI, NgoMIV, PdiI
CpoI*	CG↓GACCG	CGGACGACCG	Bsh1285I
CpoI*	CG↓GTCCG	CGGTCGTCCG	Bsh1285I
Csp6I	G↓TAC	GTATAC	Bst1107I, Hpy8I, XmiI
CviAII	C↓ATG	CATATG	NdeI
Eco130I*	C↓CATGG	CCATGCATGG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
Eco130I*	C↓CTAGG	CCTAGCTAGG	AluI, CviJI, [FspBI]
Eco52I	C↓GGCCG	CGGCCGGCCG	[Bsh1285I], [BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], [Eco52I], FseI, HpaII, MspI, NgoMIV, PdiI
Eco81I*	CC↓TCAGG	CCTCATCAGG	[MnlI]
Eco88I	C↓YCGRG	CYCGRYCGRG	Bsh1285I
Eco88I*	C↓CCGAG	CCCGACCGAG	Bsh1285I
Eco88I*	C↓CCGGG	CCCGGCCGGG	[BcnI], [Bme1390I], Bsh1285I, [BssKI], BsuRI, CfrI, CviJI, Eco52I, [HpaII], [MspI]
Eco88I*	C↓TCGAG	CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, MboI, PvuI, [TaqI]
Eco88I*	C↓TCGGG	CTCGGTCGGG	Bsh1285I
Eco91I	G↓GTNACC	GGTNACGTNACC	MaeII, [MaeIII], TaiI
Eco91I*	G↓GTAACC	GGTAACGTAACC	MaeII, [MaeIII], TaiI
Eco91I*	G↓GTCACC	GGTCACGTCACC	AjiI, MaeII, [MaeIII], [NmuCI], TaiI
Eco91I*	G↓GTGACC	GGTGACGTGACC	[HphI], MaeII, [MaeIII], [NmuCI], TaiI
Eco91I*	G↓GTTACC	GGTTACGTTACC	MaeII, [MaeIII], TaiI
EcoO109I*	RG↓GCCCY	RGGCCGCCCY	[BsuRI], [CviJI], SatI, SsiI, TauI
EcoO109I*	RG↓GGCCY	RGGGCGGCCY	[BsuRI], [CviJI], SatI, TauI
EcoRI	G↓AATTC	GAATTAATTC	PdmI, [TasI], Tru1I, VspI
EcoRII	↓CCWGG	CCWGGCCWGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
EcoRII*	↓CCAGG	CCAGGCCAGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
EcoRII*	↓CCTGG	CCTGGCCTGG	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
FatI	↓CATG	CATGCATG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
FspBI	C↓TAG	CTATAG	BfmI
Hin1I	GR↓CGYC	GRCGCGYC	Bsh1236I
Hin6I	G↓CGC	GCGCGC	Bsh1236I, Cac8I, [HhaI], [Hin6I], PauI
HindIII	A↓AGCTT	AAGCTAGCTT	[AluI], BmtI, Cac8I, [CviJI], FspBI, NheI
HpaII	C↓CGG	CCGCGG	BseDI, Bsh1236I, BtgI, Cfr42I, MspA1I, SsiI
KasI	G↓GCGCC	GGCGCGCGCC	Bsh1236I, Cac8I, [HhaI], [Hin6I], PauI
Kpn2I	T↓CCGGA	TCCGGCCGGA	Bsh1285I, BsuRI, CfrI, CviJI, Eco52I, [HpaII], [MspI]
MaeII	A↓CGT	ACGCGT	AflIII, Bsh1236I, MluI
MaeIII	↓GTNAC	GTNACGTNAC	MaeII, [MaeIII], TaiI
MaeIII*	↓GTAAC	GTAACGTAAC	MaeII, [MaeIII], TaiI
MaeIII*	↓GTCAC	GTCACGTCAC	AjiI, MaeII, [MaeIII], [NmuCI], TaiI
MaeIII*	↓GTGAC	GTGACGTGAC	MaeII, [MaeIII], [NmuCI], TaiI
MaeIII*	↓GTTAC	GTTACGTTAC	MaeII, [MaeIII], TaiI
MboI	↓GATC	GATCGATC	[Bsp143I], Bsu15I, [DpnI], [MboI], TaqI
MluI	A↓CGCGT	ACGCGCGCGT	[Bsh1236I], Cac8I, HhaI, Hin6I, PauI
MspI	C↓CGG	CCGCGG	BseDI, Bsh1236I, BtgI, Cfr42I, MspA1I, SsiI
MunI	C↓AATTG	CAATTAATTG	[TasI], Tru1I, VspI
MvaI	CC↓WGG	CCWWGG	BseDI, Eco130I
MvaI*	CC↓AGG	CCAAGG	BseDI, Eco130I
MvaI*	CC↓TGG	CCTTGG	BseDI, Eco130I
NarI	GG↓CGCC	GGCGCGCC	Bsh1236I, Cac8I, [HhaI], [Hin6I], PauI, SgsI
NcoI	C↓CATGG	CCATGCATGG	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
NgoMIV	G↓CCGGC	GCCGGCCGGC	Bsh1285I, BsuRI, [Cac8I], [Cfr10I], CfrI, CviJI, Eco52I, [HpaII], [MspI], [NgoMIV], [PdiI]
NheI	G↓CTAGC	GCTAGCTAGC	AluI, [BmtI], [Cac8I], CviJI, [FspBI], [NheI]
NmuCI	↓GTSAC	GTSACGTSAC	MaeII, [MaeIII], [NmuCI], TaiI

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

Restriction enzyme	Recognition sequence	Newly generated sequence after reaction	Restriction enzymes that cleave the newly generated recognition sequence
NmuCI*	↓GTCAC	GTCACGTCAC	AjiI, MaeII, [MaeIII], [NmuCI], TaiI
NmuCI*	↓GTGAC	GTGACGTGAC	MaeII, [MaeIII], [NmuCI], TaiI
NotI	GC↓GGCCGC	GCGGCCGGCCGC	[Bsh1285I], [BsuRI], Cac8I, Cfr10I, [CfrI], [CviJI], [Eco52I], FseI, HpaII, MspI, NgoMIV, PdiI, [SatI], [SsiI], [TauI]
PagI	T↓CATGA	TCATGCATGA	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I
PasI*	CC↓CAGGG	CCCAGCAGGG	BseYI, EcoP15I
PauI	G↓CGCGC	GCGCGCGCGC	[Bsh1236I], [Cac8I], [HhaI], [Hin6I], [PauI]
Pfl23II	C↓GTACG	CGTACGTACG	[Csp6I], Eco105I, MaeII, [Pfl23II], Ppu21I, [RsaI], TaiI
PfoI	T↓CCNGGA	TCCNGGCCNGGA	[Bme1390I], [BssKI], BsuRI, CviJI
PfoI*	T↓CCAGGA	TCCAGGCCAGGA	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
PfoI*	T↓CCCGGA	TCCCGGCCCGGA	[BcnI], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpaII], [MspI]
PfoI*	T↓CCGGGA	TCCGGGCCGGGA	[BcnI], [Bme1390I], [BssKI], BsuRI, Cfr13I, CviJI, [HpaII], [MspI]
PfoI*	T↓CCTGGA	TCCTGGCCTGGA	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
PscI	A↓CATGT	ACATGCATGT	BfrBI, [CviAII], [FatI], [Hin1II], HpyCH4V, Mph1103I, [XceI]
Psp1406I	AA↓CGTT	AACGCGTT	AflIII, Bsh1236I, MluI
PspXI	VC↓TCGAGB	VCTCGATCGAGB	Bsh1285I, Bsp143I, DpnI, MboI, PvuI, [TaqI]
PsuI	R↓GATCY	RGATCGATCY	[Bsp143I], Bsu15I, [DpnI], [MboI], TaqI
PsyI	GACN↓NNGTC	GACNNNNGTC	BoxI
PsyI*	GACN↓ANGTC	GACNAANGTC	BoxI
PsyI*	GACN↓CNGTC	GACNCCNGTC	BoxI
PsyI*	GACN↓GNGTC	GACNGGNGTC	BoxI
PsyI*	GACN↓TNGTC	GACNTTNGTC	BoxI
SalI	G↓TCGAC	GTCGATCGAC	Bsh1285I, Bsp143I, DpnI, MboI, PvuI, [TaqI]
SanDI*	GG↓GACCC	GGGACGACCC	[FaqI]
SatI	GC↓NGC	GCNNGC	Cac8I
SatI*	GC↓AGC	GCAAGC	Cac8I
SatI*	GC↓CGC	GCCCGC	Cac8I, SmuI, [SsiI]
SatI*	GC↓GGC	GCGGGC	Cac8I
SatI*	GC↓TGC	GCTTGC	Cac8I
SexAI	A↓CCWGGT	ACCWGGCCWGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
SexAI*	A↓CCAGGT	ACCAGGCCAGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
SexAI*	A↓CCTGGT	ACCTGGCCTGGT	[Bme1390I], [BssKI], BsuRI, CviJI, [EcoRII], [MvaI]
SgrAI	CR↓CCGGYG	CRCCGGCCGGYG	Bsh1285I, BsuRI, [Cfr10I], CfrI, CviJI, Eco52I, [HpaII], [MspI]
SgsI	GG↓CGCGCC	GGCGCGCGCGCC	[Bsh1236I], [Cac8I], [HhaI], [Hin6I], [PauI]
SmoI*	C↓TCGAG	CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, MboI, PvuI, [TaqI]
SmoI*	C↓TTAAG	CTTAATTAAG	PacI, TasI, [Tru1I]
SsiI	C↓CGC	CCGCGC	Bsh1236I, HhaI, Hin6I, [SsiI]
TaqI	T↓CGA	TCGCGA	Bsh1236I, Bsp68I, Hpy188III
TasI	↓AATT	AATTAATT	[TasI], Tru1I, VspI
TatI	W↓GTACW	WGTACGTACW	[Csp6I], Eco105I, MaeII, Ppu21I, [RsaI], TaiI
Tru1I	T↓TAA	TTATAA	PsiI
TseI	G↓CWGC	GCWGCWGC	[SatI], [TseI]
TseI*	G↓CAGC	GCAGCAGC	[BseXI], EcoP15I, [SatI], [TseI]
TseI*	G↓CTGC	GCTGCTGC	[SatI], [TseI]
VspI	AT↓TAAT	ATTATAAT	PsiI
XapI	R↓AATTY	RAATTAATTY	[TasI], Tru1I, VspI
XbaI	T↓CTAGA	TCTAGCTAGA	AluI, CviJI, [FspBI]
XhoI	C↓TCGAG	CTCGATCGAG	Bsh1285I, Bsp143I, DpnI, MboI, PvuI, [TaqI]
XmaJI	C↓CTAGG	CCTAGCTAGG	AluI, CviJI, [FspBI]
XmiI*	GT↓CGAC	GTCGCGAC	Bsh1236I, Bsp68I, Hpy188III
XmiI*	GT↓CTAC	GTCTCTAC	Alw26I

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 www.fermentas.com/doubledigest to plan your experiments.

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 C.
S = C or G;

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
AjiI* (CAC↓GTC)	Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC)	MnlI
	Eco105I (TAC↓GTA), Ppu21I* (YAC↓GTA)	MaeII, Ppu21I, TaiI
	Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTG)	Eco72I, MaeII, Ppu21I, TaiI
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	HpaII, MspI
	PdiI (GCC↓GGC)	BceAI
	ZraI (GAC↓GTC)	AjiI, MaeII, TaiI
AjiI* (GAC↓GTG)	DpnI (GA↓TC)	HinfI
	Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC)	MnlI
	Eco105I (TAC↓GTA), Eco72I (CAC↓GTG), Ppu21I* (YAC↓GTA), Ppu21I* (YAC↓GTG)	MaeII, TaiI
	Eco47III (AGC↓GCT), FspAI* (RTGC↓GCAC), FspAI* (RTGC↓GCAT), NsbI (TGC↓GCA)	CseI
	EheI (GGC↓GCC)	CseI, Hin1I
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	HpaII, MspI
	PdiI (GCC↓GGC)	BceAI
	ZraI (GAC↓GTC)	AatII, Hin1I, MaeII, TaiI, ZraI
AluI (AG↓CT)	Bst1107I (GTA↓TAC)	Csp6I, RsaI
	BsuRI (GG↓CC), CviJI* (RG↓CC), Eco147I (AGG↓CCT), Eco47III (AGC↓GCT), MlsI (TGG↓CCA)	CviJI
	CviJI* (RG↓CT), Ecl136II (GAG↓CTC), MbiI* (CCG↓CTC), MspA1I* (CMG↓CTG), PvuII (CAG↓CTG)	AluI, CviJI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, MboI
	EheI (GGC↓GCC)	BsuRI, CviJI
BfrBI (ATG↓CAT)	Bst1107I (GTA↓TAC)	Csp6I, RsaI
	DraI (TTT↓AAA), HincII* (GTY↓AAC), KspAI (GTT↓AAC), MssI (GTTT↓AAAC), SmiI (ATTT↓AAAT)	TspDTI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, MboI
	Eco47III (AGC↓GCT)	CviJI
	EheI (GGC↓GCC)	BsuRI, CviJI
	HpyCH4V (TG↓CA)	HpyCH4V
Bsh1236I (CG↓CG)	Bsp68I (TCG↓CGA), MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	Bsh1236I
	Bst1107I (GTA↓TAC)	Csp6I, RsaI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, MboI
	Eco47III (AGC↓GCT)	CviJI
	EheI (GGC↓GCC)	BsuRI, CviJI
Bsp68I (TCG↓CGA)	Bsh1236I (CG↓CG), MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	Bsh1236I
	Bst1107I (GTA↓TAC)	Csp6I, RsaI
	DraI (TTT↓AAA), HincII* (GTY↓AAC), KspAI (GTT↓AAC), MssI (GTTT↓AAAC), RsaI (GT↓AC), ScaI (AGT↓ACT), SmiI (ATTT↓AAAT), SspI (AAT↓ATT)	TaqI
	Eco32I (GAT↓ATC)	Bsp143I, DpnI, MboI, TaqI
	Eco47III (AGC↓GCT)	CviJI
	EheI (GGC↓GCC)	BsuRI, CviJI

(continued on next page)

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
	HincII* (GTY↓GAC)	Hpy188I
Bst1107I (GTA↓TAC)	**AluI** (AG↓CT), BfrBI (ATG↓CAT), **Bsh1236I** (CG↓CG), **Bsp68I** (TCG↓CGA), **BsuRI** (GG↓CC), CviJI* (RG↓CC), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **Eco147I** (AGG↓CCT), HpyCH4V (TG↓CA), **MbiI*** (CCG↓CTC), **MbiI*** (GAG↓CGG), **MlsI** (TGG↓CCA), MspA1I* (CMG↓CGG), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**Csp6I**, **RsaI**
	Eco47III (AGC↓GCT)	**AluI**, CviJI
	EheI (GGC↓GCC)	CviJI
	HincII* (GTY↓AAC), **KspAI** (GTT↓AAC)	**Hpy8I**
	HincII* (GTY↓GAC)	**Hpy8I**, **XmiI**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	MaeIII
	SspI (AAT↓ATT)	**TasI**
BsuRI (GG↓CC)	**AluI** (AG↓CT), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **Eco47III** (AGC↓GCT), **MbiI*** (CCG↓CTC), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **MlsI** (TGG↓CCA)	**BsuRI**, CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, **Cfr13I**, CviJI
	FspAI* (RTGC↓GCAC)	**BseSI**, **SduI**
	HincII* (GTY↓GAC)	**FaqI**
CviJI* (AG↓CY)	**AluI** (AG↓CT), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**AluI**, CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), **Eco147I** (AGG↓CCT), **Eco47III** (AGC↓GCT), **MlsI** (TGG↓CCA)	CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI
CviJI* (GG↓CY)	**AluI** (AG↓CT), **Ecl136II** (GAG↓CTC), **Eco47III** (AGC↓GCT), **MbiI*** (CCG↓CTC), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), **Eco147I** (AGG↓CCT), **MlsI** (TGG↓CCA)	**BsuRI**, CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, **Cfr13I**, CviJI
	FspAI* (RTGC↓GCAC)	**BseSI**, **SduI**
	HincII* (GTY↓GAC)	**FaqI**
DpnI (GA↓TC)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	**HinfI**, PleI, **SchI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**HinfI**
	Eco32I (GAT↓ATC)	**HinfI**, **PfeI**
	Eco47III (AGC↓GCT)	**AluI**, CviJI
	EheI (GGC↓GCC)	CviJI
	FspAI* (RTGC↓GCAC)	**Alw21I**, **SduI**
	SspI (AAT↓ATT)	**TasI**
DraI (TTT↓AAA)	**Bsp68I** (TCG↓CGA)	**TaqI**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓AAC), **KspAI** (GTT↓AAC), PsiI (TTA↓TAA)	**Tru1I**
	MssI (GTTT↓AAAC), **SmiI** (ATTT↓AAAT)	**DraI**, **Tru1I**
Ecl136II (GAG↓CTC)	**AluI** (AG↓CT), CviJI* (RG↓CT), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**AluI**, CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **Eco47III** (AGC↓GCT), **MlsI** (TGG↓CCA)	CviJI
	DpnI (GA↓TC)	**HinfI**, PleI, **SchI**
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI
	MbiI* (CCG↓CTC)	**AluI**, **Alw21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
Eco105I (TAC↓GTA)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	MaeII, **TaiI**
	AjiI* (GAC↓GTG), **Eco72I** (CAC↓GTG), **Ppu21I*** (YAC↓GTG)	MaeII, **Ppu21I**, **TaiI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	BceAI
	Ppu21I* (YAC↓GTA)	**Eco105I**, MaeII, **Ppu21I**, **TaiI**
Eco147I (AGG↓CCT)	**AluI** (AG↓CT), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **Eco47III** (AGC↓GCT), **MbiI*** (CCG↓CTC), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **MlsI** (TGG↓CCA)	**BsuRI**, CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, **Cfr13I**, CviJI
	FspAI* (RTGC↓GCAC)	**BseSI**, **SduI**
	HincII* (GTY↓GAC)	**FaqI**

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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
Eco32I (GAT↓ATC)	**AluI** (AG↓CT), BfrBI (ATG↓CAT), **Bsh1236I** (CG↓CG), **BsuRI** (GG↓CC), CviJI* (RG↓CC), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **Eco147I** (AGG↓CCT), HpyCH4V (TG↓CA), **MbiI*** (CCG↓CTC), **MbiI*** (GAG↓CGG), **MlsI** (TGG↓CCA), MspA1I* (CMG↓CGG), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**Bsp143I**, **DpnI**, **MboI**
	Bsp68I (TCG↓CGA)	**Bsp143I**, **DpnI**, **MboI**, **TaqI**
	DpnI (GA↓TC)	**HinfI**, **PfeI**
	FspAI* (RTGC↓GCAC), **NsbI** (TGC↓GCA)	HpyCH4V
	FspAI* (RTGC↓GCAT)	BfrBI, HpyCH4V, **Mph1103I**
	PsiI (TTA↓TAA)	**Tru1I**
Eco47III (AGC↓GCT)	**AluI** (AG↓CT), BfrBI (ATG↓CAT), **Bsh1236I** (CG↓CG), **Bsp68I** (TCG↓CGA), **BsuRI** (GG↓CC), CviJI* (RG↓CC), CviJI* (RG↓CT), **Eco147I** (AGG↓CCT), HpyCH4V (TG↓CA), **MlsI** (TGG↓CCA), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC), **DpnI** (GA↓TC), PsiI (TTA↓TAA)	**AluI**, CviJI
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	CviJI, **MnlI**
	Eco32I (GAT↓ATC)	**LweI**
	EheI (GGC↓GCC)	**Bsp143II**, **HhaI**, **Hin6I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	**HhaI**, **Hin6I**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	CviJI, **HpaII**, **MspI**
	PdiI (GCC↓GGC)	**SatI**, **TauI**
	SrfI (GCCC↓GGGC)	Cac8I
Eco72I (CAC↓GTG)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	**AjiI**, MaeII, **TaiI**
	AjiI* (GAC↓GTG), **Ppu21I*** (YAC↓GTG)	**Eco72I**, MaeII, **Ppu21I**, **TaiI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco105I (TAC↓GTA), **Ppu21I*** (YAC↓GTA)	MaeII, **Ppu21I**, **TaiI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	BceAI
EheI (GGC↓GCC)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	**Hin1I**
	AluI (AG↓CT), BfrBI (ATG↓CAT), **Bsh1236I** (CG↓CG), **Bsp68I** (TCG↓CGA), CviJI* (RG↓CT), HpyCH4V (TG↓CA), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**BsuRI**, CviJI
	Bst1107I (GTA↓TAC), **DpnI** (GA↓TC), PsiI (TTA↓TAA)	CviJI
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **MlsI** (TGG↓CCA)	**BsuRI**, **Cfr13I**, CviJI
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**BsuRI**, CviJI, **MnlI**
	Eco32I (GAT↓ATC)	**LweI**
	Eco47III (AGC↓GCT)	**Bsp143II**, **HhaI**, **Hin6I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	**HhaI**, **Hin6I**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**BsuRI**, CviJI, **HpaII**, **MspI**
	PdiI (GCC↓GGC)	**SatI**, **TauI**
	SrfI (GCCC↓GGGC)	Cac8I
FspAI* (ATGC↓GCAY)	**DraI** (TTT↓AAA), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC), **MssI** (GTTT↓AAAC), **RsaI** (GT↓AC), **ScaI** (AGT↓ACT), **SmiI** (ATTT↓AAAT)	HpyCH4V
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	BfrBI, HpyCH4V, **LweI**, **Mph1103I**
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC)	**HhaI**, **Hin6I**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	NsbI (TGC↓GCA)	**HhaI**, **Hin6I**, **NsbI**
	PdiI (GCC↓GGC)	**SatI**, **TauI**
	SrfI (GCCC↓GGGC)	Cac8I
	SspI (AAT↓ATT)	BfrBI, HpyCH4V, **Mph1103I**
FspAI* (GTGC↓GCAY)	**BsuRI** (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **MlsI** (TGG↓CCA)	**BseSI**, **SduI**
	DpnI (GA↓TC)	**Alw21I**, **SduI**
	DraI (TTT↓AAA), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC), **MssI** (GTTT↓AAAC), **SmiI** (ATTT↓AAAT), **SspI** (AAT↓ATT)	HpyCH4V
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	HpyCH4V, **LweI**
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC)	**HhaI**, **Hin6I**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	NsbI (TGC↓GCA)	**HhaI**, **Hin6I**, **NsbI**
	PdiI (GCC↓GGC)	**SatI**, **TauI**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	SrfI (GCCC↓GGGC)	Cac8I
HincII* (GTC↓RAC)	**Bsp68I** (TCG↓CGA)	Hpy188I
	Bst1107I (GTA↓TAC)	**Hpy8I**, **XmiI**
	DpnI (GA↓TC)	**Alw26I**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	KspAI (GTT↓AAC)	**HincII**, **Hpy8I**

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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
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	MaeIII, **NmuCI**
HincII* (GTT↓RAC)	**Bsp68I** (TCG↓CGA)	**TaqI**
	Bst1107I (GTA↓TAC)	**Hpy8I**
	DraI (TTT↓AAA), **MssI** (GTTT↓AAAC), PsiI (TTA↓TAA), **SmiI** (ATTT↓AAAT)	**Tru1I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	KspAI (GTT↓AAC)	**HincII**, **Hpy8I**, **KspAI**, **Tru1I**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	MaeIII
HpyCH4V (TG↓CA)	BfrBI (ATG↓CAT)	HpyCH4V
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	Eco47III (AGC↓GCT)	CviJI
	EheI (GGC↓GCC)	**BsuRI**, CviJI
KspAI (GTT↓AAC)	**Bsp68I** (TCG↓CGA)	**TaqI**
	Bst1107I (GTA↓TAC)	**Hpy8I**
	DraI (TTT↓AAA), **MssI** (GTTT↓AAAC), PsiI (TTA↓TAA), **SmiI** (ATTT↓AAAT)	**Tru1I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓AAC)	**HincII**, **Hpy8I**, **KspAI**, **Tru1I**
	HincII* (GTY↓GAC)	**HincII**, **Hpy8I**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	MaeIII
MbiI* (CCG↓CTC)	**AjiI*** (CAC↓GTC), **AjiI*** (GAC↓GTG), **Eco105I** (TAC↓GTA), **Eco72I** (CAC↓GTG), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **HincII*** (GTY↓GAC), **NsbI** (TGC↓GCA), **Ppu21I*** (YAC↓GTA), **Ppu21I*** (YAC↓GTG), ZraI (GAC↓GTC)	**HpaII**, **MspI**
	AluI (AG↓CT), BfrBI (ATG↓CAT), **BsuRI** (GG↓CC), CviJI* (RG↓CC), CviJI* (RG↓CT), **Eco147I** (AGG↓CCT), HpyCH4V (TG↓CA), **MlsI** (TGG↓CCA)	**SsiI**
	Bsh1236I (CG↓CG), **Bsp68I** (TCG↓CGA)	**Bsh1236I**, **SsiI**
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	Ecl136II (GAG↓CTC)	**MbiI**, **SsiI**
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	Eco47III (AGC↓GCT)	CviJI, **HpaII**, **MspI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI, **HpaII**, **MspI**
	MspA1I* (CMG↓CGG)	**BseDI**, **Bsh1236I**, BtgI, **Cfr42I**, MspA1I, **SsiI**
	MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	MspA1I, **SsiI**
	PdiI (GCC↓GGC)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	SmaI (CCC↓GGG), SrfI (GCCC↓GGGC)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **HpaII**, **MspI**
MbiI* (GAG↓CGG)	**AluI** (AG↓CT), CviJI* (RG↓CT), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	**AluI**, CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **Eco47III** (AGC↓GCT), **MlsI** (TGG↓CCA)	CviJI
	DpnI (GA↓TC)	**HinfI**, PleI, **SchI**
	Ecl136II (GAG↓CTC)	**AluI**, **Alw21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI
MlsI (TGG↓CCA)	**AluI** (AG↓CT), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **Eco47III** (AGC↓GCT), **MbiI*** (CCG↓CTC), MspA1I* (CMG↓CTG), **PvuII** (CAG↓CTG)	CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT)	**BsuRI**, CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, **Cfr13I**, CviJI
	FspAI* (RTGC↓GCAC)	**BseSI**, **SduI**
	HincII* (GTY↓GAC)	**FaqI**
MspA1I* (CAG↓CKG)	**AluI** (AG↓CT), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC)	**AluI**, CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **Eco47III** (AGC↓GCT), **MlsI** (TGG↓CCA)	CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI
	MbiI* (GAG↓CGG)	MspA1I
	PvuII (CAG↓CTG)	**AluI**, CviJI, MspA1I, **PvuII**
MspA1I* (CCG↓CKG)	**AjiI*** (CAC↓GTC), **AjiI*** (GAC↓GTG), **Eco105I** (TAC↓GTA), **Eco72I** (CAC↓GTG), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **HincII*** (GTY↓GAC), **NsbI** (TGC↓GCA), **Ppu21I*** (YAC↓GTA), **Ppu21I*** (YAC↓GTG), ZraI (GAC↓GTC)	**HpaII**, **MspI**
	AluI (AG↓CT), BfrBI (ATG↓CAT), **BsuRI** (GG↓CC), CviJI* (RG↓CC), CviJI* (RG↓CT), **Eco147I** (AGG↓CCT), HpyCH4V (TG↓CA), **MlsI** (TGG↓CCA)	**SsiI**

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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
	Bsh1236I (CG↓CG), **Bsp68I** (TCG↓CGA)	**Bsh1236I**, **SsiI**
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MbiI**, **SsiI**
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	Eco47III (AGC↓GCT)	CviJI, **HpaII**, **MspI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI, **HpaII**, **MspI**
	MbiI* (GAG↓CGG)	**BseDI**, **Bsh1236I**, BtgI, **Cfr42I**, MspA1I, **SsiI**
	PdiI (GCC↓GGC)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	PvuII (CAG↓CTG)	MspA1I, **SsiI**
	SmaI (CCC↓GGG), SrfI (GCCC↓GGGC)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **HpaII**, **MspI**
MssI (GTTT↓AAAC)	**Bsp68I** (TCG↓CGA)	**TaqI**
	DraI (TTT↓AAA), **SmiI** (ATTT↓AAAT)	**DraI**, **Tru1I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓AAC), **KspAI** (GTT↓AAC), PsiI (TTA↓TAA)	**Tru1I**
	RsaI (GT↓AC), **ScaI** (AGT↓ACT)	**Hpy8I**
NsbI (TGC↓GCA)	**DraI** (TTT↓AAA), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC), **MssI** (GTTT↓AAAC), **RsaI** (GT↓AC), **ScaI** (AGT↓ACT), **SmiI** (ATTT↓AAAT), **SspI** (AAT↓ATT)	HpyCH4V
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	HpyCH4V, **LweI**
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC)	**HhaI**, **Hin6I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT)	**HhaI**, **Hin6I**, **NsbI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	**SatI**, **TauI**
	SrfI (GCCC↓GGGC)	Cac8I
PdiI (GCC↓GGC)	**DpnI** (GA↓TC), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	BccI
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	**SatI**, **SsiI**, **TauI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG), **SmaI** (CCC↓GGG), SrfI (GCCC↓GGGC)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
Ppu21I* (CAC↓GTR)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	**AjiI**, MaeII, **TaiI**
	AjiI* (GAC↓GTG), **Eco72I** (CAC↓GTG)	**Eco72I**, MaeII, **Ppu21I**, **TaiI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco105I (TAC↓GTA)	MaeII, **Ppu21I**, **TaiI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	BceAI
Ppu21I* (TAC↓GTR)	**AjiI*** (CAC↓GTC), ZraI (GAC↓GTC)	MaeII, **TaiI**
	AjiI* (GAC↓GTG), **Eco72I** (CAC↓GTG)	MaeII, **Ppu21I**, **TaiI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco105I (TAC↓GTA)	**Eco105I**, MaeII, **Ppu21I**, **TaiI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	BceAI
PsiI (TTA↓TAA)	**DraI** (TTT↓AAA), **Eco32I** (GAT↓ATC), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC), **MssI** (GTTT↓AAAC), **RsaI** (GT↓AC), **ScaI** (AGT↓ACT), **SmiI** (ATTT↓AAAT)	**Tru1I**
	Eco47III (AGC↓GCT)	**AluI**, CviJI
	EheI (GGC↓GCC)	CviJI
	SspI (AAT↓ATT)	**TasI**, **Tru1I**
PvuII (CAG↓CTG)	**AluI** (AG↓CT), CviJI* (RG↓CT), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC)	**AluI**, CviJI
	Bst1107I (GTA↓TAC)	**Csp6I**, **RsaI**
	BsuRI (GG↓CC), CviJI* (RG↓CC), **Eco147I** (AGG↓CCT), **Eco47III** (AGC↓GCT), **MlsI** (TGG↓CCA)	CviJI
	Eco32I (GAT↓ATC)	**Bsp143I**, **DpnI**, **MboI**
	EheI (GGC↓GCC)	**BsuRI**, CviJI
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	MspA1I
	MspA1I* (CMG↓CTG)	**AluI**, CviJI, MspA1I, **PvuII**
RsaI (GT↓AC)	**Bsp68I** (TCG↓CGA)	**TaqI**
	Bst1107I (GTA↓TAC), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC)	MaeIII
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**Alw26I**
	FspAI* (RTGC↓GCAC)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	FspAI* (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓GAC)	MaeIII, **NmuCI**
	MssI (GTTT↓AAAC)	**Hpy8I**
	PsiI (TTA↓TAA)	**Tru1I**
	ScaI (AGT↓ACT)	**Csp6I**, **RsaI**

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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
ScaI (AGT↓ACT)	**Bsp68I** (TCG↓CGA)	**TaqI**
	Bst1107I (GTA↓TAC), **HincII*** (GTY↓AAC), **KspAI** (GTT↓AAC)	MaeIII
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**Alw26I**
	FspAI* (RTGC↓GCAC)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	FspAI* (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓GAC)	MaeIII, **NmuCI**
	MssI (GTTT↓AAAC)	**Hpy8I**
	PsiI (TTA↓TAA)	**Tru1I**
	RsaI (GT↓AC)	**Csp6I**, **RsaI**
SmaI (CCC↓GGG)	**DpnI** (GA↓TC), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	BccI
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	**SmuI**, **SsiI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **HpaII**, **MspI**
	PdiI (GCC↓GGC)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	SrfI (GCCC↓GGGC)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **Cfr9I**, **Eco88I**, **HpaII**, **MspI**, **SmaI**
SmiI (ATTT↓AAAT)	**Bsp68I** (TCG↓CGA)	**TaqI**
	DraI (TTT↓AAA), **MssI** (GTTT↓AAAC)	**DraI**, **Tru1I**
	FspAI* (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	HpyCH4V
	HincII* (GTY↓AAC), **KspAI** (GTT↓AAC), PsiI (TTA↓TAA)	**Tru1I**
SrfI (GCCC↓GGGC)	**DpnI** (GA↓TC), **Ecl136II** (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco32I (GAT↓ATC)	BccI
	Eco47III (AGC↓GCT), **EheI** (GGC↓GCC), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	Cac8I, **SmuI**, **SsiI**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **HpaII**, **MspI**
	PdiI (GCC↓GGC)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	SmaI (CCC↓GGG)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **Cfr9I**, **Eco88I**, **HpaII**, **MspI**, **SmaI**
SspI (AAT↓ATT)	**Bsp68I** (TCG↓CGA)	**TaqI**
	Bst1107I (GTA↓TAC), **DpnI** (GA↓TC)	**TasI**
	FspAI* (RTGC↓GCAC), **NsbI** (TGC↓GCA)	HpyCH4V
	FspAI* (RTGC↓GCAT)	BfrBI, HpyCH4V, **Mph1103I**
	PsiI (TTA↓TAA)	**TasI**, **Tru1I**
ZraI (GAC↓GTC)	**AjiI*** (CAC↓GTC)	**AatII**, **Hin1I**, MaeII, **TaiI**, ZraI
	AjiI* (GAC↓GTG), **Eco105I** (TAC↓GTA), **Eco72I** (CAC↓GTG), **Ppu21I*** (YAC↓GTA), **Ppu21I*** (YAC↓GTG)	MaeII, **TaiI**
	DpnI (GA↓TC)	**HinfI**
	Ecl136II (GAG↓CTC), **MbiI*** (CCG↓CTC)	**MnlI**
	Eco47III (AGC↓GCT), **FspAI*** (RTGC↓GCAC), **FspAI*** (RTGC↓GCAT), **NsbI** (TGC↓GCA)	**CseI**
	EheI (GGC↓GCC)	**CseI**, **Hin1I**
	MbiI* (GAG↓CGG), MspA1I* (CMG↓CGG)	**HpaII**, **MspI**
	PdiI (GCC↓GGC)	BceAI

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 www.fermentas.com/doubledigest 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 C.
S = C or G;

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
AatII (GACGT↓C)	**TaiI** (ACGT↓)	MaeII, **TaiI**
Acc65I (G↓GTACC)	**BshNI*** (G↓GTACC)	**Acc65I**, **BshNI**, **BspLI**, **Csp6I**, **KpnI**, **RsaI**
	Bsp1407I (T↓GTACA), **Pfl23II** (C↓GTACG), **TatI*** (W↓GTACA), **TatI*** (W↓GTACT)	**Csp6I**, **RsaI**
AflIII* (A↓CATGT)	BtgI* (C↓CATGG), **Eco130I*** (C↓CATGG), FatI (↓CATG), **NcoI** (C↓CATGG), **PagI** (T↓CATGA)	CviAII, FatI, **Hin1II**
	PscI (A↓CATGT)	AflIII, CviAII, FatI, **Hin1II**, **PscI**, **XceI**
AflIII* (A↓CGCGT)	BtgI* (C↓CGCGG)	**Bsh1236I**
	MluI (A↓CGCGT)	AflIII, **Bsh1236I**, **MluI**
	PauI (G↓CGCGC), **SgsI** (GG↓CGCGCC)	**Bsh1236I**, **HhaI**, **Hin6I**
AflIII* (A↓CGTGT)	BtgI* (C↓CGTGG)	MaeII, **TaiI**
Alw21I* (GAGCA↓C)	**SduI*** (GAGCA↓C)	**Alw21I**, **SduI**
Alw21I* (GAGCT↓C)	**Eco24I*** (GAGCT↓C), **SacI** (GAGCT↓C), **SduI*** (GAGCT↓C)	**AluI**, **Alw21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
Alw21I* (GTGCA↓C)	**BseSI*** (GTGCA↓C), **SduI*** (GTGCA↓C)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	Mph1103I (ATGCA↓T)	HpyCH4V
	PstI (CTGCA↓G), **SdaI** (CCTGCA↓GG)	BsgI, HpyCH4V
Alw21I* (GTGCT↓C)	**SduI*** (GTGCT↓C)	**Alw21I**, **SduI**
Alw44I (G↓TGCAC)	**BfmI*** (C↓TGCAG)	BsgI, HpyCH4V
ApaI (GGGCC↓C)	**BseSI*** (GGGCC↓C), **Eco24I*** (GGGCC↓C), **SduI*** (GGGCC↓C)	**ApaI**, **BseSI**, **Bsp120I**, **BspLI**, **BsuRI**, **Cfr13I**, CviJI, **Eco24I**, **SduI**
BamHI (G↓GATCC)	**BclI** (T↓GATCA), **Bsp143I** (↓GATC), **MboI** (↓GATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	BglII (A↓GATCT), **PsuI*** (R↓GATCT)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**, **PsuI**
	PsuI* (R↓GATCC)	**BamHI**, **Bsp143I**, **BspLI**, **BspPI**, **DpnI**, **MboI**, **PsuI**
BbeI (GGCGC↓C)	**Bsp143II*** (RGCGC↓C)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI
	Bsp143II* (RGCGC↓T)	**Bsp143II**, **HhaI**, **Hin6I**
BclI (T↓GATCA)	**BamHI** (G↓GATCC), **BglII** (A↓GATCT), **Bsp143I** (↓GATC), **MboI** (↓GATC), **PsuI*** (R↓GATCC), **PsuI*** (R↓GATCT)	**Bsp143I**, **DpnI**, **MboI**
BcuI (A↓CTAGT)	**Eco130I*** (C↓CTAGG), **NheI** (G↓CTAGC), **XbaI** (T↓CTAGA), **XmaJI** (C↓CTAGG)	**FspBI**
BfmI* (C↓TGCAG)	**Alw44I** (G↓TGCAC)	HpyCH4V
BglII (A↓GATCT)	**BamHI** (G↓GATCC), **PsuI*** (R↓GATCC)	**Bsp143I**, **DpnI**, **MboI**, **PsuI**
	BclI (T↓GATCA), **Bsp143I** (↓GATC), **MboI** (↓GATC)	**Bsp143I**, **DpnI**, **MboI**
	PsuI* (R↓GATCT)	**BglII**, **Bsp143I**, **DpnI**, **MboI**, **PsuI**
BsaWI* (A↓CCGGW)	**BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, **BshTI**, **Cfr10I**, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	**Cfr10I**, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**

(continued on next page)

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
	Kpn2I (T↓CCGGA)	BsaWI, **HpaII**, **MspI**
BsaWI* (T↓CCGGW)	**BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	**HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	Kpn2I (T↓CCGGA)	BsaWI, **HpaII**, Hpy188III, **Kpn2I**, **MspI**
BseSI* (GGGCA↓C)	**SduI*** (GGGCA↓C)	**BseSI**, **SduI**
BseSI* (GGGCC↓C)	**ApaI** (GGGCC↓C), **Eco24I*** (GGGCC↓C), **SduI*** (GGGCC↓C)	**ApaI**, **BseSI**, **Bsp120I**, **BspLI**, **BsuRI**, **Cfr13I**, CviJI, **Eco24I**, **SduI**
BseSI* (GTGCA↓C)	**Alw21I*** (GTGCA↓C), **SduI*** (GTGCA↓C)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	Mph1103I (ATGCA↓T)	HpyCH4V
	PstI (CTGCA↓G), **SdaI** (CCTGCA↓GG)	BsgI, HpyCH4V
BseSI* (GTGCC↓C)	**SduI*** (GTGCC↓C)	**BseSI**, **SduI**
Bsh1285I* (CGAT↓CG)	PacI (TTAAT↓TAA)	**Tru1I**
	PvuI (CGAT↓CG), SgfI (GCGAT↓CGC)	**Bsh1285I**, **Bsp143I**, **DpnI**, **MboI**, **PvuI**
BshNI* (G↓GCGCC)	KasI (G↓GCGCC)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI
BshNI* (G↓GTACC)	**Acc65I** (G↓GTACC)	**Acc65I**, **BshNI**, **BspLI**, **Csp6I**, **KpnI**, **RsaI**
	Bsp1407I (T↓GTACA), **Pfl23II** (C↓GTACG), **TatI*** (W↓GTACA), **TatI*** (W↓GTACT)	**Csp6I**, **RsaI**
BshTI (A↓CCGGT)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	BsaWI, **HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **Cfr10I*** (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, **BshTI**, **Cfr10I**, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	**Cfr10I**, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
Bsp119I (TT↓CGAA)	**Bsu15I** (AT↓CGAT), **TaqI** (T↓CGA), **XmiI*** (GT↓CGAC)	**TaqI**
Bsp120I (G↓GGCCC)	**CfrI*** (Y↓GGCCA), **CfrI*** (Y↓GGCCG), **Eco52I** (C↓GGCCG)	**BsuRI**, **Cfr13I**, CviJI
	NotI (GC↓GGCCGC)	**BsuRI**, **Cfr13I**, CviJI, **SatI**, **SsiI**, **TauI**
Bsp1407I (T↓GTACA)	**Acc65I** (G↓GTACC), **BshNI*** (G↓GTACC), **Pfl23II** (C↓GTACG)	**Csp6I**, **RsaI**
	TatI* (W↓GTACA)	**Bsp1407I**, **Csp6I**, **RsaI**, **TatI**
	TatI* (W↓GTACT)	**Csp6I**, **RsaI**, **TatI**
Bsp143I (↓GATC)	**BamHI** (G↓GATCC), **BclI** (T↓GATCA), **BglII** (A↓GATCT), **MboI** (↓GATC), **PsuI*** (R↓GATCC), **PsuI*** (R↓GATCT)	**Bsp143I**, **DpnI**, **MboI**
Bsp143II* (AGCGC↓Y)	BbeI (GGCGC↓C)	**Bsp143II**, **HhaI**, **Hin6I**
Bsp143II* (GGCGC↓Y)	BbeI (GGCGC↓C)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI
BspTI (C↓TTAAG)	**SmoI*** (C↓TTAAG)	**BspTI**, **SmoI**, **Tru1I**
Bsu15I (AT↓CGAT)	**Bsp119I** (TT↓CGAA), **TaqI** (T↓CGA), **XmiI*** (GT↓CGAC)	**TaqI**
BtgI* (C↓CACGG)	AflIII* (A↓CACGT)	MaeII, **TaiI**
BtgI* (C↓CATGG)	AflIII* (A↓CATGT), FatI (↓CATG), **PagI** (T↓CATGA), **PscI** (A↓CATGT)	CviAII, FatI, **Hin1II**
	Eco130I* (C↓CATGG), **NcoI** (C↓CATGG)	**BseDI**, BtgI, CviAII, **Eco130I**, FatI, **Hin1II**, **NcoI**
BtgI* (C↓CGCGG)	AflIII* (A↓CGCGT), **MluI** (A↓CGCGT)	**Bsh1236I**, **SsiI**
	PauI (G↓CGCGC), **SgsI** (GG↓CGCGCC)	**Bsh1236I**, **HhaI**, **Hin6I**, **SsiI**
Cfr10I* (A↓CCGGY)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	BsaWI, **HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, **BshTI**, **Cfr10I**, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	**Cfr10I**, **HpaII**, **MspI**
Cfr10I* (G↓CCGGY)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	**HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), SgrAI* (CR↓CCGGTG)	**Cfr10I**, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	Cac8I, **Cfr10I**, **HpaII**, **MspI**, NgoMIV, **PdiI**
Cfr42I (CCGC↓GG)	**Bsh1285I*** (CGGC↓CG)	**SsiI**
Cfr9I (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)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	Eco88I* (C↓CCGGG)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **Cfr9I**, **Eco88I**, **HpaII**, **MspI**, **SmaI**
CfrI* (C↓GGCCR)	**Bsp120I** (G↓GGCCC)	**BsuRI**, **Cfr13I**, CviJI
	Eco52I (C↓GGCCG)	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**
	NotI (GC↓GGCCGC)	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**, **SatI**, **SsiI**, **TauI**
CfrI* (T↓GGCCR)	**Bsp120I** (G↓GGCCC)	**BsuRI**, **Cfr13I**, CviJI
	Eco52I (C↓GGCCG)	**BsuRI**, **CfrI**, CviJI
	NotI (GC↓GGCCGC)	**BsuRI**, **CfrI**, CviJI, **SatI**, **SsiI**, **TauI**
CpoI* (CG↓GACCG)	**Eco47I*** (G↓GACC), **Psp5II*** (RG↓GACCT)	**Cfr13I**, **Eco47I**

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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
	Psp5II* (RG↓GACCC), SanDI* (GG↓GACCC)	**BspLI**, **Cfr13I**, **Eco47I**
CpoI* (CG↓GTCCG)	**Eco47I*** (G↓GTCC), **Psp5II*** (RG↓GTCCT)	**Cfr13I**, **Eco47I**
	Psp5II* (RG↓GTCCC), SanDI* (GG↓GTCCC)	**BspLI**, **Cfr13I**, **Eco47I**
Eco130I* (C↓CATGG)	AflIII* (A↓CATGT), FatI (↓CATG), **PagI** (T↓CATGA), **PscI** (A↓CATGT)	CviAII, FatI, **Hin1II**
	BtgI* (C↓CATGG), **NcoI** (C↓CATGG)	**BseDI**, BtgI, CviAII, **Eco130I**, FatI, **Hin1II**, **NcoI**
Eco130I* (C↓CTAGG)	**BcuI** (A↓CTAGT), **NheI** (G↓CTAGC), **XbaI** (T↓CTAGA)	**FspBI**
	XmaJI (C↓CTAGG)	**BseDI**, **Eco130I**, **FspBI**, **XmaJI**
Eco24I* (GAGCC↓C)	**SduI*** (GAGCC↓C)	CviJI, **Eco24I**, **SduI**
Eco24I* (GAGCT↓C)	**Alw21I*** (GAGCT↓C), **SacI** (GAGCT↓C), **SduI*** (GAGCT↓C)	**AluI**, **Alw21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
Eco24I* (GGGCC↓C)	**ApaI** (GGGCC↓C), **BseSI*** (GGGCC↓C), **SduI*** (GGGCC↓C)	**ApaI**, **BseSI**, **Bsp120I**, **BspLI**, **BsuRI**, **Cfr13I**, CviJI, **Eco24I**, **SduI**
Eco24I* (GGGCT↓C)	**SduI*** (GGGCT↓C)	CviJI, **Eco24I**, **SduI**
Eco47I* (G↓GACC)	**CpoI*** (CG↓GACCG), **Psp5II*** (RG↓GACCT)	**Cfr13I**, **Eco47I**
	Psp5II* (RG↓GACCC), SanDI* (GG↓GACCC)	**BspLI**, **Cfr13I**, **Eco47I**
Eco47I* (G↓GTCC)	**CpoI*** (CG↓GTCCG), **Psp5II*** (RG↓GTCCT)	**Cfr13I**, **Eco47I**
	Psp5II* (RG↓GTCCC), SanDI* (GG↓GTCCC)	**BspLI**, **Cfr13I**, **Eco47I**
Eco52I (C↓GGCCG)	**Bsp120I** (G↓GGCCC)	**BsuRI**, **Cfr13I**, CviJI
	CfrI* (Y↓GGCCA)	**BsuRI**, **CfrI**, CviJI
	CfrI* (Y↓GGCCG)	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**
	NotI (GC↓GGCCGC)	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**, **SatI**, **SsiI**, **TauI**
Eco88I* (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)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG)	**BcnI**, **Bme1390I**, **BseDI**, BssKI, **Cfr9I**, **Eco88I**, **HpaII**, **MspI**, **SmaI**
Eco88I* (C↓TCGAG)	PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), **SmoI*** (C↓TCGAG), **XhoI** (C↓TCGAG)	**Eco88I**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**TaqI**
EcoRI (G↓AATTC)	**MunI** (C↓AATTG), **TasI** (↓AATT)	**TasI**
	XapI* (R↓AATTC)	**EcoRI**, **TasI**, **XapI**
	XapI* (R↓AATTT)	**TasI**, **XapI**
EcoRII* (↓CCAGG)	SexAI* (A↓CCAGGT)	**Bme1390I**, BssKI, **EcoRII**, **MvaI**
EcoRII* (↓CCTGG)	SexAI* (A↓CCTGGT)	**Bme1390I**, BssKI, **EcoRII**, **MvaI**
FatI (↓CATG)	AflIII* (A↓CATGT), BtgI* (C↓CATGG), **Eco130I*** (C↓CATGG), **NcoI** (C↓CATGG), **PagI** (T↓CATGA), **PscI** (A↓CATGT)	CviAII, FatI, **Hin1II**
Hin1I* (GA↓CGYC)	**Hin6I** (G↓CGC), **SsiI*** (C↓CGC)	**CseI**
	MaeII (A↓CGT), **Psp1406I** (AA↓CGTT)	MaeII, **TaiI**
	NarI (GG↓CGCC)	**CseI**, **Hin1I**
Hin1I* (GG↓CGYC)	**Hin6I** (G↓CGC), **SsiI*** (C↓CGC)	**HhaI**, **Hin6I**
	NarI (GG↓CGCC)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI
Hin1II (CATG↓)	**PaeI** (GCATG↓C), **XceI*** (RCATG↓C), **XceI*** (RCATG↓T)	CviAII, FatI, **Hin1II**
Hin6I (G↓CGC)	**Hin1I*** (GR↓CGCC), NarI (GG↓CGCC), **SsiI*** (C↓CGC)	**HhaI**, **Hin6I**
HpaII (C↓CGG)	**Hin1I*** (GR↓CGCC), **Hin6I** (G↓CGC), NarI (GG↓CGCC), **SsiI*** (C↓CGC)	**SsiI**
	MspI (C↓CGG), **SsiI*** (G↓CGG)	**HpaII**, **MspI**
KasI (G↓GCGCC)	**BshNI*** (G↓GCGCC)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI
Kpn2I (T↓CCGGA)	BsaWI* (W↓CCGGA)	BsaWI, **HpaII**, Hpy188III, **Kpn2I**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT), SgrAI* (CR↓CCGGTG)	BsaWI, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC), SgrAI* (CR↓CCGGCG)	**HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
MaeII (A↓CGT)	**Hin1I*** (GR↓CGTC), **Psp1406I** (AA↓CGTT)	MaeII, **TaiI**
MboI (↓GATC)	**BamHI** (G↓GATCC), **BclI** (T↓GATCA), **BglII** (A↓GATCT), **Bsp143I** (↓GATC), **PsuI*** (R↓GATCC), **PsuI*** (R↓GATCT)	**Bsp143I**, **DpnI**, **MboI**
MluI (A↓CGCGT)	AflIII* (A↓CGCGT)	AflIII, **Bsh1236I**, **MluI**
	BtgI* (C↓CGCGG)	**Bsh1236I**
	PauI (G↓CGCGC), **SgsI** (GG↓CGCGCC)	**Bsh1236I**, **HhaI**, **Hin6I**
Mph1103I (ATGCA↓T)	**Alw21I*** (GTGCA↓C), **BseSI*** (GTGCA↓C), **PstI** (CTGCA↓G), **SdaI** (CCTGCA↓GG), **SduI*** (GTGCA↓C)	HpyCH4V
MspI (C↓CGG)	**Hin1I*** (GR↓CGCC), **Hin6I** (G↓CGC), NarI (GG↓CGCC), **SsiI*** (C↓CGC)	**SsiI**
	HpaII (C↓CGG), **SsiI*** (G↓CGG)	**HpaII**, **MspI**
MunI (C↓AATTG)	**EcoRI** (G↓AATTC), **TasI** (↓AATT), **XapI*** (R↓AATTC), **XapI*** (R↓AATTT)	**TasI**
NarI (GG↓CGCC)	**Hin1I*** (GR↓CGCC)	BbeI, **BshNI**, **Bsp143II**, **BspLI**, **EheI**, **HhaI**, **Hin1I**, **Hin6I**, KasI, NarI

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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
	Hin1I* (GR↓CGTC)	**Hin1I**
	Hin6I (G↓CGC), **SsiI*** (C↓CGC)	**HhaI**, **Hin6I**
NcoI (C↓CATGG)	AflIII* (A↓CATGT), FatI (↓CATG), **PagI** (T↓CATGA), **PscI** (A↓CATGT)	CviAII, FatI, **Hin1II**
	BtgI* (C↓CATGG), **Eco130I*** (C↓CATGG)	**BseDI**, BtgI, CviAII, **Eco130I**, FatI, **Hin1II**, **NcoI**
NgoMIV (G↓CCGGC)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	**HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT), SgrAI* (CR↓CCGGTG)	**Cfr10I**, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), SgrAI* (CR↓CCGGCG)	Cac8I, **Cfr10I**, **HpaII**, **MspI**, NgoMIV, **PdiI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
NheI (G↓CTAGC)	**BcuI** (A↓CTAGT), **Eco130I*** (C↓CTAGG), **XbaI** (T↓CTAGA), **XmaJI** (C↓CTAGG)	**FspBI**
NotI (GC↓GGCCGC)	**Bsp120I** (G↓GGCCC)	**BsuRI**, **Cfr13I**, CviJI, **SatI**, **TauI**
	CfrI* (Y↓GGCCA)	**BsuRI**, **CfrI**, CviJI, **SatI**, **TauI**
	CfrI* (Y↓GGCCG), **Eco52I** (C↓GGCCG)	**Bsh1285I**, **BsuRI**, **CfrI**, CviJI, **Eco52I**, **SatI**, **TauI**
PacI (TTAAT↓TAA)	**Bsh1285I*** (CGAT↓CG), **PvuI** (CGAT↓CG), SgfI (GCGAT↓CGC)	**Tru1I**
PaeI (GCATG↓C)	**Hin1II** (CATG↓)	CviAII, FatI, **Hin1II**
	XceI* (RCATG↓C)	Cac8I, CviAII, FatI, **Hin1II**, **PaeI**, **XceI**
	XceI* (RCATG↓T)	CviAII, FatI, **Hin1II**, **XceI**
PagI (T↓CATGA)	AflIII* (A↓CATGT), BtgI* (C↓CATGG), **Eco130I*** (C↓CATGG), FatI (↓CATG), **NcoI** (C↓CATGG), **PscI** (A↓CATGT)	CviAII, FatI, **Hin1II**
PasI* (CC↓CAGGG)	TseI* (G↓CAGC)	BseYI
PauI (G↓CGCGC)	AflIII* (A↓CGCGT), BtgI* (C↓CGCGG), **MluI** (A↓CGCGT)	**Bsh1236I**, **HhaI**, **Hin6I**
	SgsI (GG↓CGCGCC)	**Bsh1236I**, Cac8I, **HhaI**, **Hin6I**, **PauI**
Pfl23II (C↓GTACG)	**Acc65I** (G↓GTACC), **BshNI*** (G↓GTACC), **Bsp1407I** (T↓GTACA), **TatI*** (W↓GTACA), **TatI*** (W↓GTACT)	**Csp6I**, **RsaI**
PscI (A↓CATGT)	AflIII* (A↓CATGT)	AflIII, CviAII, FatI, **Hin1II**, **PscI**, **XceI**
	BtgI* (C↓CATGG), **Eco130I*** (C↓CATGG), FatI (↓CATG), **NcoI** (C↓CATGG), **PagI** (T↓CATGA)	CviAII, FatI, **Hin1II**
Psp1406I (AA↓CGTT)	**Hin1I*** (GR↓CGTC), MaeII (A↓CGT)	MaeII, **TaiI**
Psp5II* (AG↓GACCY)	**CpoI*** (CG↓GACCG), **Eco47I*** (G↓GACC)	**Cfr13I**, **Eco47I**
	SanDI* (GG↓GACCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **Psp5II**
Psp5II* (AG↓GTCCY)	**CpoI*** (CG↓GTCCG), **Eco47I*** (G↓GTCC)	**Cfr13I**, **Eco47I**
	SanDI* (GG↓GTCCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **Psp5II**
Psp5II* (GG↓GACCY)	**CpoI*** (CG↓GACCG), **Eco47I*** (G↓GACC)	**BspLI**, **Cfr13I**, **Eco47I**, **FaqI**
	SanDI* (GG↓GACCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **FaqI**, **Psp5II**, SanDI
Psp5II* (GG↓GTCCY)	**CpoI*** (CG↓GTCCG), **Eco47I*** (G↓GTCC)	**BspLI**, **Cfr13I**, **Eco47I**
	SanDI* (GG↓GTCCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **Psp5II**, SanDI
PspXI* (AC↓TCGAGB)	**Eco88I*** (C↓TCGAG), **SmoI*** (C↓TCGAG), **XhoI** (C↓TCGAG)	**Eco88I**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**TaqI**
PspXI* (CC↓TCGAGB)	**Eco88I*** (C↓TCGAG), **SmoI*** (C↓TCGAG), **XhoI** (C↓TCGAG)	**Eco88I**, **MnlI**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**MnlI**, **TaqI**
PspXI* (GC↓TCGAGB)	**Eco88I*** (C↓TCGAG), **SmoI*** (C↓TCGAG), **XhoI** (C↓TCGAG)	**Eco88I**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**TaqI**
PstI (CTGCA↓G)	**AlwI21I*** (GTGCA↓C), **BseSI*** (GTGCA↓C), **Mph1103I** (ATGCA↓T), **SduI*** (GTGCA↓C)	HpyCH4V
	SdaI (CCTGCA↓GG)	**BfmI**, HpyCH4V, **PstI**
PsuI* (A↓GATCY)	**BamHI** (G↓GATCC)	**Bsp143I**, **DpnI**, **MboI**, **PsuI**
	BclI (T↓GATCA), **Bsp143I** (↓GATC), **MboI** (↓GATC)	**Bsp143I**, **DpnI**, **MboI**
	BglII (A↓GATCT)	**BglII**, **Bsp143I**, **DpnI**, **MboI**, **PsuI**
PsuI* (G↓GATCY)	**BamHI** (G↓GATCC)	**BamHI**, **Bsp143I**, **BspLI**, **BspPI**, **DpnI**, **MboI**, **PsuI**
	BclI (T↓GATCA), **Bsp143I** (↓GATC), **MboI** (↓GATC)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**
	BglII (A↓GATCT)	**Bsp143I**, **BspPI**, **DpnI**, **MboI**, **PsuI**
PvuI (CGAT↓CG)	**Bsh1285I*** (CGAT↓CG), SgfI (GCGAT↓CGC)	**Bsh1285I**, **Bsp143I**, **DpnI**, **MboI**, **PvuI**
	PacI (TTAAT↓TAA)	**Tru1I**
SacI (GAGCT↓C)	**AlwI21I*** (GAGCT↓C), **Eco24I*** (GAGCT↓C), **SduI*** (GAGCT↓C)	**AluI**, **AlwI21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
SalI (G↓TCGAC)	**Eco88I*** (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), **SmoI*** (C↓TCGAG), **XhoI** (C↓TCGAG)	**TaqI**
SanDI* (GG↓GACCC)	**CpoI*** (CG↓GACCG), **Eco47I*** (G↓GACC)	**BspLI**, **Cfr13I**, **Eco47I**, **FaqI**
	Psp5II* (RG↓GACCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **FaqI**, **Psp5II**, SanDI
	Psp5II* (RG↓GACCT)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **FaqI**, **Psp5II**
SanDI* (GG↓GTCCC)	**CpoI*** (CG↓GTCCG), **Eco47I*** (G↓GTCC)	**BspLI**, **Cfr13I**, **Eco47I**
	Psp5II* (RG↓GTCCC)	**BspLI**, **Cfr13I**, **Eco47I**, **EcoO109I**, **Psp5II**, SanDI

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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
	Psp5II* (RG↓GTCCT)	**BspLI**, **Cfr13I**, **Eco47I**, **Eco0109I**, **Psp5II**
SdaI (CCTGCA↓GG)	**Alw21I*** (GTGCA↓C), **BseSI*** (GTGCA↓C), **Mph1103I** (ATGCA↓T), **SduI*** (GTGCA↓C)	HpyCH4V
	PstI (CTGCA↓G)	**BfmI**, HpyCH4V, **PstI**
SduI* (GAGCA↓C)	**Alw21I*** (GAGCA↓C)	**Alw21I**, **SduI**
SduI* (GAGCC↓C)	**Eco24I*** (GAGCC↓C)	CviJI, **Eco24I**, **SduI**
SduI* (GAGCT↓C)	**Alw21I*** (GAGCT↓C), **Eco24I*** (GAGCT↓C), **SacI** (GAGCT↓C)	**AluI**, **Alw21I**, CviJI, **Ecl136II**, **Eco24I**, **SacI**, **SduI**
SduI* (GGGCA↓C)	**BseSI*** (GGGCA↓C)	**BseSI**, **SduI**
SduI* (GGGCC↓C)	**ApaI** (GGGCC↓C), **BseSI*** (GGGCC↓C), **Eco24I*** (GGGCC↓C)	**ApaI**, **BseSI**, **Bsp120I**, **BspLI**, **BsuRI**, **Cfr13I**, CviJI, **Eco24I**, **SduI**
SduI* (GGGCT↓C)	**Eco24I*** (GGGCT↓C)	CviJI, **Eco24I**, **SduI**
SduI* (GTGCA↓C)	**Alw21I*** (GTGCA↓C), **BseSI*** (GTGCA↓C)	**Alw21I**, **Alw44I**, **BseSI**, **Hpy8I**, HpyCH4V, **SduI**
	Mph1103I (ATGCA↓T)	HpyCH4V
	PstI (CTGCA↓G), **SdaI** (CCTGCA↓GG)	BsgI, HpyCH4V
SduI* (GTGCC↓C)	**BseSI*** (GTGCC↓C)	**BseSI**, **SduI**
SduI* (GTGCT↓C)	**Alw21I*** (GTGCT↓C)	**Alw21I**, **SduI**
SexAI* (A↓CCAGGT)	**EcoRII*** (↓CCAGG)	**Bme1390I**, BssKI, **EcoRII**, **MvaI**
SexAI* (A↓CCTGGT)	**EcoRII*** (↓CCTGG)	**Bme1390I**, BssKI, **EcoRII**, **MvaI**
SgfI (GCGAT↓CGC)	**Bsh1285I*** (CGAT↓CG), **PvuI** (CGAT↓CG)	**Bsh1285I**, **Bsp143I**, **DpnI**, **MboI**, **PvuI**
	PacI (TTAAT↓TAA)	**Tru1I**
SgrAI* (CA↓CCGGYG)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	BsaWI, **HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT)	BsaWI, **BshTI**, **Cfr10I**, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC)	**Cfr10I**, **HpaII**, **MspI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
SgrAI* (CG↓CCGGYG)	BsaWI* (W↓CCGGA), **Kpn2I** (T↓CCGGA)	**HpaII**, **MspI**
	BsaWI* (W↓CCGGT), **BshTI** (A↓CCGGT), **Cfr10I*** (R↓CCGGT)	**Cfr10I**, **HpaII**, **MspI**
	Cfr10I* (R↓CCGGC), NgoMIV (G↓CCGGC)	Cac8I, **Cfr10I**, **HpaII**, **MspI**, NgoMIV, **PdiI**
	Cfr9I (C↓CCGGG), **Eco88I*** (C↓CCGGG)	**BcnI**, **Bme1390I**, BssKI, **HpaII**, **MspI**
SgsI (GG↓CGCGCC)	AflIII* (A↓CGCGT), BtgI* (C↓CGCGG), **MluI** (A↓CGCGT)	**Bsh1236I**, **HhaI**, **Hin6I**
	PauI (G↓CGCGC)	**Bsh1236I**, Cac8I, **HhaI**, **Hin6I**, **PauI**
SmoI* (C↓TCGAG)	**Eco88I*** (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), **XhoI** (C↓TCGAG)	**Eco88I**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**TaqI**
SmoI* (C↓TTAAG)	**BspTI** (C↓TTAAG)	**BspTI**, **SmoI**, **Tru1I**
SsiI* (C↓CGC)	**Hin1I*** (GR↓CGCC), **Hin6I** (G↓CGC), NarI (GG↓CGCC)	**SsiI**
	HpaII (C↓CGG), **MspI** (C↓CGG)	**HpaII**, **MspI**
SsiI* (G↓CGG)	**Hin1I*** (GR↓CGCC), **Hin6I** (G↓CGC), NarI (GG↓CGCC)	**HhaI**, **Hin6I**
TaiI (ACGT↓)	**AatII** (GACGT↓C)	MaeII, **TaiI**
TaqI (T↓CGA)	**Bsp119I** (TT↓CGAA), **Bsu15I** (AT↓CGAT), **XmiI*** (GT↓CGAC)	**TaqI**
TasI (↓AATT)	**EcoRI** (G↓AATTC), **MunI** (C↓AATTG), **XapI*** (R↓AATTC), **XapI*** (R↓AATTT)	**TasI**
TatI* (A↓GTACW)	**Acc65I** (G↓GTACC), **BshNI*** (G↓GTACC), **Pfl23II** (C↓GTACG)	**Csp6I**, **RsaI**
	Bsp1407I (T↓GTACA)	**Csp6I**, **RsaI**, **TatI**
TatI* (T↓GTACW)	**Acc65I** (G↓GTACC), **BshNI*** (G↓GTACC), **Pfl23II** (C↓GTACG)	**Csp6I**, **RsaI**
	Bsp1407I (T↓GTACA)	**Bsp1407I**, **Csp6I**, **RsaI**, **TatI**
Tru1I (T↓TAA)	**VspI** (AT↓TAAT)	**Tru1I**
VspI (AT↓TAAT)	**Tru1I** (T↓TAA)	**Tru1I**
XapI* (A↓AATTY)	**EcoRI** (G↓AATTC)	**TasI**, **XapI**
	MunI (C↓AATTG), **TasI** (↓AATT)	**TasI**
XapI* (G↓AATTY)	**EcoRI** (G↓AATTC)	**EcoRI**, **TasI**, **XapI**
	MunI (C↓AATTG), **TasI** (↓AATT)	**TasI**
XbaI (T↓CTAGA)	**BcuI** (A↓CTAGT), **Eco130I*** (C↓CTAGG), **NheI** (G↓CTAGC), **XmaJI** (C↓CTAGG)	**FspBI**
XceI* (ACATG↓Y)	**Hin1II** (CATG)	CviAII, FatI, **Hin1II**
	PaeI (GCATG↓C)	CviAII, FatI, **Hin1II**, **XceI**
XceI* (GCATG↓Y)	**Hin1II** (CATG)	CviAII, FatI, **Hin1II**
	PaeI (GCATG↓C)	Cac8I, CviAII, FatI, **Hin1II**, **PaeI**, **XceI**
XhoI (C↓TCGAG)	**Eco88I*** (C↓TCGAG), PspXI* (VC↓TCGAGC), PspXI* (VC↓TCGAGG), PspXI* (VC↓TCGAGT), **SmoI*** (C↓TCGAG)	**Eco88I**, **SmoI**, **TaqI**, **XhoI**
	SalI (G↓TCGAC)	**TaqI**
XmaJI (C↓CTAGG)	**BcuI** (A↓CTAGT), **NheI** (G↓CTAGC), **XbaI** (T↓CTAGA)	**FspBI**
	Eco130I* (C↓CTAGG)	**BseDI**, **Eco130I**, **FspBI**, **XmaJI**
XmiI* (GT↓CGAC)	**Bsp119I** (TT↓CGAA), **Bsu15I** (AT↓CGAT), **TaqI** (T↓CGA)	**TaqI**