About us.

SibEnzyme Ltd. (Siberian Enzyme, SE),
is a privately owned Russian company founded in 1991.
SibEnzyme Ltd. is located in Academtown near Novosibirsk city – the capital of Siberia and a geographic center of Russia.

SibEnzyme's primary focus is production of enzymes and related products for molecular biology, PCR and genetic engineering. SE product line includes more than 200 enzymes, a set of perfect DNA ladders,
high quality enzymatic dNTPs and DNA preparations.

SibEnzyme Ltd. is one of the leading companies in research, development and production of restriction endonucleases and new DNA endonucleases.
A new type of methyl-directed DNA endonucleases
has been discovered and characterized in our laboratories.
These new enzymes are a good instrument to detect DNA methylation status in epigenetics studies and medical applications.

Information about SibEnzyme products may be found at
www.sibenzyme.com

Scientific publications with detailed study of new enzymes are available at
www.science.sibenzyme.com

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NEW New product MGQ Mammalian Genome Qualified NP New package

Restriction Endonucleases

Restriction endonucleases are bacterial site-specific DNA-endonucleases that recognize and cleave with high specificity short (usually 4-8 base pairs) sequences in double stranded DNA. Type II restriction endonucleases cut both strands of substrate DNA in the presence of Mg^{2+} ions near or within the recognition sequence.

SibEnzyme Ltd. is one of the leading companies in research, development and production of restriction endonucleases. At present SibEnzyme Ltd. has **more then 200 commercially available restriction endonucleases** in the product list including many unique enzymes. 56 of these enzymes have been cloned and are purified from recombinant sources.

To simplify the reaction of DNA hydrolysis with restriction endonucleases and to set up **double digestion** reaction, a special reaction universal **Buffer "ROSE"** was designed. Over 150 enzymes reveal high activity in this buffer. The information about activity of restriction endonucleases in "ROSE" buffer is available in section "Info/SE-buffers activity".

Fast digestion of different types of DNA (mainly plasmid DNA) may be performed with **Turbo qualified Restriction Enzymes** (marked with symbol **T** , ex:E003T/E004T). Currently **25** Turbo SE restriction enzymes are commercially available. They may be used for a short time (10-15 min) DNA digestion as well as in a standard reaction. The reaction with most of these enzymes may be performed using optimal or universal ("**ROSE"**) Buffer (both are supplied with Turbo enzymes).

Furthermore, to allow the performance of a limited number of reactions (up to 50) with minimum expenses, SE 20 most commonly used restriction endonucleases are now supplied in lesser quantities (marked with a symbol "m", ex: E003m). The symbol indicates that these enzymes are available in **minimal** packages at optimal prices.

Restriction Endonucleases: Quality Controls

Unit Determination

One unit of restriction endonuclease is defined as the amount of enzyme required to digest 1 µg of substrate DNA in a total reaction volume of 50 µl in 1 hour using the optimal SE-Buffer provided.

Quality Controls

The results of all quality control assays are reported on the Certificate of analysis provided with each enzyme.

Ligation of DNA fragments

DNA fragments are produced by an excessive over-digestion of substrate DNA with each restriction endonuclease. These fragments are then ligated with T4 DNA Ligase at a 5' termini concentration of 0.1-1.0 µM. The ligated fragments are then recut with the same restriction endonuclease. Ligation can only occur if the 3' and 5' termini are left intact, and only those molecules with a perfectly restored recognition site can be recleaved. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact and the enzyme preparation is free of detectable exonucleases and phosphatases. An example of FblI ligation is presented in the figure.

16-hour assay for nuclease contamination

All SibEnzyme restriction endonucleases are incubated for 16 hours in optimal buffer with 1 µg of substrate DNA in a volume of 50 µl. The characteristic DNA fragments pattern produced by the enzyme in 1 hour is compared to the pattern produced from an excess of enzyme incubated for 16 hours. A sharp, unaltered pattern under these conditions is an indication that the enzyme preparation is free of detectable levels of nonspecific DNAses. The maximum number of units which can be incubated for 16 hours is indicated.

Assay for exonuclease and phosphatase contamination

All restriction endonucleases are incubated for 3 hours with 5'-[^{32}P]-labeled synthetic oligonucleotides (single-stranded and double-stranded) in a volume of 20 µl. After incubation of these labeled oligonucleotides with an enzyme, denatured reaction products are separated on a polyacrylamide gel and then analysed by phospho-imaging. No detectable degradation of single-stranded and double-stranded oligonucleotides indicates that the enzyme preparation is free of exonuclease and phosphatase contamination.

Turbo qualified Restriction Endonucleases

Turbo qualified Restriction Endonucleases may be used for 10-15 minutes DNA digestion as well as in a standard reaction. The reaction with the most of these enzymes may be performed using optimal or universal ("**ROSE**") Buffer (both are supplied with Turbo enzymes).

No	Turbo enzyme	Prototype	Sequence	Cat.No	Digestion time (plasmid, min.)			Recommended buffer
					5	10	15	
1	Acc65I	Acc65I, KpnI	G↑GTACC	E003T/ E004T		+	+	ROSE
2	AhlI	SpeI	A↑CTAGT	E173T/ E174T		+	+	ROSE
3	AluI	AluI	AG↑CT	E015T/ E016T		+	+	ROSE
4	ApaI	ApaI	GGGCC↑C	E019T/ E020T		+	+	ROSE
5	AsiGI	AgeI	A↑CCGGT	E235T/ E236T		+	+	ROSE
6	BamHI	BamHI	G↑GATCC	E021T/ E022T	+	+	+	ROSE
7	BglII	BglII	A↑GATCT	E027T/ E028T		+	+	ROSE
8	Bsa29I	ClaI	AT↑CGAT	E205T/ E206T	+	+	+	ROSE
9	Bsp19I	NcoI	C↑CATGG	E047T/ E048T	+	+	+	ROSE
10	CciNI	NotI	GC↑GGCCGC	E203T/ E204T			+	ROSE
11	EcoRI	EcoRI	G↑AATTC	E057T/ E058T	+	+	+	ROSE
12	EcoRV	EcoRV	GAT↑ATC	E059T/ E060T	+	+	+	ROSE
13	FauNDI	NdeI	CA↑TATG	E009T/ E010T	+	+	+	ROSE
14	HindIII	HindIII	A↑AGCTT	E073T/ E074T	+	+	+	ROSE
15	HinfI	HinfI	G↑ANTC	E075T/ E076T	+	+	+	ROSE
16	HpaI	HpaI	GTT↑AAC	E077T/ E078T		+	+	ROSE
17	MluI	MluI	A↑CGCGT	E085T/ E086T		+	+	ROSE
18	PceI	StuI	AGG↑CCT	E105T/ E106T		+	+	ROSE
19	Psp124BI	SacI	GAGCT↑C	E107T/ E108T	+	+	+	ROSE
20	PstI	PstI	CTGCA↑G	E109T/ E110T	+	+	+	ROSE
21	SalI	SalI	G↑TCGAC	E115T/ E116T		+	+	O
22	Sfr274I	XhoI	C↑TCGAG	E125T/ E126T		+	+	ROSE
23	SmaI	SmaI	CCC↑GGG	E177T/ E178T		+	+	ROSE
24	SphI	SphI	GCATG↑C	E129T/ E130T		+	+	ROSE
25	XbaI	XbaI	T↑CTAGA	E141T/ E142T	+	+	+	ROSE

Minimal packages at optimal price.

No	Cat. No	Enzyme	Qty u/pack.	Price EUR/ 1 pack
1	E021m	BamHI	500	2
2	E057m	EcoR I	500	2
3	E059m	EcoR V	500	2
4	E067m	Hae III	500	2
5	E073m	Hind III	500	2
6	E201m	Hind II	250	2
7	E075m	Hinf I	500	2
8	E161m	Hpa II	250	2
9	E109m	Pst I	500	2
10	E125m	Sfr274 I	500	2
No	**Cat. No**	**Enzyme**	**Qty u/pack.**	**Price EUR/ 1 pack**
11	E003m	Acc65 I	500	5
12	E019m	Apa I	500	5
13	E027m	Bgl II	500	5
14	E009m	FauND I	500	5
15	E077m	Hpa I	250	5
16	E069m	HspA I	500	5
17	E107m	Psp124B I	500	5
18	E177m	Sma I	500	5
19	E139m	Vsp I	500	5
20	E141m	Xba I	500	5

NEW New product MGQ Mammalian Genome Qualified NP New package

Aat II (prototype Aat II)

Isolated from an *E.coli* strain that carries the cloned Aat II gene from *Acetobacter aceti*

GACGT^C **E287** **500 u.a.**
C^TGCAG **E288** **2500 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA;
200 µg/ml BSA; 1 mM DTT; and
50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Aat II, about 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Aat II for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	10-25	25-50	100

Abs I (prototype Abs I)

Isolated from *Arthrobacter species* 7M06

CC^TCGAGG **E535** **50 u.a.**
GGAGCT^CC **E536** **250 u.a.**

Concentration: 500 - 1 000 units/ml
Assayed on pUC19SE/DriI digest
Reagents Supplied with Enzyme:
10×SE Buffer AbsI
Reaction conditions: 1× SE Buffer AbsI
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA;0.05% Triton X-100 and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Abs I, ~90% of the DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of DNA with 2 units of Abs I for 16 hours.
Star activity: A long incubation time may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	10-25	0	50-75	0-10

Acc16 I (prototype Mst I)

Isolated from *Acinetobacter calcoaceticus* 16

TGC^GCA **E001** **200 u.a.**
ACG^CGT **E002** **1000 u.a.**

Concentration: 5 000-10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1× SE Buffer W
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Acc16 I, ~90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Acc16 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	25-50	100	75-100

Acc36 I (prototype BspM I)

Isolated from *Acinetobacter calcoaceticus* 36

ACCTGC(N)$_4$^ **E289** **100 u.a.**
TGGACG(N)$_8$^ **E290** **500 u.a.**

Concentration: 2000-5000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 100 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Acc36I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 3 units of Acc36 I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	50-75	50-75	100

Acc65 I (prototype Kpn I)

Isolated from *Acinetobacter calcoaceticus* 65

G^GTACC **E003** **1000 u.a.**
CCATG^G **E004** **5000 u.a.**

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA (dcm$^-$)
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1× SE Buffer W
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **GGTACCWGG**
Acc65I is a neoschizomer of KpnI.

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Acc65 I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Acc65 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	75-100	100	10-25

AccB1 I (prototype HgiC I)

Isolated from *Acinetobacter calcoaceticus* B1

G^GYRCC E163 500 u.a.
CCRYG^G E164 2500 u.a.

Concentration: 5 000 -10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer K, BSA
Reaction conditions:1× SE Buffer K +BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with AccB1 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of AccB1 I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	10-25	10-25	75-100	50-75

AccB7 I (prototype PflM I)

Isolated from *Acinetobacter calcoaceticus* B7

CCANNNN^NTGG E179 200 u.a.
GGTN^NNNNACC E180 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA (dcm–)
Reagents supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **CCANNNCCTGG or CCAGGNNNTGG**

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with AccB7 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of AccB7 I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	100	25-50	50-75	50-75

AccBS I (prototype BsrB I)

Isolated from *Acinetobacter calcoaceticus* BS

GAG^CGG E007 1000 u.a.
CTC^GCC E008 5000 u.a.

Concentration: 5000 – 20 000 units/ml
Assayed on: λ DNA
Reagents supplied with Enzyme:
10× SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with AccBS I, 90% of the DNA fragments can be ligated. Of these, 50% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of AccBS I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	25-50	100

AclI (prototype AclI)

Isolated from *Acinetobacter calcoaceticus*

AA^CGTT E011 200 u.a.
TTGC^AA E012 1000 u.a.

Concentration: 3 000 – 5 000 units/ml
Assayed on: λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction condition:
1xSE Buffer Y + BSA
Incubate at 37°C.
Blocked by CpG methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 0,05% Triton X-100; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with AclI, 90% of the DNA fragments can be ligated and recut with this enzyme.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of AclI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	0-10	0-10	0-10	100

AclW I (prototype Bin I)

Isolated from *Acinetobacter calcoaceticus* W2131

GGATC(N)$_4$^ E211 100 u.a.
CCTAG(N)$_5$^ E212 500 u.a.

Concentration: 1000 - 3000 units/ml
Assayed on λ DNA (dam–)
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^{m}ATC): **GG^{m}ATC**
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;200 μg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with AclW I, about 50% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 6 units of AclW I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	0-10	0-10	100

NEW New product MGQ Mammalian Genome Qualified NP New package

Aco I (prototype Cfr I)

Isolated from *Acinetobacter calcoaceticus*

Y^GGCCR **E499** **100 u.a.**
RCCGG^Y **E500** **500 u.a.**

Concentration: 500 – 2 000 units/ml
Assayed on: λ DNA(dam⁻,dcm⁻)
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction condition: 1xSE Buffer G
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation ($C^{m}CWGG$): CC**TGG**CC**R.**

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 100 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Aco I, >90% of λ DNA fragments can be ligated with T4 DNA Ligase at 16°C and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of Aco I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	50-75	25-50	75-100

Acs I (prototype Apo I)

Isolated from *Arthrobacter citreus*

R^AATTY **E013** **500 u.a.**
YTTAA^R **E014** **2500 u.a.**

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction condition: 1xSE Buffer W+BSA
Incubate at 50°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:20 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
10 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Acs I, >95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 50 units of Acs I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	50-75	100	10-25

Acu I (prototype Eco57 I)

Isolated from an *E.coli* strain that carries the cloned Acu I gene from *Acinetobacter calcoaceticus S*RW4

CTGAAG(N)$_{16}$^ **E451** **50 u.a.**
GACTTC(N)$_{14}$^ **E452** **250 u.a.**

Concentration: 1 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE BufferY, BSA, SAM
Reaction conditions:
1×SE Buffer Y+BSA+SAM
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml, and SAM should be added to a final concentration 0.01 mM.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol. Store at -20° C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Acu I, about 80% of the DNA fragments can be ligated. Of these, 80% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of Acu I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	50-75	75-100	75-100

Afe I (prototype Eco47 III)

Isolated from an *E.coli* strain that carries the cloned Afe I gene from *Alcaligenes faecalis* T2774

AGC^GCT **E213** **200 u.a.**
TCG^CGA **E214** **1000 u.a.**
For high concentration
E214X **1000 u.a.**

Concentration: 10 000 and 50 000 units/ml
Assayed on λ DNA (BamHI-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; and 50% glycerol.
Store at -20°C
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Afe I, > 80% of pBR322 DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of the λ DNA with 40 units of Afe I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	75-100	75-100	100

Ags I (prototype Ags I)

Isolated from *Agrococcus species* 25

TTS^AA **E573** **200 u.a.**
AA^STT **E574** **1000 u.a.**

Concentration: 5000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y + BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with AgsI, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Ags I for 16 hours.
Do not use BSA for long incubation

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	10-25	10-25	100

Ahl I (prototype Spe I)

Isolated from *Alteromonas haloplanktis* SP

A^CTAGT E173 1000 u.a.
TGATC^A E174 5000 u.a.

Concentration: 10 000 - 30 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme: 10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65°C,80°C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Ahl I, >90% of T7 DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of T7 DNA with 40 units of Ahl I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	25-50	25-50	75-100

Ajn I (prototype EcoR II)

Isolated from *Acinetobacter johnsonii* R2

^CCWGG E473 200 u.a.
GGWCC^ E474 1000 u.a.

Concentration: 500 – 3 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y Incubate at 55°C.
Note: At 37°C activity is about 10% from maximum.
Not blocked by overlapping *dcm*-methylation (C^{m}CWGG): **CCWGG**

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with AjnI, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 6 units of Ajn I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	10-25	10-25	25-50	100

Alu I (prototype Alu I)

Isolated from an *E.coli* strain that carries the cloned AluI gene from *Arthrobacter luteus*

MGQ

AG^CT E015 200 u.a.
TC^GA E016 1000 u.a.

Concentration: 2 000 – 5000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Alu I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 4 units of Alu I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	10-25	50-75	100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

AluB I (prototype Alu I)

Isolated from *Arthrobacter luteus* B

AG^CT E549 200 u.a.
TC^GA E550 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B + BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.
Note:. AluB I is able to cleave some methylated DNA substrates.
AluB I is an isoschizomer of Alu I
See: http://science.sibenzyme.com/article8_article_30_1.phtml

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with AluB I, 80% of λ DNA fragments can be ligated with T4 DNA Ligase and can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of AluB I for 16 hours.
Do not use BSA for long incubation

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	10-25	10-25	75-100

Ama87 I (prototype Ava I)

Isolated from *Alteromonas macleodii* 87

C^YCGRG E017 1000 u.a.
GRGCY^C E018 5000 u.a.

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer W, BSA
Reaction conditions: 1xSE Buffer W+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM KH_2PO_4 (pH 7.2); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Ama87 I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Ama87 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	50-75	75-100	100	0-10

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Aox I (prototype Aox I)

Isolated from *Arthrobacter oxydans* 25K

NEW

^PuG(5mC)Py	E569	50 u.a.
Py(5mC)GPu^	E570	250 u.a.

See page 52 for more information about this enzyme.

Apa I (prototype Apa I)

Isolated from *Acetobacter pasteurianus*

GGGCC^C	E019	1000 u.a.
C^CCGGG	E020	5000 u.a.

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA (dcm⁻, BamHI-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions:1× SE Buffer Y+BSA
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **GGGCCCWGG**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Apa I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Apa I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	25-50	0-10	0-10	100

Ars I (prototype Ars I)

Isolated from *Arthrobacter* species NTS

^(N)$_8$GAC(N)$_6$TTYG(N)$_{11}$^	E575	50 u.a.
^(N)$_{13}$CTG(N)$_6$AARC(N)$_6$^	E576	250 u.a.

Concentration: 500 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y + BSA
Incubate at 30°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM KH_2PO_4 (pH 7.4); 200 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Ars I, about 70% of the DNA fragments can be ligated. Of these, 80% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of DNA with 1 units of Ars I for 16 hours.
Do not use BSA for long incubation

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0	0	0	0	100

AsiG I (prototype Age I)

Isolated from *Arthrobacter* species G

A^CCGGT	E235	100 u.a.
TGGCC^A	E236	500 u.a.

Concentration: 3 000 –5000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction condition: 1× SE Buffer O
Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA;
100 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with AsiG I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of AsiG I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	10-25

AspA2 I (prototype Avr II)

Isolated from *Arthrobacter* species A2

MGQ

C^CTAGG	E245	500 u.a.
GGATC^C	E246	2500 u.a.

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA (Hind III–digest)
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions:1× SE Buffer W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
100 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with AspA2 I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of the DNA with 20 units of AspA2 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	50-75	75-100	100	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

AspLE I (prototype Hha I)

Isolated from *Arthrobacter* species LE3860

GCG^C C^GCG | E221 E222 | 500 u.a. 2500 u.a.

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1× SE Buffer O Incubate at 37°C.
Blocked by CG methylation
5'-G(5mC)GC-3'/3-CG(5mC)G-5' or
5'-G(5mC)GC-3'/3'-CGCG-5'
Not blocked by methylation
5'-GCG(5mC)-3'/3'-(5mC)GCG-5' or
5'-GCG(5mC)-3'/3'-CGCG-5'.
Cut hemimethylated site:
5'- G(5mC)GC-3' / 3'-CGCG-5'

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 100 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with AspLE I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of AspLE I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	75-100	100	50-75	25-50

AspS9 I (prototype Sau96 I)

MGQ

Isolated from *Arthrobacter* species S9

G^GNCC CCNG^G | E117 E118 | 1000 u.a. 5000 u.a.

Concentration: 10 000 -30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer W
Reaction condition: 1×SE Buffer W Incubate at 37°C**.**
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **GGNCCWGG**

Storage buffer:10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 200 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with AspS9 I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 30 units of AspS9 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	75-100	100	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

AsuC2 I (prototype Cau II)

Isolated from *Actinobacillus suis* CA

CC^SGG GGS^CC | E257 E258 | 2000 u.a. 10000 u.a.

Concentration: 20 000 - 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with AsuC2 I, ~20% of the DNA fragments can be ligated. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 50 units of AsuC2 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-50	50-75	10-25	25-50	100

AsuHP I (prototype Hph I)

MGQ

Isolated from *Actinobacillus suis* HP

GGTGA(N)$_8$^ CCACT(N)$_7$^ | E231 E232 | 200 u.a. 1000 u.a.

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA (dam-)
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1× SE Buffer O Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^{m}ATC): **GGTGATC**.
Enzyme may cleave at N$_9$/N$_8$ depending on the sequence between the recognition and cleave sites.

Storage buffer:10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with AsuHP I, about 30% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of AsuHP I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	50-75	100	75-100	25-50

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

AsuNH I (prototype Nhe I)

Isolated from *Actinobacillus suis* NH

G^CTAGC CGATC^G | E063 E064 | 1000 u.a. 5000 u.a.

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme: 10×SE Buffer Y, BSA
Reaction conditions: 1× SE Buffer Y+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 100 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with AsuNH I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of the DNA with 20 units of AsuNHI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	0-10	0-10	100

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BamH I (prototype BamH I)

Isolated from an *E.coli* strain that carries the cloned BamHI gene from *Bacillus amyloliquefaciens* H

G^GATCC **E021** **4000 u.a.**
CCTAG^G **E022** **20000 u.a.**

For high concentration
E021X **4000 u.a.**
E022X **20000 u.a.**

Concentration: 20 000 and 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer G, BSA
Reaction conditions: 1× SE Buffer G+BSA Incubate at 37°C.
Not blocked by overlapping *dam*-methylation (G^{m}ATC): **GGATCC**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0,1 mM EDTA;
100 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 50-fold overdigestion with BamH I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of BamH I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	100	75-100	75-100	25-50

Bar I (prototype Bar I)

Isolated from *Bacillus sphaericus*

^(N)$_7$GAAG(N)$_6$TAC(N)$_{12}$^ **E547** **100 u.a.**
^(N)$_{12}$CTTC(N)$_6$ATG(N)$_7$^ **E548** **500 u.a.**

Concentration: 500 - 2 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme: 10×SE Buffer 2K
Reaction conditions: 1× SE Buffer 2K Incubate at 37°C.

Storage buffer:20 mM KH_2PO_4 (pH 7.4); 100 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;200 μg/ml BSA, 50% glycerol.Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bar I, about 90% of T7 DNA fragments can be ligated and 95% of these can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of T7 DNA with 2 units of Bar I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0	0-10	25-50	50-75	10-25

Bbv12 I (prototype HgiA I)

Isolated from *Bacillus brevis* 12

GWGCW^C **E023** **200 u.a.**
C^WCGWG **E024** **1000 u.a.**

Concentration: 1 000 –5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1× SE Buffer O Incubate at 37°C.

Storage buffer:10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80°C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Bbv12 I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of Bbv12 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	100	75-100	10-25

Bgl I (prototype Bgl I)

Isolated from *Bacillus globigii*

GCCNNNN^NGGC **E025** **500 u.a.**
CGGN^NNNNCCG **E026** **2500 u.a.**

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10 × SE Buffer 2W
Reaction conditions: 1 × SE Buffer 2W Incubate at 37°C.
Not blocked by *dcm*-methylation (C^{m}CWGG): G**CCWGG**NNGGC.

Storage buffer:10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bgl I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of Bgl I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	0-10	75-100	25-50

Bgl II (prototype Bgl II)

Isolated from an *E.coli* strain that carries the cloned Bgl II gene from *Bacillus globigii*

A^GATCT **E027** **1000 u.a.**
TCTAG^A **E028** **5000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1× SE Buffer O Incubate at 37°C.
Not blocked by overlapping *dam*-methylation (G^{m}ATC): **AGATCT**.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM NaCl; 0,1 mM EDTA;1 mM DTT; 200 μ g/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65°C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bgl II, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Bgl II for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	100	25-50	10-25

Bis I (prototype Bis I)

Isolated from *Bacillus subtilis* T30

G(5mC)^NGC	E485	40 u.a.
CGN^(5mC)G	E486	200 u.a.

See page 52 for more information about this enzyme.

Bls I (prototype Bls I)

Isolated from *Bacillus simplex* 23

DNA sequence with at least two 5mC:		
PuPyN^PuPy	E533	100 u.a.
PyPu^NPyPu	E534	500 u.a.

See page 53 for more information about this enzyme.

Bme18 I (prototype Ava II)

Isolated from *Bacillus megaterium* 18

G^GWCC	E029	1000 u.a.
CCWG^G	E030	5000 u.a.

Concentration: 5 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 37°C.
Cleaved of DNA is impaired by overlapping dcm-methylation ($C^{m}CWGG$):
GGWCCWGG.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bme18 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Bme18 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	10-25

Bmt I (prototype Nhe I)

Isolated from an *E.coli* strain that carries the cloned Bmt I gene from *Bacillus megaterium* S2

GCTAG^C	E457	1000 u.a.
C^GATCG	E458	5000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 37°C.
BmtI is a neoschizomer of NheI.

Storage buffer:10mM Tris-HCl(pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
200 µg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Bmt I about 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Bmt I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	50-75	50-75	100	75-100

Bmu I (prototype Bfi I)

Isolated from *Bacillus megaterium* S2

$ACTGGG(N)_5$^	E487	50 u.a.
$TGACCC(N)_4$^	E488	250 u.a.

Concentration: 500 - 1000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.
Enzyme is active in presence of EDTA.

Storage buffer:10mM Tris-HCl(pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bmu I about 75% of the DNA fragments can be ligated and 95% of these can be recut.
Overnight digest with BmuI is not recommended. A 50 µl reaction containing 1 µg of λ DNA and 0.5 units of enzyme incubated for 4 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	10-25	100

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Bpm I (prototype Gsu I)

Isolated from *Bacillus pumilus*

CTGGAG(N)$_{16}$^ E467 50 u.a.
GACCTC(N)$_{14}$^ E468 250 u.a.

Concentration: 200 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions: 1×SE Buffer W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0,1 mM EDTA;
10 mM 2-mercaptoethanol;
200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bpm I about 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 0,4 units of Bpm I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	100	50-75

Bpu10 I (prototype Bpu10 I)

Isolated from an *Escherichia coli* strainthat carries plasmids pBpu10IA and pBpu10IB

MGQ

CC^TNAGC E149 200 u.a.
GGANT^CG E150 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer K
Reaction conditions: 1×SE Buffer K
Incubate at 37°C.

Storage buffer:10mM Tris-HCl(pH 7.5);
50 mM NaCl; 0.1 mM EDTA; 1 mM DTT;
50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Bpu10 I, 80% of the DNA fragments can be ligated. Of these, 90% can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of DNA with 5 units of enzyme for 16 hour.
Note: High enzyme concentration may result in star activity or incomplete DNA cleavage. We recommend increasing the incubation time instead of using an excess of Bpu10 I.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	50-75	50-75	25-50

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Bpu14 I (prototype Asu II)

Isolated from *Bacillus pumilus* 14

TT^CGAA E033 1000 u.a.
AAGC^TT E034 5000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 37°C.

Storage buffer:10mM Tris-HCl(pH 7.5);
100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bpu14 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Bpu14 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	25-50	25-50	75-100

Bsa29 I (prototype Cla I)

Isolated from *Bacillus stearothermophilus* 29

AT^CGAT E205 1000 u.a.
TAGC^TA E206 5000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA (dam$^-$)
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^{m}ATC): **GATCGATC**.
Blocked by CG methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5);
250 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Bsa29 I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Bsa29 I for 16 hour.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	100	50-75	50-75	75-100

Bsc4 I (prototype BsiY I)

Isolated from *Bacillus schlegelii* 4

CCNNNNN^NNGG E219 500 u.a.
GGNN^NNNNNCC E220 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions: 1×SE Buffer W+BSA
Incubate at 55°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Bsc4 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 30 units of Bsc4 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	50-75	100	25-50

Bse1 I (prototype Bsr I)

Isolated from *Bacillus stearothermophilus* 1

ACTGGN^ E035 1000 u.a.
TGAC^CN E036 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 65°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
100 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bse1 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Bse1 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	10-25	100

Bse118 I (prototype Cfr10 I)

Isolated from *Bacillus stearothermophilus* 118

R^CCGGY E039 200 u.a.
YGGCC^R E040 1000 u.a.

Concentration: 2 000-5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 65°C.

Storage buffer: 10 mM KH_2PO_4 (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
100 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bse118 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of Bse118 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	50-75	100	75-100	25-50

Bse21 I (prototype Sau I)

Isolated from *Bacillus* species 21

MGQ

CC^TNAGG E037 500 u.a.
GGANT^CC E038 2500 u.a.

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.

Storage buffer: 10 mM KH_2PO_4 (pH 7.4); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bse21 I, about 50% of the DNA fragments can be ligated (by using of high concentration T4 DNA Ligase and 10% PEG). Of these, >90% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 30 units of Bse21 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	10-25	25-50	100

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Bse3D I (prototype BsrD I)

Isolated from *Bacillus stearothermophilus* 3D

MGQ

GCAATGNN^ E253 200 u.a.
CGTTAC^NN E254 1000 u.a.

Concentration: 5 000 - 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 60°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 200 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bse3D I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Bse3D I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	100	25-50	50-75	75-100

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Bse8 I (prototype BsaB I)

Isolated from *Bacillus* species 8

GATNN^NNATC E147 1000 u.a.
CTANN^NNTAG E148 5000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 60°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl;. 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
100 µg/ml BSA; and 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Bse8 I, 80% of the DNA fragments can be ligated and recut.
Overdigestion assay: Long incubation is not recommended owing to occurrence of star activity.
Star activity is observed at a greater than 5-fold overdigestion of 1 µg substrate with Bse8 I.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	100	75-100	75-100	50-75

BseP I (prototype BseP I)

Isolated from *Bacillus stearothermophilus* P

G^CGCGC E181 200 u.a.
CGCGC^G E182 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 50°C.
Blocked by CG methylation.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0,1 mM EDTA;
10 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BseP I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BseP I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	75-100	50-75	50-75

BseX3 I (prototype Xma III)

Isolated from *Bacillus stearothermophilus* X3

C^GGCCG E263 200 u.a.
GCCGG^C E264 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 50°C.
Blocked by CG methylation.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BseX3 I,
> 90% of the DNA fragments can be ligated. Of these, 80% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BseX3 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	50-75	10-25

BslF I (prototype Fin I)

Isolated from *Bacillus stearothermophilus* FI

GGGAC(N)$_{10}$^ E479 100 u.a.
CCCTG(N)$_{14}$^ E480 500 u.a.

Concentration: 1 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 37°C.
BslF I also **cleaves the sequence** GGGAC(11/15).
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with BslFI I, 90% of the DNA fragments can be ligated Of these, 95% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of BslF I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	10-25	25-50	100

Bso31 I (prototype Eco31 I)

Isolated from *Bacillus stearothermophilus* 31

GGTCTC(N)$_1$^ E285 200 u.a.
CCAGAG(N)$_5$^ E286 1000 u.a.

Concentration: 5 000 – 10 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions: 1×SE Buffer O+BSA
Incubate at 55°C.
Not blocked by methylation GGTCT^{m}C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
100 µg/ml BSA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Bso31 I, 90% of the DNA fragments can be ligated. Of these, 80% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 5 units of Bso31 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	75-100	100	75-100	25-50

Bsp13 I (prototype BspM II)

Isolated from *Bacillus* species 13

T^CCGGA
AGGCC^T

E183 1000 u.a.
E184 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA (dam⁻)
Reagents Supplied with Enzyme:
10×SE Buffer 2K
Reaction conditions: 1× SE Buffer 2K
Incubate at 50°C.
Blocked by overlapping *dam*-methylation ($G^{m}ATC$):
TCCGGATC and **GATCCGGA.**

Storage buffer:10mM Tris-HCl(pH 7.5); 200 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA; 50% glycerol.
Store at -20°C
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bsp13 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Bsp13 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	50-75	0-10

Bsp1720 I (prototype Esp I)

Isolated from *Bacillus* species 1720

GC^TNAGC
CGANT^CG

E185 500 u.a.
E186 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction mixture: 1× SE Buffer G
Incubate at 37°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
50% glycerol.Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bsp1720I, about 80% of the DNA fragments can be ligated. Of these, 95 % can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of Bsp1720 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	50-75	50-75	75-100

Bsp19 I (prototype Nco I)

Isolated from *Bacillus* species 19

C^CATGG
GGTAC^C

E047 1000 u.a.
E048 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer 2W, BSA
Reaction conditions:
1×SE Buffer 2W+BSA
Incubate at 37°C.
Bsp19I **cuts hemimethylated site 5`-(5mC)CATGG-3`/3`-GGTACC-5`** and **doesn't cut methylated sites 5`-(5mC)CATGG-3`/3`-GGTAC(5mC)-5`** and **5`-(4mC)CATGG-3`/3`-GGTAC(4mC)-5`**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bsp19 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Bsp19 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	50-75	75-100	10-25

BspAC I (prototype Aci I)

MGQ

Isolated from *Bacillus* species AC

C^CGC
GGC^G

E501 200 u.a.
E502 1000 u.a.

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions:1×SE Buffer O+BSA
Incubate at 37°C.
Blocked by CG methylation.
Note: BspACI has a non-palindromic recognition site.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM KH_2PO_4 (pH 7.2); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BspAC I, > 95% of λ DNA fragments can be ligated with T4 DNA Ligase at 16°C and 50% of these can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of BspAC I at 37°C for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	10-25

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BspFN I (prototype FnuD II)

Isolated from *Bacillus* species FN

CG^CG
GC^GC

E557 500 u.a.
E558 2500 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions:1×SE Buffer Y
Incubate at 37°C.
Blocked by CG methylation.

Storage buffer: 20 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA;
50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BspFN I,
> 95% of λ DNA fragments can be ligated with T4 DNA Ligase and recut
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of BspFN I at 37°C for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	75-100	50-75	100

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BssEC I (prototype Sec I)

Isolated from *Bacillus stearothermophilus* EC

MGQ

C^CNNGG E273 200 u.a.
GGNNC^C E274 1000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 60°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0,1 mM EDTA;
200 µg/ml BSA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BssECI 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BssEC I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	50-75	75-100	100

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BssNA I (prototype Sna I)

Isolated from *Bacillus stearothermophilus* NA

GTA^TAC E261 1000 u.a.
CAT^ATG E262 5000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions:
1× SE Buffer W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
100 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C, 80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BssNA I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BssNA I for 16 hours
Star activity: High enzyme concentration results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	75-100	100	75-100

BssT1 I (prototype Sty I)

Isolated from *Bacillus stearothermophilus* T1

C^CWWGG E207 1000 u.a.
GGWWC^C E208 5000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer 2K
Reaction conditions: 1× SE Buffer 2K
Incubate at 60°C.

Storage buffer:10mM Tris-HCl(pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with BssT1 I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of BssT1 I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	25-50	75-100	10-25

Bst2B I (prototype Bsi I)

Isolated from *Bacillus stearothermophilus* 2B

C^TCGTG E043 200 u.a.
GAGCA^C E044 1000 u.a.

Concentration: 5 000 - 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 60°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bst2B I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Bst2B I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	25-50	10-25	25-50	100

Bst2U I (prototype BstN I)

Isolated from *Bacillus stearothermophilus* 2U

MGQ

CC^WGG E051 1000 u.a.
GGW^CC E052 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1× SE Buffer G+BSA
Incubate at 60°C.
Not blocked by overlapping *dcm*-methylation ($C^{m}CWGG$): **CCWGG**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer:10mM Tris-HCl(pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (80° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bst2U I, none of the DNA fragments can be ligated.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Bst2U I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	50-75	50-75	10-25

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NEW New product MGQ Mammalian Genome Qualified NP New package

Bst4C I (prototype Tsp4C I)

Isolated from *Bacillus stearothermophilus* 4C

ACN^GT E265 500 u.a.
TG^NCA E266 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 65°C.

Storage buffer 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Bst4C I 50% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Bst4C I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	10-25	25-50	100

Bst6 I (prototype Ksp632 I)

Isolated from *Bacillus stearothermophilus* 6

MGQ

CTCTTC(N)$_1$^ E239 200 u.a.
GAGAAG(N)$_4$^ E240 1000 u.a.

Concentration: 1 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions:
1× SE Buffer Y+BSA
Incubate at 65°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C (*see note).
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Bst6 I, 80% of the DNA fragments can be ligated and 80% of those can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of Bst6I for 16 hours.
Do not use BSA for long incubation.
***Note: For long term storage (more than 30 days), store at -70ºC .**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	50-75	75-100	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

BstAC I (prototype Acy I)

Isolated from *Bacillus stearothermophilus* AC

GR^CGYC E093 500 u.a.
CYGC^RG E094 2500 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 40-fold overdigestion with BstAC I, 95% of λ DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of BstACI for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	50-75	100	75-100

BstAF I (prototype Afl II)

Isolated from *Bacillus stearothermophilus* AF

C^TTAAG E135 1000 u.a.
GAATT^C E136 5000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W , BSA
Reaction conditions:
1×SE Buffer W + BSA
Incubate at 55°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstAF I, about 40% of the DNA fragments can be ligated and 95% of those can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of BstAF I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	75-100	100	25-50

NEW New product MGQ Mammalian Genome Qualified NP New package

BstAP I (prototype ApaB I)

Isolated from *Bacillus stearothermophilus* AP

GCANNNN^NTGC **E259** **200 u.a.**
CGTN^NNNNACG **E260** **1000 u.a.**

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 60°C.
BstAPI is a neoschizomer of ApaBI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; and 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstAP I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of BstAPI for 16 hours.
Star activity: High enzyme concentration (>5 units for 16 hours) on 1µg of DNA may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	75-100	100	25-50

BstAU I (prototype Bsp1407 I)

Isolated from *Bacillus stearothermophilus* AU

T^GTACA **E267** **1000 u.a.**
ACATG^T **E268** **5000 u.a.**

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
100 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstAU I about 90% of λ DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstAUI for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	50-75	25-50	100	25-50

BstBA I (prototype BsaA I)

Isolated from *Bacillus stearothermophilus* BA

YAC^GTR **E237** **500 u.a.**
RTG^CAY **E238** **2500 u.a.**

Concentration: 5 000 - 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction Conditions:
1×SE Buffer W+BSA
Incubate at 65°C.
Blocked by CG methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
10 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstBA I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstBA I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	75-100	100	25-50

BstC8 I (prototype Cac8 I)

Isolated from *Bacillus stearothermophilus* BA

GCN^NGC **E305** **500 u.a.**
CGN^NCG **E306** **2500 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.
Incubation at 37^0C results in 50% activity.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
10 mM 2-mercaptoethanol;
200 µg/ml BSA; and 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstC8 I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstC8 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	50-75	75-100	100

BstDE I (prototype Dde I)

Isolated from *Bacillus stearothermophilus* DE

MGQ

C^TNAG E227 500 u.a.
GANT^C E228 2500 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 60°C.
Incubation at 37^{0}C results in 10% activity.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 30-fold overdigestion with BstDE I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 60 units of BstDE I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	50-75	10-25

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

BstDS I (prototype Dsa I)

Isolated from *Bacillus stearothermophilus* DS

C^CRYGG E083 1000 u.a.
GGYRC^C E084 5000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 65°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA; and 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstDS I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstDS I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	75-100	50-75	25-50	100

BstEN I (prototype EcoN I)

Isolated from *Bacillus stearothermophilus* EN

CCTNN^NNNAGG E103 200 e.a.
GGANNN^NNTCC E104 1000 e.a.

Concentration: 2 000 –5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 65°C.
Not blocked by overlapping *dcm*-methylation (C^{m}CWGG):
CCWGGNNNAGG or
CCTNNNCCAGG.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with BstEN I, about 60% of the DNA fragments can be ligated. Of these, 90 % can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of BstEN I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	25-50	25-50	100

BstF5 I (prototype Fok I)

Isolated from *Bacillus stearothermophilus* F5

GGATGNN^ E031 500 u.a.
CCTAC^NN E032 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 65°C.
BstF5I is a neoschizomer of FokI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstF5 I,
> 90% of of the λ DNA fragments can be ligated. Of these, 95 % can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstF5 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	25-50	50-75	100

BstFN I (prototype FnuD II)

Isolated from *Bacillus stearothermophilus* FN

MGQ

CG^CG E283 300 u.a.
GC^GC E284 1500 u.a.

Concentration: 2 000 - 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 60°C.
Blocked by CG methylation.

Storage buffer: 20 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0,1 mM EDTA;
10 mM $MgCl_2$; 7 mM 2-mercaptoethanol;
200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstFN I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BstFN I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	25-50	25-50	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

BstH2 I (prototype Hae II)

Isolated from *Bacillus stearothermophilus* H2

RGCGC^Y **E171** **500 u.a.**
Y^CGCGR **E172** **2500 u.a.**

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions:
1×SE Buffer Y+BSA
Incubate at 65°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM KH_2PO_4 (pH 7.2); 100 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with BstH2 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of BstH2 I for 16 hours.
Star activity: High enzyme concentration results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	0-10	10-25	100

BstHH I (prototype Hha I)

Isolated from *Bacillus stearothermophilus* HH

GCG^C **E143** **2000 u.a.**
C^GCG **E144** **10000 u.a.**

Concentration: 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 50°C.
Blocked by CG methylation
5'-G(5mC)GC-3'/3-CG(5mC)G-5' or
5'-G(5mC)GC-3'/3'-CGCG-5'
Not blocked by methylation
5'-GCG(5mC)-3'/3'-(5mC)GCG-5' or
5'-GCG(5mC)-3'/3'-CGCG-5'.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65°C, 80° C for 20 minutes)

Ligation/recutting assay: After 40-fold overdigestion with BstHH I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 100 units of BstHH I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	25-50	50-75	100

BstKT I (prototype Mbo I)

Isolated from *Bacillus stearothermophilus* KT

GAT^C **E151** **200 u.a.**
C^TAG **E152** **1000 u.a.**

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA (dam^-)
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1 × SE Buffer W
Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^mATC): **GATC.**
Not blocked by CG methylation.
Cut hemimethylated site:
5'- G^mATC-3' / 5'-GATC-3'
BstKTI is a neoschizomer of MboI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstKT I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of BstKT I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	100	50-75

BstMA I (prototype BsmA I)

Isolated from *Bacillus stearothermophilus* MA

MGQ **GTCTC$(N)_1$^** **E291** **2000 u.a.**
CAGAG$(N)_5$^ **E292** **10 000 u.a.**

Concentration: 30 000 – 100 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions:
1×SE Buffer W+BSA
Incubate at 55°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 100 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 50-fold overdigestion with BstMA I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 100 units of BstMA I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	50-75	100	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

BstMB I (prototype Mbo I)

Isolated from *Bacillus stearothermophilus* MB

^GATC E119 200 u.a.
CTAG^ E120 1000 u.a.

Concentration: 5 000 - 10 000 units/ml
Assayed on λ DNA (dam⁻)
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 65°C.
Blocked by overlapping *dam*-methylation (G^mATC): **GATC.**
Not blocked by CG methylation.
Not cut hemimethylated site:
5'- G^mATC-3' / 3'-CTAG-5'

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstMB I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstMB I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	10-25

BstMC I (prototype Mcr I)

Isolated from *Bacillus stearothermophilus* MC

CGRY^CG E071 500 u.a.
GC^YRGC E072 2500 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 50°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstMC I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BstMC I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	10-25	10-25	50-75

BstMW I (prototype Mwo I)

Isolated from *Bacillus stearothermophilus* MW

GCNNNNN^NNGC E459 500 u.a.
CGNN^NNNNNCG E460 2500 u.a.

Concentration: 2 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.
Incubation at 37° results in 20% activity.

Storage buffer: 10 mM Tris-HCl(pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, and 50% glycerol.
Store at -20°C (*see note).
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstMW I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BstMW I for 16 hours.
***Note: For long term storage (more than 7 days), store at -70ºC .**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	25-50	50-75	100

BstNS I (prototype Nsp I)

Isolated from *Bacillus stearothermophilus* NS

RCATG^Y E251 200 u.a.
Y^GTACR E252 1000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol;; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation :
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstNS I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of BstNS I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	10-25	75-100

BstPA I (prototype PshA I)

Isolated from *Bacillus stearothermophilus* PA

GACNN^NNGTC E299 1000 u.a.
CTGNN^NNCAG E300 5000 u.a.

Concentration: 10 000 –20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 65°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation :
No (65° C, 80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstPA I, < 5% of the DNA fragments can be ligated.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BstPA I for 16 hours at 25°C.
Star activity: Incubation at **65°C** for 16 hours results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	25-50	50-75	50-75	100

NEW New product MGQ Mammalian Genome Qualified NP New package

BstSC I (prototype ScrF I)

Isolated from *Bacillus stearothermophilus* SC

MGQ

^CCNGG E307 100 u.a.
GGNCC^ E308 500 u.a.

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA (dcm-)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.
Incubation at 37^{0}C results in 10% activity.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **CCWGG.**
BstSCI is a neoschizomer of ScrFI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstSC I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 3 units of BstSC I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	50-75	50-75	100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

BstSF I (prototype Sfe I)

Isolated from *Bacillus stearothermophilus* SF

C^TRYAG E197 200 u.a.
GAYRT^C E198 1000 u.a.

Concentration: 2 000 - 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions: 1×SE Buffer O+BSA
Incubate at 60°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No(65°C, 80°C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with BstSF I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of BstSF I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	25-50	100	50-75	50-75

BstSL I (prototype BseS I)

Isolated from *Bacillus stearothermophilus* S

GKGCM^C E561 500 u.a.
C^MCGKG E562 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G + BSA
Incubate at 55°C.
Not blocked by overlapping *dcm*-methylation (C^{m}CWGG):
GKGCCCWGG
Blocked by **GKG^{m}CMC** methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstSL I, ~80% of the DNA fragments can be ligated. Of these, 95% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of BstSL I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	50-75	75-100	75-100

BstSN I (prototype SnaB I)

Isolated from *Bacillus stearothermophilus* SN

TAC^GTA E065 200 u.a.
ATG^CAT E066 1000 u.a.

Concentration: 5 000 – 10 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 100 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstSN I, ~70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 5 units of BstSN I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	0-10	10-25	50-75

BstV1 I (prototype Bbv I)

Isolated from *Bacillus stearothermophilus* V1

GCAGC(N)$_8$^ E303 100 u.a.
CGTCG(N)$_{12}$^ E304 500 u.a.

Concentration: 1 000 – 2 000 units/ml
Assayed on pBR322 DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 55°C.
Incubation at 37° results in 10% activity.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with BstV1 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 ug of the DNA with 2 units of enzyme for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	75-100	75-100	75-100

BstV2 I (prototype Bbv II)

Isolated from *Bacillus stearothermophilus* V2

MGQ

GAAGAC(N)$_2$^ E297 200 u.a.
CTTCTG(N)$_6$^ E298 1000 u.a.

Concentration: 5 000 – 15 000 units/ml
Assayed on λ DNA
Reagents Supplied with Buffer:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 55°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65°C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BstV2 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of BstV2 I for 16 hours.
Star activity: High enzyme concentration results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	25-50	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

BstX I (prototype BstX I)

Isolated from *Bacillus stearothermophilus* X

CCANNNNN^NTGG E465 200 u.a.
GGTN^NNNNNACC E466 1000 u.a.

Concentration: 5 000 -15 000 units/ml
Assayed on λ DNA
Reagents Supplied with Buffer:
10×SE Buffer O
Reaction conditions: 1×SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstX I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of BstX I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	100	75-100	25-50

BstX2 I (prototype Xho II)

Isolated from an *E.coli* strain that carries the cloned BstX2I gene from *Bacillus stearothermophilus* X2

R^GATCY E229 500 u.a.
YCTAG^R E230 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 60°C.
Not blocked by overlapping *dam*-methylation (G^{m}ATC): R**GATCY.**

Storage buffer: 10 mM Tris-HCl (pH 7.6); 100 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with BstX2 I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of BstX2 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	0-10	10-25	25-50

Bsu I (prototype BciVI)

Isolated from *Bacillus sphaericus*

GTATCC(N)$_6$ E581 200 u.a.
CATAGG(N)$_5$ E582 1000 u.a.

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at **37**°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 0,05% Triton X-100, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with BsuI 10% of the DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 6 units of BsuI for 16 hours at 37°C.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	10-25	25-50	100

BsuR I (prototype Hae III)

Isolated from *Bacillus subtilis* R

GG^CC E053 1000 u.a.
CC^GG E054 5000 u.a..

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with BsuR I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of BsuR I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	50-75	50-75

NEW New product MGQ Mammalian Genome Qualified NP New package

Btr I (prototype Btr I)

Isolated from *Bacillus stearothermophilus* SE-U62

CAC^GTC **E277** **100 u.a.**
GTG^CAG **E278** **500 u.a.**

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions: 1×SE Buffer O+BSA
Incubate at 60°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Btr I, 80% of the DNA fragments can be ligated. Of these, 90 % can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of Btr I for 16 hours.
Star activity: High enzyme concentration results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	100	75-100	75-100

Cci I (prototype BspH I)

Isolated from *Curtobacterium citreum*

T^CATGA **E565** **1000 u.a.**
AGTAC^T **E566** **5000 u.a.**

Concentration: 20000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions:
1×SE Buffer W + BSA
Incubate at 55°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Cci I, > 90% of the DNA fragments can be ligated and recut with.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Cci I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	25-50	100	75-100

CciN I (prototype Not I)

Isolated from *Curtobacterium citreum* N

GC^GGCCGC **E203** **200 u.a.**
CGCCGG^CG **E204** **1000 u.a.**

Concentration: 2000 –5000 units/ml
Assayed on Ad2 DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.
Blocked by CG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with CciN I, > 95% of the DNA fragments can be ligated and recut with.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of Ad2 DNA with 10 units of CciN I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	75-100	100

Dra I (prototype Aha III)

Isolated from *Deinococcus radiophilus*

TTT^AAA **E055** **1000 u.a.**
AAA^TTT **E056** **5000 u.a..**

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 100 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with DraI ~70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 30 units of Dra I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	75-100	75-100

Dra III (prototype Dra III)

Isolated from an *E.coli* strain that carries the cloned DraIII gene from *Deinococcus radiophilus*

CACNNN^GTG **E309** **500 u.a.**
GTG^NNNCAC **E310** **2500 u.a.**

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer 2K, BSA
Reaction conditions: 1×SE Buffer 2K+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Dra III, about 70% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of DraIII for 16 hours.
Star activity: High concentration of enzyme may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	75-100	50-75

Dri I (prototype Eam1105 I)

Isolated from *Deinococcus radiophilus* EA

GACNNN^NNGTC **E193** **200 u.a.**
CTGNN^NNNCAG **E194** **1000 u.a.**

Concentration: 5 000 -10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with DriI, about 5% of the DNA fragments can be ligated. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Dri I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	10-25	10-25	100

DseD I (prototype Drd I)

Isolated from *Deinococcus* species D2

GACNNNN^NNGTC **E241** **500 u.a.**
CTGNN^NNNNCAG **E242** **2500 u.a.**

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65°C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with DseD I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of DseD I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	50-75	100

EcoICR I (prototype Sac I)

Isolated from *Escherichia coli* ICR

GAG^CTC **E469** **200 u.a.**
CTC^GAG **E470** **1000 u.a.**

Concentration: 2 000 - 10 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme: 10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.
EcoICRI is a neoschizomer of SacI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with EcoICR I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of EcoICR I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	0-10	0-10	75-100

EcoR I (prototype EcoR I)

Isolated from an *E.coli* strain that carries the cloned EcoR I gene from *Escherichia coli*

MGQ

G^AATTC **E057** **5000 u.a.**
CTTAA^G **E058** **25000 u.a.**

For high concentration
E057X **5000 u.a.**
E058X **25000 u.a.**

Concentration: 20 000 and 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
5×SE Buffer EcoR I, BSA
Reaction conditions:
1×SE Buffer EcoRI+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 50-fold overdigestion with EcoR I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of EcoR I for 16 hours.
Star activity: High concentration of enzyme results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	75-100	75-100	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

EcoR V (prototype EcoR V)

Isolated from an *E.coli* strain that carries the cloned EcoR V gene from *Escherichia coli*

GAT^ATC **E059** **2000 u.a.**
CTA^TAG **E060** **10000 u.a.**

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions: 1×SE Buffer W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with EcoR V, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of EcoR V for 16 hours.
Star activity: High enzyme concentration results in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	25-50	50-75	100	25-50

Ege I (prototype Nar I)

Isolated from *Enterobacter gergoviae*

GGC^GCC **E243** **200 u.a.**
CCG^CGG **E244** **1000 u.a.**

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.
EgeI is a neoschizomer of NarI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Ege I, 70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of the DNA with 10 units of Ege I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	10-25	50-75	75-100

Erh I (prototype Sty I)

Isolated from *Erwinia rhapontici*

C^CWWGG **E061** **1000 u.a.**
GGWWC^C **E062** **5000 u.a.**

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer 2W, BSA
Reaction conditions:
1 ×SE Buffer 2W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Erh I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 50 units of ErhI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	25-50	75-100	10-25

Fae I (prototype Nla III)

Isolated from *Flavobacterium aquatile* N3

CATG^ E495 50 u.a.
^GTAC E496 250 u.a.

Concentration: 500 - 2 000 units/ml
Assayed on pUC19 DNA
Reagents Supplied with Enzyme:
10×SE Buffer FaeI, BSA
Reaction conditions:
1 ×SE Buffer FaeI+BSA
Incubate at 37°C.
Blocked by $C^{m}ATG$ methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Fae I,>90% of the DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of pUC19 DNA with 1 units of Fae I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	10-25	10-25	75-100

Fai I (prototype Fai I)

Isolated from *Flavobacterium aquatile* B15

YA^TR E551 50 u.a.
RT^AY E552 250 u.a.

FaiI cleaves 4 expected recognition sites as well as several other sites with a weaker activity
Concentration: 2 000 units/ml
One unit is defined as the amount of enzyme required to cleave 1 pmol of the double-stranded oligonucleotide with the following structure
5'- CGAGTTCA^TAGCTGGGCCCAAC -3'
3'- GCTCAAGT^ATCGACCCGGGTTG -5'
in 1 hour at 50°C in a total reaction volume of 20 μl.
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions:1 ×SE Buffer B
Incubate at **50**°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Fai I, about 90% of the pUC19 DNA fragments can be ligated with DNA Ligase and recut.
Note! ***In the case of long incubation with Fai I DNA can be digested to small oligos.***

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	25-10	25-50

Fal I (prototype Fal I)

Isolated from *Flavobacterium aquatile* Ob10

$^(N)_8AAGN_5CTT(N)_{13}^$ E153 100 u.a.
$^(N)_{13}TTCN_5GAA(N)_8^$ E154 500 u.a.

Concentration: 1 000 - 3 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, SAM
Reaction conditions:
1 ×SE Buffer W+SAM
Incubate at 37°C.
To obtain 100% activity, SAM should be added to a final concentration 0.01 mM.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Fal I, 20% of the DNA fragments can be ligated. Of these, 80 % can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of Fal I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	25-50	75-100	100	50-75

Fat I (prototype Nla III)

Isolated from an *E.coli strain* that carries the cloned Fat I gene from *Flavobacterium aquatile* NL3

MGQ

^CATG E155 100 u.a.
GTAC^ E156 500 u.a.

Concentration: 2 000 - 5 000 units/ml
Assayed on DNA pUC19
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 55°C.
Blocked by $^{m}CATG$ methylation.
FatI is a neoschizomer of NlaIII.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Fat I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of the DNA with 3 units of Fat I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	100	25-50	10-25	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Fau I (prototype Fau I)

Isolated from an *E.coli strain* that carries the cloned Fau I gene from *Flavobacterium aquatili*

CCCGC(N)$_4$^ **E209** **100 u.a.**
GGGCG(N)$_6$^ **E210** **500 u.a.**

Concentration: 2 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 55°C.
Blocked by CG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA and 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Fau I, > 90% of the DNA fragments can be ligated. Of these, 95 % can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 4 units of Fau I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	25-50	0-10	0-10	50-75

FauND I (prototype Nde I)

Isolated from an *E.coli strain* that carries the cloned FauND I gene from *Flavobacterium aquatili* ND

MGQ

CA^TATG **E009** **1000 u.a.**
GTAT^AC **E010** **5000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1× SE Buffer Y+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 1mM DTT; 200 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with FauND I, 80% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of FauND I for 16 hours.
Note: *Sensitive to impurities present in some DNA preparations. For example, DNA purified by standard miniprep procedures is cleaved at lower rate.*
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	10-25	50-75	100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

Fbl I (prototype Acc I)

Isolated from an *E.coli* strain that carries the cloned Fbl I gene from *Flavobacterium balustinum*

GT^MKAC **E271** **100 u.a.**
CAKM^TG **E272** **500 u.a.**

Concentration: 1 000 – 2 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Fbl I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of Fbl I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	0-10	50-75	100

Fok I (prototype Fok I)

Isolated from an *E.coli* strain that carries the cloned Fok I gene from *Flavobacterium okeanokoites*

GGATG(N)$_9$^ **E247** **100 u.a.**
CCTAC(N)$_{13}$^ **E248** **500 u.a.**

Concentration: 1 000 – 2 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: SE Buffer Y
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT ; 200 μg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Fok I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 1 units of Fok I for 16 hours.
Note:*Overdigestions of > 5 units of Fok I per 1μg of DNA and incubation times > 2 hours are not recommended.*

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	25-50	25-50	100

FriO I (prototype Ban II)

Isolated from *Flavobacterium rigense* O

GRGCY^C **E157** **1000 u.a.**
C^YCGRG **E158** **5000 u.a.**

Concentration: 10 000 - 40 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions:1×SE Buffer Y+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with FriO I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of FriOI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	10-25	0-10	100

Fsp4H I (prototype Fnu4H I)

Isolated from an *E.coli strain* that carries the cloned Fsp4H I gene from *Flavobacterium* species 4H

MGQ

GC^NGC **E095** **200 u.a.**
CGN^CG **E096** **1000 u.a.**

Concentration: 3 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Fsp4H I, about 5% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of Fsp4H I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	10-25	25-50	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Gla I (prototype GlaI)

Isolated from *Glacial ice bacterium* GL 29

Pu(5mC)^GPy **E493** **100 u.a.**
PyG^(5mC)Pu **E494** **500 u.a.**

See page 54 for more information about this enzyme.

Glu I (prototype GluI)

Isolated from *Glacial ice bacterium* GL 24

G(5mC)^NG(5mC) **E519** **100 u.a.**
(5mC)GN^(5mC)G **E520** **500 u.a.**

See page 55 for more information about this enzyme.

Gsa I (prototype BseY I)

Isolated from *Geobacillus stearothermophilus* Y

CCCAG^C **E563** **1000 u.a.**
G^GGTCG **E564** **5000 u.a.**

Concentration: 10 000-20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions: 1×SE Buffer W+BSA
Incubate at 70°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol;200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Gsa I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Gsa I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	75-100	100	75-100

Hae III (prototype Hae III)

Isolated from an *E.coli* strain that carries the cloned Hae III gene from *Haemophilus aegyptius*

MGQ

GG^CC **E067** **2000 u.a.**
CC^GG **E068** **10000 u.a.**

For high concentration
E067X **2000 u.a.**
E068X **10000 u.a.**

Concentration: 10 000 and 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Hae III, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Hae III for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	50-75	50-75

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_27_1.phtml

Hga I (prototype Hga I)

Isolated from *E.coli* strain that carries the cloned HgaI gene from *Haemophilus gallinarum*

GACGC(N)$_5$^ **E461** **50 u.a.**
CTGCG(N)$_{10}$^ **E462** **250 u.a.**

Concentration: 1 000 units/ml
Assayed on pBR322 DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1× SE Buffer B
Incubate at 37°C.
Blocked by CG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Hga I, > 90% of the DNA fragments can be ligated and recut.
Note: Incubation with >2 units of HgaI per 1 µg of DNA and digestion > 1 hour is not recommended.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	10-25	25-50	50-75

Hind II (prototype Hind II)

Isolated from an *E.coli* strain that carries the cloned Hind II gene from *Haemophilus influenzae* Rd

GTY^RAC **E201** **1000 u.a.**
CAR^YTG **E202** **5000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Hind II, > 60% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Hind II for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	25-50	75-100

Hind III (prototype Hind III)

MGQ

Isolated from an *E.coli* strain that carries the cloned Hind III gene from *Haemophilus influenzae* Rd

A^AGCTT **E073** **5000 u.a.**
TTCGA^A **E074** **25000 u.a.**

For high concentration
E073X **5000 u.a.**
E074X **25000 u.a.**

Concentration: 20 000 and 100 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions:
1 ×SE Buffer W+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 50-fold overdigestion with Hind III, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of Hind III for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	0-10	100	0-10

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Hinf I (prototype Hinf I)

MGQ

Isolated from an *E.coli* strain that carries the cloned Hinf I gene from *Haemophilus influenzae*

G^ANTC **E075** **2000 u.a.**
CTNA^G **E076** **10000 u.a.**

For high concentration
E076X **10000 u.a.**

Concentration: 20 000 and 40 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation :
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Hinf I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of Hinf I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	75-100	100	75-100	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

Hpa I (prototype Hpa I)

Isolated from an *E.coli* strain that carries the cloned Hpa I gene from *Haemophilus parainfluenzae*

GTT^AAC **E077** **500 u.a.**
CAA^TTG **E078** **2500 u.a.**

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Hpa I, 60% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of Hpa I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	50-75	10-25	25-50	100

Hpa II (prototype Hpa II)

Isolated from an *E.coli* strain that carries the cloned Hpa II gene from *Haemophilus parainfluenzae*

MGQ

C^CGG **E161** **500 u.a.**
GGC^C **E162** **2500 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.
Blocked by CG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Hpa II, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Hpa II for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	25-50	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

HpySE526 I(prototype Mae II)

Isolated from an *E.coli* strain that carries the cloned HpySE526 I gene from *Helicobacter pylori* SE526.

NEW

A^CGT **E583** **200 u.a.**
TGC^A **E584** **1000 u.a.**

Concentration: 5 000 units/ml
Assayed on pUC19 DNA
Reagents Supplied with Enzyme:
10×SE BufferY
ReactionConditions: 1× SE Buffer Y
Incubate at 37°C.
Blocked by CG methylation

Storage buffer: 10 mM Tris-HCl (pH 7.6); 100 mM NaCl; 0.1 mM EDTA; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with HpySE526 I about 95% of the DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of HpySE526 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	10-25	25-50	100

HspA I (prototype Hha I)

Isolated from an *E.coli* strain that carries the cloned HspA I gene from *Haemophilus* species A1

MGQ

G^CGC **E069** **1000 u.a.**
CGC^G **E070** **5000 u.a.**

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE BufferY
ReactionConditions: 1× SE Buffer Y
Incubate at 37°C.
Blocked by CG methylation
5'-G(5mC)GC-3'/3'-CG(5mC)G-5'.
Not blocked by methylation
5'-GCG(5mC)-3'/3'-CGCG-5' or
5'-GCG(5mC)-3'/3'-(5mC)GCG-5'.
HspAI is a neoschizomer of HhaI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with HspA I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of HspA I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	25-50	25-50	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Kpn I (prototype Kpn I)

Isolated from an *E.coli* strain that carries the cloned Kpn I gene from *Klebsiella pneumonia*

MGQ

GGTAC^C E079 2000 u.a.
C^CATGG E080 10000 u.a.

For high concentration
E079X 2000 u.a.
E080X 10000 u.a.

Concentration: 20 000 and 40 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
Not blocked by overlapping *dcm*-methylation (C^{m}CWGG): **GGTACCWGG**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT ; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Kpn I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Kpn I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	25-50	25-50	25-50	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

Kro I (prototype Kro I)

Isolated from *Kocurea rosea* 307

G^C(5mC)GGC E541 50 u.a.
CGG(5mC)C^G E542 250 u.a.

See page 56 for more information about this enzyme.

Ksp22 I (prototype Bcl I)

Isolated from *Kurthia* species 22

MGQ

T^GATCA E081 1000 u.a.
ACTAG^T E082 5000 u.a.

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA (dam-)
Reagents Supplied with Enzyme:
10×SE Buffer 2K, BSA
Reaction conditions: 1×SE Buffer 2K+BSA
Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^{m}ATC): **TGATCA**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Ksp22 I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Ksp22 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	50-75	50-75	25-50

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Kzo9 I (prototype Mbo I)

Isolated from *Kurthia zopfii* 9

MGQ

^GATC E187 200 u.a.
CTAG^ E188 1000 u.a.

Concentration: 1 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1×SE Buffer G
Incubate at 37°C.
Not blocked by overlapping d*am*-methylation (G^{m}ATC): **GATC**.
Blocked by CG methylation.
Cleaved of DNA is impaired by overlapping CG methylation: **GAT^{m}CG**.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Kzo9 I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of Kzo9I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	50-75	50-75	50-75

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_27_1.phtml

Lmn I (prototype Lmn I)

Isolated from *Lysinibacillus manganicus* An22

NEW

GCTCCN^ E593 50 e.a.
CGAG^GN E594 250 e.a.

Concentration: 500 – 1 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.

Storage buffer: 20 mM Tris-HCl (pH 7.5); 250 mM NaCl; 100 μg/ml BSA; 7 mM 2-mercaptoethanol; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Lmn I 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of Lmn I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	50-75	50-75	75-100

Mab I (prototype SexA I)

Isolated from *Microbacterium arborescens* SE

A^CCWGGT E121 200 u.a.
TGGWCC^A E122 1000 u.a.

Concentration: 1 000 – 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W, BSA
Reaction conditions: 1×SE Buffer W+BSA
Incubate at 37°C.
Not blocked by overlapping *dcm*-methylation
(C^{m}C(A/T)GG): **A<u>CCWGG</u>T.**
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Mab I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Mab I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	75-100	100	50-75

Mal I (prototype Dpn I)

Isolated from *Marinococus albus* I

G(mA)^TC E489 50 u.a.
CT^(mA)G E490 250 u.a.

See page 56 for more information about this enzyme.

Mbo II (prototype Mbo II)

Isolated from an *E.coli* strain that carries the cloned Mbo II gene from *Moraxella bovis*

GAAGA(N)$_8$^ E471 200 u.a.
CTTCT(N)$_7$^ E472 1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA (dam$^-$)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.
Blocked by overlappin *dam*-methylation
(G^{m}ATC): **GAA<u>GA</u>TC**.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA;50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Mbo II, 60% of the DNA fragments can be ligated and recut. In presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 ug of DNA with 5 units of enzyme for 16 hours at 37°C.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	50-75	100

Mfe I (prototype Mfe I)

Isolated from an *E.coli* strain that carries the cloned MfeI gene from *Mycoplasma fermentans*

C^AATTG E295 1000 u.a.
GTTAA^C E296 5000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Mfe I, about 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Mfe I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	10-25	25-50	75-100

Mhl I (prototype Sdu I)

Isolated from *Micrococcus halobius* SD

GDGCH^C E049 500 u.a.
C^HCGDG E050 2500 u.a.

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1 × SE Buffer W
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Mhl I, >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Mhl I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	75-100	100	10-25

Mlu I (prototype Mlu I)

Isolated from *Micrococcus luteus*

A^CGCGT E085 1000 u.a.
TGCGC^A E086 5000 u.a.

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Mlu I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Mlu I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	100	25-50	10-25

Mly113 I (prototype Nar I)

Isolated from *Micrococcus lylae* 113

GG^CGCC **E189** **200 u.a.**
CCGC^GG **E190** **1000 u.a.**

Concentration: 3 000 – 5 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1× SE Buffer B
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Mly113 I, > 80% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 10 units of Mly113 I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	25-50	10-25	10-25	50-75

MnlI (prototype Mnl I)

Isolated from an *E.coli* strain that carries the cloned MnlI gene from *Moraxella nonliquefaciens*

CCTC(N)$_7$^ **E481** **500 u.a.**
GGAG(N)$_6$^ **E482** **2500 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 37°C.
Blocked by overlapping CG methylation:
CCT^{m}CG.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA, 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with MnlI, about 50% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of MnlI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	25-50	75-100

Mox20 I (prototype Bal I)

NEW MGQ

Isolated from *Microbacterium oxydans*

TGG^CCA **E301** **1000 u.a.**
ACC^GGT **E302** **5000 u.a.**

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA (dcm-)
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1×SE Buffer O
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG) : **TGGCCAGG**.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Mox20 I, 80% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of Mox20 I for 16 hours..

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	25-50

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

MroN I (prototype Nae I)

Isolated from *Micrococcus roseus* NO

G^CCGGC **E087** **500 u.a.**
CGGCC^G **E088** **2500 u.a.**

Concentration: 2 000 –10 000 units/ml
Assayed on Adenovirus-2 DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.
MroNI is a neoschizomer of NaeI.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65°C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with MroN I, > 90% of the Ad-2 DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of Ad-2 DNA with 5 units of MroN I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	0-10	10-25

MroX I (prototype Xmn I)

MGQ

Isolated from *Micrococcus roseus* X

GAANN^NNTTC **E249** **200 u.a.**
CTTNN^NNAAG **E250** **1000 u.a.**

Concentration: 5 000 - 15 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction mixture: 1×SE Buffer W
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with MroX I, 50% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of MroX I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	50-75	100	25-50

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

Msp I (prototype Hpa II)

Isolated from *Moraxella* species

MGQ

C^CGG E091 1000 u.a.
GGC^C E092 5000 u.a.

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1×SE Buffer B Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Msp I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of DNA with 20 units of Msp I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	50-75	75-100	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_27_1.phtml

Msp20 I (prototype Bal I)

Isolated from *Micrococcus* species 20

Replaced by Mox20 I

TGG^CCA
ACC^GGT

MspA1 I (prototype NspB II)

Isolated from *Moraxella* species A1

CMG^CKG E191 500 u.a.
GKC^GMC E192 2500 u.a.

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y, BSA
Reaction conditions: 1× SE BufferY + BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 20 mM Tris-HCl (pH 7.6); 300 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 10 mM $MgCl_2$, 200 µg/ml BSA, 50% glycerol. Store at -20°C (*see note).
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with MspA1 I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of MspA1 I for 16 hours.
Do not use BSA for long incubation
***Note: Store For long term storage (more than 30 days), store at -70ºC .**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	75-100	10-25	25-50	100

MspR9 I (prototype ScrF I)

Isolated from *Moraxella* species R9

CC^NGG E175 1000 u.a.
GGN^CC E176 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA (dcm-)
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1×SE Buffer O Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^mCWGG): **CCWGG.**

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoetyanol; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with MspR9 I none of the DNA fragments can be ligated.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 30 units of MspR9I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	100	50-75	75-100

Mte I (prototype Mte I)

Isolated from *Microbacterium testaceum* 17B

G(5mC)G(5mC)^NG(5mC)G(5mC) E553 500 u.a.
(5mC)G(5mC)GN^(5mC)G(5mC)G E554 2500 u.a.

See page 57 for more information about this enzyme.

Nru I (prototype Nru I)

Isolated from *Nocardia rubra*

TCG^CGA E099 500 u.a.
AGC^GCT E100 2500 u.a.

Concentration: 5 000 - 10 000 units/ml
Assayed on λ DNA (dam-)
Reagents Supplied with Enzyme: 10×SE Buffer W
Reaction conditions: 1× SE Buffer W Incubate at 37°C.
Blocked by overlapping *dam*-methylation (G^mATC): **TCGCGATC**.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Nru I, 20% of the DNA fragments can be ligated. Of these, 90% can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Nru I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	75-100	100	10-25

NEW New product MGQ Mammalian Genome Qualified NP New package

PalA I (prototype Asc I)

Isolated from *Pseudomonas alcaligenes* BS17

GG^CGCGCC E483 100 u.a.
CCGCGC^GG E484 500 u.a.

Concentration: 500 – 2 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction condition: 1×SE Buffer Y
Incubate at 37°C.
Blocked by CpG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with PalAI, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of PalAI for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	10-25	0	0	100

Pce I (prototype Stu I)

Isolated from *Planococcus citreus* 55

AGG^CCT E105 1000 u.a.
TCC^GGA E106 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction Condition: 1xSE Buffer Y
Incubate at 50°C.
Incubation at 37°C results in 50% activity.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Pce I 70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Pce I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	50-75	25-50	100

Pci I (prototype BspLU11 I)

Isolated from an *E.coli* strain that carries the cloned Pci I gene from *Planococcus citreus* SE-F45

MGQ

A^CATGT E275 300 u.a.
TGTAC^A E276 1500 u.a.

Concentration: 10 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1×SE Buffer O
Incubate at 37°C.
Blocked by ^{m}ACATGT methylation.
Not blocked by AC^{m}ATGT methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Pci I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 20 units of Pci I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	100	75-100	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

PciS I (prototype Sap I)

Isolated from *Planococcus citreus* S

GCTCTTC(N)$_1$^ E497 50 u.a.
CGAGAAG(N)$_4$^ E498 250 u.a.

Concentration: 500 - 2 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with PciS I, 90% of the DNA fragments can be ligated with T4 DNA Ligase at 16°C and 95% of these can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 1 units of PciS I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	0-10	0-10	75-100

Pcs I (prototype Pcs I)

Isolated from *Paracoccus carotinifaciens* 3K

(5mC)GNNNNN^NN(5mC)G E505 50 u.a.
G(5mC)NN^NNNNNG(5mC) E506 250 u.a.

See page 57 for more information about this enzyme.

Pct I (prototype Bsm I)

Isolated from *Planococcus citreus* SM

MGQ

GAATGCN^ E045 1000 u.a.
CTTAC^GN E046 5000 u.a.

Concentration: 10 000 - 40 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1×SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Pct I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20units of Pct I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	100	75-100	10-25

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Pkr I (prototype Pkr I)

Isolated from *Planomicrobium koreense* 78k

NEW

DNA sequence with at least three 5mC: G(5mC)N^G(5mC) (5mC)G^N(5mC)G

E579 50 u.a.
E580 250 u.a.

See page 58 for more information about this enzyme.

Ple19 I (prototype Pvu I)

Isolated from *Pseudomonas lemoignei* 19

CGAT^CG
GC^TAGC

E195 100 u.a.
E196 500 u.a.

Concentration: 2 000 - 5 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y Incubate at 37°C.
Not blocked by overlapping *dam*-methylation (G^{m}ATC): **CGATCG.**

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Ple19 I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of Ple19 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	25-50	100

Pps I (prototype Ple I)

Isolated from *Pseudomonas pseudoalcaligenes*

GAGTC(N)$_4$^
CTCAG(N)$_5$^

E269 25 u.a.
E270 125 u.a.

Concentration: 500 – 1 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA , 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Pps I, 20% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 unit of Pps I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	10-25	0-10	25-50	100

Psi I (prototype Psi I)

Isolated from *Pseudomonas* species SE-G49

TTA^TAA
AAT^ATT

E279 200 u.a.
E280 1000 u.a.

Concentration: 5 000 - 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1×SE Buffer B Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Psi I, about 50% of the DNA fragments can be ligated. Of these, 95% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Psi I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	25-50	10-25	25-50	75-100

Psp124B I (prototype Sac I)

Isolated from *Pseudomonas* species 124B

GAGCT^C
C^TCGAG

E107 1000 u.a.
E108 5000 u.a.

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme: 10×SE Buffer G
Reaction mixture: 1×SE Buffer G Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Psp124B I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Psp124B I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	10-25	0-10	75-100

Psp6 I (prototype EcoR II)

Isolated from *Pseudomonas* species 6

^CCWGG
GGWCC^

E453 100 u.a.
E454 500 u.a.

Concentration: 1 000 – 3 000 units/ml
Assayed on λ DNA (dcm-)
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1×SE Buffer B Incubate at 55°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **CCWGG.**

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with Psp6 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 6 units of Psp6 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	25-50	75-100

NEW New product MGQ Mammalian Genome Qualified NP New package

PspC I (prototype PmaC I)

Isolated from *Pseudomonas* species C

CAC^GTG **E475** **2000 u.a.**
GTG^CAC **E476** **10000 u.a.**

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 10 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C (*see note).
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with PspC I, > 90% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of PspC I for 16 hours.
Do not use BSA for long incubation.
***Note: For long term storage (more than 30 days), store at -70ºC .**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	0	0	50-75

PspE I (prototype BstE II)

Isolated from *Pseudomonas* species E

G^GTNACC **E169** **2000 u.a.**
CCANTG^G **E170** **10000 u.a.**

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with PspE I >90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of PspE I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	25-50	50-75	50-75

PspL I (prototype Spl I)

Isolated from *Pseudomonas* species L

C^GTACG **E223** **200 u.a.**
GCATG^C **E224** **1000 u.a.**

Concentration: 2 000 – 5 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 100 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with PspL I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 2 units of PspL I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	10-25	100

PspN4 I (prototype Nla IV)

Isolated from *Pseudomonas* species N4

GGN^NCC **E089** **1000 u.a.**
CCN^NGG **E090** **5000 u.a.**

Concentration: 10 000 - 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 37°C.
Blocked by methylation
5'-GGNN(5mC)C-3'/3'-C(5mC)NNGG-5' or.
5'-GGNN(5mC)C-3'/3'-CCNNGG-5'

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with PspN4 I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 30 units of PspN4 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	10-25	25-50	100

PspOM I (prototype Apa I)

Isolated from an *E.coli* strain that carries the cloned PspOM I gene from *Pseudomonas* species OM2164

G^GGCCC **E215** **1500 u.a.**
CCCGG^G **E216** **7500 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA (Bam HI-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C
PspOMI is a neoschizomer of ApaI.

Storage buffer: 20 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with PspOM I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of PspOM I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	10-25	0-10	0-10	100

PspPP I (prototype PpuM I)

Isolated from *Pseudomonas* species PP

RG^GWCCY **E255** **100 u.a.**
YCCWG^GR **E256** **500 u.a.**

Concentration: 2 000 - 5 000 units/ml
Assayed on λ DNA (dcm-,HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 37°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **RGGW<u>CCT</u>GG**.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with PspPP I, >70% of the DNA fragments can be ligated and 80% of these can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 5 units of PspPP I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	0	0-10	100

Psp X I (prototype PspX I)

Isolated from an *E.coli* strain that carries the cloned PspX I gene from *Pseudomanas* species A1-1

VC^TCGAGB **E477** **200 u.a.**
BGAGCT^CV **E478** **1000 u.a.**

For high concentration
E478X **1000 u.a**

Concentration: 10 000 and 50 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA.
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA; 7 mM 2 -mercaptoethanol, 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with PspX I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of PspXI for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	50-75	25-50	75-100	100

PsrI (prototype Psr I)

Isolated from *Pseudomonas stutzeri* N2

^(N)$_7$GAAC(N)$_6$TAC(N)$_{12}$^ **E131** **100 u.a.**
^(N)$_{12}$CTTG(N)$_6$ATG(N)$_7$^ **E132** **500 u.a.**

Concentration: 1 000 – 3 000units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction mixture: 1×SE Buffer Y+BSA
Incubate at 30°C.
Incubation at 37°C results in 20% activity.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Psr I, > 70% of the DNA fragments can be ligated. Of these, 80% of these can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of T7 DNA with 2 units of Psr I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	0	0-10	100

Pst I (prototype Pst I)

Isolated from an *E.coli* strain that carries the cloned PstI gene from *Providencia stuartii*

CTGCA^G **E109** **4000 u.a.**
G^ACGTC **E110** **20000 u.a.**

For high concentration
E109X **4000 u.a.**
E110X **20000 u.a.**

Concentration: 20 000 and 50 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions: 1× SE Buffer O+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Pst I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Pst I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	25-50	25-50

NEW New product MGQ Mammalian Genome Qualified NP New package

PstN I (prototype AlwN I)

Isolated from *Bacillus sphaericus*

CAGNNN^CTG **E571** **500 u.a.**
GTC^NNNGAC **E572** **2500 u.a.**

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at **37**°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with PstNI > 95% of Lambda DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of PstNI for 16 hours at 37°C.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	50-75	10-25	25-50	100

Pvu II (prototype Pvu II)

Isolated from an *E.coli* strain that carries the cloned Pvu II gene from *Proteus vulgaris*

MGQ

CAG^CTG **E111** **2000 u.a.**
GTC^GAC **E112** **10000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1× SE Buffer G+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Pvu II, 70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of Pvu II for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	100	25-50	25-50	25-50

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Rga I (prototype Sgf I)

Isolated from *Rhizoblum galegae*

GCGAT^CGC **E491** **200 u.a.**
CGC^TAGCG **E492** **1000 u.a.**

Concentration: 5 000 - 10 000 units/ml
Assayed on Ad2 DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.
Not blocked by overlapping *dam*-methylation (G^{m}ATC): **GC<u>GATC</u>GC.**

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Rga I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of Ad2 DNA with 10 units of Rga I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	10-25	25-50	100

Rig I (prototype Fse I)

Isolated from *Rhizobium yangligense*

GGCCGG^CC **E529** **100 u.a.**
CC^GGCCGG **E530** **500 u.a.**

Concentration: 1 000 - 5 000 units/ml
Assayed on Adenovirus-2 DNA
Reagents Supplied with Enzyme:
10×SE Buffer RigI, BSA
Reaction conditions:
1×SE Buffer RigI+BSA
Incubate at 37°C.
Blocked by mCG or GmC methylation:
5'-GGC(m5C)GGCC-3'/3'-CCGG(m5C)CGG-5' or 5'-GG(m5C)CGG(m5C)C-3'/ 3'-C(m5C)GGC(m5C)GG-5'
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol.
Store at -20°C(*see note)
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Rig I, > 95% of Ad2 DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of Ad2 DNA with 6 units of Rig I for 16 hours.
Do not use BSA for long incubation.
***Note: For long term storage (more than 7 days), store at -70ºC .**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	0-10	10-25	50-75

Rsa I (prototype Rsa I)

Isolated from *Rhodopseudomonas sphaeroides*

MGQ

GT^AC	E113	1000 u.a.
CA^TG	E114	5000 u.a.

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1× SE Buffer B Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Rsa I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of Rsa I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	0-10	50-75	75-100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

RsaN I (prototype Rsa I)

Isolated from *Rhodopseudomonas sphaeroides* N

G^TAC	E555	200 u.a.
CAT^G	E556	1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer B
Reaction conditions: 1× SE Buffer B Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with RsaN I, > 90% of λ DNA fragments can be ligated with T4 DNA Ligase and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of RsaN I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	50-75	50-75	75-100

Rsr2 I (prototype Rsr II)

Isolated from *Rhodobacter sphaeroides* 12

CG^GWCCG	E281	1000 u.a.
GCCWG^GC	E282	5000 u.a.

Concentration: 10 000 - 30 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Rsr2 I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 60 units of Rsr2 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	0-10	10-25	100

Sal I (prototype Sal I)

Isolated from an *E.coli* strain that carries the cloned Sal I gene from *Streptomyces albus*

G^TCGAC	E115	2000 u.a.
CAGCT^G	E116	10000 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme: 10×SE Buffer O
Reaction conditions: 1× SE Buffer O Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Sal I, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Sal I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	100	25-50	0-10

Sbf I (prototype Sse8387 I)

Isolated from *Streptomyces* species Bf61

CCTGCA^GG	E101	200 u.a.
GG^ACGTCC	E102	1000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme: 10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Sbf I, > 90% of the DNA fragments can be ligated. Of these, 90% can be recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Sbf I for 16 hours.
Star activity: High enzyme concentration may result in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	0-10	0-10	100

Set I (prototype Set I)

Isolated from an *E.coli* strain that carries the cloned Set I gene from *Streptomyces werraensis 37*

ASST^ E537 200 u.a.
^TSSA E538 1000 u.a.

For high concentration
E538X 1000 u.a.

Set I is a restriction endonuclease with 4 expected recognition sites as well as relaxed non-cognate sites (star sites).
Concentration: 5 000 and 20 000 units/ml
One unit is defined as the amount of enzyme required to cleave 1 pmol of the double-stranded oligonucleotide with the following structure
5'-CGAGTTTAT**AGCT**GGGCCCAAC-3'
3'-GCTCAAATA**TCGA**CCCGGGTTG-5'
in 1 hour at 50°C in a total reaction volume of 20 µl.
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 55°C.

Storage buffer: 10 mM Tris-HCl (pH 7.6); 100 mM NaCl; 0.1 mM EDTA; 1mM DTT, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Set I, 50% of the pBR322 DNA fragments can be ligated with T4 DNA Ligase and recut.
Note! ***In the case of long incubation with Set I DNA can be digested to small oligos.***

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	75-100	75-100	100

SfaN I (prototype SfaN I)

Isolated from an *E.coli* strain that carries the cloned SfaN I gene from *Streptococcus faecalis* N

MGQ

GCATC(N)$_5$^ E165 500 u.a.
CGTAG(N)$_9$^ E166 2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with SfaN I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of SfaN I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	75-100	0-10

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Sfi I (prototype Sfi I)

Isolated from an *E.coli* strain that carries the cloned SfiI gene from *Streptomyces fimbriatus*

GGCCNNNN^NGGCC E123 1000 u.a.
CCGGN^NNNNCCGG E124 5000 u.a.

For high concentration
E123X 1000 u.a.
E124X 5000 u.a.

Concentration: 10 000 and 40 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 50°C.
Blocked by overlapping *dcm*-methylation (C^{m}CWGG): **GGCCWGGNNGGCC.**
Not blocked by overlapping *dcm*-methylation
(C^{m}CWGG): **GGCCNNNNNGGCCWGG.**
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Sfi I, about 70% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 20 units of Sfi I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	100	25-50	25-50	25-50

Sfr274 I (prototype Xho I)

Isolated from *Streptomyces fradiae* 274

C^TCGAG E125 2000 u.a.
GAGCT^C E126 10000 u.a.

Concentration: 10 000 – 30 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 50°C.
Note: At 37°C activity is about 70% from maximum.
Blocked by CTCG^{m}AG methylation.
Not blocked by CT ^{m}CGAG methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Sfr274 I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Sfr274 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	50-75	50-75	75-100

Sfr303 I (prototype Sac II)

Isolated from *Streptomyces fradiae* 303

CCGC^GG **E127** **1000 u.a.**
GG^CGCC **E128** **5000 u.a.**

Concentration: 5 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Sfr303 I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Sfr303 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	10-25	10-25	75-100

Sma I (prototype Sma I)

Isolated from an *E.coli* strain that carries the cloned Sma I gene from *Serratia marcescens*

CCC^GGG **E177** **2000 u.a.**
GGG^CCC **E178** **10000 u.a.**

Concentration: 20 000 units/ml
Assayed on λ DNA (Hind III-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1× SE Buffer Y
Incubate at 25°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Sma I,
> 90% of the DNA fragments can be ligated by high concentration T4 DNA Ligase and 10% PEG, and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 40 units of Sma I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	0-10	0-10	0-10	100

Smi I (prototype Swa I)

Isolated from *Streptococcus milleri* S

ATTT^AAAT **E225** **1000 u.a.**
TAAA^TTTA **E226** **5000 u.a.**

Concentration: 10 000 - 30 000 units/ml
Assayed on T7 DNA (Ssp I-digest)
Reagents Supplied with Enzyme:
10×SE Buffer O, BSA
Reaction conditions: 1×SE Buffer O+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 100 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Smi I, about 80% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of T7 DNA with 30 units of Smi I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	100	75-100	25-50

SmiM I (prototype Msl I)

Isolated from *Sphingobacterium mizutae* M

CAYNN^NNRTG **E293** **500 u.a.**
GTRNN^NNYAC **E294** **2500 u.a.**

Concentration: 5 000 – 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 250 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; and 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat Inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with SmiM I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of DNA with 20 units of SmiM I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	75-100	100	10-25

Sph I (prototype Sph I)

Isolated from an *E.coli* strain that carries the cloned SphI gene from *Streptomyces phaeochromogenes*

GCATG^C **E129** **500 u.a.**
C^GTACG **E130** **2500 u.a.**

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer G, BSA
Reaction conditions: 1×SE Buffer G+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 100 μg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Sph I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 10 units of Sph I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	100	75-100	75-100	50-75

NEW New product MGQ Mammalian Genome Qualified NP New package

Sse9 I (prototype Tsp509 I)

Isolated from an *E.coli* strain that carries the cloned Sse9I gene from *Sporosarcina* species 9

MGQ

^AATT	E217	500 u.a.
TTAA^	E218	2500 u.a.

Concentration: 5 000 units/ml
Assayed on pBR322 DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 55°C.
Incubation at 37°C results in 75% activity.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tis-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol. Store at -20° C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with Sse9 I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of Sse9 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	75-100	50-75	50-75	75-100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Ssp I (prototype Ssp I)

Isolated from an *E.coli* strain that carries the cloned SspI gene from *Sphaerotilus* species

AAT^ATT	E041	500 u.a.
TTA^TAA	E042	2500 u.a.

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer K, BSA
Reaction conditions: 1×SE Buffer K+BSA
Incubate at 37°C.
Blocked by A^{m}ATATT methylation.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with SspI 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Ssp I for 16 hours.
Star activity: High enzyme concentration may result in star activity.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	25-50	50-75	75-100

SspM I (prototype Mae I)

Isolated from *Sporosarcina species* M

NEW

C^TAG	E591	100 u.a.
GAT^C	E592	500 u.a.

Concentration: 1 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at **55**°C.
At 37°C activity is 75% from maximum.

Storage buffer: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0,1 mM EDTA; 0,01%Triton X-100; 500 µg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (80° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with SspMI 5% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of SspM I for 16 hours.
***Note: For long term storage (more than 30 days), store at -70ºC ..**

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	25-50	10-25	50-75	100

Taq I (prototype Taq I)

Isolated from an *E.coli* strain that carries the cloned Taq I gene from *Thermus aquaticus*

MGQ

T^CGA	E133	2000 u.a.
AGC^T	E134	10000 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 65°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 300 mM NaCl; 0,1 mM EDTA; 1 mM DTT ; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Taq I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Taq I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	75-100	50-75	100

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Tru9 I (prototype Mse I)

Isolated from an *E.coli* strain that carries the cloned Tru9 I gene from *Thermus ruber* 9

MGQ

T^TAA E199 500 u.a.
AAT^T E200 2500 u.a.

Concentration: 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 65°C.
Blocked by TTA^{m}A methylation.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Tru9 I, > 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 40 units of Tru9 I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	25-50	25-50	100	50-75

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

TseF I (prototype Tsp45 I)

Isolated from an *E.coli* strain that carries the cloned TseF I gene from *Thermus species* F35

NEW

^GTSAC E589 200 e.a.
CASTG^ E590 1000 e.a.

Concentration: 5 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 65°C.

Storage buffer: 10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with TseF I, about 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of TseFI for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	0-10	25-50	50-75

Tth111 I (prototype Tth111 I)

Isolated from an *E.coli* strain that carries the cloned Tth111 I gene from *Thermus thermophilus* 111

GACN^NNGTC E097 400 u.a.
CTGNN^NCAG E098 2000 u.a.

Concentration: 5 000 units/ml
Assayed on λ DNA (HindIII-digest)
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 65°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 500 mM NaCl; 0,1 mM EDTA; 1 mM DTT ; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Tth111 I, about 10% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 5 units of Tth111 I for 16 hours.
Star activity: Long incubation or conditions of high enzyme concentration may result in star activity. Star activity is observed at a greater than 5-fold overdigestion of 1 µg substrate with Tth111I for 1 hour.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	10-25	10-25	100

Vne I (prototype ApaL I)

Isolated from an *E.coli* strain that carries the cloned VneI gene from *Vibrio nereis* 18

G^TGCAC E137 1000 u.a.
CACGT^G E138 5000 u.a.

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer O
Reaction conditions: 1× SE Buffer O
Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Vne I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Vne I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	100	25-50	25-50

Vsp I (prototype Vsp I)

Isolated from an *E.coli* strain that carries the cloned Vsp I gene from *Vibrio* species 343

MGQ

AT^TAAT **E139** **1000 u.a.**
TAAT^TA **E140** **5000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Buffer:
10×SE Buffer W
Reaction conditions: 1×SE Buffer W
Incubate at 37°C.
Blocked by ATTA^{m}AT methylation.

Storage buffer: 10 mM Tris HCl (pH 7.6); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Vsp I, 70% of the DNA fragments can be ligated. Of these, 90% can be recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 20 units of Vsp I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	0-10	10-25	50-75	100	25-50

Qualified for mammalian genome studies: http://science.sibenzyme.com/article8_article_28_1.phtml

Xba I (prototype Xba I)

Isolated from an *E.coli* strain that carries the cloned Xba I gene from *Xanthomonas badrii*

MGQ

T^CTAGA **E141** **2000 u.a.**
AGATC^T **E142** **10000 u.a.**

For high concentration
E141X **2000 u.a.**
E142X **10000 u.a.**

Concentration:
20 000 and 50 000 units/ml
Assayed on λ DNA (dam^{-}/ Hind III-digest)
Reagents Supplied with Enzyme:
10×SE Buffer O**,** BSA
Reaction conditions: 1×SE Buffer O+BSA
Incubate at 37°C.
Blocked by overlapping d*am*-methylation (G^{m}ATC): **TCTAGA**TC.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0,1 mM EDTA; 1 mM DTT;- 200 µg/ml BSA; 50% glycerol.
Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 20-fold overdigestion with Xba I, > 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of DNA with 40 units of Xba I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	100	50-75	75-100

Qualified for human genome studies: http://science.sibenzyme.com/article8_article_31_1.phtml

Xma I (prototype Xma I)

Isolated from an *E.coli* strain that carries the cloned *XmaI* gene from *Xanthomonas malvacearum*

C^CCGGG **E233** **300 u.a.**
GGGCC^C **E234** **1500 u.a.**

Concentration: 3000 units/ml
Assayed on Adenovirus-2 DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y
Reaction conditions: 1×SE Buffer Y
Incubate at 37°C.

Storage buffer: 20 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol. Store at -20° C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 3-fold overdigestion with XmaI, 95% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 3 units of Xma I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	50-75	0	0-10	100

Zra I (prototype Aat II)

Isolated from an *E.coli* strain that carries the cloned Zra I gene from *Zoogloea ramigera*11

GAC^GTC **E463** **200 u.a.**
CTG^CAG **E464** **1000 u.a.**

Concentration: 10 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B
Reaction conditions: 1×SE Buffer B
Incubate at 37°C.
ZraI is a neoschizomer of AatII.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 200 µg/ml BSA; 50% glycerol. Store at -20° C.
Diluent: SE Buffer A
Heat Inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Zra I, about 90% of the DNA fragments can be ligated and recut. In the presence of 10% PEG ligation is better.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 10 units of Zra I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	25-50	25-50	75-100

Zrm I (prototype Sca I)

Isolated from *Zoogloea ramigera* SCA

AGT^ACT **E005** **1000 u.a.**
TCA^TGA **E006** **5000 u.a.**

Concentration: 10 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer Y, BSA
Reaction conditions: 1×SE Buffer Y+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Zrm I, 70% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Zrm I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	25-50	0-10	0-10	100

Zsp2 I (prototype Ava III)

Isolated from *Zoogloea* species 2

ATGCA^T **E145** **1000 u.a.**
T^ACGTA **E146** **5000 u.a.**

Concentration: 5 000 – 20 000 units/ml
Assayed on λ DNA
Reagents Supplied with Enzyme:
10×SE Buffer B, BSA
Reaction conditions: 1×SE Buffer B+BSA
Incubate at 37°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μg/ml.

Storage buffer: 10 mM Tis-HCl (pH 7.5); 200 mM KCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 μg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 10-fold overdigestion with Zsp2 I,
> 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 μg of λ DNA with 20 units of Zsp2 I for 16 hours.
Do not use BSA for long incubation.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	100	50-75	25-50	25-50	25-50

NEW New product MGQ Mammalian Genome Qualified NP New package

Nickases

Nicking Endonuclease N.Bst9 I

GAGTCNNNN^NN	**E401**	**100 u.a.**
CTCAGNNNNNN	**E402**	**500 u.a.**

Isolated from *Bacillus stearothermophilus* T9

Concentration: 2 000 – 5 000 units/ml
Assayed on T7 DNA
Reagents Supplied with Enzyme:
10×SE Buffer N.Bst9I
Reaction conditions: 1×SE Buffer N.Bst9 I
Incubate at 55°C.
Incubation at 37°C results in 20% activity.

Storage buffer: 10 mM Tis-HCl (pH 7.5); 50 mM KCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. Store at -20°C.
Diluent: SE Buffer A
Heat inactivation:
No (65° C for 20 minutes)
Yes (80° C for 20 minutes)

Ligation/recutting assay: After 5-fold overdigestion with N.Bst9 I, 90% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of T7 DNA with 2 units of N.Bst9I for 16 hours.
Star activity: High enzyme concentration results in star activity.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	75-100	100	100	50-75

Methyl-directed DNA endonucleases

Aox I (prototype Aox I)

Isolated from *Arthrobacter oxydans* 25K

NEW

^PuG(5mC)Py	E569	50 u.a.
Py(5mC)GPu^	E570	250 u.a.

The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA!

Concentration: 500 units/ml

Assayed on DNA pMHaeIII/DriI is a linearized plasmid pMHaeIII. pMHaeIII carries a gene of DNA-methyltransferase M.HaeIII, which methylates sites 5`-GGCC-3` producing 5`-GG(5mC)C-3`/3`-C(5mC)GG-5`.

One unit is defined as the amount of enzyme required to hydrolyze in 1 µg of linearized plasmid pMHaeIII/DriI in 1 hour at 60°C in a total reaction volume of 50 µl.

Reagents Supplied with Enzyme: 10×SE Buffer AoxI

Reaction conditions: 1× SE Buffer AoxI

Incubate at 60°C.

Storage buffer: 10 mM Tris-HCl (pH 7.4); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 60°C in a total reaction volume of 50 µl.

Heat inactivation:

No (80° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	25-50	10-25	25-50	75-100

Bis I (prototype Bis I)

Isolated from *Bacillus subtilis* T30

G(5mC)^NGC	E485	40 u.a.
CGN^(5mC)G	E486	200 u.a.

The enzyme cleaves only C5-methylated DNA and doesn't cut unmodified DNA! [1]

Concentration: 1 000 – 2 000 units/ml

Assayed on Double-stranded oligonucleotide

5’ GCTTGTACTTTA G(5mC)G G C ATTGATTCTCACCACG 3’

3' CGAACATGAAAT C G C(5mC)G TAACTAAGAGTGGTGC 5'

One unit is defined as the amount of enzyme required to cleave 1 pmol of the double-stranded oligonucleotide with the following structure

5' GCTTGTACTTTA G(5mC)G G CATTGATTCTCACCACG 3'

3' CGAACATGAAATC G C(5mC)GTAACTAAGAGTGGTGC 5'

in 1 hour at 37°C in a total reaction volume of 20 µl.

Reagents Supplied with Enzyme: 10×SE Buffer BisI

Reaction conditions: 1× SE Buffer BisI

Incubate at 37°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**

Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of pFsp4HI1 DNA (BamHI digest) with 1 unit of BisI for 16 hours at 37°C. The pFsp4HI1 plasmid carries a gene for Fsp4HI DNA-methyltransferase, which modifies DNA forming 5'-G(5mC)NGC-3'/3'-CGN(5mC)G-5'.

1.Chmuzh E.V., Kashirina J.G., Tomilova J.E., Mezentseva N.V., Dedkov V.S., Gonchar D.A., Abdurashitov M.A., Degtyarev S.Kh. A Novel Restriction Endonuclease BisI from Bacillus subtilis T30, Recognizes a Methylated DNA Sequence 5'-G(m5C)^NGC-3'.// Biotekhnologia (Moscow), No.3, p.22-26 (2005) (In Russian). Online version in English: http://science.sibenzyme.com/article8_article_7_1.phtml

Heat inactivation:

Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	25-50	50-75	75-100	50-75

NEW New product MGQ Mammalian Genome Qualified NP New package

Bls I (prototype Bls I)

Isolated from *Bacillus simplex* 23

DNA sequence with at least two 5mC:
PuPyN^PuPy
PyPu^NPyPu

E533	**100 u.a.**
E534	**500 u.a.**

The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA! [1]

Concentration: 5000 units/ml
Assayed on DNA pFsp4HI3/DriI is a linearized plasmid pFsp4HI3, which carries a gene of DNA-methyltransferase M.Fsp4HI and includes three canonical sites:
5`-G(5mC)NG(5mC)-3`/3`-CGN(5mC)G-5`[2].
One unit is defined as the amount of enzyme required to hydrolyze at least one of three canonical sites
5`-G(5mC)NG(5mC)-3`/3`-CGN(5mC)G-5`
in 1 µg of linearized plasmid pFsp4HI3 in 1 hour at 30°C
in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme: 10×SE Buffer **W**
Reaction conditions: 1× SE Buffer **W**
Incubate at 30°C.
Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol. **Store at -20°C.**

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 5 units of enzyme for 16 hours at 30°C in a total reaction volume of 50 µl.

1. Chernukhin V.A., Tomilova J.E., Chmuzh E.V., Sokolova O.O., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Bacillus simplex - producer of BlsI site specific endonuclease. // Russian Federation patent RU 2322494 C1 (2006).
2. Chmuzh E.V., Kashirina Yu.G., Tomilova Yu.E., Chernukhin V.A., Okhapkina S.S., Gonchar D. A., Dedkov V.S., Abdurashitov M. A., Degtyarev S. Kh. Gene cloning, comparative analysis of the protein structures from Fsp4HI restriction-modification system and biochemical characterization of the recombinant DNA methyltransferase M.Fsp4HI. // Molecular Biology, V.41, No 1, p. 43-50 (2007)

Heat inactivation:

Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	10-25	10-25	50-75	100	75-100

Gla I (prototype GlaI)

Isolated from Glacial ice bacterium GL 29

Pu(5mC)^GPy	**E493**	**100 u.a.**
PyG^(5mC)Pu	**E494**	**500 u.a.**

The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA and DNA with N4-methylcytosines![1]

Concentration: 10 000 units/ml

Assayed on DNA pHspAI2/GsaI is a linearized plasmid pHspAI2, which carries a gene of DNA-methyltransferase M.HspAI (recognition sequence 5`-GCGC-3`) and includes a unique GlaI recognition site:
5`-G(5mC)G(5mC)-3`/3`-(5mC)G(5mC)G-5`[2].

One unit is defined as the amount of enzyme required to hydrolyze completely a unique
5`-G(5mC)G(5mC)-3`/3`-(5mC)G(5mC)G-5`
site in 1 µg of pHspAI2 plasmid DNA, which is linearized with GsaI, in 1 hour at 30°C in a total reaction volume of 50 µl.

Reagents Supplied with Enzyme:
10×SE Buffer Y, pHspAI2/GsaI DNA.

pHspAI2/GsaI DNA is a linearized plasmid pHspAI2, which includes a gene of DNA-methyltransferase M.HspAI(recognition sequence 5'-GCGC-3') and contains a unique GlaI canonical site: 5'-G(5mC)G(5mC)-3'/3'-(5mC)G(5mC)G-5'.

Reaction conditions: 1× SE Buffer Y.
Incubate at 30°C.

Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 0,05% Triton X-100; 100 µg/ml BSA, 50% glycerol.

Store at -20°C.

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 8 units of enzyme for 16 hours at 30°C in a total reaction volume of 50 µl.

1.Chernukhin V.A., Nayakshina T.N., Tomilova J.E., Mezentseva N.V., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Glacial ice bacterium I - producer of GlaI restriction endonuclease. // Russian Federation patent RU 2287012 C1 (2006).
2.Chernukhin V.A., Najakshina T.N., Abdurashitov M.A., Tomilova J.E., Mezentzeva N.V., Dedkov V.S., Mikhnenkova N.A., Gonchar D.A., Degtyarev S. Kh A novel restriction endonuclease GlaI recognizes methylated sequence 5'-G(5mC)^GC-3'.// Biotechnologia V.4. p.31-35(2006) (In Russian). Online version in English: http://science.sibenzyme.com/article8_article_11_1.phtml
3. Tarasova G. V., Nayakshina T. N., Degtyarev S. Kh. Substrate specificity of new methyl-directed DNA endonuclease GlaI . // BMC Molecular Biology 2008, 9:7.
4. Tomilova J.E., Chernukhin V.A., Degtyarev S.Kh. Dependence of site-specific endonuclease GlaI activity on quantity and location of methylcytosines in the recognition sequence 5`-GCGC-3`. // Bulletin of biotechnology and physico-chemical biology V.2, No 1, p.30-39 (2006) (In Russian).
Online version in English: http://science.sibenzyme.com/article10_article_22_1.phtml
5. Chernukhin V.A, Abdurashitov M.A., Tomilov V.N., Gonchar D.A., Degtyarev S.Kh. Comparative analysis of mouse chromosomal DNA digestion with restriction endonucleases in vitro and in silico // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.3, No 4, pp 19-27, 2007. Online version in English: http://science.sibenzyme.com/article14_article_46_1.phtml
6. Abdurashitov M.A., Chernukhin V.A, Gonchar D.A., Degtyarev S.Kh. GlaI digestion of mouse γ-satellite DNA: study of primary structure and ACGT sites methylation.// BMC Genomics 2009, 10:322.
7. D. A. Gonchar, A. G. Akishev, S. Kh. Degtyarev BlsI- and GlaI-PCR assays – a new method of DNA methylation study // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.6, No 1, pp 5-12, 2010
Online version in English: http://science.sibenzyme.com/article12_article_53_1.phtml

Heat inactivation:

Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	25-50	100

The enzyme activity depending on number and position of methylated nucleotides in the recognition sequence:

recognition sequence	G(mC)G(mC) (mC)G(mC) G	R(mC)G(mC) Y G(mC) G	G (mC)R(mC) (mC) G Y G
Activity, %	100	> 25	> 6

Glu I (prototype GluI)

Isolated from Glacial ice bacterium GL 24

G(5mC)^NG(5mC)	E519	50 u.a.
(5mC)GN^(5mC)G	E520	250 u.a.

The enzyme cleaves C5-methylated DNA and does not cut unmodified DNA! [1]

Concentration: 1 000 units/ml

Assayed on DNA pFsp4HI3/DriI is a linearized plasmid pFsp4HI3, which includes a gene of DNA-methyltransferase M.Fsp4HI and contains a unique GluI site:
5`-G(5mC)NG(5mC)-3`/3`-(5mC)GN(5mC)G-5`. [2]

One unit is defined as the amount of enzyme required to hydrolyze completely a unique canonical site:
5`-G(5mC)NG(5mC)-3`/3`-(5mC)GN(5mC)G-5`
in 1 µg of linearized plasmid pFsp4HI3/DriI in 1 hour at 37°C
in a total reaction volume of 50 µl.

Reagents Supplied with Enzyme:
10×SE Buffer Y

Reaction conditions: 1× SE Buffer Y
Incubate at **37**°C.

Storage buffer: 10 mM KH_2PO_4 (pH 7.45); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol.

Store at -20°C.

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 37°C in a total reaction volume of 50 µl.

1. Chernukhin V.A., Chmuzh E.V., Tomilova J.E., Nayakshina T.N., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Glacial ice bacterium - producer of GluI site specific endonuclease. // Russian Federation patent RU 2322492 C1 (2006).

2. Chernukhin V.A., Chmuzh E.V., Tomilova Yu.E., Nayakshina T.N., Gonchar D.A., Dedkov V.S., Degtyarev S.Kh. A novel site-specific endonuclease GluI recognizes methylated DNA sequence 5'-G(5mC)^NG(5mC)-3'/3'-(5mC)GN^(5mC)G.// Bulletin of biotechnology and physico-chemical biology named by Yu.A.Ovchinnikov (Moscow), V.3, No.2, p.13-17 (2007) (In Russian).
Online version in English: http://science.sibenzyme.com/article8_article_24_1.phtml

3. Chernukhin V.A, Abdurashitov M.A., Tomilov V.N., Gonchar D.A., Degtyarev S.Kh. Comparative analysis of mouse chromosomal DNA digestion with restriction endonucleases in vitro and in silico // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.3, No 4, pp 19-27, 2007. Online version in English: http://science.sibenzyme.com/article14_article_46_1.phtml

Heat inactivation:

Yes (80° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	75-100	75-100	25-50	50-75	100

Kro I (prototype Kro I)

Isolated from *Kocurea rosea* 307

G^C(5mC)GGC **E541** **50 u.a.**
CGG(5mC)C^G **E542** **250 u.a.**

The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA! [1]

Kro I doesn`t cleave DNA modified with MspI DNA-methyltransferase

Concentration: 1 000 units/ml
Assayed on DNA pMHpaII1/DriI is a linearized plasmid pMHpaII1.
pMHpaII1 carries a gene of DNA-methyltransferase M.HpaII, which methylates sites 5`-CCGG-3` producing 5`-C(5mC)GG-3`/3`-GG(5mC)C-5`,
and includes three canonical sites
5`-GC(5mC)GGC-3`/3`-CGG(5mC)CG-5`.
One unit One unit is defined as the amount of enzyme required to hydrolyze completely 1 µg of linearized plasmid pMHpaII1 in 1 hour at 37°C in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme:
10×SE Buffer G
Reaction conditions: 1× SE Buffer G
Incubate at 37°C.
Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 200 µg /ml BSA; 50% glycerol.
Store at -20°C.

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 30°C in a total reaction volume of 50 µl.

1. Chernukhin V.A., Zhuravleva R.O., Tarasova G.V., Boltengagen A. A., Akishev A.G., Mikhnenkova N.A., Degtyarev S.Kh. Bacterial strain Kocuria rosea - producer of KroI site specific endonuclease. // Russian Federation patent RU 2394099 C1 (2010).

Heat inactivation:
Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	100	25-50	50-75	75-100

Mal I (prototype Dpn I)

Isolated from *Marinococus albus* I

G(mA)^TC **E489** **50 u.a.**
CT^(mA)G **E490** **250 u.a.**

The enzyme cleaves only methylated DNA
Concentration: 500 - 1 000 units/ml
Assayed on
pBR322 DNA (dam-methylated)
One unit One unit of the enzyme is the amount required to hydrolyze 1 µg of pBR322 DNA (dam-methylated) in 1 hour at 37°C in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme:
10×SE Buffer Mal I
Reaction conditions: 1×SE Buffer Mal I
Incubate at 37°C.
Storage buffer: 10 mM Tris-HCl (pH 7.5); 100 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 100 µg/ml BSA, 50% glycerol.
Diluent: SE Buffer A
Store at -20°C.
Heat inactivation:
Yes (65° C for 20 minutes)

Ligation/recutting assay: After 2-fold overdigestion with Mal I, ~80% of the DNA fragments can be ligated and recut.
Overdigestion assay: No nonspecific activity was detected after incubation of 1 µg of λ DNA with 2 units of Mal I for 16 hours.

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	25-50	50-75	75-100	50-75

NEW New product MGQ Mammalian Genome Qualified NP New package

Mte I (prototype Mte I)

Isolated from *Microbacterium testaceum* 17B

NEW

G(5mC)G(5mC)^NG(5mC)G(5mC)	E553	500 u.a.
(5mC)G(5mC)GN^(5mC)G(5mC)G	E554	2500 u.a.

The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA ! [1]

Concentration: 10 000 units/ml
Assayed on pHspAI10/DriI+M.Fsp4HI is a plasmid pHspAI10, which is linearized with DriI, and, additionally, modified with Fsp4HI DNA methyltransferase. pHspAI10 carries a gene of HspAI DNA methyltransferase, that modifies the sequence
5`-GCGC-3`, producing 5`-G(5mC)GC-3`.
M.Fsp4HI modifies the sequence
5`-GCNGC-3`, producing 5`-G(5mC)NGC-3`.
A substrate pHspAI10/DriI+M.Fsp4HI includes one site
5`-G(5mC)G(5mC)NG(5mC)G(5mC)-3`/3`-(5mC)G(5mC)GN(5mC)G(5mC)G-5`,
which is MteI canonical site [1]. The enzyme activity depends on a number and positions of methylated nucleotides in the recognition sequence. For example, MteI cuts the recognition site with six 5-methylcytosines, but the enzyme activity is reduced for more that one order [1].
One unit is defined as the amount of enzyme required to hydrolyze completely 1 µg of linearized plasmid pHspAI10/DriI+M.Fsp4HI in 1 hour at 55°C in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme:
10×SE Buffer W
Reaction conditions: 1 x SE-buffer W
Incubate at **55**°C.
Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA, 50% glycerol.
Store at -20°C.

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 10 units of enzyme for 16 hours at 55°C in a total reaction volume of 50 µl.

1. V.A. Chernukhin, E.V. Kileva, V.A. Sokolova., D.A. Gonchar, L.N. Golikova, V.S. Dedkov, N.A. Mikhnenkova, S.Kh. Degtyarev. A new methyl-directed site-specific DNA endonuclease MteI cleaves nine nucleotides sequence 5`-G(5mC)G(5mC)^NG(5mC)GC-3`/3`-CG(5mC)GN^(5mC)G(5mC)G-5` // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.8, No 1, pp 16-26, 2012

Heat inactivation:
No (80° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	25-50	75-100	75-100	100	50-75

Pcs I (prototype Pcs I)

Isolated from *Paracoccus carotinifaciens* 3K

(5mC)GNNNNN^NN(5mC)G	E505	50 u.a.
G(5mC)NN^NNNNNG(5mC)	E506	250 u.a.

The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA ! [1]

Concentration: 1 000 units/ml
Assayed on pMHgaI/DriI is a linearized plasmid pMHgaI, which carries genes of DNA-methyltransferases M1.HgaI (recognition sequence 5`-GCGTC-3`)
and M2.HgaI (5`-GACGC-3`) and includes a unique PcsI canonical site:
5`-W(5mC)GNNNNNNN(5mC)GW-3`/ 3`-WG(5mC)NNNNNNNG(5mC)W-5` [1].
One unit defined as the amount of enzyme required to digest a unique site
5`-A(5mC)GNNNNNNN(5mC)GT-3`
in 1 µg of DNA pMHgaI/DriI in 1 hour at 37°C in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme:
10×SE Buffer Pcs I
Reaction conditions: 1 x SE-buffer PcsI
Incubate at 37°C.
Storage buffer: 10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA;
7 mM 2-mercaptoethanol; 0.1 mg/ml BSA, 50% glycerol.
Store at -20°C.

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 37°C in a total reaction volume of 50 µl.
Notes: When using a buffer other than the optimal (suppied) SEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

1. Chernukhin V.A., Nayakshina T.N., Tarasova M.V., Golikova L.N., Akishev A.G., Dedkov V.S., Mikhnenkova N.A., Degtyarev S.Kh. Bacterial strain Paracoccus carotinifaciens 3K- producer of PcsI site specific endonuclease. // Russian Federation patent RU 2377294 C1 (2009).

Heat inactivation:
Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	25-50	0	10-25	50-75

Pkr I (prototype Pkr I)

Isolated from
Planomicrobium koreense 78k

NEW

DNA sequence with at least three 5mC: G(5mC)N^G(5mC) (5mC)G^N(5mC)G

E579	**50 u.a.**
E580	**250 u.a.**

The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA! [1]

Concentration: 1000 units/ml
Assayed on DNA pFsp4HI3/DriI is a linearized plasmid pFsp4HI3, which carries a gene of DNA-methyltransferase M.Fsp4HI and includes three sites:
5`-G(5mC)NG(5mC)-3`/3`-CGN(5mC)G-5` [2].
One unit is defined as the amount of enzyme required to hydrolyze completely 1 µg of linearized plasmid pFsp4HI3 in 1 hour at 37°C in a total reaction volume of 50 µl
Reagents Supplied with Enzyme: 10×SE Buffer **Y**
Reaction conditions: 1× SE Buffer **Y**
Incubate at 37°C.
Storage buffer: 20 mM Tris-HCl (pH 7.4); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**

Overdigestion assay: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 2 units of enzyme for 16 hours at 37°C in a total reaction volume of 50 µl.

1. V.A. Chernukhin, T.N. Nayakshina, D.A. Gonchar, Ju.E. Tomilova, M.V.Tarasova, V.S. Dedkov, N.A. Mikhnenkova, S.Kh. Degtyarev A new site-specific methyl-directed DNA endonuclease PkrI recognizes and cuts methylatedDNA sequence 5`-GCN^GC-3`/3`-CG^NCG-5` carrying at least three 5-methylcytosines. // Bulletin of biotechnology and physico-chemical biology named by Yu.A.Ovchinnikov (Moscow), V.7, No.3, p.35-42 (2011). (In Russian).
2. Chmuzh E.V., Kashirina Yu.G., Tomilova Yu.E., Chernukhin V.A., Okhapkina S.S., Gonchar D. A., Dedkov V.S., Abdurashitov M. A., Degtyarev S. Kh. Gene cloning, comparative analysis of the protein structures from Fsp4HI restriction-modification system and biochemical characterization of the recombinant DNA methyltransferase M.Fsp4HI. // Molecular Biology, V.41, No 1, p. 43-50 (2007)

Heat inactivation:
Yes (65° C for 20 minutes)

SE Buffers	B	G	O	W	Y
Activity in SE Buffers (% of max)	50-75	75-100	10-25	25-50	100

DNA – Methyltransferases

M3.BstF5I

Isolated from *E.coli* strain, that carries the cloned M3.BstF5I gene from *Bacillus stearothermophilus* F5

CH_3
I
5'-GGATG-3'
3'-CCTAC-5'

M007 **1000 u**

Description: M3.BstF5I Methylase modifies the adenin residue (mA) in the recognition secuence 5'-GGATG-3'
Concentration: 10000 units/ml
Reagents Supplied: 10 × SEBuffer K, SAM
Incubate at 60°C.

Storage Conditions: 10 mM Tis-HCl (pH 7.5); 50 mM NaCl; 50% glycerol**. Store at -20°C**.
Unit Definition: One unit of the enzyme is the amount required to protect 1 µg of Lambda DNA in 1 hour at 60°C in a total reaction volume of 20 µl against cleavage by BstF5 I restriction endonuclease.

1.Golikova L.N., Netesova N.A., Gutorov V.V., Belavin P.A., Abdurashitov M.A., Gonchar D.A., Degtyarev S.Kh. Multiplicity of site-specific DNA-methyltransferases of the BstF5I restriction modification system from Bacillus stearothermophilus F5.// Molecular Biology(Moscow), V.34, No.3, p.443-447 (2000) (In Russian).

M.Fsp4HI

Isolated from *E.coli* strain, that carries the cloned M.Fsp4HI gene from *Flavobacterium* species 4H

CH_3
I
5'-GCNGC-3'
3'-CGNCG-5'
I
CH_3

M001 **100 u**

Description: M.Fsp4HI Methylase modifies the internal cytosine residue (C5) in the recognition secuence 5'-GCNGC-3'.
Concentration: 500-1000 units/ml
Reagents Supplied: 10 × SEBuffer B, SAM
Incubate at 30°C.

Storage Conditions: 10 mM Tis-HCl (pH 7.6); 100 mM NaCl; 0.1 mM EDTA; 10 mM 2-mercaptoethanol; 50% glycerol**. Store at -20°C**.
Unit Definition: One unit of the enzyme is the amount required to protect 1 µg of Lambda DNA in 1 hour at 30°C in a total reaction volume of 20 µl against cleavage by Fsp4HI restriction endonuclease.

1. Chmuzh E.V., Kashirina Yu.G., Tomilova Yu.E., Chernukhin V.A., Okhapkina S.S., Gonchar D. A., Dedkov V.S., Abdurashitov M. A., Degtyarev S. Kh. Gene cloning, comparative analysis of the protein structures from Fsp4HI restriction-modification system and biochemical characterization of the recombinant DNA methyltransferase M.Fsp4HI. // Molecular Biology, V.41, No 1, p.43-50 (2007) (In Russian).

M.HspAI

Isolated from *E.coli* strain, that carries the cloned M.HspAI gene from *Haemophilus* species AI

CH_3
I
5'-GCGC-3'
3'-CGCG-5'
I
CH_3

M003 **100 u**

Description: M.HspAI Methylase modifies the internal cytosine residue (C5) in the recognition secuence 5'-GCGC-3'.
Concentration: 1000-3000 units/ml
Reagents Supplied: 10 × SEBuffer B, SAM
Incubate at 37°C.

Storage Conditions: 10 mM Tis-HCl (pH 7.6); 100 mM NaCl; 0.1 mM EDTA; 10 mM 2-mercaptoethanol; 50% glycerol.. **Store at -20°C**.
Unit Definition: One unit of the enzyme is the amount required to protect 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 20 µl against cleavage by HspAI restriction endonuclease.

1. Chernukhin V.A., Najakshina T.N., Abdurashitov M.A., Tomilova J.E., Mezentzeva N.V., Dedkov V.S., Mikhnenkova N.A., Gonchar D.A., Degtyarev S. Kh A novel restriction endonuclease GlaI recognizes methylated sequence 5'-G(5mC)^GC-3'.// Biotechnologia V.4. p.31-35 (2006) (In Russian). Online version in English: http://science.sibenzyme.com/article8_article_11_1.phtml

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

Polymerases

DNA Polymerase I, Large (Klenow) Fragment

E325 **200 u**
E326 **1000 u**

Isolated from *E.coli* strain that carries the cloned DNA Polymerase I, Large (Klenow) Fragment gene

Description: DNA Polymerase I, Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3'→5' exonuclease activity, but has lost 5'→3' exonuclease activity.
Applications:
- dideoxy sequencing;
- polishing ends;
- second strand cDNA synthesis

Concentration: 5 000 units/ml
Reagents Supplied with Enzyme: 10 × Klenow Buffer
Storage Conditions: 10 mM KH_2PO_4 (pH 7.5); 50 mM KCl; 0.5 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol.
Store at -20°C.

Heat Inactivation: 75°C for 20 minutes.
Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTPs into an acid-insoluble material in 30 minutes at 37°C.
Unit Assay Conditions: 1 × Klenow Buffer, 33 µM dNTP including [^{3}H]-dTTP and 70 µg/ml denatured calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

M-MuLV Reverse Transcriptase RNase H -

E317 **5000 u**
E318 **25000 u**

Isolated from *E.coli* strain that carries the recombinant plasmid

Description: Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase is an RNA directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. The absence of RNase H activity enhances the synthesis of long cDNAs and therefore the enzyme is recommended for preparing long cDNAs.
Application: first strand cDNA synthesis.
Concentration: 50 000–200 000 units/ml
Reagents Supplied with Enzyme:
10 × M-MulV Reverse Transcriptase Buffer
Dilution Buffer M-MulV Reverse Transcriptase

Storage Conditions: 10 mM KH_2PO_4 (pH 7.5); 0,1 mM EDTA; 200 mM NaCl; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**
Unit Definition: One unit is the amount of the enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo(dT).
Unit Assay Conditions: 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 10 mM DTT, 0.5 mM [^{3}H]-dTTP, 0.4 mM poly(rA)•oligo(dT) 12-18.
Quality Control: Purified free of detectable levels of RNase, endonuclease and exonuclease activities.
Note: High enzyme concentration may lead to RT-PCR inhibition. In this case the enzyme preparation should be diluted in 5, 10 or 20 times with M-MuLV Reverse Transcriptase dilution Buffer

T4 DNA Polymerase

E339 **200 u.**
E340 **1000 u**

Isolated from *E.coli* strain that carries the recombinant plasmid

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5'→3' direction and requires the presence of template and primer. This enzyme has a 3'→5' exonuclease activity.
Applications:
- polishing ends;
- probe labeling using replacement synthesis.

Concentration: 2 000 – 5 000 units/ml
Reagents Supplied with Enzyme: 10 × T4 DNA Polymerase Buffer
Storage Conditions: 20 mM Tris-HCl (pH 7.5); 50 mM KCl; 10 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**

Heat Inactivation: 75°C for 10 minutes.
Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-precipitable material in 30 minutes at 37°C.
Unit Assay Conditions: 1 × T4 DNA Polymerase Reaction Buffer, 33 µM dNTPs including [^{3}H]-dTTP and 70 µg/ml denatured calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

NEW New product NP New package

SP-Taq DNA Polymerase

Isolated from *E.coli* strain that carries the cloned Taq DNA polymerase gene from Thermus aquaticus

NEW

E333 **200 u**
E334 **1000 u**

Description: SP-*Taq* DNA Polymerase is a fraction of *Taq* DNA Polymerase which was specially treated and additionally purified. It doesn` t contain PCR detected DNA contaminations. Enzyme is suitable for different manipulations in the field of PCR-diagnostics.
Applications:
- nick translation;
- primer extension reaction.

Concentration: 5 000 units/ml
Reagents Supplied with Enzyme: 10 × Taq-DNA-polymerase buffer.
Storage conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5 % Triton X-100; 50% glycerol.
Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at **72°C.**
Unit Assay Conditions: 60 mM Tris-HCl (pH 8.5 at 25ºC); 25 mM KCl; 1.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, 200 µM dATP, dCTP, dGTP, 50 mkM H-TTP, 12.5 µg activated Calf Thymus DNA in a total reaction volume of 50 µl.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

Taq DNA Polymerase with Standard Taq Buffer

Isolated from *E.coli* strain that carries the cloned Taq DNA polymerase gene from Thermus aquaticus

E331 **200 u**
E332 **1000 u**

Description: *Taq* DNA polymerase is a thermostable DNA polymerase that possesses a 5'→3' polymerase activity and a double strand specific 5'→3' exonuclease activity.
Applications:
- nick translation;
- primer extension reaction.

Concentration: 5 000 units/ml
Reagents Supplied with Enzyme: 10 × Taq-DNA-polymerase buffer.
Storage conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5 % Triton X-100; 50% glycerol.
Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at **72°C.**
Unit Assay Conditions: 1 × Taq-DNA-polymerase buffer, 200 µM dNTPs including [^{3}H]-dTTP and 250 µg/ml activated calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

Taq DNA Polymerase with Standard Taq Buffer (Mg^{2+} free)

Isolated from *E.coli* strain that carries the cloned Taq DNA polymerase gene from Thermus aquaticus

E341 **200 u**
E342 **1000 u**

Description: *Taq* DNA polymerase is a thermostable DNA polymerase that possesses a 5'→3' polymerase activity and a double strand specific 5'→3' exonuclease activity.
Applications:
- nick translation;
- primer extension reaction.

Concentration: 5 000 units/ml
Reagents Supplied with Enzyme:
10 × Taq-DNA-polymerase buffer (Mg^{2+} free).
Storage conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5 % Triton X-100; 50% glycerol.
Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at **72°C.**
Unit Assay Conditions: 1 × Taq-DNA-polymerase buffer, 200 µM dNTPs including [^{3}H]-dTTP and 250 µg/ml activated calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

Taq DNA Polymerase with AS Buffer

Isolated from *E.coli* strain that carries the cloned Taq DNA polymerase gene from Thermus aquaticus

E337 **200 u**
E338 **1000 u**

Description: *Taq* DNA polymerase is a thermostable DNA polymerase that possesses a 5'→3' polymerase activity and a double strand specific 5'→3' exonuclease activity.
Applications:
- nick translation;
- primer extension reaction.

Concentration: 5 000 units/ml
Reagents Supplied with Enzyme:
10 × AS (Ammonium Sulfate) buffer.
Storage conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5 % Triton X-100; 50% glycerol.
Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at **72°C.**
Unit Assay Conditions: 1 × AS (Ammonium Sulfate) buffer, 200 µM dNTPs including [^{3}H]-dTTP and 250 µg/ml activated calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

TaqSE DNA Polymerase

Isolated from *E.coli* strain that carries the recombinant plasmids.

E313 **200 u**
E314 **1000 u**

Description: TaqSE DNA polymerase is a complex mix of thermostable DNA polymerase that possesses a 5'→3' polymerase activity, 3'→5'exonuclease (proofreading) activity and a double strand specific 5'→3' exonuclease activity.
It may increase yield of reaction product compare to Taq DNA polymerase.
Application: long high fidelity primer extension reaction.
Concentration: 5 000 units/ml
Reagents Supplied with Enzyme: 10 × Taq-DNA-polymerase buffer.
Storage conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5 % Triton X-100; 50% glycerol.
Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at **72°C.**
Unit Assay Conditions: 1 × Taq-DNA-polymerase buffer, 200 µM dNTPs including [^{3}H]-dTTP and 250 µg/ml activated calf thymus DNA.
Quality Control: Purified free of contaminating endonucleases and exonucleases.

Hot Start Taq-DNA Polymerase

E351 **200 u**
E352 **1000 u**

Description: Hot Start Taq DNA Polymerase is complex mixture of a thermostable 94 kD Taq DNA Polymerase purified from E.coli recombinant strain expressing *Thermus aquaticus* polymerase gene and specific monoclonal antibodies from mouse.
Hot Start Taq DNA Polymerase is inactive under conditions of amplification reaction preparation. It can eliminate amplification artefacts such as primer-dimer formation and mispriming during preamplification stage and thus may provide improved specificity when compared to standard DNA polymerases.
An advantage of Hot Start Taq DNA Polymerase is the absence of additional heating step for polymerase activation. Heat activation of enzyme occurs during the first denaturation step. An inactive complex of Hot Start Taq DNA Polymerase dissociates automatically over +70°C, allowing activation of DNA polymerase.
Applications: -Highly specific PCR;
-Multiplex PCR (highly recommended);
-High sensitivity applications.
Concentration: 5 000 units/ml
Reagents Supplied with Enzyme:
10 × Hot Start Taq-DNA-polymerase buffer.
Supplementary material is 50 mM $MgCl_2$.

Storage and dilution buffer: 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0,1 mM EDTA, 1 mM DTT, 50 % glycerol, 0.5 % Nonidet P-40, 0.5 % Tween-20. **Store at -20°C.**
Unit Definition: One unit of activity is the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74 °C.
Notes: The recommended amount of enzyme is 1 u per 50µl of a total reaction volume.

Pfu DNA Polymerase

Isolated from *E.coli* strain that carries the cloned D*NA Polymerase* gene from *Pyrococcus furiosus.*

E353 **200 u**
E354 **1000 u**

Description: Pfu DNA Polymerase catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions. The enzyme also exhibits 3'→5'exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. Products of reaction have blunt ends.
Application: Pfu DNA Polymerase useful for high fidelity synthesis and polishing ends.
Concentration: 5 000 units/ml
Reagents Supplied with Enzyme:
10 × Pfu DNA polymerase buffer, 50 × BSA (5 mg/ml BSA).
Storage conditions: 10 mM K_2HPO_4 (pH 7.4); 0,1 mM DTT, 0.1 mM EDTA; 0.5 % Tween 20; 50% glycerol.
Store at –20°C.
To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 µg/ml.

Unit Definition: One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE- 81) in 30 minutes at **72°C.**
Unit Assay Conditions: 1 × Pfu DNA polymerase buffer, 0.1 mg/ml BSA, 200µg/ml activated calf thymus DNA, 0.2 mM of each dNTP, 0.4MBq/ ml [^{3} H]- dTTP in 50 µl reaction mix.
Quality Control: Purified free of contaminating endonucleases and exonucleases.
Notes: 1. Do **not** use dU-contained templates.
2. This enzyme is **not** recommended for a number of experiments dealing with very low-annealing temperature amplification approaches (e.g. RAPDs, Random Amplified Polymorphic DNAs).
3. Prepare PCR mix at **0°C**.
Set in amplificator preheated to **95°C.**
4. The recommended amount of enzyme is 2.5 u per 50µl of a total reaction volume.

NEW New product NP New package

T7 RNA Polymerase

Isolated from *E.coli* strain that carries the cloned T7 gene I

E355	**5000 u**
E356	**25000 u**

Description: T7 RNA Polymerase catalyzes the synthesis of RNA in the 5'→ 3' direction in the presence of a DNA template containing a T7 phage promoter.

Application: -Radiolabeled RNA probe preparation
-RNA generation for *in vitro* translation
-RNA generation for studies of RNA structure, processing and catalysis

Concentration: 100 000 units/ml

Reagents Supplied with Enzyme: 10 × T7 RNA polymerase buffer

Storage conditions: 50 mM Tris-HCl (pH 7.5); 100 mM NaCl; 20 mM 2-mercaptoethanol; 1 mM EDTA; 50% Glycerol; 0.1% Triton X-100.

Store at –20°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol NTP into an acid-insoluble material in 60 minutes at 37°C.

Quality Control: Purified free of contaminating endonucleases and exonucleases.

DNA Modifying Enzymes

Alkaline Phosphatase, Calf Intestinal

Calf intestinal mucosa

E327 **100 u**
E328 **500 u**

Description: *:* Alkaline phosphatase catalyzes the removal of 5' phosphate groups from DNA, RNA and ribo- and deoxyribonucleoside triphosphates.
Applications:
- removing 5' and 3' phosphoryl groups from nucleic acids;
- preparing templates for 5' end labeling;
- preventing fragments from self ligating;
- dephosphorylation of proteins

Concentration: 10 000 units/ml.
Reagents Supplied with Enzyme: 10 × SEBuffer O

Storage conditions: 10 mM Tris-HCl (pH 8.2); 50 mM KCl;1 mM $MgCl_2$, 0,1 mM $ZnCl_2$, 50% glycerol.
Store at -20°C.
Unit Definition: One unit is the amount of enzyme that hydrolyzes 1 µmol of p-nitrophenylphosphate to p-nitrophenol in a total reaction volume of 1 ml in 1 min at 37ºC.
Unit Assay Conditions:1 M diethanolamine-HCl(pH 9.8), 0.5 mM $MgCl_2$ and 10 mM p-nitrophenylphosphate. These conditions are only used for quantitating enzyme activity.
Quality Control: Purified free of contaminating exonuclease, endonuclease, ribonuclease activities.

Thermolabile Alkaline Phosphatase

Isolated from *E.coli* strain that carries the cloned *Alkaline Phosphatase* gene from *Alteromonas undina* P2

E365 **200 u**
E366 **1000 u**

Description: *:* Thermolabile Alkaline phosphatase catalyzes the removal of 5' phosphate groups from DNA, RNA and ribo- and deoxyribonucleoside triphosphates.
Concentration: 5 000 units/ml.
Reagents Supplied with Enzyme: 10 × SEBuffer W
Heat inactivation: Yes (65° C for 20 minutes)

Storage conditions: 20 mM Tris-HCl (pH 7.6); 0,1 mM $ZnCl_2$, 50% glycerol.
Store at -20°C.
Unit Definition: One unit is the amount of enzyme that will dephosphorylate 1 µg of pUC19 DNA (linearized with Hind III) in 30 minutes at 25ºC.
Dephosphorylation is defined as >95% inhibition of recirculation in a self-ligation reaction that is measured by transformation into *E.coli.*
Quality Control: Purified free of contaminating exonuclease, endonuclease, ribonuclease activities.

Endonuclease I

Isolated from *Proteus vulgaris* 84

E323 **4000 u**
E324 **20000 u**

Description: Endonuclease I hydrolyzes double- and single-stranded nucleic acids to oligonucleotides of 3-5 nucleotide in length with 5'-terminal phosphates.
Application: DNA and RNA degradation.
Concentration: 10 000 – 50 000 units/ml.
Reagents Supplied with Enzyme: 10 × Endonuclease I Buffer.

Storage Conditions: 10 mM Tris-HCl (pH 7.4); 250 mM NaCl; 0,2 mM EDTA; 7 mM 2-mercaptoethanol; 100 µg/ml BSA; 50% glycerol.
Store at -20°C.
Unit Definition: One unit is the amount of enzyme that hydrolyze 1 µg of Lambda DNA in 30 minutes at 37°C.
Unit Assay Conditions: 20 mM Glycine-NaOH (pH 9.5), 100 mM NaCl, 25 mM $MgCl_2$; 1 mM 2-mercaptoethanol.
Quality Control: Purified free of contaminating phosphatase activity.

Exonuclease III (*E. coli*)

Isolated from a recombinant source

E345 **5000 u**
E346 **25000 u**

Description: Exonuclease III (*E. coli*) catalyzes the stepwise removal of mononucleotides from 3'-hydroxyl termini of double stranded DNA. Exonuclease III activity depends partially on helical structure and displays sequence dependence (C>A=T>G). Temperature, salt concentration and the ratio of enzyme to DNA greatly affect enzyme activity, requiring reaction conditions to be tailored to specific applications.
Applications:
- unidirectional nested deletions;
- site-directed mutagenesis;
- preparation of strand-specific probes;
- preparation of single-stranded substrates for dideoxy sequencing.

Concentration: 40 000 – 100 000 units/ml.
Reagents Supplied with Enzyme: 10 × Exonuclease III Buffer **.**

Storage Conditios: 10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0.5 mM EDTA; 1 mM 2-mercaptoethanol; 50% glycerol**. Store at -20°C.**
Unit Definition: One unit is the amount of enzyme required to produce 1 nmol of acid soluble total nucleotide in 30 minutes at 37°C.
Unit Assay Conditions:50 mM Tris-HCl(pH 7.5 at 25°C); 1 mM $MgCl_2$; 1 mM DTT,
0.15 mM sonicated pancreatic DNA.

NEW New product NP New package

RNA Ligase T4

Isolated from *E.coli* strain that carries the cloned *RNA Ligase* gene from bacteriophage T4

E349 **1000 u**
E350 **5000 u**

Description: RNA Ligase catalyzes ligation of a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor through the formation of a 3'→5'phosphodiester bond, with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.
Applications:
- labeling of 3'-termini of RNA with 5'-[^{32}P] pCp;
- inter- and intra-molecular joining of RNA and DNA molecules.
Concentration: 10 000 units/ml.
Reagents Supplied with Enzyme: 10 × T4 RNA Ligase Buffer.

Storage conditions: 10 mM Tris-HCl(pH 7.4); 50 mM KCl; 0.1 mM EDTA; 1 mM DTT; 50% glycerol. **Store at -20°C.**
Unit Definition: One unit is defined as the amoumt of enzyme required to convert of 1 pmol of [^{3}H]ATP in AMP –Ligase complex in 15 minutes at 25°C.
Unit Assay Conditions: 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM DTT, 0.2 µM [^{3}H]ATP.
Quality Control: Free of contaminating single-stranded DNA exonuclease, endonuclease, ribonuclease and phosphatase activities.

T4 Polynucleotide Kinase

Isolated from *E.coli* strain that carries the cloned *Polynucleotide Kinase* gene from bacteriophage T4

E311 **500 u**
E312 **2500 u**

Description: T4 polynucleotide Kinase catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5' hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA) and nucleoside 3'-monophosphates. The enzyme also catalyzes the removal of 3'- phosphoryl groups from 3'- phosphoryl polynucleotides, deoxynucleoside 3'- monophosphates and deoxynucleoside 3'-diphosphates.
Applications:
- end-labeling DNA or RNA for probes and DNA sequencing;
- addition of 5'- phosphates to oligonucleotides to allow subsequent ligation;
- removal of 3'- phosphoryl groups.
Concentration: 10 000 units/ml.
Reagents Supplied with Enzyme:
10 × T4 Polynucleotide Kinase Buffer.

Storage conditions: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 1 mM DTT; 50% glycerol. **Store at -20°C.**
Unit Definition One unit is the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in 30 minutes at 37°C.
Unit Assay Conditions: 1 × T4 Polynucleotide Kinase Buffer, 66 µM [γ- ^{32}P] ATP (5×10^6 cpm/µmol) and 0.26 mM 5'- hydroxyl-terminated salmon sperm DNA.
Quality Control: Free of exonuclease, phosphatase, endonuclease and RNase activities.

T4 DNA Ligase

Isolated from *E.coli* strain that carries the cloned *DNA Ligase* gene from bacteriophage T4

E319 **10000 u**
E320 **50000 u**

Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt-end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.
Concentration: 50 000 - 200 000 units/ml
Applications:
- cloning of restriction fragments;
- joining linkers and adapters to blunt-ended DNA;
Reagents Supplied with Enzyme: 10 × T4 DNA Ligase Buffer.

Storage conditions: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at-20°C.**
Unit Definition: One unit is defined as the amoumt of enzyme required to give 50% ligation of Hind III fragments of Lambda DNA (5' DNA termini concentration of 0.12 µM [300 µg/ml]) in 20 µl of 1 × T4 DNA Ligase Reaction Buffer in 30 minutes **at 16°C.**
Quality Control: Free of contaminating exonuclease and endonuclease.
Notes on Use: ATP is an essential cofactor for the reaction.

T4 DNA Ligase Concentrated

Isolated from *E.coli* strain that carries the cloned *DNA Ligase* gene from bacteriophage T4

E329 **50000 u**
E330 **250000 u**

Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt-end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.
Concentration: 2 000 000 units/ml.
Applications:
- cloning of restriction fragments;
- joining linkers and adapters to blunt-ended DNA.
Reagents Supplied with Enzyme: 10 × T4 DNA Ligase Buffer.

Storage conditions: 10 mM Tris-HCl (pH 7.5); 50 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol. **Store at -20°C.**
Unit Definition: One unit is defined as the amoumt of enzyme required to give 50% ligation of Hind III fragments of Lambda DNA (5' DNA termini concentration of 0.12 µM [300 µg/ml]) in 20 µl of 1 × T4 DNA Ligase Reaction Buffer in 30 minutes **at 16°C.**
Quality Control: Free of contaminating exonuclease and endonuclease.
Notes on Use: ATP is an essential cofactor for the reaction.

Tli-Inorganic Pyrophosphatase

Isolated from *E.coli* strain that carries the cloned Inorganic pyrophosphatase gene from Thermococcus litoralis.

E315 **100 u**
E316 **500 u**

Description: Tli-Inorganic Pyrophosphatase catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate:
$P_2O_7^{-4}+H_2O \rightarrow 2HPO_4^{-2}$. Enzyme is extremely thermostable.
Concentration: 1 000 units/ml.
Reagents Supplied with Enzyme:
10 × Inorganic Pyrophosphatase Buffer.

Storage Conditions: 20 mM Tris-HCl (pH 7.6); 100 mM KCl; 0.1 mM EDTA; 0.2% Tween-20; 50% glycerol. **Store at -20°C.**
Unit Definition: One unit is the amount of enzyme that will generate 40 nmoles of phosphate per minute from pyrophosphate under standard reaction conditions (a 10 minute reaction at **75°C** in 50 mM Tris-HCl(pH 8.5), 1 mM $MgCl_2$,0.32 mM PPi, reaction volume of 0.5 ml).
Quality Control: Free of exonuclease and endonuclease activities.

Uracil-DNA Glycosylase (UDG)

Isolated from a recombinant source

E335 **1000 u**
E336 **5000 u**

Description: E.coli uracil-DNA glycosylase (UDG) catalyzes the release of free uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).
Applications:
- site-directed mutagenesis;
- as a probe for protein-DNA interaction studies.

Treatment of 1 µg of uracil-containing DNA with 1 unit of UDG for 10 minutes at 37°C renders the DNA incapable of being copied by DNA polymerase. The enzyme can be 95% heat killed by incubation at 95°C for 10 minutes.
Concentration: 20 000 – 50 000 units/ml.
Reagents Supplied with Enzyme: 10 × UDG Buffer **.**

Storage Conditios: 10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 1 mM EDTA; 1 mM DTT; 50% glycerol. **Store at -20°C**.
Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4 – 10^5 cpm/µg) in 30 minutes at 37°C.
Notes on Use: UDG is active over a broad pH range with an optimum at pH 8.0, does not require divalent cation, and is inhibited by high ionic strength (>200 mM).

NEW New product NP New package

DNA Ladders

1 Kb DNA Ladder

M11 50 mkg
M12 250 mkg

Description: The 1 Kb DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 13 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
10 000	60 ng	**3000**	200 ng	750	60 ng
8000	60 ng	2500	70 ng	**500**	30 ng
6000	60 ng	2000	60 ng	250	20 ng
5000	60 ng	1500	50 ng		
4000	60 ng	**1000**	210 ng		

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 1 Kb DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

50 Kb DNA Ladder

M29 50 mkg
M30 250 mkg

Description: The 50 Kb DNA Ladder has a number of proprietary plasmids and phages DNA which are digested to completion with appropriate restriction enzymes to yield 17 bands suitable for use as molecular weight standards for agarose pulsed-field gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
48502	70	6000	45	1500	35
39936	70	5000	45	**1000**	150
24730	70	4000	45	750	45
15206	70	**3000**	140	**500**	20
10000	45	2500	50	250	10
8000	45	2000	45		

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 50 Kb DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 0,5-1 mkg of the DNA Ladder per line.

100 bp DNA Ladder

M15 50 mkg
M16 250 mkg

Description: The 100 bp DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 10 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
1 000	190 ng	600	110 ng	200	40 ng
900	170 ng	**500**	130 ng	**100**	40 ng
800	150 ng	400	80 ng		
700	90 ng	300	40 ng		

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 100 bp DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

100 bp + 50 bp DNA Ladder

M33 50 mkg
M34 250 mkg

Description: The 100 bp + 50bp DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 11 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
1 000	160 ng	600	100 ng	200	30 ng
900	140 ng	**500**	170 ng	**100**	30 ng
800	120 ng	400	70 ng	**50**	20 ng
700	110 ng	300	50 ng		

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 100 bp + 50 bp DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

100 bp + 1.5 Kb DNA Ladder

M23 50 mkg
M24 250 mkg

Description: The 100 bp + 1.5 Kb DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 11 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
1500	150 ng	700	70 ng	300	30 ng
1000	200 ng	600	80 ng	200	20 ng
900	120 ng	**500**	150 ng	**100**	20 ng
800	110 ng	400	50 ng		

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 100 bp + 1.5 Kb DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

100 bp + 1.5 Kb + 3 Kb DNA Ladder

M27 50 mkg
M28 250 mkg

Description: The 100 bp + 1.5 Kb + 3 Kb DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 12 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
3000	70 ng	800	120 ng	400	60 ng
1500	60 ng	700	80 ng	300	40 ng
1000	140 ng	600	90 ng	200	30 ng
900	130 ng	**500**	120 ng	**100**	30 ng

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 100 bp + 1.5 Kb + 3 Kb DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

100 bp + 2 Kb + 3 Kb DNA Ladder

M25 50 mkg
M26 250 mkg

Description: The 100 bp + 2 Kb + 3 Kb DNA Ladder has a number of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 12 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Base Pairs	DNA Mass	Base Pairs	DNA Mass	Base Pairs	DNA Mass
3000	120 ng	800	110 ng	400	50 ng
2000	120 ng	700	70 ng	300	30 ng
1000	160 ng	600	80 ng	200	20 ng
900	120 ng	**500**	100 ng	**100**	20 ng

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: The 100 bp + 2 Kb + 3 Kb DNA Ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.
We recommend loading 1 mkg of the DNA Ladder per line.

NEW New product

Lambda DNA - Hind III Digest

M01 100 mkg
M02 500 mkg

Description: The Hind III digest of lambda DNA yields 8 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)			
23130	6557	2322	564
9416	4361	2027	125

Concentration: 500 mkg/ml
Storage buffer (TE): 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA. **Store at -20°C.**
Usage recommendation: Dilute in TE buffer (10 – 20 times) before loading.
We recommend loading 1 mkg of the DNA Ladder per line.

Lambda DNA - BssT1 I (Sty I) Digest

M05 100 mkg
M06 500 mkg

Description: The BssT1 I (Sty I) digest of lambda DNA yields 11 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)			
19329	4254	1882	421
7743	3472	1489	74
6223	2690	925	

Concentration: 500 mkg/ml
Storage buffer (TE): 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA. **Store at -20°C.**
Usage recommendation: Dilute in TE buffer (10 – 20 times) before loading.
We recommend loading 1 mkg of the DNA Ladder per line.

Lambda DNA - Bme18 I (Ava II) Digest

M03 100 mkg
M04 500 mkg

Description: The Bme18 I (Ava II) digest of lambda DNA yields 36 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)					
8126	2134	985	511	272	67
6555	2005	974	433	242	45
6442	1951	894	398	215	42
3676	1612	597	345	151	32
2605	1420	590	310	88	28
2555	1284	513	308	73	23

Concentration: 500 mkg/ml
Storage buffer (TE): 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA. **Store at -20°C.**
Usage recommendation: Dilute in TE buffer (10 – 20 times) before loading.
We recommend loading 1 mkg of the DNA Ladder per line.

Lambda DNA - Bgl I Digest

M17 100 mkg
M18 500 mkg

Description: The Bgl I digest of lambda DNA yields 30 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)					
16179	1650	1138	562	366	126
9649	1446	790	499	267	115
3009	1441	773	489	210	91
2481	1249	669	447	186	51
2256	1203	621	404	126	9

Concentration: 500 mkg/ml
Storage buffer (TE): 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA. **Store at -20°C.**
Usage recommendation: Dilute in TE buffer (10 – 20 times) before loading.
We recommend loading 1 mkg of the DNA Ladder per line.

Lambda DNA – BstE II Digest

M09 100 mkg
M10 500 mkg

Description: The BstEII digest of lambda DNA yields 14 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)					
8454	5686	3675	1371	224	
7242	4822	2323	1264	117	
6369	4324	1929	702		

Concentration: 500 mkg/ml
Storage buffer (TE): 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA. **Store at -20°C.**
Usage recommendation: Dilute in TE buffer (10 – 20 times) before loading.
We recommend loading 1 mkg of the DNA Ladder per line.

NEW New product

pBR322 DNA - BsuR I (HaeIII) Digest

M21 50 mkg
M22 250 mkg

Description: The BsuR I digest of pBR322 DNA yields 22 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)					
587	434	192	104	57	11
540	267	184	89	51	8
502	234	124	80	21	
458	213	123	64	18	

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: We recommend loading 1 mkg of the DNA Ladder per line.

pBR322 DNA - Alu I Digest

M19 50 mkg
M20 250 mkg

Description: The Alu I digest of pBR322 DNA yields 9 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)		
908	521	257
659	403	226
656	281	100

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: We recommend loading 1 mkg of the DNA Ladder per line.

pUC19 DNA – Kzo9 I (Sau3AI) Digest

M13 50 mkg
M14 250 mkg

Description: The Kzo9 I digest of pUC19 DNA yields 15 fragments suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)			
955	141	46	12
585	105	36	11
341	78	18	8
258	75	17	

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: We recommend loading 1 mkg of the DNA Ladder per line.

pUC19 DNA - Msp I Digest

M07 50 mkg
M08 250 mkg

Description: The Msp I digest of pUC19 DNA yields 12 bands suitable for use as molecular weight standards for agarose gel electrophoresis.

Length of the DNA fragments (Base Pairs)			
501	331	147	67
489	242	111	**34**
404	190	110	26

Concentration: 200 mkg/ml
Storage buffer: 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 50 mM NaCl. **Store at -20°C.**
Usage recommendation: We recommend loading 1 mkg of the DNA Ladder per line.

NEW New product

Plasmid and Phage DNAs

Lambda DNA (dam$^-$, dcm$^-$)

Isolated from bacteriophage lambda (cl857ind 1 Sam 7) obtained from heat inducible lysogen *E.coli* strain (dam-, dcm-)

D10 **500 mkg**

Description: Duplex DNA is 48502 base pairs in length. The molecular weight is 31.5x10^6 daltons.
Concentration: 500 mkg/ml
Storage Buffer: 10 mM Tris-HCl (pH8.0) and 1 mM EDTA. **Store at -20°C.**

Lambda DNA

Isolated from bacteriophage lambda (cl857ind 1 Sam 7) obtained from heat inducible lysogen *E.coli* strain (dam+, dcm+).

D11 **500 mkg**

Description: Duplex DNA is 48502 base pairs in lenght. The molecular weight is 31.5x10^6 daltons.
Concentration: 500 mkg/ml
Storage Buffer: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. **Store at -20°C.**

T7 Phage DNA

Isolated from T7 phage obtained from infected *E.coli* strain

D02 **500 mkg**

Description: Duplex DNA is 39936 base pairs in lenght. The molecular weight is 26x10^6 daltons.
Concentration: 500 mkg/ml.
Storage Buffer: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. **Store at -20°C.**

pBR322 DNA

Isolated from *E.coli* XL1-Blue (dam+, dcm+).

D03 **50 mkg**
D04 **250 mkg**

Description: pBR322 is commonly used plasmid cloning vector in E.coli. The molecule is a double-stranded circle, 4361 base pairs in lenght. pBR322 contains the genes for resistance to ampicillin and tetracycline, and may be amplified with chloramphenicol. The molecular weight of pBR322 is 2.83x10^6 daltons.
Concentration: 200 mkg /ml
Storage Buffer: 10 mM Tris-HCl (pH8.0) and 1 mM EDTA. **Store at -20°C.**

pHspAI2/GsaI DNA

pHspAI2 is isolated from *E.coli* (dam+,dcm+) by a standard plasmid purification procedure.

D09 **10 mkg**

Description: DNA pHspAI2/GsaI is a linearized plasmid pHspAI2, which includes a gene of DNA-methyltransferase M.HspAI (recognition sequence 5'-GCGC-3') and contains a unique GlaI canonical site 5’-G(5mC)G(5mC)-3’/3’-(5mC)G(5mC)G-5’.
Concentration: 200 mkg /ml
Storage Buffer: 10 mM Tris-HCl (pH8.0) and 1 mM EDTA. **Store at -20°C.**

pUC19 DNA

isolated from *E.coli* XL1-Blue (dam+, dcm+)

D05 **50 mkg**
D06 **250 mkg**

Description: pUC19 is commonly used plasmid cloning vector in E.coli. The molecule is a small double-stranded circle, 2686 base pairs in lenght, and has a high copy number. pUC19 carries a 54 base-pair multiple cloning site polylinker that contains unique sites for 13 different hexanucleotide-specific restriction endonucleases. The molecular weight of pUC19 is 1.75x10^6 daltons
Concentration: 200 mkg /ml
Storage Buffer: 10 mM Tris-HCl (pH8.0) and 1 mM EDTA. **Store at -20°C.**

NEW New product

Human Genomic DNA

Genomic DNA HeLa

D07 **10 mkg**

Isolated from HeLa cervix adenocarcinoma cells.

Description: Human female HeLa genomic DNA.
Cells were grown to confluency in DMEM – 90%, plus fetal bovine serum – 10%.
Genomic DNA was isolated by a standard protocol [1].
Concentration: 200 mkg /ml.
Quality Control: Purified free of contaminating proteins and RNA
Storage Buffer: 10 mM Tris-HCl (pH 7.8), 1 mM EDTA. **Store at -20°C.**
Note: Avoid multiple freeze/thaw cycles.

1. Sambrook J. and Russell D. (2001) *Molecular Cloning: A Laboratory Manual*, (3rd ed.), pp. 6.4-6.12. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Genomic DNA Jurkat

D08 **10 mkg**

Isolated from Jurkat acute T-cell leukemia cells.

Description: Human male Jurkat genomic DNA.
Cells were grown to confluency in RPMI-1640 – 90%, plus fetal bovine serum – 10%.
Genomic DNA was isolated by a standard protocol [1].
Concentration: 200 mkg /ml.
Quality Control: Purified free of contaminating proteins and RNA
Storage Buffer: 10 mM Tris-HCl (pH 7.8), 1 mM EDTA. **Store at -20°C.**
Note: Avoid multiple freeze/thaw cycles.

1. Sambrook J. and Russell D. (2001) *Molecular Cloning: A Laboratory Manual*, (3rd ed.), pp. 6.4-6.12. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Genomic DNA L-68

D014 **10 mkg**

Isolated from L-68 fibroblast cells from lung.

Description: Human embryo L-68 genomic DNA.
Cells were grown to confluency in DMEM – 90%, plus fetal bovine serum – 10%.
Genomic DNA was isolated by a standard protocol [1].
Concentration: 200 mkg /ml.
Quality Control: Purified free of contaminating proteins and RNA
Storage Buffer: 10 mM Tris-HCl (pH 7.8), 1 mM EDTA. **Store at -20°C.**
Note: Avoid multiple freeze/thaw cycles.

1. Sambrook J. and Russell D. (2001) *Molecular Cloning: A Laboratory Manual*, (3rd ed.), pp. 6.4-6.12. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Genomic DNA Raji

D13 **10 mkg**

Isolated from Raji Burkitt's lymphoma cells.

Description: Human male Raji genomic DNA.
Cells were grown to confluency in RPMI-1640 – 90%, plus fetal bovine serum – 10%.
Genomic DNA was isolated by a standard protocol [1].
Concentration: 200 mkg /ml.
Quality Control: Purified free of contaminating proteins and RNA
Storage Buffer: 10 mM Tris-HCl (pH 7.8), 1 mM EDTA. **Store at -20°C.**
Note: Avoid multiple freeze/thaw cycles.

1. Sambrook J. and Russell D. (2001) *Molecular Cloning: A Laboratory Manual*, (3rd ed.), pp. 6.4-6.12. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Genomic DNA U-937

D12 **10 mkg**

Isolated from U-937 histiocytic lymphoma cells.

Description: Human male U-937 genomic DNA.
Cells were grown to confluency in RPMI-1640 – 90%, plus fetal bovine serum – 10%.
Genomic DNA was isolated by a standard protocol [1].
Concentration: 200 mkg /ml.
Quality Control: Purified free of contaminating proteins and RNA
Storage Buffer: 10 mM Tris-HCl (pH 7.8), 1 mM EDTA. **Store at -20°C.**
Note: Avoid multiple freeze/thaw cycles.

1. Sambrook J. and Russell D. (2001) *Molecular Cloning: A Laboratory Manual*, (3rd ed.), pp. 6.4-6.12. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

NEW New product

dNTPs(enzymatic)

dATP

40 mM water solution | **N011** | **5 µmoles**
N012 | **25 µmoles**

Description: 2'-deoxyadenosine 5'-triphosphate sodium salt
Concentration: 40 mM
Method of production: enzymatic synthesis.
dATP are Qualified free of nucleases and phosphatases.
15 Kb and longer DNA fragments may be amplified in PCR with this dNTP(enzymatic).
Store at -20°C.

dCTP

40 mM water solution | **N013** | **5 µmoles**
N014 | **25 µmoles**

Description: 2'-deoxycytidine 5'-triphosphate sodium salt
Concentration: 40 mM
Method of production: enzymatic synthesis.
dCTP are Qualified free of nucleases and phosphatases.
15 Kb and longer DNA fragments may be amplified in PCR with this dNTP(enzymatic).
Store at -20°C.

dGTP

40 mM water solution | **N015** | **5 µmoles**
N016 | **25 µmoles**

Description: 2'-deoxyguanosine 5'-triphosphate sodium salt
Concentration: 40 mM
Method of production: enzymatic synthesis.
dGTP are Qualified free of nucleases and phosphatases.
15 Kb and longer DNA fragments may be amplified in PCR with this dNTP(enzymatic).
Store at -20°C.

dTTP

40 mM water solution | **N017** | **5 µmoles**
N018 | **25 µmoles**

Description: 2'-deoxythymidine 5'-triphosphate sodium salt
Concentration: 40 mM
Method of production: enzymatic synthesis.
dTTP are Qualified free of nucleases and phosphatases.
15 Kb and longer DNA fragments may be amplified in PCR with this dNTP(enzymatic).
Store at -20°C.

dUTP

100 mM water solution | **N031** | **5 µmoles**
N032 | **25 µmoles**

Description: 2'-deoxyuridine 5'-triphosphate sodium salt
Concentration: 100 mM
Method of production: enzymatic synthesis.
dUTP are Qualified free of nucleases and phosphatases.
15 Kb and longer DNA fragments may be amplified in PCR with this dNTP(enzymatic).
Store at -20°C.

dNTP Mix

0,5 µmol of each Water solution | **N024** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 0,5 mM.
Quantity: 1.0 ml contains 0,5 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP Mix

2,5 µmol of each 40 mM water solution | **N026** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 2,5 mM.
Quantity: 1.0 ml contains 2,5 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP Mix

4 µmol of each 40 mM water solution | **N027** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 4 mM.
Quantity: 1.0 ml contains 4 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP Mix

10 µmol of each 40 mM water solution | **N025** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 10 mM.
Quantity: 1.0 ml contains 10 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP solution set (enzymatic)

40 mM of each Water solution. | **N030** | **4 x 25 µmoles**

Description: Deoxynucleotide solution set: Four separate solutions of ultrapure nucleotides (dATP, dCTP, dGTP and dTTP). Each nucleotide is supplied at a concentration of 40 mM aqueous solution .
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pairs with T7 phage DNA as a template.
Store at -20°C.

 New product

dNTPs(chemical)

dATP

NEW

100 mM water solution. | **N001** **5 µmoles** | **N002** **25 µmoles**

Description: 2'-deoxyadenosine 5'-triphosphate lithium salt
Concentration: 100 mM
Method of production: chemical synthesis.
Purity of dATP is 98-99% according to HPLC analysis (Column ProntoSIL-120-5-C18AQ).

The product is free of DNases, RNases and phosphatases.
Coefficient of extinction: 15200 M^{-1} X cm^{-1} at λ_{max}=259 nm
Functional test: The product has been tested in PCR for 0.1-15 kb fragments in length.
Store at -20°C.

dCTP

NEW

100 mM water solution. | **N003** **5 µmoles** | **N004** **25 µmoles**

Description: 2'-deoxyadenosine 5'-triphosphate lithium salt
Concentration: 100 mM
Method of production: chemical synthesis.
Purity of dATP is 98-99% according to HPLC analysis (Column ProntoSIL-120-5-C18AQ).

The product is free of DNases, RNases and phosphatases.
Coefficient of extinction: 9300 M^{-1} X cm^{-1} at λ_{max}=271 nm
Functional test: The product has been tested in PCR for 0.1-15 kb fragments in length.
Store at -20°C.

dGTP

NEW

100 mM water solution. | **N005** **5 µmoles** | **N006** **25 µmoles**

Description: 2'-deoxyadenosine 5'-triphosphate lithium salt
Concentration: 100 mM
Method of production: chemical synthesis.
Purity of dATP is 98-99% according to HPLC analysis (Column ProntoSIL-120-5-C18AQ).

The product is free of DNases, RNases and phosphatases.
Coefficient of extinction: 13700 M^{-1} X cm^{-1} at λ_{max}=253 nm
Functional test: The product has been tested in PCR for 0.1-15 kb fragments in length.
Store at -20°C.

dTTP

NEW

100 mM water solution. | **N007** **5 µmoles** | **N008** **25 µmoles**

Description: 2'-deoxyadenosine 5'-triphosphate lithium salt
Concentration: 100 mM
Method of production: chemical synthesis.
Purity of dATP is 98-99% according to HPLC analysis (Column ProntoSIL-120-5-C18AQ).

The product is free of DNases, RNases and phosphatases.
Coefficient of extinction: 9600 M^{-1} X cm^{-1} at λ_{max}=267 nm
Functional test: The product has been tested in PCR for 0.1-15 kb fragments in length.
Store at -20°C.

dNTP Mix

NEW

0,5 µmol of each 100 mM water solution. | **N020** **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP, dTTP, each at a final concentration of 0,5 mM.
Concentration: 100 mM
Method of production: chemical synthesis.
Quantity: 1.0 ml contains 0,5 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

NEW New product

dNTP Mix

NEW

2,5 µmol of each 100 mM water solution. | **N021** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 2,5 mM.
Concentration: 100 mM
Method of production: chemical synthesis.
Quantity: 1.0 ml contains 2,5 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP Mix

NEW

4 µmol of each 100 mM water solution. | **N023** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 4 mM.
Concentration: 100 mM
Method of production: chemical synthesis.
Quantity: 1.0 ml contains 4 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP Mix

NEW

10 µmol of each 100 mM water solution. | **N022** | **1 ml**

Description: Deoxynucleotide solution mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP, each at a final concentration of 10 mM.
Concentration: 100 mM
Method of production: chemical synthesis.
Quantity: 1.0 ml contains 10 µmol of each dATP, dCTP, dGTP and dTTP.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pair nucleotides with T7 phage DNA as a template.
Store at -20°C.

dNTP solution set (enzymatic)

NEW

100 mM of each water solution. | **N028** | **4 x 25 µmoles**

Description: Deoxynucleotide solution set: Four separate solutions of ultrapure nucleotides (dATP, dCTP, dGTP and dTTP). Each nucleotide is supplied at a concentration of 100 mM aqueous solution .
Concentration: 100 mM
Method of production: chemical synthesis.
Application: For use in molecular biology and biotechnology, including PCR, long-PCR, and for labeling and sequencing DNA. PCR-diagnostics for medicine.
Quality: Molecular Biology Grade.
Functional test: The product has been tested for receiving products with length more than 15,000 base pairs with T7 phage DNA as a template.
Store at -20°C.

NEW New product

Substrates

S-Adenosyl – L - methionine (SAM)

S005 **100 mkl**

Source: fermentative synthesis with subsequent purification

Description: S-Adenosyl-L-methionine (SAM) is a substrate for methyltransferases and a cofactor for some restriction endonucleases. SAM provided in 5 mM H_2SO_4 and 10% EtOH.

Formula: $C_{15}H_{23}N_6O_5S$

Molecular weight: 399,5

Concentration: 32 mM

Use and Storage: Store at -20°C.

The SAM solution should be thawed on ice and added into the reaction mixture immediately before the incubation. For use in a methylation reaction the SAM should be diluted to a final concentration of 80 µM.

Quality Control: the SAM is tested in a reaction of methylation of phage λ DNA with DNA-methyltransferase M.HspAI (SE #003).

NEW New product

DNA Quick Ligation Kit NEW K006 20 reactions

Description: The Quick Ligation Kit includes:
1. Quick T4 DNA Ligase (recombinant, 2000 u/µl) - 20µl
2. 2 x Quick Ligation Buffer - 200µl (132 mM Tris-HCI (pH 7.6 at 25°C); 20 mM $MgCI_2$, 2 mM DTT; 2 mM ATP; 15% PEG 6000).

Mix thoroughly before use.

Ligation reaction protocol (for 20 µl)
1. Mix:
   H_2O - calculated quantity
   2 x Quick Ligation Buffer - 10µl
   Add DNA
   Add 1 µl Quick T4 DNA Ligase
2. Incubate at room temperature (+25°C) for 5 min.
3. Cool the mixture on ice, store at -20°C.

Notes on Use: Before the first use the 2 x SE-DNA Quick Ligation Buffer should be divided into small aliquots and store at -20°C.
Avoid defrosting this Buffer more than 2-3 times.
The Buffer can be stored at +4°C during 7 days.
The efficiency of ligation starts to decrease after 2 hours of incubation and is reduced by up to 75% if the reaction is allowed to go overnight at 25°C.
Before using the products of a Quick Ligation reaction for electrotransformation, it is necessary to reduce the PEG concentration.

Store at -20°C.

GLAD-PCR assay Kit NEW K009 200 reactions

GLAD-PCR assay Kit was developed for 200 GLAD-PCR reactions.
The following reagents are supplied with this product:
1. dH_2O -3 mL,
2. 10x SE TMN Buffer - 150 µL,
3. DMSO - 30 µL,
4. BSA, 10 mg/mL - 80 µL,
5. GlaI (10 u.a./µL) - 10 µL,
6. Universal adapter, double-stranded (10 µM) - 100 µL,
7. ATP, 10 mM - 100 µL,
8. DNA ligase T4 concentrated (2000 u.a./µL) - 10 µL,
9. 10X SE GLAD Buffer - 400 µL,
10. $MgCl_2$, 50 mM - 110 µL,
11. dNTP Mix, 10 mM each - 80 µL,
12. SP Taq DNA Polymerase, 5 u.a./µL - 15 µL,
13. Control DNA Raji, 9 ng/µL - 70 µL,
14. Control DNA HeLa, 9 ng/µL - 70 µL,
15. Control DNA Lambda, 9 ng/µL - 70 µL,
16. Control URB1 mix (oligonucleotide primers + TaqMan probe, 10 µM each) - 25 µL,
17. Control CEBPD mix (oligonucleotide primers + TaqMan probe, 10 µM each) - 25 µL

Additional services are available with GLAD-PCR assay Kit order:
1. Primers and TaqMan probes design (free for up to 3 RCGY sites);
2. Primes and TaqMan probes synthesis - price upon request;
3. GLAD PCR assay with synthesized oligonucleotides on selected DNA from malignant cell lines (see SE list of products) - price upon request.

Reaction requires:
1. DNA sample,
2. Genome primer and TaqMan probe designed for DNA region of interest,
3. Hybrid primer (includes constant part complimentary to universal adapter and tetranucleotide part which is complimentary to DNA at the point of GlaI hydrolysis). Hybrid primers may be ordered by request (see Cat.# K010/1, K010/3, K010/5 and K010/32).

All reagents for the control experiments are included.
The time of assay including DNA hydrolysis, ligation of the adaptor and PCR is less than 4 hours.

Store at -20°C.

NEW New product

GLAD-PCR assay Kit

NEW

K010/1
K010/3
K010/5
K010/32

200 reactions

GLAD-PCR assay Kit was developed for 200 GLAD-PCR reactions.
The following reagents are supplied with this product:
1. dH_2O -3 mL,
2. 10x SE TMN Buffer - 150 µL,
3. DMSO - 30 µL,
4. BSA, 10 mg/mL - 80 µL,
5. GlaI (10 u.a./µL) - 10 µL,
6. Universal adapter, double-stranded (10 µM) - 100 µL,
7. ATP, 10 mM - 100 µL,
8. DNA ligase T4 concentrated (2000 u.a./µL) - 10 µL,
9. 10X SE GLAD Buffer - 400 µL,
10. $MgCl_2$, 50 mM - 110 µL,
11. dNTP Mix, 10 mM each - 80 µL,
12. SP Taq DNA Polymerase, 5 u.a./µL - 15 µL,
13. Control DNA Raji, 9 ng/µL - 70 µL,
14. Control DNA HeLa, 9 ng/µL - 70 µL,
15. Control DNA Lambda, 9 ng/µL - 70 µL,
16. Control URB1 mix (oligonucleotide primers + TaqMan probe, 10 µM each) - 25 µL,
17. Control CEBPD mix (oligonucleotide primers + TaqMan probe, 10 µM each) - 25 µL
18. Hybrid primers[1] (10 µM) - 170 µL.

[1] - You can choose 1 (Cat.# K010/1), 3 (Cat.# K010/3), 5 (Cat.# K010/5) of hybrid primers, or order full range of 32 hybrid primers (Cat.# K010/1).
The list of 32 hybrid primers (HP1-HP32) you can find in the Instruction manual

Additional services are available with GLAD-PCR assay Kit order:
1. Primers and TaqMan probes design (free for up to 3 RCGY sites);
2. Primes and TaqMan probes synthesis - price upon request;
3. GLAD PCR assay with synthesized oligonucleotides on selected DNA from malignant cell lines (see SE list of products) - price upon request.

Reaction requires:
1. DNA sample,
2. Genome primer and TaqMan probe designed for DNA region of interest,
3. Hybrid primer (includes constant part complimentary to universal adapter and tetranucleotide part which is complimentary to DNA at the point of GlaI hydrolysis). Hybrid primers may be ordered by request. The Kits K010/1, K010/3, K010/5 and K010/32 includes 1, 3, 5 of chosen primers, or full range of 32 hybrid primers, respectively. The concentration of each hybrid primer is 10 µM, quantity - 170 µL. All reagents for the control experiments are included. The time of assay including DNA hydrolysis, ligation of the adaptor and PCR is less than 4 hours.

Store at -20°C.

Buffers composition

Cat #	Buffer	Contents ×1
B001	**B:**	10 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 1 mM DTT.
B002	**G:**	10 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 50 mM NaCl; 1 mM DTT.
B003	**O:**	50 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 100 mM NaCl; 1 mM DTT.
B004	**W:**	10 mM Tris-HCl (pH 8.5 at 25^{o} C); 10 mM $MgCl_2$; 100 mM NaCl; 1 mM DTT.
B005	**Y:**	33 mM Tris- acetate (pH 7.9 at 25^{o} C); 10 mM magnesium acetate; 66 mM potassium acetate; 1 mM DTT.
B006	**2W:**	20 mM Tris-HCl (pH 8.5 at 25^{o} C); 10 mM $MgCl_2$; 200 mM NaCl; 1 mM DTT.
B007	**K:**	10 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 100 mM KCl; 1 mM DTT.
B008	**2K**:	10 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 200 mM KCl; 1 mM DTT.
B009	**FaeI:**	33 mM Tris- acetate (pH 8.3 at 25^{o} C); 10 mM magnesium acetate; 66 mM potassium acetate; 1 mM DTT.
B010	**AbsI:**	10 mM Tris-HCl (pH 9.0 at 25^{o} C); 10 mM $MgCl_2$; 50 mM KCl; 1 mM DTT.
B020	**AoxI**	10 mM Tris-HCl (pH 7.5 at 25°C); 200 mM KCl; 0,1 mM EDTA, 7 mM 2-mercaptoethanol; 200 μg/ml BSA, 50% glycerol.
B011	**EcoRI:**	100 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 50 mM NaCl; 1 mM DTT.
B012	**BisI**	10 mM Tris-HCl (pH 9.0 at 25^{o} C); 10 mM $MgCl_2$; 150 mM KCl; 1 mM DTT.
B016	**MalI**	20 mM Tris-HCl (pH 9.0 at 25^{o} C); 10 mM $MgCl_2$; 150 mM NaCl; 1 mM DTT.
B017	**N·Bst9I:**	10 mM Tris-HCl (pH 8.5 at 25^{o} C); 10 mM $MgCl_2$; 150 mM KCl; 1 mM DTT.
B019	**PcsI**	10 mM Tris-HCl (pH 8.3 at 25^{o} C); 20 mM NaCl; 3 mM $MgCl_2$; 1 mM DTT.
B018	**RigI:**	10 mM Tris-HCl (pH 8.5 at 25^{o} C); 5 mM $MgCl_2$; 1 mM DTT
B021	**ROSE**	
B022	**GLAD:**	50 mM Tris-SO_4, pH 9.0 at 25°C; 30 mM KCl; 10 mM $[NH_4]_2SO_4$
B301	**T4-Polynucleotide Kinase**:	50 mM Tris-HCl (pH 7.6 at 25°C); 10 mM $MgCl_2$; 5 mM DTT.
B302	**T4-DNA Ligase**:	50 mM Tris-HCl (pH 7.8 at 25°C); 10 mM $MgCl_2$;10 mM DTT; 1 mM ATP. Storage conditions: by small portions avoiding repeated defrosting to prevent ÀTP decomposition.
B303	**T4-RNA Ligase:**	50 mM Tris-HCl (pH 7.8 at 25^{0}C); 10 mM $MgCl_2$; 10 mM DTT; 1 mM ATP.
B304	**DNA polymerase I E.coli (Klenow Fragment)**:	50 mM Tris-HCl (pH 7.6 at 25°C); 10 mM $MgCl_2$; 5 mM DTT.
B309	**Hot Start Taq DNA-polymerase:**	67 mM Tris-HCl (pH 8.8 at 25 C), 16.6 mM $(NH_4)_2SO_4$, 0.01 % Tween-20. Supplementary material is 50 mM $MgCl_2$.
B310	**Pfu DNA- polymerase:**	20 mM Tris-HCl (pH 8.8 at 25^{o} C), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100.
B321	**AS (Ammonium Sulfate):**	67 mM Tris-HCl (pH 8.8 at 25 C), 16.6 mM $(NH_4)_2SO_4$, 0.01 % Tween-20. Supplementary material is 50 mM $MgCl_2$.
B305	**Taq-DNA- polymerase , TaqSE-DNA- polymerase**:	60 mM Tris-HCl (pH 8.5 at 25°C); 1.5 mM $MgCl_2$; 25 mM KCl; 10 mM 2- mercaptoethanol; 0.1% Triton X-100.
B306	**Taq-DNA- polymerase (Mg^{2+} – free):**	60 mM Tris-HCl (pH 8.5 at 25°C); 25 mM KCl; 10 mM 2- mercaptoethanol; 0.1% Triton X-100.
B311	**T4- DNA- polymerase**:	67 mM Tris-HCl (pH 8.8 at 25°C); 6.7 mM $MgCl_2$; 16.7 mM $(NH_4)_2SO_4$; 1 mM DTT.
B319	**T7- RNA- polymerase**:	50 mM Tris-HCl (pH 7.5 at 25°C); 6 mM $MgCl_2$; 10 mM DTT; 2 mM spermidine.
B312	**M-MuLV reverse transcriptase**	50 mM Tris-HCl (pH 8.3 at 25°C); 75 mM KCl; 3 mM $MgCl_2$, 10 mM DTT.
B313	**Inorganic pyrophosphatase**	50 mM Tris-HCl (pH 8.5 at 25^{o} C); 1 mM $MgCl_2$
B314	**BAL-31 nuclease**:	20 мМ Tris-HCl (pH 8.0 at 25°C); 600 mM NaCl; 12 mM $CaCl_2$; 12 mM $MgCl_2$; 1 mM EDTA.
B315	**Mung Bean nuclease**	50 mM sodium acetate; 30 mM NaCl; 1 mM $ZnSO_4$; (pH 5.0 at 25°C).
B316	**Exonuclease III**:	50 mM Tris-HCl (pH 7.6 at 25°C); 1 mM $MgCl_2$.
B317	**Endonuclease I**:	20 mM Glycin -NaOH (pH 9.5 at 25^{o} C); 25 mM $MgCl_2$; 100mM NaCl; 1 mM 2- mercaptoethanol.
B318	**Uracil-DNA-glycosylase**:	20 mM Tris-HCl (pH 8.0 at 25^{o} C); 1 mM EDTA; 1 mM DTT.
B003	**Alkaline phosphatase**:	50 mM Tris-HCl (pH 7.6 at 25^{o} C); 10 mM $MgCl_2$; 100 mM NaCl; 1 mM DTT.

B100	**A (Storage and dilution buffer)** :	10 mM Tris-HCl (pH 7.6 at 25^{o} C); 50 mM KCl; 0,1 mM EDTA; 200 µg/ml BSA; 1 mM DTT; 50% glycerol.
B102	**M-MuLV Reverse Transcriptase dilution Buffer**	10 mM KH_2PO_4 (pH 7.5); 0,1 mM EDTA; 200 mM NaCl; 7 mM 2-mercaptoethanol; 50% glycerol
B307	**$MgCl_2$, 50 mM water solution**	50 mM $MgCl_2$; 500 µl
B101	**BSA (for Restrictases)**	10 mg/ml BSA. ; 500 µl

SE Buffer Activity Chart for Restriction Enzymes

Enzyme	Recognition sequence	SE Buffer	BSA	Activity (% from maximum)					Optimum t, ^{0}C	Inactivation,20min
				B	G	O	W	Y		
Aat II	GACGT^C	Y	-	10-25	25-50	10-25	25-50	100	37	65°C
Abs I	CC^TCGAGG	*	-	75-100	10-25	0	50-75	0-10	37	65°C
Acc16 I	TGC^GCA	W	-	50-75	75-100	25-50	100	75-100	37	65°C
Acc36 I	ACCTGC(4/8)	Y	-	25-50	25-50	50-75	50-75	100	37	65°C
Acc65 I	G^GTACC	W	-	10-25	25-50	75-100	100	10-25	37	65°C
AccB1 I	G^GYRCC	K	+	50-75	10-25	10-25	75-100	50-75	37	65°C
AccB7 I	CCANNNN^NTGG	G	-	10-25	100	25-50	50-75	50-75	37	65°C
AccBS I	GAG^CGG	Y	-	75-100	75-100	25-50	25-50	100	37	65°C
Acl I	AA^CGTT	Y	+	0-10	0-10	0-10	0-10	100	37	65°C
AclW I	GGATC(4/5)	Y	+	75-100	50-75	0-10	0-10	100	37	65°C
Aco I	Y^GGCCR	G	-	50-75	100	50-75	25-50	75-100	37	65°C
Acs I	R^AATTY	W	+	25-50	50-75	50-75	100	10-25	50	80°C
Acu I	CTGAAG(16/14)	Y+SAM	+	25-50	50-75	50-75	75-100	100	37	65°C
Afe I	AGC^GCT	Y	-	10-25	25-50	75-100	75-100	100	37	65°C
Ags I	TTS^AA	Y	+	75-100	50-75	10-25	10-25	100	37	65°C
Ahl I	A^CTAGT	B	+	100	75-100	25-50	25-50	75-100	37	No
Ajn I	^CCWGG	Y	-	25-50	10-25	10-25	25-50	100	55	65°C
Alu I	AG^CT	Y	-	75-100	75-100	10-25	50-75	100	37	65°C
AluB I	AG^CT	B	+	100	75-100	10-25	10-25	75-100	37	65°C
Ama87 I	C^YCGRG	W	+	10-25	50-75	75-100	100	0-10	37	65°C
Aox I	^PuG(5mC)Py	*	-	75-100	25-50	10-25	25-50	75-100	60	No
Apa I	GGGCC^C	Y	+	50-75	25-50	0-10	0-10	100	37	65°C
Ars I	(8/13)GAC(N)$_6$TTYG(11/6)	Y	+	0	0	0	0	100	30	65°C
AsiG I	A^CCGGT	O	-	10-25	25-50	100	75-100	10-25	37	65°C
AspA2 I	C^CTAGG	W	+	10-25	50-75	75-100	100	75-100	37	80°C
AspLE I	GCG^C	O	-	0-25	75-100	100	50-75	25-50	37	80°C
AspS9 I	G^GNCC	W	-	50-75	50-75	75-100	100	50-75	37	65°C
AsuC2 I	CC^SGG	Y	-	75-100	50-75	10-25	25-50	100	37	65°C
AsuHP I	GGTGA(8/7)	O	-	10-25	50-75	100	75-100	25-50	37	65°C
AsuNH I	G^CTAGC	Y	+	75-100	50-75	0-10	0-10	100	37	65°C
BamH I	G^GATCC	G	+	25-50	100	75-100	75-100	25-50	37	65°C
Bar I	(7/12)GAAG(N)$_6$TAC(12/7)	2K	-	0	0-10	25-50	50-75	10-25	37	65°C
Bbv12 I	GWGCW^C	O	-	0-10	10-25	100	75-100	10-25	37	80°C
Bgl I	GCCNNNN^NGGC	2W	-	50-75	50-75	0-10	75-100	25-50	37	65°C
Bgl II	A^GATCT	O	-	0-10	10-25	100	25-50	10-25	37	80°C
Bis I	G(5mC)^NGC	*	-	10-25	25-50	50-75	75-100	50-75	37	65°C
Bls I	PuPyN^PuPy	W	-	10-25	10-25	50-75	100	75-100	30	65°C
Bme18 I	G^GWCC	O	-	10-25	25-50	100	75-100	10-25	37	65°C
Bmt I	GCTAG^C	W	-	10-25	50-75	50-75	100	75-100	37	65°C
BmuI	ACTGGG(5/4)	Y	-	75-100	75-100	25-50	50-75	100	37	65°C
Bpm I	CTGGAG(16/14)	W	+	25-50	50-75	75-100	100	50-75	37	65°C
Bpu10 I	CC^TNAGC	K	-	10-25	25-50	50-75	50-75	25-50	37	80°C
Bpu14 I	TT^CGAA	G	-	50-75	100	25-50	25-50	75-100	37	65°C
Bsa29 I	AT^CGAT	G	+	25-50	100	50-75	50-75	75-100	37	65°C
Bsc4 I	CCNNNNN^NNGG	W	+	75-100	75-100	50-75	100	25-50	55	80°C
Bse1 I	ACTGG(1/-1)	Y	-	75-100	75-100	25-50	10-25	100	65	80°C
Bse118 I	R^CCGGY	O	-	0-10	50-75	100	75-100	25-50	65	80°C
Bse21 I	CC^TNAGG	Y	-	50-75	50-75	10-25	25-50	100	37	80°C
Bse3D I	GCAATG(2/0)	G	-	10-25	100	25-50	50-75	75-100	60	80°C
Bse8 I	GATNN^NNATC	G	-	25-50	100	75-100	75-100	50-75	60	80°C
BseP I	G^CGCGC	G	-	50-75	100	75-100	50-75	50-75	50	65°C
BseX3 I	C^GGCCG	O	-	10-25	25-50	100	50-75	10-25	50	80°C
BslF I	GGGAC(10/14)	Y	+	25-50	25-50	10-25	25-50	100	37	80°C
Bso31 I	GGTCTC(1/5)	O	+	25-50	75-100	100	75-100	25-50	55	80°C
Bsp13 I	T^CCGGA	2K	-	25-50	50-75	75-100	50-75	0-10	50	65°C
Bsp1720 I	GC^TNAGC	G	-	50-75	100	50-75	50-75	75-100	37	80°C
Bsp19 I	C^CATGG	2W	+	0-10	10-25	50-75	75-100	10-25	37	65°C

* - supplied with its own unique reaction buffer that is different from the five standard SE-Buffers.

SE Buffer Activity Chart for Restriction Enzymes

Enzyme	Recognition sequence	SE Buffer	BSA	Activity (% from maximum)					Optimum t,^{0}C	Inactivation,20min
				B	G	O	W	Y		
BspAC I	CCGC(-3/-1)	O	+	10-25	25-50	100	75-100	10-25	37	65°C
BspFN I	CG^CG	Y	-	50-75	75-100	75-100	50-75	100	37	65°C
BssEC I	C^CNNGG	Y	-	50-75	50-75	50-75	75-100	100	60	80°C
BssNA I	GTA^TAC	W	+	50-75	50-75	75-100	100	75-100	37	No
BssT1 I	C^CWWGG	2K	-	10-25	25-50	25-50	75-100	10-25	60	80°C
Bst2B I	CTCGTG(-5/-1)	Y	+	75-100	25-50	10-25	25-50	100	60	80°C
Bst2U I	CC^WGG	G	+	75-100	100	50-75	50-75	10-25	60	80°C
Bst4C I	ACN^GT	Y	-	75-100	75-100	10-25	25-50	100	65	80°C
Bst6 I	CTCTTC(1/4)	Y	+	75-100	75-100	50-75	75-100	100	65	80°C
BstAC I	GR^CGYC	W	-	75-100	75-100	50-75	100	75-100	37	80°C
BstAF I	C^TTAAG	W	+	10-25	25-50	75-100	100	25-50	55	80°C
BstAP I	GCANNNN^NTGC	W	-	25-50	25-50	75-100	100	25-50	60	80°C
BstAU I	T^GTACA	W	-	10-25	50-75	25-50	100	25-50	37	80°C
BstBA I	YAC^GTR	W	+	25-50	25-50	75-100	100	25-50	65	80°C
BstC8 I	GCN^NGC	Y	-	10-25	25-50	50-75	75-100	100	55	80°C
BstDE I	C^TNAG	G	-	75-100	100	25-50	50-75	10-25	60	80°C
BstDS I	C^CRYGG	Y	-	0-10	75-100	50-75	25-50	100	65	80°C
BstEN I	CCTNN^NNNAGG	Y	-	50-75	50-75	25-50	25-50	100	65	80°C
BstF5 I	GGATG(2/0)	Y	-	75-100	50-75	25-50	50-75	100	65	80°C
BstFN I	CG^CG	Y	-	75-100	50-75	25-50	25-50	100	60	80°C
BstH2 I	RGCGC^Y	Y	+	50-75	50-75	0-10	10-25	100	65	80°C
BstHH I	GCG^C	Y	+	75-100	50-75	25-50	50-75	100	50	No
BstKTI	GAT^C	W	-	25-50	50-75	75-100	100	50-75	37	65°C
BstMA I	GTCTC(1/5)	W	+	25-50	50-75	50-75	100	75-100	55	65°C
BstMB I	^GATC	O	-	10-25	25-50	100	75-100	10-25	65	80°C
BstMC I	CGRY^CG	B	+	100	75-100	10-25	10-25	50-75	50	80°C
BstMW I	GCNNNNN^NNGC	Y	-	10-25	25-50	25-50	50-75	100	55	80°C
BstNS I	RCATG^Y	B	+	100	50-75	10-25	10-25	75-100	37	65°C
BstPA I	GACNN^NNGTC	Y	-	50-75	25-50	50-75	50-75	100	65	No
BstSC I	^CCNGG	Y		50-75	50-75	50-75	50-75	100	55	80°C
BstSF I	C^TRYAG	O	+	75-100	25-50	100	50-75	50-75	60	No
BstSL I	GKGCM^C	G	+	50-75	100	50-75	75-100	75-100	55	65°C
BstSN I	TAC^GTA	B	-	100	50-75	0-10	10-25	50-75	37	80°C
BstV1I	GCAGC(8/12)	G	-	75-100	100	75-100	75-100	75-100	55	80°C
BstV2 I	GAAGAC(2/6)	Y	+	75-100	75-100	25-50	25-50	100	55	65°C
BstX I	CCANNNNN^NTGG	O	-	10-25	10-25	100	75-100	25-50	37	65°C
BstX2 I	R^GATCY	G	-	75-100	100	0-10	10-25	25-50	60	80°C
Bsu I	GTATCC(6/5)	Y	-	75-100	50-75	10-25	25-50	100	37	65°C
BsuR I	GG^CC	G	-	75-100	100	25-50	50-75	50-75	37	80°C
Btr I	CACGTC(-3/-3)	O	+	75-100	75-100	100	75-100	75-100	60	80°C
Cci I	T^CATGA	W	+	0-10	10-25	25-50	100	75-100	55	80°C
CciN I	GC^GGCCGC	Y	-	25-50	50-75	75-100	75-100	100	37	65°C
Dra I	TTT^AAA	G	+	75-100	100	25-50	75-100	75-100	37	65°C
Dra III	CACNNN^GTG	2K	+	25-50	50-75	75-100	75-100	50-75	37	65°C
Dri I	GACNNN^NNGTC	Y	-	75-100	75-100	10-25	10-25	100	37	65°C
DseD I	GACNNNN^NNGTC	Y	+	75-100	75-100	25-50	50-75	100	37	80°C
EcoICR I	GAG^CTC	G	+	75-100	100	0-10	0-10	75-100	37	65°C
EcoR I	G^AATTC	*	+	50-75	75-100	75-100	100	50-75	37	65°C
EcoR V	GAT^ATC	W	+	0-10	25-50	50-75	100	25-50	37	80°C
Ege I	GGC^GCC	B	+	100	75-100	10-25	50-75	75-100	37	65°C
Erh I	C^CWWGG	2W	+	10-25	25-50	25-50	75-100	10-25	37	65°C
Fae I	CATG^	*	+	25-50	50-75	10-25	10-25	75-100	37	65°C
Fai I	YA^TR	B	-	100	50-75	10-25	25-50	25-50	50	80°C
Fal I	(8/13)AAG(N)$_5$CTT(13/8)	W+SAM	-	0-10	25-50	75-100	100	50-75	37	65°C
Fat I	^CATG	G	-	10-25	100	25-50	10-25	50-75	55	65°C
Fau I	CCCGC(4/6)	B	-	100	25-50	0-10	0-10	50-75	55	65°C
FauND I	CA^TATG	Y	+	50-75	75-100	10-25	50-75	100	37	65°C
Fbl I	GT^MKAC	Y	-	50-75	75-100	0-10	50-75	100	55	80°C
Fok I	GGATG(9/13)	Y	-	50-75	50-75	25-50	25-50	100	37	65°C

* - supplied with its own unique reaction buffer that is different from the five standard SE-Buffers.

SE Buffer Activity Chart for Restriction Enzymes

Enzyme	Recognition sequence	SE Buffer	BSA	Activity (% from maximum)					Optimum t,^{0}C	Inactivation,20min
				B	G	O	W	Y		
FriO I	GRGCY^C	Y	+	75-100	75-100	10-25	0-10	100	37	65°C
Fsp4H I	GC^NGC	Y	-	50-75	75-100	10-25	25-50	100	37	65°C
Fbl I	GT^MKAC	Y	-	50-75	75-100	0-10	50-75	100	55	80°C
Fok I	GGATG(9/13)	Y	-	50-75	50-75	25-50	25-50	100	37	65°C
FriO I	GRGCY^C	Y	+	75-100	75-100	10-25	0-10	100	37	65°C
Fsp4H I	GC^NGC	Y	-	50-75	75-100	10-25	25-50	100	37	65°C
Gla I	Pu(5mC)^GPy	Y	-	75-100	75-100	25-50	25-50	100	30	65°C
Glu I	G(5mC)^NG(5mC)	Y	-	75-100	75-100	25-50	50-75	100	37	80°C
Gsa I	CCCAG^C	W	+	10-25	25-50	75-100	100	75-100	70	No
Hae III	GG^CC	G	-	75-100	100	25-50	50-75	50-75	37	80°C
Hga I	GACGC(5/10)	B	-	100	75-100	10-25	25-50	50-75	37	65°C
Hind II	GTY^RAC	G	+	75-100	100	25-50	25-50	75-100	37	65°C
Hind III	A^AGCTT	W	+	10-25	25-50	0-10	100	0-10	37	80°C
Hinf I	G^ANTC	O	-	25-50	75-100	100	75-100	75-100	37	80°C
Hpa I	GTT^AAC	Y	-	0-10	50-75	10-25	25-50	100	37	65°C
Hpa II	C^CGG	B	-	100	50-75	10-25	25-50	50-75	37	80°C
HpySE526I	A^CGT	Y	-	75-100	75-100	10-25	25-50	100	37	65°C
HspA I	G^CGC	Y	-	50-75	50-75	25-50	25-50	100	37	80°C
Kpn I	GGTAC^C	B	+	100	25-50	25-50	25-50	75-100	37	80°C
Kro I	G^C(5mC)GGC	G	-	50-75	100	25-50	50-75	75-100	37	65°C
Ksp22 I	T^GATCA	2K	+	50-75	100	50-75	50-75	25-50	37	65°C
Kzo9 I	^GATC	G	-	50-75	100	50-75	50-75	50-75	37	65°C
Lmn I	GCTCCN^	B	-	100	75-100	50-75	50-75	75-100	37	65°C
Mab I	A^CCWGGT	W	+	25-50	50-75	75-100	100	50-75	37	65°C
Mal I	G(mA)^TC	*	-	25-50	25-50	50-75	75-100	50-75	37	65°C
Mbo II	GAAGA(8/7)	Y	-	75-100	75-100	25-50	50-75	100	37	65°C
Mfe I	C^AATTG	B	+	100	75-100	10-25	25-50	75-100	37	No
Mhl I	GDGCH^C	W	-	10-25	25-50	75-100	100	10-25	37	80°C
Mlu I	A^CGCGT	O	-	0-10	10-25	100	25-50	10-25	37	65°C
Mly113 I	GG^CGCC	B	-	100	25-50	10-25	10-25	50-75	37	65°C
Mnl I	CCTC(7/6)	G	+	75-100	100	25-50	25-50	75-100	37	65ºC
Mox20 I	TGG^CCA	O	-	10-25	25-50	100	75-100	25-50	37	No
MroN I	G^CCGGC	B	-	100	50-75	10-25	0-10	10-25	37	80°C
MroX I	GAANN^NNTTC	W	-	50-75	50-75	50-75	100	25-50	37	65°C
Msp I	C^CGG	B	-	100	75-100	50-75	75-100	75-100	37	65°C
MspA1 I	CMG^CKG	Y	+	10-25	75-100	10-25	25-50	100	37	65°C
MspR9 I	CC^NGG	O	-	50-75	50-75	100	50-75	75-100	37	80°C
Mte I	G(5mC)G(5mC)^NG(5mC)G(5mC)	W	-	25-50	75-100	75-100	100	50-75	55	No
Nru I	TCG^CGA	W	-	0-10	10-25	75-100	100	10-25	37	80°C
PalA I	GG^CGCGCC	Y	-	25-50	10-25	0	0	100	37	65ºC
Pce I	AGG^CCT	Y	-	75-100	75-100	50-75	25-50	100	50	80°C
Pci I	A^CATGT	O	-	50-75	75-100	100	75-100	50-75	37	65°C
PciS I	GCTCTTC(1/4)	B	-	100	50-75	0-10	0-10	75-100	37	65°C
Pcs I	(5mC)GNNNNN^NN(5mC)G	*	-	50-75	25-50	0	10-25	50-75	37	65°C
Pct I	GAATGC(1/-1)	O	-	25-50	50-75	100	75-100	10-25	37	65°C
Pkr I	G(5mC)N^G(5mC)	Y	-	50-75	75-100	10-25	25-50	100	37	65°C
Ple19 I	CGAT^CG	Y	-	75-100	75-100	25-50	25-50	100	37	65°C
Pps I	GAGTC(4/5)	Y	+	50-75	10-25	0-10	25-50	100	37	65°C
Psi I	TTA^TAA	B	-	100	25-50	10-25	25-50	75-100	37	65°C
Psp124B I	GAGCT^C	G	-	75-100	100	10-25	0-10	75-100	37	80°C
Psp6I	^CCWGG	B	-	100	50-75	10-25	25-50	75-100	55	80°C
PspC I	CAC^GTG	B	+	100	50-75	0	0	50-75	37	65°C
PspE I	G^GTNACC	B	-	100	50-75	25-50	50-75	50-75	37	65°C
PspL I	C^GTACG	Y	+	75-100	75-100	25-50	10-25	100	37	65°C
PspN4 I	GGN^NCC	Y	-	10-25	10-25	10-25	25-50	100	37	65°C
PspOM I	G^GGCCC	Y	-	75-100	10-25	0-10	0-10	100	37	65°C
PspPP I	RG^GWCCY	Y	+	50-75	25-50	10-25	10-25	100	37	65°C
PspX I	VC^TCGAGB	Y	+	50-75	50-75	25-50	75-100	100	37	80°C
Psr I	(7/12)GAAC(N)$_6$TAC(12/7)	Y	+	10-25	10-25	0	0-10	100	30	65°C
Pst I	CTGCA^G	O	+	10-25	25-50	100	25-50	25-50	37	80°C

* - supplied with its own unique reaction buffer that is different from the five standard SE-Buffers.

SE Buffer Activity Chart for Restriction Enzymes

Enzyme	Recognition sequence	SE Buffer	BSA	Activity (% from maximum)					Optimum t,^{0}C	Inactivation,20min
				B	G	O	W	Y		
PstN I	CAGNNN^CTG	Y	-	75-100	50-75	10-25	25-50	100	37	65°C
Pvu II	CAG^CTG	G	+	25-50	100	25-50	25-50	25-50	37	80°C
Rga I	GCGAT^CGC	Y	-	75-100	50-75	10-25	25-50	100	55	80°C
Rig I	GGCCGG^CC	*	+	75-100	50-75	0-10	10-25	50-75	37	65°C
Rsa I	GT^AC	B	-	100	50-75	0-10	50-75	75-100	37	80°C
RsaN I	G^TAC	B	-	100	75-100	50-75	50-75	75-100	37	80°C
Rsr2 I	CG^GWCCG	Y	+	50-75	75-100	0-10	10-25	100	37	65°C
Sal I	G^TCGAC	O	-	0-10	10-25	100	25-50	0-10	37	65°C
Sbf I	CCTGCA^GG	Y	-	75-100	50-75	0-10	0-10	100	37	80°C
Set I	ASST^	Y	-	25-50	25-50	75-100	75-100	100	55	80°C
SfaN I	GCATC(5/9)	O	-	10-25	25-50	100	75-100	0-10	37	80°C
Sfi I	GGCCNNNN^NGGCC	G	+	75-100	100	25-50	25-50	25-50	50	65°C
Sfr274 I	C^TCGAG	B	-	100	75-100	50-75	50-75	75-100	50	65°C
Sfr303 I	CCGC^GG	B	-	100	50-75	10-25	10-25	75-100	37	65°C
Sma I	CCC^GGG	Y	-	0-10	0-10	0-10	0-10	100	25	65°C
Smi I	ATTT^AAAT	O	+	25-50	25-50	100	75-100	25-50	37	65°C
SmiM I	CAYNN^NNRTG	W	-	10-25	10-25	75-100	100	10-25	37	65°C
Sph I	GCATG^C	G	+	25-50	100	75-100	75-100	50-75	37	65°C
Sse9 I	^AATT	B	+	100	75-100	50-75	50-75	75-100	55	65°C
Ssp I	AAT^ATT	K	+	75-100	50-75	25-50	50-75	75-100	37	65°C
SspM I	C^TAG	Y	-	50-75	25-50	10-25	50-75	100	55	No
Taq I	T^CGA	Y	+	50-75	75-100	75-100	50-75	100	65	80°C
Tru9 I	T^TAA	W	-	75-100	25-50	25-50	100	50-75	65	80°C
TseF I	^GTSAC	B	-	100	50-75	0-10	25-50	50-75	65	No
Tth111 I	GACN^NNGTC	Y	-	75-100	50-75	10-25	10-25	100	65	80°C
Vne I	G^TGCAC	O	-	10-25	25-50	100	25-50	25-50	37	65°C
Vsp I	AT^TAAT	W		0-10	10-25	50-75	100	25-50	37	65°C
Xba I	T^CTAGA	O	+	75-100	75-100	100	50-75	75-100	37	65°C
Xma I	C^CCGGG	Y	-	75-100	50-75	0	0-10	100	37	65°C
Zra I	GAC^GTC	B	-	100	50-75	25-50	25-50	75-100	37	80°C
Zrm I	AGT^ACT	Y	+	50-75	25-50	0-10	0-10	100	37	65°C
Zsp2 I	ATGCA^T	B	+	100	50-75	25-50	25-50	25-50	37	65°C
N·Bst9 I	GAGTC(4/-)	*	-	10-25	75-100	100	100	50-75	55	80°C

* - supplied with its own unique reaction buffer that is different from the five standard SE-Buffers.

Isoschizomers

Enzyme	SE Enzyme
AanI	PsiI
AasI	DseDI
AatI	PceI
AatII	AatII
AbsI	AbsI
AccI	FblI
AccII	BstFNI
AccIII	Bsp13I
Acc16I	Acc16I
Acc36I	Acc36I
Acc65I	Acc65I
	KpnI^
AccB1I	AccB1I
AccB7I	AccB7I
AccBSI	AccBSI
AciI	BspACI
AclI	AclI
AclWI	AclWI
AcoI	AcoI
AcsI	AcsI
AcuI	AcuI
AcvI	PspCI
AcyI	BstACI
AdeI	DraIII
AfaI	RsaI
AfeI	AfeI
AflII	BstAFI
AgeI	AsiGI
AgsI	AgsI
AhaIII	DraI
AhdI	DriI
AhlI	AhlI
AjnI	AjnI
	Bst2UI^
	Psp6I
AluI	AluI
	AluBI
AluBI	AluBI
AlwI	AclWI
Alw21I	Bbv12I
Alw26I	BstMAI
Alw44I	VneI
AlwNI	PstNI
Ama87I	Ama87I
Aor51HI	AfeI
AoxI	AoxI
ApaI	ApaI
	PspOMI^
ApaBI	BstAPI^
ApaLI	VneI
ApoI	AcsI
ArsI	ArsI
AscI	PalAI
AseI	VspI
AsiGI	AsiGI
AsiSI	AsiSI
AspI	Tth111I
Asp700I	MroXI
Asp718I	Acc65I
	KpnI^
AspA2I	AspA2I
AspEI	DriI
AspHI	Bbv12I
AspLEI	AspLEI
	BstHHI
	HspAI^
AspS9I	AspS9I
AsuI	AspS9I
AsuII	Bpu14I
AsuC2I	AsuC2I
AsuHPI	AsuHPI
AsuNHI	AsuNHI
	BmtI^
AvaI	Ama87I
AvaII	Bme18I
AvaIII	Zsp2I^
AviII	Acc16I
AvrII	AspA2I
AxyI	Bse21I
BalI	Mox20I
BamHI	BamHI
BanI	AccB1I
BanII	FriOI
BanIII	Bsa29I
BarI	BarI
BbeI	EgeI^
	Mly113I^^
BbrPI	PspCI
BbsI	BstV2I
BbuI	SphI
BbvI	BstV1I
BbvII	BstV2I
Bbv12I	Bbv12I
BclI	Ksp22I
BciVI	BsuI
BcnI	AsuC2I
BcuI	AhlI
BfiI	BmuI
BfmI	BstSFI
BfrI	BstAFI
BfrBI	Zsp2I^
BfuI	BsuI
BfuAI	Acc36I
BfuCI	BstMBI
	BstKTI^
	Kzo9I
BglI	BglI
BglII	BglII
BinI	AclWI
BisI	BisI
BlnI	AspA2I
BlpI	Bsp1720I
BlsI	BlsI
Bme18I	Bme18I
Bme1390I	MspR9I
	BstSCI^
BmgBI	BtrI
BmtI	BmtI
	AsuNHI^
BmyI	MhlI
BoxI	BstPAI
BpiI	BstV2I
BpmI	BpmI
Bpu10I	Bpu10I
Bpu14I	Bpu14I
Bpu1102I	Bsp1720I
BpuAI	BstV2I
BsaI	Bso31I
Bsa29I	Bsa29I
BsaAI	BstBAI
BsaBI	Bse8I
BsaHI	BstACI
BsaJI	BssECI
BsaMI	PctI
Bsc4I	Bsc4I
Bse1I	Bse1I
Bse8I	Bse8I
Bse21I	Bse21I
Bse118I	Bse118I
BseAI	Bsp13I
BseBI	AjnI^
	Bst2UI
	Psp6I^
BseCI	Bsa29I
BseDI	BssECI
Bse3DI	Bse3DI
BseGI	BstF5I
BseGI	FokI^
BseJI	Bse8I
BseLI	Bsc4I
BseMI	Bse3DI
BseNI	Bse1I
BsePI	BsePI
BseSI	BstSLI
BseXI	BstV1I
BseX3I	BseX3I
BseYI	GsaI
Bsh1236I	BstFNI
Bsh1285I	BstMCI
BshFI	BsuRI
	HaeIII
BshNI	AccB1I
BshTI	AsiGI
BsiI	Bst2BI
BsiEI	BstMCI
BsiHKAI	Bbv12I
BsiHKCI	Ama87I
BsiSI	HpaII
	MspI
BsiWI	PspLI
BsiYI	Bsc4I
BslI	Bsc4I
BslFI	BslFI
BsmFI	BslFI^
BsmI	PctI
BsmAI	BstMAI
Bso31I	Bso31I
BsoBI	Ama87I
BsoMAI	BstMAI
Bsp13I	Bsp13I
Bsp19I	Bsp19I
Bsp68I	NruI
Bsp106I	Bsa29I
Bsp119I	Bpu14I
Bsp120I	PspOMI
	ApaI^
Bsp143I	BstMBI
	Kzo9I
	BstKTI^
Bsp143II	BstH2I
Bsp1286I	MhlI
Bsp1407I	BstAUI
Bsp1720I	Bsp1720I
BspACI	BspACI
BspANI	BsuRI
	HaeIII
BspCI	Ple19I
BspDI	Bsa29I
BspEI	Bsp13I
BspFNI	BspFNI
BspHI	CciI
BspLI	PspN4I
BspLU11I	PciI
BspMI	Acc36I
BspMII	Bsp13I
BspMAI	PstI
BspPI	AclWI
BspTI	BstAFI
BspT104I	Bpu14I
BspT107I	AccB1I
BspTNI	Bso31I
BspXI	Bsa29I
BsrI	Bse1I
BsrBI	AccBSI
BsrDI	Bse3DI
BsrFI	Bse118I
BsrGI	BstAUI
BsrSI	Bse1I
BssAI	Bse118I
BssECI	BssECI
BssHI	Sfr274I
BssHII	BsePI
BssKI	MspR9I^
	BstSCI
BssNAI	BssNAI
BssSI	Bst2BI
BssT1I	BssT1I
	ErhI
Bst6I	Bst6I
Bst98I	BstAFI
Bst1107I	BssNAI
BstACI	BstACI
BstAFI	BstAFI
BstAPI	BstAPI
BstAUI	BstAUI
BstBI	Bpu14I
Bst2BI	Bst2BI
BstBAI	BstBAI
Bst4CI	Bst4CI
BstC8I	BstC8I
BstDEI	BstDEI
BstDSI	BstDSI
BstEII	PspEI
BstENI	BstENI
BstF5I	BstF5I
	FokI^
BstFNI	BstFNI
BstH2I	BstH2I
BstHHI	BstHHI
	AspLEI
	HspAI^
BstKTI	BstKTI
	BstMBI^
	Kzo9I^
BstMBI	BstKTI^
	BstMBI
	Kzo9I
BstMCI	BstMCI
BstMWI	BstMWI
BstNI	AjnI^
	Bst2UI
	Psp6I^
BstNSI	BstNSI
BstOI	AjnI^
	Bst2UI
	Psp6I^
BstPI	PspEI
BstPAI	BstPAI
BstSCI	BstSCI
	MspR9I^
BstSFI	BstSFI
BstSLI	BstSLI
BstSNI	BstSNI
BstUI	BstFNI
Bst2UI	AjnI^
	Bst2UI
	Psp6I^
BstV1I	BstV1I
BstV2I	BstV2I
BstXI	BstXI
BstX2I	BstX2I
BstYI	BstX2I
BstZI	BseX3I
BstZ17I	BssNAI
BsuI	BsuI
Bsu15I	Bsa29I
Bsu36I	Bse21I
BsuRI	BsuRI
	HaeIII
BsuTUI	Bsa29I
BtgI	BstDSI
BtrI	BtrI
BveI	Acc36I
Cac8I	BstC8I
CaiI	PstNI
CauII	AsuC2I
CciI	CciI
CciNI	CciNI
CelII	Bsp1720I
CfoI	AspLEI
	BstHHI
	HspAI^
CfrI	AcoI
Cfr9I	XmaI
	SmaI^
Cfr10I	Bse118I
Cfr13I	AspS9I
Cfr42I	Sfr303I
ClaI	Bsa29I
CpoI	Rsr2I
CspI	Rsr2I
Csp6I	RsaI^
Csp45I	Bpu14I
CspAI	AsiGI
CviAII	FaeI^^
	FatI^
DdeI	BstDEI
DpnI	MalI
DpnII	BstMBI
	Kzo9I
	BstKTI^
DraI	DraI
DraIII	DraIII
DrdI	DseDI
DriI	DriI
DsaI	BstDSI
DseDI	DseDI
EaeI	AcoI
EagI	BseX3I
Eam1104I	Bst6I
Eam1105I	DriI
EarI	Bst6I
Ecl136II	EcoICRI
	Psp124BI^
EclHKI	DriI
EclXI	BseX3I
Eco24I	FriOI
Eco31I	Bso31I
Eco32I	EcoRV
Eco47I	Bme18I
Eco47III	AfeI
Eco52I	BseX3I
Eco57I	AcuI
Eco72I	PspCI
Eco81I	Bse21I
Eco88I	Ama87I
Eco91I	PspEI
Eco105I	BstSNI
Eco130I	BssT1I
	ErhI

Isoschizomers

Enzyme	SE Enzyme
Eco147I	PceI
EcoICRI	EcoICRI
	Psp124BI^
EcoNI	BstENI
EcoO65I	PspEI
EcoRI	EcoRI
EcoRII	AjnI
	Bst2UI^
	Psp6I
EcoRV	EcoRV
EcoT14I	BssT1I
	ErhI
EcoT22I	Zsp2I
EcoT38I	FriOI
EgeI	EgeI
	Mly113I^
EheI	EgeI
	Mly113I^
ErhI	BssT1I
	ErhI
EspI	Bsp1720I
FaeI	FaeI
FaiI	FaiI
FalI	FalI
FatI	FaeI^
	FatI
FauI	FauI
FauNDI	FauNDI
FbaI	Ksp22I
FblI	FblI
FinI	BslFI
FnuDII	BspFNI
	BstFNI
Fnu4HI	Fsp4HI
FokI	FokI
	BstF5I^
FriOI	FriOI
FseI	RigI
FspI	Acc16I
Fsp4HI	Fsp4HI
FunI	AfeI
FunII	EcoRI
Gla I	Gla I
GluI	GluI
GsaI	GsaI
GsuI	BpmI
HaeII	BstH2I
HaeIII	BsuRI
	HaeIII
HapII	HpaII
	MspI
HgaI	HgaI
HgiAI	Bbv12I
HgiCI	AccB1I
HgiJII	FriOI
HhaI	AspLEI
	BstHHI
	HspAI^
Hin1I	BstACI
Hin6I	AspLEI^
	BstHHI^
	HspAI
HinP1I	AspLEI^
	BstHHI^
	HspAI

Enzyme	SE Enzyme
HincII	HindII
HindII	HindII
HindIII	HindIII
HinfI	HinfI
HpaI	HpaI
HpaII	HpaII
	MspI
HphI	AsuHPI
HpyCH4III	Bst4CI
HpyF10VI	BstMWI
HpySE526I	HpySE526I
Hsp92I	BstACI
Hsp92II	FatI^
HspAI	AspLEI^
	BstHHI^
	HspAI
ItaI	Fsp4HI
KasI	EgeI^
	Mly113I^^
KpnI	KpnI
	Acc65I^
Kpn2I	Bsp13I
KroI	KroI
KspI	Sfr303I
Ksp22I	Ksp22I
Ksp632I	Bst6I
KspAI	HpaI
Kzo9I	BstMBI
	Kzo9I
	BstKTI^
Lmn I	Lmn I
LweI	SfaNI
MabI	MabI
Mae I	SspMI
MaeII	HpySE526I
MalI	MalI
MamI	Bse8I
MbiI	AccBSI
MboI	BstMBI
	Kzo9I
	BstKTI^
MboII	MboII
McrI	BstMCI
MfeI	MfeI
MflI	BstX2I
MhlI	MhlI
MlsI	Msp20I
MluI	MluI
MluNI	Msp20I
MlyI	PpsI^
Mly113I	EgeI^
	Mly113I
MnlI	MnlI
Mox20I	Mox20I
Mph1103I	Zsp2I
MroI	Bsp13I
MroNI	MroNI
MroXI	MroXI
MscI	Msp20I
MseI	Tru9I
MslI	SmiMI
MspI	HpaII
	MspI
MspA1I	MspA1I
MspCI	BstAFI
MspR9I	BstSCI^
	MspR9I
MstI	Acc16I
MteI	MteI
MunI	MfeI

Enzyme	SE Enzyme
MvaI	AjnI^
	Bst2UI
	Psp6I^
Mva1269I	PctI
MvnI	BstFNI
MwoI	BstMWI
NaeI	MroNI^
NarI	EgeI^
	Mly113I
NciI	AsuC2I
NcoI	Bsp19I
NdeI	FauNDI
NdeII	BstMBI
	Kzo9I
	BstKTI^
NgoMIV	MroNI
NheI	AsuNHI
	BmtI^
NlaIII	FaeI
	FatI^
NlaIV	PspN4I
NotI	CciNI
NruI	NruI
NsbI	Acc16I
NsiI	Zsp2I
NspI	BstNSI
NspIII	Ama87I
NspV	Bpu14I
NspBII	MspA1I
PaeI	SphI
PaeR7I	Sfr274I
PalI	BsuRI
	HaeIII
PauI	BsePI
PceI	PceI
PciI	PciI
PciSI	PciSI
PcsI	PcsI
PctI	PctI
PdiI	MroNI^
PdmI	MroXI
Pfl23II	PspLI
PflBI	AccB7I
PflFI	Tth111I
PflMI	AccB7I
PhoI	BsuRI
	HaeIII
PinAI	AsiGI
PkrI	PkrI
PleI	PpsI
Ple19I	Ple19I
PmaCI	PspCI
PmlI	PspCI
PpsI	PpsI
PpuMI	PspPPI
PpuXI	PspPPI
PshAI	BstPAI
PshBI	VspI
PsiI	PsiI
Psp5II	PspPPI
Psp6I	AjnI
	Bst2UI^
	Psp6I
Psp1406I	AclI
PspAI	SmaI^
	XmaI

Enzyme	SE Enzyme
Psp124BI	EcoICRI^
	Psp124BI
PspCI	PspCI
PspEI	PspEI
PspGI	AjnI
	Bst2UI^
	Psp6I
PspLI	PspLI
PspN4I	PspN4I
PspOMI	ApaI^
	PspOMI
PspPI	AspS9I
PspPPI	PspPPI
PspXI	PspXI
PsrI	PsrI
PstI	PstI
PsuI	BstX2I
PsyI	Tth111I
PvuI	Ple19I
PvuII	PvuII
RgaI	RgaI
RigI	RigI
RsaI	RsaI
	RsaNI^
RsaNI	RsaNI
RsrII	Rsr2I
Rsr2I	Rsr2I
SacI	EcoICRI^
	Psp124BI
SacII	Sfr303I
SalI	SalI
SapI	PciSI
SatI	Fsp4HI
SauI	Bse21I
Sau96I	AspS9I
Sau3AI	BstMBI
	Kzo9I
	BstKTI^
SbfI	SbfI
ScaI	ZrmI
SchI	PpsI^
ScrFI	BstSCI^
	MspR9I
SdaI	SbfI
SduI	MhlI
SecI	BssECI
SetI	SetI
SexAI	MabI
SfaNI	SfaNI
SfcI	BstSFI
SfeI	BstSFI
SfiI	SfiI
SfoI	EgeI
	Mly113I^
Sfr274I	Sfr274I
Sfr303I	Sfr303I
SfuI	Bpu14I
SgfI	AsiSI
	RgaI
SgrBI	Sfr303I
SinI	Bme18I
SlaI	Sfr274I
SmaI	SmaI
	XmaI^
SmiI	SmiI
SmiMI	SmiMI
SmuI	FauI

Enzyme	SE Enzyme
SnaI	BssNAI
SnaBI	BstSNI
SpaHI	SphI
SpeI	AhlI
SphI	SphI
SplI	PspLI
Sse9I	Sse9I
Sse8387I	SbfI
SseBI	PceI
SsiI	BspACI
SspI	SspI
SspBI	BstAUI
SspMI	SspMI
SstI	EcoICRI^
	Psp124BI
StuI	PceI
StyI	BssT1I
	ErhI
StyD4I	BstSCI
	MspR9I^
SunI	PspLI
SwaI	SmiI
TaaI	Bst4CI
Taq I	TaqI
TasI	Sse9I
TelI	Tth111I
TliI	Sfr274I
Tru1I	Tru9I
Tru9I	Tru9I
TseFI	TseFI
Tsp45I	TseFI
Tsp4CI	Bst4CI
Tsp509I	Sse9I
TspEI	Sse9I
Tth111I	Tth111I
Van91I	AccB7I
VneI	VneI
VpaK11BI	Bme18I
VspI	VspI
XagI	BstENI
XapI	AcsI
XbaI	XbaI
XceI	BstNSI
XhoI	Sfr274I
XhoII	BstX2I
XmaI	SmaI^
	XmaI
XmaIII	BseX3I
XmaCI	SmaI^
	XmaI
XmaJI	AspA2I
XmiI	FblI
XmnI	MroXI
ZhoI	Bsa29I
ZraI	AatII^
	ZraI
ZrmI	ZrmI
Zsp2I	Zsp2I

Alphabetized List of SE Recognition Sequences

AA^CGTT	Acl I	C^CGG	Hpa II	GACNN^NNGTC	BstPA I
A^AGCTT	Hind III	C^CGG	Msp I	GACNNN^NNGTC	Dri I
(8/13)AAGN$_5$CTT(13/8)	Fal I	CC^NGG	MspR9 I	GACNNNN^NNGTC	DseD I
AAT^ATT	Ssp I	^CCNGG	BstSC I	(8/13)GAC(N)$_6$TTYG(11/6)	Ars I
^AATT	Sse9 I	C^CNNGG	BssEC I	(5/4)GACTC	Pps I
A^CATGT	Pci I	CCNNNNN^NNGG	Bsc4 I	(5/1)GAGAC	BstMA I
A^CCGGT	AsiG I	C^CRYGG	BstDS I	(5/1)GAGACC	Bso31 I
ACCTGC(4/8)	Acc36 I	CC^SGG	AsuC2 I	GAG^CGG	AccBS I
A^CCWGGT	Mab I	C^CTAGG	AspA2 I	GAG^CTC	EcoICR I
A^CGCGT	Mlu I	CCTC(7/6)	MnlI	GAGCT^C	Psp124B I
A^CGT	HpySE526I	CC^TCGAGG	Abs I	(6/7)GAGG	Mnl I
ACN^GT	Bst4C I	CCTGCA^GG	Sbf I	GAGTC(4/5)	Pps I
A^CTAGT	Ahl I	CC^TNAGC	Bpu10 I	G^ANTC	Hinf I
ACTGG(1/-1)	Bse1 I	CC^TNAGG	Bse21 I	GAT^ATC	EcoR V
ACTGGG(5/4)	Bmu I	CCTNN^NNNAGG	BstEN I	G(mA)^TC	Mal I
A^GATCT	Bgl II	^CCWGG	Psp6 I	^GATC	BstMB I
AGC^GCT	Afe I	CC^WGG	Bst2U I	^GATC	Kzo9 I
AG^CT	Alu I	C^CWWGG	BssT1 I	GAT^C	BstKTI
AG^CT	AluB I	C^CWWGG	Erh I	(5/4)GATCC	AclW I
AGG^CCT	Pce I	CGAT^CG	Ple19 I	(9/5)GATGC	SfaN I
AGG^CGG	AccBS I	CG^CG	BspFN I	GATNN^NNATC	Bse8 I
AGT^ACT	Zrm I	CG^CG	BstFN I	GCAATG(2/0)	Bse3D I
ASST^	Set I	C^GGCCG	BseX3 I	GCAGC(8/12)	BstV1 I
AT^CGAT	Bsa29 I	CG^GWCCG	Rsr2 I	(8/4)GCAGGT	Acc36 I
ATGCA^T	Zsp2 I	(5mC)GNNNNN^NN(5mC)G	Pcs I	GCANNNN^NTGC	BstAP I
AT^TAAT	Vsp I	CGRY^CG	BstMC I	GCATC(5/9)	SfaN I
ATTT^AAAT	Smi I	C^GTACG	PspL I	GCATG^C	Sph I
C^AATTG	Mfe I	CMG^CKG	MspA I	(-1/1)GCATTC	Pct I
C^ACGAG	Bst2B I	C^TAG	SspM I	G^CCGGC	MroN I
CAC^GTC	Btr I	(14/16)CTCCAG	Bpm I	GCCNNNN^NGGC	Bgl I
CAC^GTG	PspC I	C^TCGAG	Sfr274 I	GCGAT^CGC	AsiS I
CACNNN^GTG	Dra III	C^TCGTG	Bst2B I	GCGAT^CGC	Rga I
CAG^CTG	Pvu II	CTCTTC(1/4)	Bst6 I	G^CGC	HspA I
CAGNNN^CTG	PstN I	CTGAAG(16/14)	Acu I	GCG^C	AspLE I
CA^TATG	FauND I	CTGCA^G	Pst I	GCG^C	BstHH I
(13/9)CATCC	Fok I	CTGGAG(16/14)	Bpm I	G^CGCGC	BseP I
(0/2)CATCC	BstF5 I	C^TNAG	BstDE I	G(5mC)G(5mC)^NG(5mC)G(5mC)	Mte I
CATG^	Fae I	C^TRYAG	BstSF I	(-1/-3)GCGG	BspAC I
^CATG	Fat I	C^TTAAG	BstAF I	GC^GGCCGC	CciN I
(0/2)CATTGC	Bse3D I	(14/16)CTTCAG	Acu I	(6/4)GCGGG	Fau I
CAYNN^NNRTG	SmiM I	C^YCGRG	Ama87 I	(10/5)GCGTC	Hga I
(-1/1)CCAGT	Bse1 I	(7/12)GAAC(N)$_6$TAC(12/7)	Psr I	GC^NGC	Fsp4H I
CCANNNN^NTGG	AccB7 I	GAAGAC(2/6)	BstV2 I	G^C(5mC)GGC	Kro I
CCANNNNN^NTGG	BstX I	(4/1)GAAGAG	Bst6 I	G(5mC)^NGC	Bis I
C^CATGG	Bsp19 I	(4/1)GAAGAGC	PciS I	G(5mC)N^G(5mC)	Pkr I
CCCAG^C	Gsa I	(7/12)GAAG(N)$_6$TAC(12/7)	Bar I	G(5mC)^NG(5mC)	GluI
(4/5)CCCAGT	Bmu I	GAANN^NNTTC	MroX I	GCN^NGC	BstC8 I
CCCGC(4/6)	Fau I	GAATGC(1/-1)	Pct I	GCNNNNN^NNGC	BstMW I
CCC^GGG	Sma I	G^AATTC	EcoR I	G^CTAGC	AsuNH I
C^CCGGG	Xma I	GACGC(5/10)	Hga I	GCTAG^C	Bmt I
CCGC(-3/-1)	BspAC I	GACGT^C	Aat II	GCTCCN^	Lmn
CCG^CCT	AccBS I	GAC^GTC	Zra I	GCTCTTC(1/4)	PciS I
CCGC^GG	Sfr303 I	GAC^GTG	Btr I	(12/8)GCTGC	BstV1 I
		GACN^NNGTC	Tth111 I		

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

Alphabetized List of SE Recognition Sequences

G^CTGGG	Gsa I	G^GYRCC	AccB1 I	R^GATCY	BstX2 I
GC^TNAGC	Bst1720 I	GKGCM^C	BstSL I	RGCGC^Y	BstH2 I
GC^TNAGG	Bpu10 I	GR^CGYC	BstAC I	RG^GWCCY	PspPP I
GDGCH^C	Mhl I	GRGCY^C	FriO I	TAC^GTA	BstSN I
GGATC(4/5)	AclW I	GT^AC	Rsa I	(7/8)TCACC	AsuHP I
G^GATCC	BamH I	G^TAC	RsaN I	T^CATGA	Cci I
GGATG(2/0)	BstF5 I	(7/12)GTA(N)$_6$CTTC(12/7)	Bar I	T^CCGGA	Bsp13 I
GGATG(9/13)	Fok I	(7/12)GTA(N)$_6$GTTC(12/7)	Psr I	T^CGA	Taq I
GG^CC	BsuR I	GTA^TAC	BssNA I	TCG^CGA	Nru I
GG^CC	Hae III	GTATCC(6/5)	Bsu I	T^CTAGA	Xba I
GGCCGG^CC	Rig I	(14/10)GTCCC	BslF I	T^GATCA	Ksp22 I
GGCCNNNN^NGGCC	Sfi I	G^TCGAC	Sal I	TGC^GCA	Acc16 I
GG^CGCC	Mly113 I	GTCTC(1/5)	BstMA I	TGG^CCA	Mox20 I
GGC^GCC	Ege I	(6/2)GTCTTC	BstV2 I	T^GTACA	BstAU I
GG^CGCGCC	PalA I	G^TGCAC	Vne I	T^TAA	Tru9 I
GGGAC(10/14)	BslF I	GT^MKAC	Fbl I	TTA^TAA	Psi I
G^GGCCC	PspOM I	^GTSAC	TseF I	TT^CGAA	Bpu14 I
GGGCC^C	Apa I	GTT^AAC	Hpa I	TTS^AA	Ags I
G^GNCC	AspS9 I	GTY^RAC	Hind II	TTT^AAA	Dra I
GGN^NCC	PspN4 I	GWGCW^C	Bbv12 I	VC^TCGAGB	PspX I
G^GTACC	Acc65 I	Pu(5mC)^GPy	Gla I	YAC^GTR	BstBA I
GGTAC^C	Kpn I	^PuG(5mC)Py	Aox I	YA^TR	Fai I
GGTCTC(1/5)	Bso31 I	PuPyN^PuPy	Bls I	Y^GGCCR	Aco I
GGTGA(8/7)	AsuHP I	R^AATTY	Acs I		
G^GTNACC	PspE I	RCATG^Y	BstNS I		
G^GWCC	Bme18 I	R^CCGGY	Bse118 I		

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