Restriction Endonucleases

TECHNICAL GUIDE

UPDATE 2017/18

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Cut Smarter *with* Restriction Enzymes *from* NEB®

Looking to bring CONVENIENCE to your workflow?

Simplify Reaction Setup and Double Digestion with CutSmart® Buffer

Over 210 restriction enzymes are 100% active in a single buffer, CutSmart Buffer, making it significantly easier to set up your double digest reactions. Since CutSmart Buffer includes BSA, there are fewer tubes and pipetting steps to worry about. Additionally, many DNA modifying enzymes are 100% active in CutSmart Buffer, eliminating the need for subsequent purification.

For more information, visit **www.NEBCutSmart.com**

Speed up Digestions with Time-Saver™ Qualified Restriction Enzymes

> 190 of our restriction enzymes are able to digest DNA in 5–15 minutes, and can safely be used overnight with no loss of sample. For added convenience and flexibility, most of these are supplied with CutSmart Buffer.

For more information, visit **www.neb.com/timesaver**

Bring Flexibility to your Workflow

NEB offers the largest selection of restriction enzymes commercially available. With an evergrowing list to choose from, currently at 285 enzymes – including traditional restriction enzymes, nicking endonucleases, homing endonucleases and methylation-sensitive enzymes for epigenetics studies.

Improve your analysis with our Purple Gel Loading Dye

Our Gel Loading Dye, Purple (6X), which is supplied with most restriction enzymes and all HF enzymes, sharpens bands and eliminates the UV shadow seen with other dyes. This solution contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.

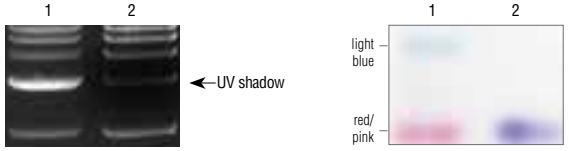
Activity of DNA Modifying Enzymes in CutSmart Buffer: **Clone Smarter!**

Enzyme	Activity in CutSmart	Required Supplements
Phosphatases:		
Alkaline Phosphatase (CIP)	+ + +	
Antarctic Phosphatase	+ + +	Requires Zn^{2+}
Quick CIP	+ + +	
Shrimp Alkaline Phosphatase (rSAP)	+ + +	
Ligases:		
T4 DNA Ligase	+ + +	Requires ATP
E. coli DNA Ligase	+ + +	Requires NAD
T3 DNA Ligase	+ + +	Requires ATP + PEG
T7 DNA Ligase	+ + +	Requires ATP + PEG
Polymerases:		
T4 DNA Polymerase	+ + +	
DNA Polymerase I, Large (Klenow) Frag.	+ + +	
DNA Polymerase I	+ + +	
DNA Polymerase Klenow Exo^-	+ + +	
Bst DNA Polymerase	+ + +	
phi29 DNA Polymerase	+ + +	
T7 DNA Polymerase (unmodified)	+ + +	
Transferases/Kinases:		
T4 Polynucleotide Kinase	+ + +	Requires ATP + DTT
T4 PNK (3′ phosphatase minus)	+ + +	Requires ATP + DTT
CpG Methyltransferase (M. SssI)	+ + +	
GpC Methyltransferase (M. CviPI)	+	Requires DTT
T4 Phage β-glucosyltransferase	+ + +	
Nucleases, other:		
DNase I (RNase free)	+ + +	Requires Ca^{2+}
Endonuclease III (Nth), recombinant	+ + +	
Endonuclease VIII	+ + +	
Exonuclease III	+ + +	
Lambda Exonuclease	+ +	
McrBC	+ + +	
Micrococcal Nuclease	+ +	Requires Ca^{2+}
$RecJ_f$	+ + +	
T5 Exonuclease	+ + +	
T7 Exonuclease	+ + +	
USER™ Enzyme, recombinant	+ + +	

+ + + full functional activity + + 50–100% functional activity + 0–50% functional activity

A selection of DNA modifying enzymes were assayed in CutSmart Buffer and functional activity was compared to the activity in their supplied buffers. Reactions were set up according to the recommended reaction conditions, with CutSmart Buffer (plus required supplement) replacing the supplied buffer.

Tech Tip: When supplements are required, one can simply add the supplied buffer of the respective modifying enzyme at 1× concentration to the CutSmart Buffer to achieve appropriate activity for most applications – no change of buffers needed.



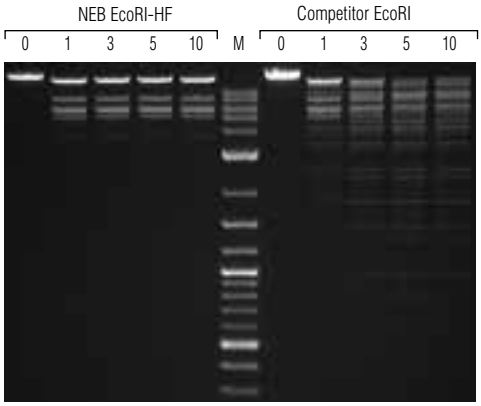
The Gel Loading Dye, Purple (6X) (Lane 1) as supplied with all top selling restriction enzymes does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

Looking to optimize PERFORMANCE in your reaction?

Choose a High-Fidelity (HF®) Restriction Enzyme

NEB's High-Fidelity (HF) enzymes have the same specificity as the native enzymes, with the added benefit of reduced star activity, rapid digestion (5-15 minutes), and 100% activity in CutSmart Buffer. Enjoy the improved performance of NEB's engineered enzymes at the same price as the native enzymes!

For more information, visit **www.neb.com/HF**



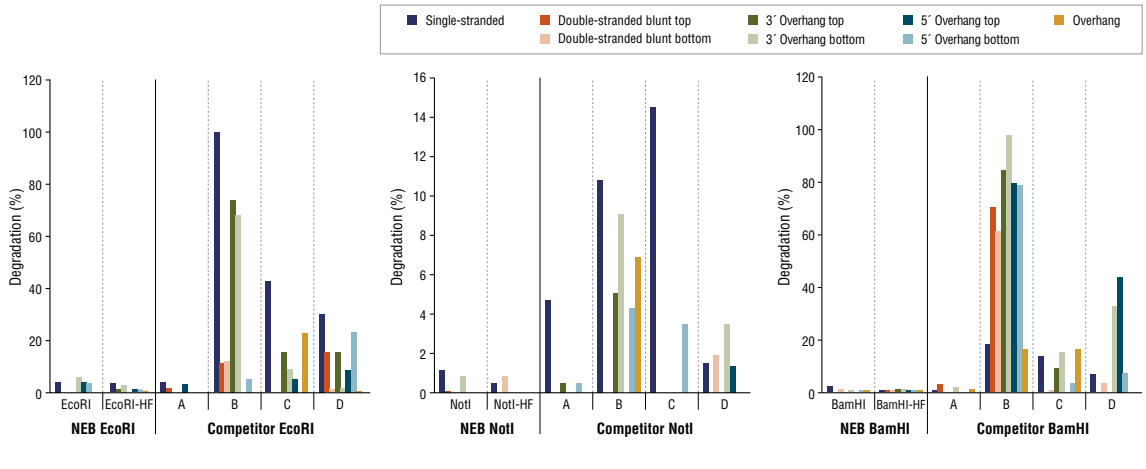
EcoRI-HF (NEB #R3101) shows no star activity in overnight digests, even when used at higher concentrations. 50 µl reactions were set up using 1 µg of Lambda DNA, the indicated amount of enzyme and the recommended reaction buffer. Reactions were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB# N3232).

Benefit from Industry-leading Quality

NEB's reputation as a leader in enzyme technologies stems from the quality and reliability of our restriction enzymes. All of our restriction enzymes undergo stringent quality control testing, ensuring the highest levels of purity and lot-to-lot consistency.

HIGHLIGHTS

- Industry-leading product quality
- State-of-the-art production and purification
- Over 40 years of experience
- Stringent quality control testing
- Lot-to-lot consistency
- ISO 9001- and 13485-certified



Restriction Enzyme Competitor Study: Nuclease Contamination

EcoRI, NotI, and BamHI from multiple suppliers were tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3'overhang or 5' overhang containing oligonucleotides. The percent degradation is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.

Visit **NEBCutSmart.com** for information on the smarter choice of restriction enzymes.

High Fidelity (HF) Enzymes

– Engineered for performance!

NEB exclusively offers High-Fidelity (HF) restriction enzymes that have been engineered by NEB's R&D team for superior performance. HF enzymes have the same specificity as the native enzymes but offer dramatically reduced star activity (i.e. degradation of end-product and off-target cleavage, see p. 5).

In addition to reduced star activity, all HF enzymes work optimally in CutSmart Buffer, which has the highest level of enzyme compatibility and will simplify double digest reactions.

They are all Time-Saver qualified and digest substrate DNA in 5–15 minutes and are flexible enough to digest overnight. As a free bonus, HF enzymes are supplied with our **Gel Loading Dye, Purple (6X) (#B7024)**, which sharpens bands and eliminates UV shadow. Lastly, they are available at the same price as the native enzymes.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- The full list of HF restriction enzymes available
- Online tutorials on how to avoid star activity and setting up digests using the Time-Saver protocol

The following table indicates the HF Factor, which refers to the X-fold increase in fidelity that is achieved by choosing an HF enzyme vs. the native counterpart. It clearly illustrates the added flexibility, higher reliability and greater confidence for all digests and cloning work flows that is offered by using an HF restriction enzyme.

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	HF FACTOR*
AgeI-HF	#R3552	CutSmart	≥ 8
AgeI	#R0552	1.1	
ApoI-HF	#R3566	CutSmart	25
ApoI	#R0566	3.1	
BamHI-HF	#R3136	CutSmart	≥ 125
BamHI	#R0136	3.1	
BbsI-HF	#R3539	CutSmart	1
BbsI	#R0539	2.1	
BclI-HF	#R3160	CutSmart	16
BclI	#R0160	3.1	
BmtI-HF	#R3658	CutSmart	31,250
BmtI	#R0658	3.1	
BsaI-HF	#R3535	CutSmart	≥ 250
BsaI	#R0535	CutSmart	
BsiWI-HF	#R3553	CutSmart	1
BsiWI	#R0553	3.1	
BsrGI-HF	#R3575	CutSmart	≥ 62
BsrGI	#R0575	2.1	
BstEII-HF	#R3162	CutSmart	≥ 125
BstEII	#R0162	3.1	
BstZ17I-HF	#R3594	CutSmart	25
BstZ17I**	#R0594	CutSmart	
DraIII-HF	#R3510	CutSmart	≥ 1,000
DraIII**	N/A	3.1	
EagI-HF	#R3505	CutSmart	2
EagI	#R0505	3.1	
EcoRI-HF	#R3101	CutSmart	64
EcoRI	#R0101	U	
EcoRV-HF	#R3195	CutSmart	≥ 64
EcoRV	#R0195	3.1	
HindIII-HF	#R3104	CutSmart	≥ 2,000
HindIII	#R0104	2.1	
KpnI-HF	#R3142	CutSmart	≥ 62,500
KpnI	#R0142	1.1	

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	HF FACTOR*
MfeI-HF	#R3589	CutSmart	≥ 16
MfeI	#R0589	CutSmart	
MluI-HF	#R3198	CutSmart	2
MluI	#R0198	3.1	
NcoI-HF	#R3193	CutSmart	≥ 530
NcoI	#R0193	3.1	
NheI-HF	#R3131	CutSmart	≥ 266
NheI	#R0131	2.1	
NotI-HF	#R3189	CutSmart	≥ 16
NotI	#R0189	3.1	
NruI-HF	#R3192	CutSmart	64
NruI	#R0192	3.1	
NsiI-HF	#R3127	CutSmart	20
NsiI	#R0127	3.1	
PstI-HF	#R3140	CutSmart	33
PstI	#R0140	3.1	
PvuI-HF	#R3150	CutSmart	≥ 32
PvuI	#R0150	3.1	
PvuII-HF	#R3151	CutSmart	32
PvuII	#R0151	2.1	
SacI-HF	#R3156	CutSmart	≥ 266
SacI	#R0156	1.1	
SalI-HF	#R3138	CutSmart	≥ 8,000
SalI	#R0138	3.1	
SbfI-HF	#R3642	CutSmart	32
SbfI	#R0642	CutSmart	
ScaI-HF	#R3122	CutSmart	62
ScaI**	#R0122	3.1	
SpeI-HF	#R3133	CutSmart	≥ 16
SpeI	#R0133	CutSmart	
SphI-HF	#R3182	CutSmart	250
SphI	#R0182	2.1	
SspI-HF	#R3132	CutSmart	16
SspI	#R0132	U	
StyI-HF	#R3500	CutSmart	125
StyI	#R0500	3.1	

† Wild type enzymes were tested in supplied buffer for comparisons.
* Wei, H. et al (2008) Nucleic Acids Reseach 36, e50.
** No longer available.

Avoiding Star Activity

Tips for preventing unwanted cleavage in restriction enzyme digests

Under non-standard reaction conditions, some restriction enzymes are capable of degrading end-product and cleaving sequences which are similar but not identical to their defined recognition sequence. This altered specificity has been termed "star activity". It has been suggested that star activity is a general property of restriction endonucleases (1) and that any restriction endonuclease will cleave noncanonical sites under certain extreme conditions, some of which are listed below. Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog, on the supplied card and on our website.

CONDITIONS THAT CONTRIBUTE TO STAR ACTIVITY	STEPS THAT CAN BE TAKEN TO INHIBIT STAR ACTIVITY
High glycerol concentration (> 5% v/v)	Restriction enzymes are stored in 50% glycerol, therefore the amount of enzyme added should not exceed 10% of the total reaction volume. Use the standard 50 µl reaction volume to reduce evaporation during incubation.
High concentration of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	Use the fewest units possible to achieve digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
Non-optimal buffer	Whenever possible, set up reactions in the recommended buffer. Buffers with differing ionic strengths and pHs may contribute to star activity.
Prolonged reaction time	Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (2), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (3)]	Make sure the reaction is free of any organic solvents, such as alcohols, that might be present in the DNA preparation.
Substitution of Mg^{2+} with other divalent cations (Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+})	Use Mg^{2+} as the divalent cation. Other divalent cations may not fit correctly into the active site of the restriction enzyme, possibly interfering with proper recognition.

Note: The relative significance of each of these altered conditions will vary from enzyme to enzyme.

New England Biolabs recommends setting up restriction enzyme digests in a 50 µl reaction volume. However, different methods may require smaller reaction volumes. When performing restriction enzyme digests in smaller reaction volumes, extra care must be taken to follow the steps listed above to avoid star activity. Alternatively, using our line of **High Fidelity (HF) restriction enzymes** will allow greater flexibility in reaction setup. Please visit **www.neb.com/HF** frequently to learn about additions to the HF restriction enzyme product line.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Video tutorials on how to avoid star activity, and for setting up restriction enzyme digests
- The full list of HF enzymes available
- Troubleshooting guides

RESTRICTION ENZYME TYPES

- Type I enzymes are multisubunit proteins that cut DNA randomly at a distance from their recognition sequence.
- Type II enzymes cut DNA at defined positions close to or within their recognition sequence and are commonly used in the laboratory. There are over ten subtypes with different types of recognition sites, cleavage sites and cofactor requirements.
- The most common Type II enzymes cleave within their recognition site (e.g., BamHI, EcoRI); sites can be symmetric or asymmetric.
- Type IIS enzymes cleave outside their recognition sequence (e.g., BsaI, BsmBI) and are invaluable for emerging technologies in the biotechnology industry, including Golden Gate Assembly
- Type IIM enzymes recognize methylated targets (e.g., DpnI).
- Type III enzymes are large, combination restriction-and-modification enzymes that cleave outside their recognition sequences and require two sequences in opposite orientations to cleave one DNA molecule.
- Type IV enzymes recognize modified DNA (methylated, hydroxymethylated, etc.). They require two sites and cleave non-specifically.
- Isoschizomers are restriction enzymes that recognize the same sequence as the prototype.
- Neoschizomers are isoschizomers with different cleavage sites.

Learn more about restriction enzyme types in our online tutorials.

References:
1. Nasri, M. and Thomas, D. (1986) *Nucleic Acids Res.* 14, 811.
2. Nasri, M. and Thomas, D. (1987) *Nucleic Acids Res.* 15, 7677.
3. Tikchonenko, T.I., *et al.* (1978) *Gene,* 4, 195–212.

Time-Saver Qualified Restriction Enzymes

Whether you are quickly screening large numbers of clones or setting up overnight digests, you will benefit from the high quality of our enzymes. Typically, a restriction digest involves the incubation of 1 µl of enzyme with 1 µg of purified DNA in a final volume of 50 µl for 1 hour. However, to speed up the screening process, choose one of NEB's enzymes that are Time-Saver qualified. **> 190 of our enzymes will digest 1 µg of substrate DNA in 5-15 minutes using 1 µl of enzyme under recommended reaction conditions, and can also be used safely in overnight digestions.** Unlike other suppliers, there is no special formulation, change in concentration or need to buy more expensive, new lines of enzymes to achieve digestion in 5-15 minutes. Nor do you have to worry if you incubate too long.

In an effort to provide you with as much information as possible, NEB has tested all of its enzymes on unit assay substrate, as well as plasmid substrate and PCR fragments. We recommend that this data be used as a guide, as it is not definitive for all plasmids. Restriction enzymes can often show site preference, presumably determined by the sequence flanking the recognition site. In addition, supercoiled DNA may have varying rates of cleavage. For more information, visit **www.neb.com/TimeSaver**. Note that there are some enzymes indicated below that can cut in 5-15 minutes, but cannot be incubated overnight. These are not Time-Saver qualified.

Since all of our enzymes are rigorously tested for nuclease contamination, you can also safely set up digests for long periods of time without sample degradation. NEB Time-Saver qualified enzymes offer power and flexibility – the power to digest in 5-15 minutes and the flexibility to withstand overnight digestions with no loss of substrate.

Chart Legend

- ● digests in 5 minutes
- ■ digests in 15 minutes
- ▲ not completely digested in 15 minutes

NT not tested

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
AatII	■	▲	●
AccI	■	▲	▲
Acc65I	●	▲	●
AciI	●	●	●
AclI	●	■	▲
AcuI	■	▲	▲
AflIII	●	●	●
AgeI-HF	●	●	●
AhdI	●	●	■
AluI	●	▲	●
AlwNI	●	●	▲
ApaI	●	●	●
ApaLI	●	●	▲
ApeKI	●	■	▲
ApoI	●	●	●
ApoI-HF	●	●	▲
AscI	●	●	NT
AseI	●	●	NT
AvaI	●	▲	▲
AvaII	●	●	●
AvrII	●	NT	NT
BaeI	■	●	▲
BaeGI	●	▲	▲

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
BamHI	●	●	▲
BamHI-HF	●	●	●
BbsI	■	▲	▲
BbsI-HF	●	▲	▲
BbvI	●	▲	▲
BccI	■	▲	▲
BceAI	■	■	▲
BciVI	●	■	▲
BclI	●	▲	▲
BclI-HF	●	▲	▲
BcoDI	●	●	▲
BfuAI	●	●	▲
BfuCI	■	▲	●
BglI	●	●	▲
BglII	●	■	▲
BlpI	●	●	●
BmgBI	●	●	▲
BmrI	■	▲	■
BmtI-HF	●	●	▲
BpuEI	●	●	▲
BsaI	●	●	▲
BsaI-HF	●	●	▲
BsaAI	●	●	■

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
BsaHI	■	■	●
BsaWI	■	▲	▲
BsaXI	●	▲	▲
BseRI	●	●	■
BsgI	●	●	▲
BsiEI	●	▲	▲
BsiWI	●	●	▲
BsiWI-HF	●	●	▲
BsmI	●	■	■
BsmAI	●	●	▲
BsmBI	●	▲	●
BsmFI	■	▲	▲
BsoBI	●	●	▲
Bsp1286I	●	■	●
BspCNI	●	●	▲
BspEI	■	▲	▲
BspHI	●	▲	▲
BspQI	■	●	●
BsrI	●	●	▲
BsrBI	●	■	▲
BsrDI	●	■	▲
BsrFαI	●	■	▲
BsrGI	●	▲	▲
BsrGI-HF	■	▲	▲
BssHII	●	●	▲
BssSαI	●	▲	▲
BstBI	●	▲	▲
BstEII	●	●	▲
BstEII-HF	●	●	▲
BstNI	●	●	●
BstUI	●	●	▲
BstXI	●	●	▲
BstYI	●	●	▲
BstZ17I-HF	■	●	▲
Bsu36I	●	●	▲
BtgI	●	▲	■
BtsαI	●	●	■
BtsCI	●	■	▲
Cac8I	■	▲	▲
ClaI	●	●	▲
CspCI	●	●	▲
CviAII	■	●	●

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
CviQI	●	●	●
DdeI	●	■	■
DpnI	●	●	▲
DpnII	■	▲	●
DraI	●	●	■
DraIII-HF	●	●	▲
DrdI	■	●	●
EagI	●	▲	▲
EagI-HF	■	■	▲
EarI	■	■	▲
Eco53KI	●	●	■
EcoNI	●	■	●
EcoO109I	●	▲	▲
EcoP15I	■	▲	▲
EcoRI	●	●	▲
EcoRI-HF	●	●	●
EcoRV	●	●	▲
EcoRV-HF	●	●	▲
Fnu4HI	●	■	■
FokI	●	●	●
FseI	●	●	▲
FspI	■	▲	■
HaeII	■	▲	▲
HaeIII	●	●	●
HgaI	■	▲	▲
HhaI	●	■	▲
HinclI	■	▲	●
HindIII-HF	●	●	●
HinfI	●	●	●
HinP1I	●	▲	●
HpaII	●	●	▲
HphI	●	▲	▲
Hpy166II	●	●	●
HpyAV	●	●	NT
HpyCH4IV	●	●	●
HpyCH4V	●	●	●
KpnI	●	●	●
KpnI-HF	●	●	●
MboI	●	▲	●
MboII	●	●	●
MfeI	●	●	●
MfeI-HF	●	●	●
MluI	●	●	●
MluI-HF	●	●	▲
MluCI	●	●	▲
MlyI	●	▲	●
MmeI	●	●	▲
MnlI	●	●	■
MseI	■	■	●
MslI	●	●	●
MspI	●	●	●

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
MspA1I	●	●	●
MwoI	■	▲	▲
NciI	●	●	●
NcoI	●	■	▲
NcoI-HF	●	●	●
NdeI	●	●	▲
NgoMIV	■	●	▲
NheI	●	■	▲
NheI-HF	●	●	■
NlaIII	■	▲	■
NmeAIII	●	▲	▲
NotI	●	●	▲
NotI-HF	●	●	●
NruI	●	■	▲
NruI-HF	●	■	▲
NsiI	●	●	●
NsiI-HF	●	●	■
NspI	●	■	▲
PacI	●	●	●
PaeR7I	●	▲	▲
PflFI	●	■	▲
PflMI	●	▲	▲
PmeI	●	■	NT
PmlI	●	▲	▲
PpuMI	●	▲	▲
PshAI	■	■	■
PstI	●	●	●
PstI-HF	●	●	●
PvuI	●	▲	●
PvuI-HF	●	●	●
PvuII	●	●	▲
PvuII-HF	●	●	▲
RsaI	●	●	●
SacI	●	●	▲
SacI-HF	●	●	●
SacII	●	▲	▲
SalI	●	■	▲
SalI-HF	●	●	▲
SapI	■	▲	▲
SbfI	●	●	▲
SbfI-HF	●	●	▲
ScaI-HF	●	●	▲
SfiI	●	▲	▲
SfoI	●	●	●
SmaI	●	■	■
SpeI	●	●	●
SpeI-HF	■	■	▲
SphI	●	●	▲
SphI-HF	●	●	▲
SrfI	●	●	▲
SspI	●	●	▲

ENZYME	SUBSTRATE		
	UNIT ASSAY	PLASMID	PCR
SspI-HF	●	●	▲
StuI	■	▲	▲
StyI	■	▲	▲
StyI-HF	●	●	▲
StyD4I	■	▲	▲
SwaI	■	▲	▲
TaqαI	●	●	▲
TfiI	■	●	▲
TseI	■	▲	▲
TspMI	●	■	▲
TspRI	●	■	▲
Tth111I	■	■	▲
XbaI	●	●	▲
XhoI	●	●	▲
XmaI	■	▲	■
XmnI	●	●	▲

TOOLS & RESOURCES

Visit www.neb.com/TimeSaver to find:

- The full list of Time-Saver qualified restriction enzymes available
- Video tutorials on how Time-Saver qualified enzymes speed up restriction enzyme digests

Optimizing Restriction Enzyme Reactions

There are several key factors to consider when setting up a restriction enzyme digest. Using the proper amounts of DNA, enzyme and buffer components in the correct reaction volume will allow you to achieve optimal digestion. By definition, 1 unit of restriction enzyme will completely digest 1 µg of substrate DNA in a 50 µl reaction in 60 minutes. This enzyme:DNA:reaction volume ratio can be used as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, where a 5–10 fold overdigestion is recommended to overcome variability in DNA source, quantity and purity. The Time-Saver protocol can be used for enzymes that are Time-Saver qualified and will digest DNA in 5–15 minutes (see page 6–7 for the full list). NEB offers the following tips to help you to achieve maximal success in your restriction enzyme reactions.

Standard Protocol

Restriction Enzyme	1 µl (or 10 units)*
DNA	up to 1 µg
10X NEBuffer	5 µl (1X)
Total Reaction Volume	50 µl
Incubation Temperature	Enzyme Dependent
Incubation Time	60 minutes

**Sufficient to digest all types of DNAs.*

Time-Saver Protocol:

Restriction Enzyme	1 µl
DNA	up to 1 µg
10X NEBuffer	5 µl (1X)
Total Reaction Volume	50 µl
Incubation Temperature	Enzyme Dependent
Incubation Time	5–15 minutes*

**Time-Saver qualified enzymes can also be incubated overnight with no star activity.*

Enzyme

- Keep on ice when not in the freezer
- Should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units for genomic DNA

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents, nucleases or excessive salts
- Methylation of DNA can inhibit digestion with certain enzymes

Buffer

- Use at a 1X concentration
- BSA is included in NEBuffer 1.1, 2.1, 3.1 and CutSmart Buffer. No additional BSA is needed.

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of 1 µg of substrate
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes.

Alternative Volumes for Restriction Digests

	RESTRICTION ENZYME*	DNA	10X NEBUFFER
10 µl rxn**	1 unit	0.1 µg	1 µl
25 µl rxn	5 units	0.5 µg	2.5 µl
50 µl rxn	10 units	1 µg	5 µl

* Restriction Enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed

** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation. 10 µl reaction volumes are not recommended for salt-sensitive enzymes (i.e., enzymes that exhibit low activity in NEBuffer 3.1).

Incubation Time

- Incubation time for Standard Protocol is 1 hour. Incubation for Time-Saver Protocol is 5–15 minutes.
- With many enzymes, it is possible to use fewer units and digest for up to 16 hours. For more information, visit **www.neb.com**.

Storage

- Storage at –20°C is recommended for most restriction enzymes. For a few enzymes, storage at –80°C is recommended for periods longer than 30 days. Visit **www.neb.com** for storage information.
- 10X NEBuffers should also be stored at –20°C

Stability

- All enzymes are assayed for activity every 3–6 months. The expiration date is found on the label.
- Exposure to temperatures above –20°C should be minimized whenever possible

Star Activity

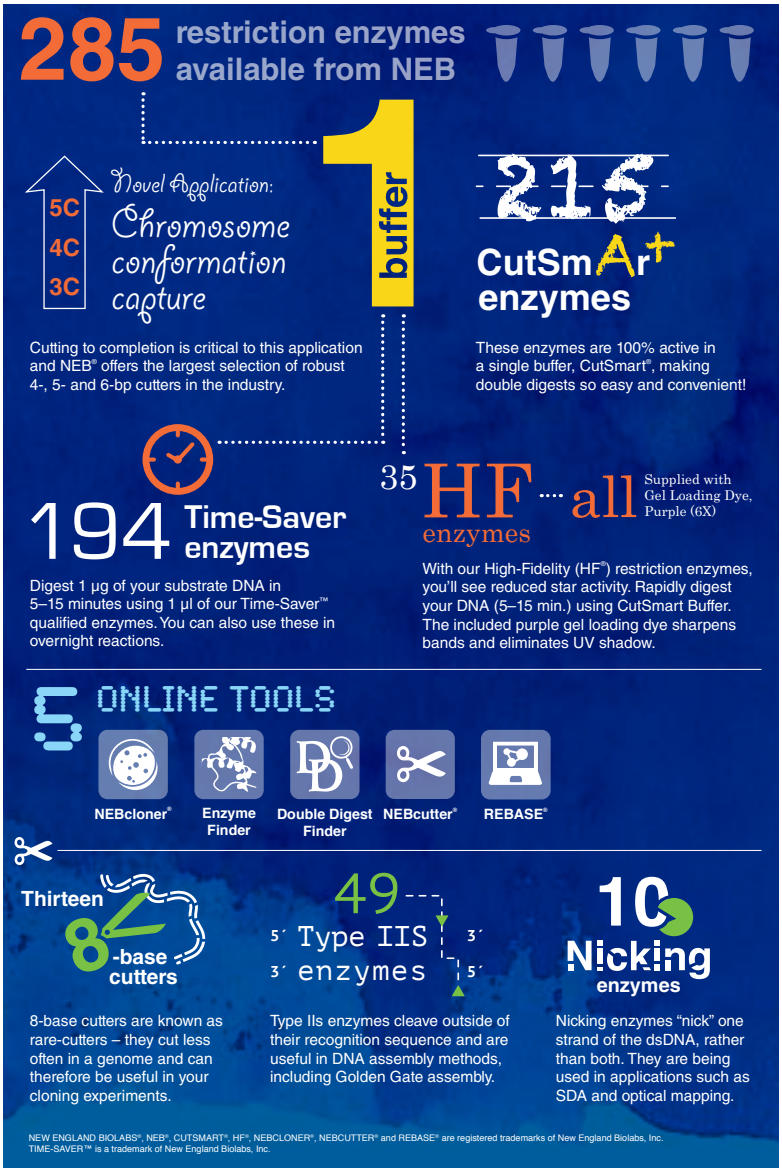
- Can occur when enzyme is used under sub-optimal conditions
- Star activity can be reduced by using a High-Fidelity (HF) enzyme, by reducing incubation time, by using a Time-Saver enzyme or by increasing reaction volume

TOOLS & RESOURCES

Visit NEBCutSmart.com to find:

- Video tutorials on setting up restriction enzyme reactions from NEB scientists

Industry leading convenience & performance in a nutshell:



Troubleshooting Guide

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Restriction enzyme(s) didn't cleave completely	Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence
		Use the recommended buffer supplied with the restriction enzyme
		Clean up the DNA to remove any contaminants that may inhibit the enzyme
		When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	Lower the number of units
		Add SDS (0.1–0.5%) to the loading buffer, or use Gel Loading Dye, Purple (6X) #B7024S
	Nuclease contamination	Use fresh, clean running buffer and a fresh agarose gel
		Clean up the DNA
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation
		DNA isolated from eukaryotic source may be blocked by CpG methylation
		Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence
		If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a *dam-/dcm-* strain (NEB #C2925)
	Salt inhibition	Enzymes that have low activity in salt-containing buffers (NEBuffer 3.1) may be salt sensitive, so clean up the DNA prior to digestion
		DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume.
	Inhibition by PCR components	Clean up the PCR fragment prior to restriction digest
	Using the wrong buffer	Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	Use at least 3–5 units of enzyme per µg of DNA
	Incubation time was too short	Increase the incubation time
	Digesting supercoiled DNA	Some enzymes have a lower activity on supercoiled DNA. Increase the number of enzyme units in the reaction.
Incomplete restriction enzyme digestion	Presence of slow sites	Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	Some enzymes require the presence of two recognition sites to cut efficiently
	DNA is contaminated with an inhibitor	Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Mini prep DNA is particularly susceptible to contaminants.
		Clean DNA with a spin column, resin or drop dialysis, or increase volume to dilute contaminant
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	Lower the number of units in the reaction
		Add SDS (0.1–0.5%) to the loading buffer, or use Gel Loading Dye, Purple (6X) #B7024S
	Star activity	Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
		Decrease the number of enzyme units in the reaction
		Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v.
		Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity.
		Use the recommended buffer supplied with the restriction enzyme.
	Partial restriction enzyme digest	Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer 3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion.
		DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume
		Clean-up the PCR fragment prior to restriction digest
		Use the recommended buffer supplied with the restriction enzyme
		Use at least 3–5 units of enzyme per µg of DNA and digest the DNA for 1–2 hours

FAQS

Q. Do restriction enzymes cleave single-stranded DNA?

A. Although some restriction enzymes have been reported to cleave ssDNA, it is unclear whether cleavage occurs on a ssDNA molecule or on two ssDNA molecules which transiently anneal at a region of partial homology (1–3). For this reason, we hesitate to make unreserved claims about a restriction enzyme's ability to cut ssDNA.

Q. How stable are restriction enzymes?

A. All restriction enzymes from NEB are assayed for activity every 3–6 months. Most are very stable when stored at -20°C in the recommended storage buffer. Exposure to temperatures above -20°C should be minimized whenever possible.

Q. Is extended digestion (incubation times > 1 hour) recommended?

A. The unit definition of our restriction enzymes is based on a 1 hour incubation. Incubation time may be shortened if additional units of restriction enzyme are added to the reaction or if a Time-Saver qualified restriction enzyme is used (5–15 minutes). Conversely, longer incubation times are often used to allow a reaction to proceed to completion with fewer units of enzyme. This is contingent on how long a particular enzyme can survive (maintain activity) in a reaction. Additional information on extended digestion can be found at **www.neb.com**.

References
1. Blakesley, R.W., Wells, R.D. (1975) *Nature* 257, 421–422.
2. Blakesley, R.W., et al. (1977) *J. Biol. Chem.* 252, 7300–7306.
3. Yoo, O.J., Agarwal, K.L, (1980) *J. Biol. Chem.* 255, 10559–10562.

Double Digestion

Digesting a DNA substrate with two restriction enzymes simultaneously (double digestion) is a common timesaving procedure. Over 210 restriction enzymes are 100% active in CutSmart Buffer, making double digestion simple. If you are using an enzyme that is not supplied with CutSmart Buffer, the Performance Chart for Restriction Enzymes rates the percentage activity of each restriction endonuclease in the four standard NEBuffers.

Setting up a Double Digest

- Double digests with CutSmart restriction enzymes can be set up in CutSmart Buffer. Otherwise, choose an NEBuffer that results in the most activity for both enzymes. If star activity is a concern, consider using one of our High-Fidelity (HF) enzymes.
- Set up reaction according to recommended protocol. The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity. For example, in a 50 µl reaction, the total amount of enzyme added should not exceed 5 µl.
- If two different incubation temperatures are necessary, choose the optimal reaction buffer and set up reaction accordingly. Add the first enzyme and incubate at the desired temperature. Then, heat inactivate the first enzyme, add the second enzyme and incubate at the recommended temperature.
- Depending on an enzyme's activity rating in a non-optimal NEBuffer, the number of units or incubation time may be adjusted to compensate for the slower rate of cleavage.

Setting up a Double Digest with a unique buffer

- NEB currently supplies three enzymes with unique buffers: EcoRI, SspI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI has an HF version which is supplied with CutSmart Buffer.

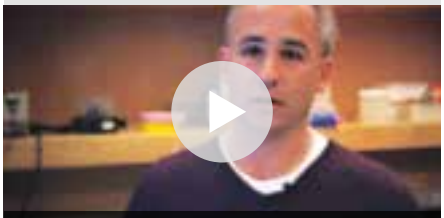
Setting up a Sequential Digest

- If there is no buffer in which the two enzymes both exhibit > 50% activity, a sequential digest can be performed.
- Set up a reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer and incubate to completion.
- Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.
- Add the second enzyme and incubate to complete the second reaction.
- Alternatively, a spin column can be used to isolate the DNA prior to the second reaction.

TOOLS & RESOURCES

Visit www.neb.com/nebtools for:

- Help choosing double digest conditions using NEB's **Double Digest Finder** or **NEBCloner®**



TIPS FOR SETTING UP DOUBLE DIGESTS

Double Digest Finder
see page 16

DNA Methylation & Restriction Digests

DNA methyltransferases (MTases) that transfer a methyl group from S-adenosylmethionine to either adenine or cytosine residues are found in a wide variety of prokaryotes and eukaryotes. Methylation should be considered when digesting DNA with restriction endonucleases because cleavage can be blocked or impaired when a particular base in the recognition site is methylated.

Prokaryotic Methylation

In prokaryotes, MTases have most often been identified as elements of restriction/modification systems that act to protect host DNA from cleavage by the corresponding restriction endonuclease. Most laboratory strains of *E. coli* contain three site-specific DNA methyltransferases.

- Dam methyltransferases–methylation at the N^6 position of the adenine in the sequence GATC (1,2).
- Dcm methyltransferases–methylation at the C^5 position of cytosine in the sequences CCAGG and CCTGG (1,3).
- EcoKI methylase–methylation of adenine in the sequences AAC(N^6A)GTGC and GCAC(N^6A)GTT.

Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm MTase if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from dam^+ *E. coli* is completely resistant to cleavage by MboI, which cleaves at GATC sites.

Not all DNA isolated from *E. coli* is methylated to the same extent. While pBR322 DNA is fully modified (and is therefore completely resistant to MboI digestion), only about 50% of λ DNA Dam sites are methylated, presumably because the methylase does not have the opportunity to methylate the DNA fully before it is packaged into the phage head. As a result, enzymes blocked by Dam or Dcm modification will yield partial digestion patterns with λ DNA.

Restriction sites that are blocked by Dam or Dcm methylation can be un-methylated by cloning your DNA into a dam^-, dcm^- strain of *E. coli*, such as dam^-/dcm^- Competent *E. coli* (NEB #C2925).

Restriction sites can also be blocked if an overlapping site is present. In this case, part of the Dam or Dcm sequence is generated by the restriction enzyme sequence, followed by the flanking sequence. This situation should also be considered when designing restriction enzyme digests.

Eukaryotic Methylation

CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C^5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific and correlate with gene expression. Consequently, CpG methylation has been postulated to play a role in differentiation and gene expression (4).

Note: The effects of CpG methylation are mainly a concern when digesting eukaryotic genomic DNA. CpG methylation patterns are not retained once the DNA is cloned into a bacterial host.

Methylation Sensitivity

The table below summarizes methylation sensitivity for NEB restriction enzymes, indicating whether or not cleavage is blocked or impaired by Dam, Dcm or CpG methylation if or when it overlaps each recognition site. This table should be viewed as a guide to the behavior of the enzymes listed rather than an absolute indicator. Consult REBASE (**http://rebase.neb.com/rebase/**), the restriction enzyme database, for more detailed information and specific examples upon which these guidelines are based.

KEY POINTS TO CONSIDER

- Genomic DNA directly isolated from a mammalian source is not Dcm or Dam methylated, and is therefore not an issue when digesting mammalian DNA.
- Mammalian and plant DNA that has been cloned into a methylating *E. coli* strain will be Dam/Dcm methylated. Most commonly used laboratory *E. coli* strains methylate DNA.
- Directly isolated mammalian and plant genomic DNA are CpG methylated. Some enzymes are inhibited by CpG methylation. (See **www.neb.com** for more information).
- Most bacterial DNA (including *E. coli* DNA) is not CpG methylated. Inhibition of enzyme activity by CpG methylation is not an issue for DNA prepared from *E. coli* strains.
- DNA amplified by PCR does not contain any methylated bases.
- To avoid Dam/Dcm methylation when subcloning in bacteria, NEB offers the methyltransferase deficient cloning strain dam^-/dcm^- Competent *E. coli* (NEB #C2925) for propagation.

References

1. Marinus, M.G. and Morris, N.R. (1973) *J. Bacteriol.*, 114, 1143–1150.
2. Geier, G.E. and Modrich, P. (1979) *J. Biol. Chem.*, 254, 1408–1413.
3. May, M.S. and Hattman, S. (1975) *J. Bacteriol.*, 123, 768–770.
4. Siegfried, Z. and Cedar, H. (1997) *Curr. Biol.*, 7, r305–307.

Methylation Sensitivity – Dam, Dcm and CpG Methylation

Legend:

●	not sensitive
■	blocked
□ ol	blocked by overlapping
□ scol	blocked by some combinations of overlapping
◆	impaired
◇ ol	impaired by overlapping
◇ scol	impaired by some combinations of overlapping

Single Letter Code:

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

ENZYME	SEQUENCE	Dam	Dcm	CpG
AatII	GACGT/C	●	●	■
AbaSI		●	●	●
AccI	GT/MKAC	●	●	□ ol
Acc65I	G/GTACC	●	□ scol	□ scol
AciI	CCGC(-3/-1)	●	●	■
AclI	AA/CGTT	●	●	■
AcuI	CTGAAG(16/14)	●	●	●
AfeI	AGC/GCT	●	●	■
AflII	C/TTAAG	●	●	●
AflIII	A/CRYGT	●	●	●
AgeI	A/CCGGT	●	●	■
AgeI-HF	A/CCGGT	●	●	■
AhdI	GACNNN/NNGTC	●	●	◇ scol
AleI	CACNN/NNGTG	●	●	◇ scol
AluI	AG/CT	●	●	●
AlwI	GGATC(4/5)	■	●	●
AlwNI	CAGNNN/CTG	●	□ ol	●
ApaI	GGGCC/C	●	□ ol	□ ol
ApaLI	G/TGCAC	●	●	□ ol
ApeKI	G/CWGC	●	●	□ ol
ApoI	R/AATTY	●	●	●
ApoI-HF	R/AATTY	●	●	●
AscI	GG/CGCGCC	●	●	■
AseI	AT/TAAT	●	●	●
AsiSI	GCGAT/CGC	●	●	■
AvaI	C/YCGRG	●	●	■
AvaII	G/GWCC	●	□ ol	□ ol
AvrII	C/CTAGG	●	●	●
BaeI	(10/15)ACNNNNGTAYC(12/7)	●	●	□ scol
BaeGI	GKGCM/C	●	●	●
BamHI	G/GATCC	●	●	●
BamHI-HF	G/GATCC	●	●	●
BanI	G/GYRCC	●	□ scol	□ scol
BanII	GRGCY/C	●	●	●
BbsI	GAAGAC(2/6)	●	●	●
BbsI-HF	GAAGAC(2/6)	●	●	●
BbvI	GCAGC(8/12)	●	●	●
BbvCI	CCTCAGC(-2/-5)	●	●	◇ ol
BccI	CCATC(4/5)	●	●	●
BceAI	ACGGC(12/14)	●	●	■
BcgI	(10/12)CGANNNNNNTGC(12/10)	◇ ol	●	□ scol
BciVI	GTATCC(6/5)	●	●	●
BclI	T/GATCA	■	●	●
BclI-HF	T/GATCA	■	●	●
BcoDI	GTCTC(1/5)	●	●	◇ scol
BfaI	C/TAG	●	●	●
BfuAI	ACCTGC(4/8)	●	●	◇ ol

ENZYME	SEQUENCE	Dam	Dcm	CpG
BfuCI	/GATC	●	●	□ ol
BglI	GCCNNNN/NGGC	●	●	□ scol
BglII	A/GATCT	●	●	●
BlpI	GC/TNAGC	●	●	●
BmgBI	CACGTC(-3/-3)	●	●	■
BmrI	ACTGGG(5/4)	●	●	●
BmtI	GCTAG/C	●	●	●
BmtI-HF	GCTAG/C	●	●	●
BpmI	CTGGAG(16/14)	●	●	●
Bpu10I	CCTNAGC(-5/-2)	●	●	●
BpuEI	CTTGAG(16/14)	●	●	●
BsaI	GGTCTC(1/5)	●	◇ scol	□ scol
BsaI-HF	GGTCTC(1/5)	●	□ ol	□ scol
BsaAI	YAC/GTR	●	●	■
BsaBI	GATNN/NNATC	□ ol	●	□ scol
BsaHI	GR/CGYC	●	□ scol	■
BsaJI	C/CNNGG	●	●	●
BsaWI	W/CCGGW	●	●	●
BsaXI	(9/12)ACNNNNNCTCC(10/7)	●	●	●
BseRI	GAGGAG(10/8)	●	●	●
BseYI	CCCAGC(-5/-1)	●	●	□ ol
BsgI	GTGCAG(16/14)	●	●	●
BsiEI	CGRY/CG	●	●	■
BsiHKAI	GWGCW/C	●	●	●
BsiWI	C/GTACG	●	●	■
BsiWI-HF	C/GTACG	●	●	■
BsII	CCNNNNN/NNGG	●	□ scol	□ scol
BsmI	GAATGC(1/-1)	●	●	●
BsmAI	GTCTC (1/5)	●	●	□ scol
BsmBI	CGTCTC(1/5)	●	●	■
BsmFI	GGGAC(10/14)	●	□ ol	□ ol
BsoBI	C/YCGRG	●	●	●
Bsp1286I	GDGCH/C	●	●	●
BspCNI	CTCAG(9/7)	●	●	●
BspDI	AT/CGAT	□ ol	●	■
BspEI	T/CCGGA	□ ol	●	◆
BspHI	T/CATGA	◇ ol	●	●
BspMI	ACCTGC(4/8)	●	●	●
BspQI	GCTCTTC(1/4)	●	●	●
BsrI	ACTGG(1/-1)	●	●	●
BsrBI	CCGCTC(-3/-3)	●	●	□ scol
BsrDI	GCAATG(2/0)	●	●	●
BsrF$^{\alpha}$I	R/CCGGY	●	●	■
BsrGI	T/GTACA	●	●	●
BsrGI-HF	T/GTACA	●	●	●
BssHII	G/CGCGC	●	●	■
BssS$^{\alpha}$I	CACGAG(-5/-1)	●	●	●

METHYLATION SENSITIVITY

ENZYME	SEQUENCE	Dam	Dcm	CpG
BstAPI	GCANNNN/NTGC	●	●	□ scol
BstBI	TT/CGAA	●	●	■
BstEII	G/GTNACC	●	●	●
BstEII-HF	G/GTNACC	●	●	●
BstNI	CC/WGG	●	●	●
BstUI	CG/CG	●	●	■
BstXI	CCANNNNN/NTGG	●	□ scol	●
BstYI	R/GATCY	●	●	●
BstZ17I-HF	GTA/TAC	●	●	□ scol
Bsu36I	CC/TNAGG	●	●	●
BtgI	C/CRYGG	●	●	●
BtgZI	GCGATG(10/14)	●	●	◆
Bts$^{\alpha}$I	GCAGTG(2/0)	●	●	●
BtsIMutI	CAGTG(2/0)	●	●	●
BtsCI	GGATG(2/0)	●	●	●
Cac8I	GCN/NGC	●	●	□ scol
ClaI	AT/CGAT	□ ol	●	■
CspCI	(11/13)CAANNNNNGTGG(12/10)	●	●	●
CviAII	C/ATG	●	●	●
CviKI-1	RG/CY	●	●	●
CviQI	G/TAC	●	●	●
DdeI	C/TNAG	●	●	●
DpnI	GA/TC	●	●	□ ol
DpnII	/GATC	■	●	●
DraI	TTT/AAA	●	●	●
DraIII-HF	CACNNN/GTG	●	●	◇ ol
DrdI	GACNNNN/NNGTC	●	●	□ scol
EaeI	Y/GGCCR	●	□ ol	□ ol
EagI	C/GGCCG	●	●	■
EagI-HF	C/GGCCG	●	●	■
EarI	CTCTTC(1/4)	●	●	◇ ol
EciI	GGCGGA(11/9)	●	●	□ scol
Eco53kI	GAG/CTC	●	●	□ scol
EcoNI	CCTNN/NNNAGG	●	●	●
EcoO109I	RG/GNCCY	●	□ ol	●
EcoP15I	CAGCAG(25/27)	●	●	●
EcoRI	G/AATTC	●	●	□ scol
EcoRI-HF	G/AATTC	●	●	□ scol
EcoRV	GAT/ATC	●	●	◇ scol
EcoRV-HF	GAT/ATC	●	●	◇ scol
FatI	/CATG	●	●	●
FauI	CCCGC(4/6)	●	●	■
Fnu4HI	GC/NGC	●	●	□ ol
FokI	GGATG(9/13)	●	◇ ol	◇ ol
FseI	GGCCGG/CC	●	◇ scol	■
FspI	TGC/GCA	●	●	■
FspEI	C5mCNNNNNNNNNNN	●	●	●
HaeII	RGCGC/Y	●	●	■
HaeIII	GG/CC	●	●	●
HgaI	GACGC(5/10)	●	●	■
HhaI	GCG/C	●	●	■
HincII	GTY/RAC	●	●	□ scol
HindIII	A/AGCTT	●	●	●
HindIII-HF	A/AGCTT	●	●	●
HinfI	G/ANTC	●	●	□ scol
HinP1I	G/CGC	●	●	■
HpaI	GTT/AAC	●	●	□ scol
HpaII	C/CGG	●	●	■

ENZYME	SEQUENCE	Dam	Dcm	CpG
HphI	GGTGA(8/7)	■	■	●
Hpy99I	CGWCG/	●	●	■
Hpy166II	GTN/NAC	●	●	□ ol
Hpy188I	TCN/GA	□ ol	●	●
Hpy188III	TC/NNGA	□ ol	●	□ ol
HpyAV	CCTTC(6/5)	●	●	◇ ol
HpyCH4III	ACN/GT	●	●	●
HpyCH4IV	A/CGT	●	●	■
HpyCH4V	TG/CA	●	●	●
KasI	G/GCGCC	●	●	■
KpnI	GGTAC/C	●	●	●
KpnI-HF	GGTAC/C	●	●	●
LpnPI	C5mCDGNNNNNNNNNN	●	●	●
MboI	/GATC	■	●	◇ ol
MboII	GAAGA(8/7)	□ ol	●	●
MfeI	C/AATTG	●	●	●
MfeI-HF	C/AATTG	●	●	●
MluI	A/CGCGT	●	●	■
MluI-HF	A/CGCGT	●	●	■
MluCI	/AATT	●	●	●
MlyI	GAGTC(5/5)	●	●	●
MmeI	TCCRAC(20/18)	●	●	□ ol
MnlI	CCTC(7/6)	●	●	●
MscI	TGG/CCA	●	□ ol	●
MseI	T/TAA	●	●	●
MslI	CAYNN/NNRTG	●	●	●
MspI	C/CGG	●	●	●
MspA1I	CMG/CKG	●	●	□ ol
MspJI	5mCNNRNNNNNNNNN	●	●	●
MwoI	GCNNNNN/NNGC	●	●	□ scol
NaeI	GCC/GGC	●	●	■
NarI	GG/CGCC	●	●	■
Nb.BbvCI	CCTCAGC (none/-2)	●	●	●
Nb.BsmI	GAATGC (none/-2)	●	●	●
Nb.BsrDI	GCAATG (none/0)	●	●	●
Nb.BssSI	CACGAG	●	●	●
Nb.BtsI	GCAGTG	●	●	●
NciI	CC/SGG	●	●	◇ ol
NcoI	C/CATGG	●	●	●
NcoI-HF	C/CATGG	●	●	●
NdeI	CA/TATG	●	●	●
NgoMIV	G/CCGGC	●	●	■
NheI	G/CTAGC	●	●	□ scol
NheI-HF	G/CTAGC	●	●	□ scol
NlaIII	CATG/	●	●	●
NlaIV	GGN/NCC	●	□ ol	□ ol
NmeAIII	GCCGAG(21/19)	●	●	●
NotI	GC/GGCCGC	●	●	■
NotI-HF	GC/GGCCGC	●	●	■
NruI	TCG/CGA	□ ol	●	■
NruI-HF	TCG/CGA	□ ol	●	■
NsiI	ATGCA/T	●	●	●
NsiI-HF	ATGCA/T	●	●	●
NspI	RCATG/Y	●	●	●
Nt.AlwI	GGATC(4/-5)	■	●	●
Nt.BbvCI	CCTCAGC(-5/none)	●	●	□ scol
Nt.BsmAI	GTCTC(1/none)	●	●	■
Nt.BspQI	GCTCTTC(1/none)	●	●	●

ENZYME	SEQUENCE	Dam	Dcm	CpG
Nt.BstNBI	GAGTC(4/none)	●	●	●
PacI	TTAAT/TAA	●	●	●
PaeR7I	C/TCGAG	●	●	■
PciI	A/CATGT	●	●	●
PflFI	GACN/NNGTC	●	●	●
PflMI	CCANNNN/NTGG	●	□ ol	●
PleI	GAGTC(4/5)	●	●	□ scol
PluTI	GGCGC/C	●	●	■
PmeI	GTTT/AAAC	●	●	□ scol
PmlI	CAC/GTG	●	●	■
PpuMI	RG/GWCCY	●	□ ol	●
PshAI	GACNN/NNGTC	●	●	□ scol
PsiI	TTA/TAA	●	●	●
PspGI	/CCWGG	●	■	●
PspOMI	G/GGCCC	●	◇ scol	□ ol
PspXI	VC/TCGAGB	●	●	◆
PstI	CTGCA/G	●	●	●
PstI-HF	CTGCA/G	●	●	●
PvuI	CGAT/CG	●	●	■
PvuI-HF	CGAT/CG	●	●	■
PvuII	CAG/CTG	●	●	●
PvuII-HF	CAG/CTG	●	●	●
RsaI	GT/AC	●	●	□ scol
RsrII	CG/GWCCG	●	●	■
SacI	GAGCT/C	●	●	●
SacI-HF	GAGCT/C	●	●	□ scol
SacII	CCGC/GG	●	●	■
SalI	G/TCGAC	●	●	■
SalI-HF	G/TCGAC	●	●	■
SapI	GCTCTTC(1/4)	●	●	●
Sau3AI	/GATC	●	●	□ ol
Sau96I	G/GNCC	●	□ ol	□ ol
SbfI	CCTGCA/GG	●	●	●
SbfI-HF	CCTGCA/GG	●	●	●
ScaI-HF	AGT/ACT	●	●	●
ScrFI	CC/NGG	●	□ ol	□ ol
SexAI	A/CCWGGT	●	■	●
SfaNI	GCATC(5/9)	●	●	◇ scol
SfcI	C/TRYAG	●	●	●
SfiI	GGCCNNNN/NGGCC	●	◇ ol	□ scol
SfoI	GGC/GCC	●	□ scol	■
SgrAI	CR/CCGGYG	●	●	■
SmaI	CCC/GGG	●	●	■
SmlI	C/TYRAG	●	●	●
SnaBI	TAC/GTA	●	●	■
SpeI	A/CTAGT	●	●	●
SpeI-HF	A/CTAGT	●	●	●
SphI	GCATG/C	●	●	●
SphI-HF	GCATG/C	●	●	●
SrfI	GCCC/GGGC	●	●	■
SspI	AAT/ATT	●	●	●
SspI-HF	AAT/ATT	●	●	●
StuI	AGG/CCT	●	□ ol	●
StyI	C/CWWGG	●	●	●
StyI-HF	C/CWWGG	●	●	●
StyD4I	/CCNGG	●	□ ol	◇ ol
SwaI	ATTT/AAAT	●	●	●
Taq$^{\alpha}$I	T/CGA	□ ol	●	●

ENZYME	SEQUENCE	Dam	Dcm	CpG
TfiI	G/AWTC	●	●	□ scol
TseI	G/CWGC	●	●	□ scol
Tsp45I	/GTSAC	●	●	●
TspMI	C/CCGGG	●	●	■
TspRI	NNCASTGNN/	●	●	●
Tth111I	GACN/NNGTC	●	●	●
XbaI	T/CTAGA	□ ol	●	●
XcmI	CCANNNNN/NNNNTGG	●	●	●
XhoI	C/TCGAG	●	●	◆
XmaI	C/CCGGG	●	●	◆
XmnI	GAANN/NNTTC	●	●	●
ZraI	GAC/GTC	●	●	■

Online Tools

The Tools & Resources tab, accessible on our homepage, contains a selection of interactive technical tools for use with restriction enzymes. These tools can also be accessed directly in the footer of every web page.

NEB Tools for Restriction Enzymes

DNA Sequences and Maps Tool

With the DNA Sequences and Maps Tool, find the nucleotide sequence files for commonly used molecular biology tools, including plasmid, viral and bacteriophage vectors.

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double-digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product.

Enzyme Finder

Use this tool to select restriction enzymes by name, sequence, overhang or type. Enter your sequence using single letter code, and Enzyme Finder will identify the right enzyme for the job.

NEBioCalculator®

NEBioCalculator is a collection of calculators and converters that are useful in planning bench experiments in molecular biology laboratories.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation and transformation) of your next traditional cloning experiment. It is also very helpful with double digests! While you are there, you can also, find other relevant tools and resources to enable protocol optimization.

NEBcutter® V2.0

Identify restriction sites within your DNA sequence using NEBcutter. Choose between Type II and commercially available Type III enzymes to digest your DNA. NEBcutter also indicates cut frequency and methylation sensitivity.

REBASE®

Use this tool as a guide to the ever-changing landscape of restriction enzymes. REBASE, the Restriction Enzyme DataBASE, is a dynamic, curated database of restriction enzymes and related proteins.

Mobile Apps

NEB Tools for iPhone®, iPad® or Android™

NEB Tools brings New England Biolabs' most popular web tools to your iPhone, iPad or Android devices.

- Use Enzyme Finder to select a restriction enzyme by category or recognition sequence, or search by name to find information on any NEB enzyme. Sort your results so they make sense to you, then email them to your inbox or connect directly to www.neb.com.
- Use Double Digest Finder or NEBcloner to determine buffer and reaction conditions for experiments requiring two restriction enzymes.

When using either of these tools, look for CutSmart, HF and Time-Saver enzymes for the ultimate in convenience. NEB Tools enables quick and easy access to the most requested restriction enzyme information, and allows you to plan your experiments from anywhere.

Cleavage Close to the Ends of DNA Fragments

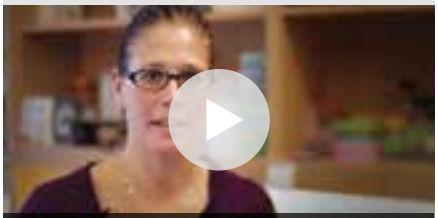
Annealed 5´ FAM-labeled oligos were incubated with the indicated enzyme (10 units/ 1pmol oligo) for 60 minutes at the recommended incubation temperature and NEBuffer. The digest was run on a TBE acrylamide gel and analyzed by fluorescent imaging. The double stranded oligos were designed to have the indicated number of base pairs from the end followed by the recognition sequence and an additional 12 bases. In some cases asymmetric cleavage was observed and interpreted as a negative result. Asymmetric cleavage decreased with increasing base pairs from the end.

Note: As a general rule and for enzymes not listed below, 6 base pairs should be added on on either side of the recognition site to cleave efficiently. The extra bases should be chosen so that palindromes and primer dimers are not formed. In most cases there is no requirement for specific bases.

TOOLS & RESOURCES

Visit www.neb.com for:

- Technical information including additional charts, protocols and technical tips related to restriction enzymes



ENZYME	BASE PAIRS FROM END 1 bp	2 bp	3 bp	4 bp	5 bp
AciI	–	+	+	++	+++
AgeI	+++	+++	+++	+++	+++
AgeI-HF	++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BglI	++	+++	+++	+++	+++
BmtI	+++	+++	+++	+++	+++
BsaI	+++	+++	+++	+++	+++
BsaI-HF	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsmBI	+++	+++	+++	+++	+++
BsrGI	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII	+++	+++	+++	+++	+++
DraIII-HF	+++	+++	+++	+++	+++
EagI	++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI	+	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI	+	++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI	+	++	+++	+++	+++
MseI	+++	+++	+++	+++	+++
NcoI	–	++	+++	+++	+++
NcoI-HF	+	++	+++	+++	+++

ENZYME	BASE PAIRS FROM END 1 bp	2 bp	3 bp	4 bp	5 bp
NdeI	+	+	+++	+++	+++
NheI	+	++	+++	+++	+++
NheI-HF	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	–	–	+	+	+++
PacI	+++	+++	+++	+++	+++
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PstI	+	+++	+++	+++	+++
PstI-HF	++	+++	+++	+++	+++
PvuI	+++	+++	++	+++	+++
PvuI-HF	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF	–	++	++	+++	+++
RsaI	+	+++	+++	+++	+++
SacI	–	++	+++	+++	+++
SacI-HF	–	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	–	++	+++	+++	+++
SalI-HF	–	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
SbfI	++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI	+++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI	+	+++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI	+	++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Chart Legend

– 0%
+ 0–20%
++ 20–50%
+++ 50–100%
NT not tested

Performance Chart for Restriction Enzymes

New England Biolabs supplies > 210 restriction enzymes that are 100% active in a single buffer, CutSmart. This results in increased efficiency, flexibility and ease-of-use, especially when performing double digests.

This performance chart summarizes the activity information of NEB restriction enzymes. To help select the best conditions for double digests, this chart shows the optimal (supplied) NEBuffer and approximate activity in the four standard NEBuffers for each enzyme. Note that BSA is included in all NEBuffers, and is not provided as a separate tube. In addition, this performance chart shows recommended reaction temperature, heat-inactivation temperature, recommended diluent buffer, methylation sensitivity and whether the enzyme is Time-Saver qualified (e.g., cleaves substrate in 5–15 minutes under recommended conditions, and can be used overnight without degradation of DNA).

Chart Legend

U	Supplied with a unique reaction buffer that is different from the four standard NEBuffers. The compatibility with the four standard NEBuffers is indicated in the chart.	**SAM**	Supplied with a separate vial of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as specified on the product data card.
RR	Recombinant	dam	dam methylation sensitivity
⏱	Time-Saver qualified	dcm	dcm methylation sensitivity
e	Engineered enzyme for maximum performance	CpG	CpG methylation sensitivity
2*site	Indicates that the restriction enzyme requires two or more sites for cleavage		

Activity Notes (see last column)

FOR STAR ACTIVITY

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

FOR LIGATION AND RECUTTING

a. Ligation is less than 10%

b. Ligation is 25% – 75%

c. Recutting after ligation is < 5%

d. Recutting after ligation is 50% – 75%

e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

NEBuffer Compositions (1X)

NEBuffer 1.1	10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 100 µg/ml BSA (pH 7.0 @ 25°C).
NEBuffer 2.1	10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 100 µg/ml BSA (pH 7.9 @ 25°C).
NEBuffer 3.1	50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 100 µg/ml BSA (pH 7.9 @ 25°C).
CutSmart Buffer	20 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, 100 µg/ml BSA (pH 7.9 @ 25°C).
Diluent A	50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA (pH 7.4 @ 25°C).
Diluent B	300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA, 50% glycerol (pH 7.4 @ 25°C).
Diluent C	50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA 50% glycerol (pH 7.4 @ 25°C).

	ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
RR ⏱	AatII	CutSmart	< 10	50*	50	100	37°	80°	B	Lambda		CpG	
RR	AbaSI	CutSmart	25	50	50	100	25°	65°	C	T4 wt Phage			e
RR ⏱	AccI	CutSmart	50	50	10	100	37°	80°	A	Lambda		CpG	
RR ⏱	Acc65I	3.1	10	75*	100	25	37°	65°	A	pBC4	dcm	CpG	
RR ⏱	AciI	CutSmart	< 10	25	100	100	37°	65°	A	Lambda		CpG	d
RR ⏱	AclI	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda		CpG	
RR ⏱	AcuI	CutSmart + SAM	50	100	50	100	37°	65°	B	Lambda			1, b, d
RR	AfeI	CutSmart	25	100	25	100	37°	65°	B	pXba		CpG	
RR ⏱	AflII	CutSmart	50	100	10	100	37°	65°	A	phiX174			
RR	AflIII	3.1	10	50	100	50	37°	80°	B	Lambda			
RR	AgeI	1.1	100	75	25	75	37°	65°	C	Lambda		CpG	
RR ⏱ e	AgeI-HF	CutSmart	100	50	10	100	37°	65°	A	Lambda		CpG	
RR ⏱	AhdI	CutSmart	25	25	10	100	37°	65°	A	Lambda		CpG	a
RR	AleI	CutSmart	< 10	< 10	< 10	100	37°	80°	B	Lambda		CpG	
RR ⏱	AluI	CutSmart	25	100	50	100	37°	80°	B	Lambda			b
RR	AlwI	CutSmart	50	50	10	100	37°	No	A	Lambda dam-	dam		1, b, d
RR ⏱	AlwNI	CutSmart	10	100	50	100	37°	80°	A	Lambda	dcm		
RR ⏱	ApaI	CutSmart	25	25	< 10	100	25°	65°	A	pXba	dam	CpG	
RR ⏱	ApaLI	CutSmart	100	100	10	100	37°	No	A	Lambda HindIII		CpG	
RR ⏱	ApeKI	3.1	25	50	100	10	75°	No	B	Lambda		CpG	
RR ⏱	ApoI	3.1	10	75	100	75	50°	80°	A	Lambda			
RR ⏱ e	ApoI-HF	CutSmart	10	100	10	100	37°	80°	B	Lambda			
RR ⏱	AscI	CutSmart	< 10	10	10	100	37°	80°	A	Lambda		CpG	
RR ⏱	AseI	3.1	< 10	50*	100	10	37°	65°	B	Lambda			3
RR	AsiSI	CutSmart	50	100	100	100	37°	80°	B	pXba (Xho digested)		CpG	2, b

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
AvaI	CutSmart	< 10	100	25	100	37°	80°	A	Lambda		CpG	
AvaII	CutSmart	50	75	10	100	37°	80°	A	Lambda	dcm	CpG	
AvrII	CutSmart	100	50	50	100	37°	No	B	Lambda HindIII			
BaeI	CutSmart + SAM	50	100	50	100	25°	65°	A	Lambda		CpG	e
BaeGI	3.1	75	75	100	25	37°	80°	A	Lambda			
BamHI	3.1	75*	100*	100	100*	37°	No	A	Lambda			3
BamHI-HF	CutSmart	100	50	10	100	37°	No	A	Lambda			
BanI	CutSmart	10	25	< 10	100	37°	65°	A	Lambda	dcm	CpG	1
BanII	CutSmart	100	100	50	100	37°	80°	A	Lambda			2
BbsI	2.1	100	100	25	75	37°	65°	B	Lambda			
BbsI-HF	CutSmart	10	10	10	100	37°	65°	B	Lambda			3
BbvI	CutSmart	100	100	25	100	37°	65°	B	pBR322			3
BbvCI	CutSmart	10	100	50	100	37°	No	B	Lambda		CpG	1, a
BccI	CutSmart	100	50	10	100	37°	65°	A	pXba			3, b
BceAI	3.1	100*	100*	100	100*	37°	65°	A	pBR322		CpG	1
BcgI	3.1 + SAM	10	75*	100	50*	37°	65°	A	Lambda	dam	CpG	e
BciVI	CutSmart	100	25	< 10	100	37°	80°	C	Lambda			b
BclI	3.1	50	100	100	75	50°	No	A	Lambda dam-	dam		
BclI-HF	CutSmart	100	100	10	100	37°	65°	B	Lambda dam-	dam		3
BcoDI	CutSmart	50	75	75	100	37°	No	B	Lambda		CpG	
BfaI	CutSmart	< 10	10	< 10	100	37°	80°	B	Lambda			2, b
BfuAI	3.1	< 10	25	100	10	50°	65°	B	Lambda		CpG	3
BfuCI	CutSmart	100	50	25	100	37°	80°	B	Lambda		CpG	
BglI	3.1	10	25	100	10	37°	65°	B	Lambda		CpG	
BglII	3.1	10	10	100	< 10	37°	No	A	Lambda			
BlpI	CutSmart	50	100	10	100	37°	No	A	Lambda			d
BmgBI	3.1	< 10	10	100	10	37°	65°	B	Lambda		CpG	3, b, d
BmrI	2.1	75	100	75	100*	37°	65°	B	Lambda HindIII			b
BmtI	3.1	100	100	100	100	37°	65°	B	pXba			2
BmtI-HF	CutSmart	50	100	10	100	37°	65°	B	pXba			
BpmI	3.1	75	100	100	100	37°	65°	B	Lambda			2
Bpu10I	3.1	10	25	100	25	37°	80°	B	Lambda			3, b, d
BpuEI	CutSmart + SAM	50*	100	50*	100	37°	65°	B	Lambda			d
BsaI	CutSmart	75*	75	100	100	37°	65°	B	pXba	dcm	CpG	3
BsaI-HF	CutSmart	50	100	25	100	37°	65°	B	pXba	dcm	CpG	
BsaAI	CutSmart	100	100	100	100	37°	No	C	Lambda		CpG	
BsaBI	CutSmart	50	100	75	100	60°	80°	B	Lambda dam-	dam	CpG	2
BsaHI	CutSmart	50	100	100	100	37°	80°	C	Lambda	dcm	CpG	
BsaJI	CutSmart	50	100	100	100	60°	80°	A	Lambda			
BsaWI	CutSmart	10	100	50	100	60°	80°	A	Lambda			
BsaXI	CutSmart	50*	100*	10	100	37°	No	C	Lambda			e
BseRI	CutSmart	100*	100	75	100	37°	80°	A	Lambda			d
BseYI	3.1	10	50	100	50	37°	80°	B	Lambda		CpG	d
BsgI	CutSmart + SAM	25	50	25	100	37°	65°	B	Lambda			d
BsiEI	CutSmart	25	50	< 10	100	60°	No	A	Lambda		CpG	
BsiHKAI	CutSmart	25	100	100	100	65°	No	A	Lambda			
BsiWI	3.1	25	50*	100	25	55°	65°	B	phiX174		CpG	
BsiWI-HF	CutSmart	50	100	10	100	37°	No	B	phiX174		CpG	3
BsII	CutSmart	50	75	100	100	55°	No	A	Lambda	dcm	CpG	b
BsmI	CutSmart	25	100	< 10	100	65°	80°	A	Lambda			
BsmAI	CutSmart	50	100	100	100	55°	No	B	Lambda		CpG	
BsmBI	3.1	10	50*	100	25	55°	80°	B	Lambda		CpG	
BsmFI	CutSmart	25	50	50	100	65°	80°	A	pBR322	dcm	CpG	1

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
BsoBI	CutSmart	25	100	100	100	37°	80°	A	Lambda			
Bsp1286I	CutSmart	25	25	25	100	37°	65°	A	Lambda			3
BspCNI	CutSmart + SAM	100	75	10	100	25°	80°	A	Lambda			b
BspDI	CutSmart	25	75	50	100	37°	80°	A	Lambda	dam	CpG	
BspEI	3.1	< 10	10	100	< 10	37°	80°	B	Lambda dam-	dam	CpG	
BspHI	CutSmart	< 10	50	25	100	37°	80°	A	Lambda	dam		
BspMI	3.1	10	50*	100	10	37°	65°	B	Lambda			
BspQI	3.1	100	100	100	100	50°	80°	B	Lambda			3
BsrI	3.1	< 10	50	100	10	65°	80°	B	phiX174			b
BsrBI	CutSmart	50	100	100	100	37°	80°	A	Lambda		CpG	d
BsrDI	2.1	10	100	75	25	65°	80°	A	Lambda			3, d
BsrF$^{\alpha}$I	CutSmart	25	25	0	100	37°	No	C	pBR322		CpG	
BsrGI	2.1	25	100	100	25	37°	80°	A	Lambda			
BsrGI-HF	CutSmart	10	100	100	100	37°	80°	A	Lambda			
BssHII	CutSmart	100	100	100	100	50°	65°	B	Lambda		CpG	
BssS$^{\alpha}$I	CutSmart	10	25	< 10	100	37°	No	B	Lambda			
BstAPI	CutSmart	50	100	25	100	60°	80°	A	Lambda		CpG	b
BstBI	CutSmart	75	100	10	100	65°	No	A	Lambda		CpG	
BstEII	3.1	10	75*	100	75*	60°	No	A	Lambda			3
BstEII-HF	CutSmart	< 10	10	< 10	100	37°	No	A	Lambda			
BstNI	3.1	10	100	100	75	60°	No	A	Lambda			a
BstUI	CutSmart	50	100	25	100	60°	No	A	Lambda		CpG	b
BstXI	3.1	< 10	50	100	25	37°	80°	B	Lambda	dcm		3
BstYI	2.1	25	100	75	100	60°	No	A	Lambda			
BstZ17I-HF	CutSmart	100	100	10	100	37°	No	A	Lambda		CpG	
Bsu36I	CutSmart	25	100	100	100	37°	80°	C	Lambda HindIII			b
BtgI	CutSmart	50	100	100	100	37°	80°	B	pBR322			
BtgZI	CutSmart	10	25	< 10	100	60°	80°	A	Lambda		CpG	3, b, d
Bts$^{\alpha}$I	CutSmart	100	100	25	100	55°	No	A	Lambda			
BtsIMutI	CutSmart	100	50	10	100	55°	80°	A	pUC19			b
BtsCI	CutSmart	10	100	25	100	50°	80°	B	Lambda			
Cac8I	CutSmart	50	75	100	100	37°	65°	B	Lambda		CpG	b
ClaI	CutSmart	10	50	50	100	37°	65°	A	Lambda dam-	dam	CpG	
CspCI	CutSmart + SAM	10	100	10	100	37°	65°	A	Lambda			e
CviAII	CutSmart	50	50	10	100	25°	65°	C	Lambda			
CviKI-1	CutSmart	25	100	100	100	37°	No	A	pBR322			1, b
CviQI	3.1	75	100*	100	75*	25°	No	C	Lambda			b
DdeI	CutSmart	75	100	100	100	37°	65°	B	Lambda			
DpnI	CutSmart	100	100	75	100	37°	80°	B	pBR322		CpG	b
DpnII	U	25	25	100*	25	37°	65°	B	Lambda dam-	dam		
DraI	CutSmart	75	75	50	100	37°	65°	A	Lambda			
DraIII-HF	CutSmart	< 10	50	10	100	37°	No	B	Lambda		CpG	b
DrdI	CutSmart	25	50	10	100	37°	65°	A	pUC19		CpG	3
EaeI	CutSmart	10	50	< 10	100	37°	65°	A	Lambda	dcm	CpG	b
EagI	3.1	10	25	100	10	37°	65°	B	pXba		CpG	
EagI-HF	CutSmart	25	100	100	100	37°	65°	B	pXba		CpG	
EarI	CutSmart	50	10	< 10	100	37°	65°	B	Lambda		CpG	b, d
EciI	CutSmart	100	50	50	100	37°	65°	A	Lambda		CpG	2
Eco53kI	CutSmart	100	100	< 10	100	37°	65°	A	pXba		CpG	3, b
EcoNI	CutSmart	50	100	75	100	37°	65°	A	Lambda			b
Eco0109I	CutSmart	50	100	50	100	37°	65°	A	Lambda HindIII	dcm		3
EcoP15I	3.1 + ATP	75	100	100	100	37°	65°	A	pUC19			e

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
EcoRI	U	25	100*	50	50*	37°	65°	C	Lambda		CpG	
EcoRI-HF	CutSmart	10	100	< 10	100	37°	65°	C	Lambda		CpG	
EcoRV	3.1	10	50	100	10	37°	80°	A	Lambda		CpG	
EcoRV-HF	CutSmart	25	100	100	100	37°	65°	B	Lambda		CpG	
FatI	2.1	10	100	50	50	55°	80°	A	pUC19			
FauI	CutSmart	100	50	10	100	55°	65°	A	Lambda		CpG	3, b, d
Fnu4HI	CutSmart	< 10	< 10	< 10	100	37°	No	A	Lambda		CpG	a
FokI	CutSmart	100	100	75	100	37°	65°	A	Lambda	dcm	CpG	3, b, d
FseI	CutSmart	100	75	< 10	100	37°	65°	B	pBC4	dcm	CpG	
FspI	CutSmart	10	100	10	100	37°	No	C	Lambda		CpG	b
FspEI	CutSmart	< 10	< 10	< 10	100	37°	80°	B	pBR322	dcm		2, e
HaeII	CutSmart	25	100	10	100	37°	80°	A	Lambda		CpG	
HaeIII	CutSmart	50	100	25	100	37°	80°	A	Lambda			
HgaI	1.1	100	100	25	100	37°	65°	A	phiX174		CpG	1
HhaI	CutSmart	25	100	100	100	37°	65°	A	Lambda		CpG	
HincII	3.1	25	100	100	100	37°	65°	B	Lambda		CpG	
HindIII	2.1	25	100	50	50	37°	80°	B	Lambda			2
HindIII-HF	CutSmart	10	100	10	100	37°	80°	B	Lambda			
HinfI	CutSmart	50	100	100	100	37°	80°	A	Lambda		CpG	
HinP1I	CutSmart	100	100	100	100	37°	65°	A	Lambda		CpG	
HpaI	CutSmart	< 10	75*	25	100	37°	No	A	Lambda		CpG	1
HpaII	CutSmart	100	50	< 10	100	37°	80°	A	Lambda		CpG	
HphI	CutSmart	50	50	< 10	100	37°	65°	B	Lambda	dam	dcm	b, d
Hpy99I	CutSmart	50	10	< 10	100	37°	65°	A	Lambda		CpG	
Hpy166II	CutSmart	100	100	50	100	37°	65°	C	pBR322		CpG	
Hpy188I	CutSmart	25	100	50	100	37°	65°	A	pBR322	dam		1, b
Hpy188III	CutSmart	100	100	10	100	37°	65°	B	pUC19	dam	CpG	3, b
HpyAV	CutSmart	100	100	25	100	37°	65°		Lambda		CpG	3, b, d
HpyCH4III	CutSmart	100	25	< 10	100	37°	65°	A	Lambda			b
HpyCH4IV	CutSmart	100	50	25	100	37°	65°	A	pUC19		CpG	
HpyCH4V	CutSmart	50	50	25	100	37°	65°	A	Lambda			
I-CeuI	CutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized			
I-SceI	CutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized			
KasI	CutSmart	50	100	50	100	37°	65°	B	pBR322		CpG	3
KpnI	1.1	100	75	< 10	50	37°	No	A	pXba			1
KpnI-HF	CutSmart	100	25	< 10	100	37°	No	A	pXba			
LpnPI	CutSmart	< 10	< 10	< 10	100	37°	65°	B	pBR322			2, e
MboI	CutSmart	75	100	100	100	37°	65°	A	Lambda dam-	dam	CpG	
MboII	CutSmart	100*	100	50	100	37°	65°	C	Lambda dam-	dam		b
MfeI	CutSmart	75	50	10	100	37°	No	A	Lambda			2
MfeI-HF	CutSmart	75	25	< 10	100	37°	No	A	Lambda			
MluI	3.1	10	50	100	25	37°	80°	A	Lambda		CpG	
MluI-HF	CutSmart	25	100	100	100	37°	No	A	Lambda		CpG	
MluCI	CutSmart	100	10	10	100	37°	No	A	Lambda			
MlyI	CutSmart	50	50	10	100	37°	65°	A	Lambda			b, d
MmeI	CutSmart + SAM	50	100	50	100	37°	65°	B	phiX174		CpG	b, c
MnlI	CutSmart	75	100	50	100	37°	65°	B	Lambda			b
MscI	CutSmart	25	100	100	100	37°	80°	C	Lambda	dcm		
MseI	CutSmart	75	100	75	100	37°	65°	A	Lambda			
MslI	CutSmart	50	50	< 10	100	37°	80°	A	Lambda			
MspI	CutSmart	75	100	50	100	37°	No	A	Lambda			
MspA1I	CutSmart	10	50	10	100	37°	65°	B	Lambda		CpG	

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is <5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

PERFORMANCE CHART

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
MspJI	CutSmart	< 10	< 10	< 10	100	37°	65°	B	pBR322			2, e
MwoI	CutSmart	< 10	100	100	100	60°	No	B	Lambda		CpG	
NaeI	CutSmart	25	25	< 10	100	37°	No	A	pXba		CpG	b
NarI	CutSmart	100	100	10	100	37°	65°	A	pXba		CpG	
Nb.BbvCI	CutSmart	25	100	100	100	37°	80°	A	pUB			e
Nb.BsmI	3.1	< 10	50	100	10	65°	80°	A	pBR322			e
Nb.BsrDI	CutSmart	25	100	100	100	65°	80°	A	pUC19			e
Nb.BssSI	3.1	10	100	100	25	37°	No	B	pUC19			
Nb.BtsI	CutSmart	75	100	75	100	37°	80°	A	phiX174			e
NciI	CutSmart	100	25	10	100	37°	No	A	Lambda		CpG	b
NcoI	3.1	100	100	100	100	37°	80°	A	Lambda			
NcoI-HF	CutSmart	50	100	10	100	37°	80°	B	Lambda			
NdeI	CutSmart	75	100	100	100	37°	65°	A	Lambda			
NgoMIV	CutSmart	100	50	10	100	37°	No	A	pXba		CpG	1
NheI	2.1	100	100	10	100	37°	65°	C	Lambda HindIII		CpG	
NheI-HF	CutSmart	100	25	< 10	100	37°	80°	C	Lambda HindIII		CpG	
NlaIII	CutSmart	< 10	< 10	< 10	100	37°	65°	B	phiX174			
NlaIV	CutSmart	10	10	10	100	37°	65°	B	pBR322	dcm	CpG	
NmeAIII	CutSmart + SAM	10	10	< 10	100	37°	65°	B	phiX174			c
NotI	3.1	< 10	50	100	25	37°	65°	C	pBC4		CpG	
NotI-HF	CutSmart	25	100	25	100	37°	65°	A	pBC4		CpG	
NruI	3.1	< 10	10	100	10	37°	No	A	Lambda	dam	CpG	b
NruI-HF	CutSmart	0	25	50	100	37°	No	A	Lambda	dam	CpG	
NsiI	3.1	10	75	100	25	37°	65°	B	Lambda			
NsiI-HF	CutSmart	< 10	20	< 10	100	37°	80°	B	Lambda			
NspI	CutSmart	100	100	< 10	100	37°	65°	A	Lambda			
Nt.AlwI	CutSmart	10	100	100	100	37°	80°	A	pUC101 dam-dcm-	dam		e
Nt.BbvCI	CutSmart	50	100	10	100	37°	80°	A	pUB		CpG	e
Nt.BsmAI	CutSmart	100	50	10	100	37	65°	A	pBR322		CpG	e
Nt.BspQI	3.1	< 10	25	100	10	50°	80°	B	pUC19			e
Nt.BstNBI	3.1	0	10	100	10	55°	80°	A	T7			
PacI	CutSmart	100	75	10	100	37°	65°	A	pNEB193			
PaeR7I	CutSmart	25	100	10	100	37°	No	A	Lambda HindIII		CpG	
PciI	3.1	50	75	100	50*	37°	80°	B	pXba			
PflFI	CutSmart	25	100	25	100	37°	65°	A	pBC4			b
PflMI	3.1	0	100	100	50	37°	65°	A	Lambda	dcm		3, b, d
PI-PspI	U	10	10	10	10	65°	No	B	pAKR XmnI			
PI-SceI	U	10	10	10	10	37°	65°	B	pBSvdeX XmnI			
PleI	CutSmart	25	50	25	100	37°	65°	A	Lambda		CpG	b, d
PluTI	CutSmart	100	25	< 10	100	37°	65°	A	pXba		CpG	
PmeI	CutSmart	< 10	50	10	100	37°	65°	A	Lambda		CpG	
PmlI	CutSmart	100	50	< 10	100	37°	65°	A	Lambda HindIII		CpG	
PpuMI	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda HindIII	dcm		
PshAI	CutSmart	25	50	10	100	37°	65°	A	Lambda		CpG	
PsiI	CutSmart	10	100	10	100	37°	65°	B	Lambda			3
PspGI	CutSmart	25	100	50	100	75°	No	A	T7	dcm		3
PspOMI	CutSmart	10	10	< 10	100	37°	65°	B	pXba	dcm	CpG	
PspXI	CutSmart	< 10	100	25	100	37°	No	B	Lambda HindIII		CpG	
PstI	3.1	75	75	100	50*	37°	80°	C	Lambda			
PstI-HF	CutSmart	10	75	50	100	37°	No	C	Lambda			
PvuI	3.1	< 10	25	100	< 10	37°	No	B	pXba		CpG	

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2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
PvuI-HF	CutSmart	25	100	100	100	37°	No	B	pXba		CpG	
PvuII	3.1	50	100	100	100*	37°	No	B	Lambda			
PvuII-HF	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda			
RsaI	CutSmart	25	50	< 10	100	37°	No	A	Lambda		CpG	
RsrII	CutSmart	25	75	10	100	37°	65°	C	Lambda		CpG	
SacI	1.1	100	50	10	100	37°	65°	A	Lambda HindIII			
SacI-HF	CutSmart	10	50	< 10	100	37°	65°	A	Lambda HindIII		CpG	
SacII	CutSmart	10	100	10	100	37°	65°	A	pXba		CpG	
SalI	3.1	< 10	< 10	100	< 10	37°	65°	A	Lambda HindIII		CpG	
SalI-HF	CutSmart	10	100	100	100	37°	65°	A	Lambda HindIII		CpG	
SapI	CutSmart	75	50	< 10	100	37°	65°	B	Lambda			
Sau3AI	1.1	100	50	10	100	37°	65°	A	Lambda		CpG	b
Sau96I	CutSmart	50	100	100	100	37°	65°	A	Lambda	dcm	CpG	
SbfI	CutSmart	50	25	< 10	100	37°	80°	A	Lambda			3
SbfI-HF	CutSmart	50	25	< 10	100	37°	80°	B	Lambda			
ScaI-HF	CutSmart	100	100	10	100	37°	80°	B	Lambda			
ScrFI	CutSmart	100	100	100	100	37°	65°	C	Lambda	dcm	CpG	2, a
SexAI	CutSmart	100	75	50	100	37°	65°	A	pBC4 dcm-	dcm		3, b, d
SfaNI	3.1	< 10	75	100	25	37°	65°	B	phiX174		CpG	3, b
SfcI	CutSmart	75	50	25	100	37°	65°	B	Lambda			3
SfiI	CutSmart	25	100	50	100	50°	No	C	Adenovirus-2	dcm	CpG	
SfoI	CutSmart	50	100	100	100	37°	No	B	Lambda HindIII	dcm	CpG	
SgrAI	CutSmart	100	100	10	100	37°	65°	A	Lambda		CpG	1
SmaI	CutSmart	< 10	< 10	< 10	100	25°	65°	B	Lambda HindIII		CpG	b
SmlI	CutSmart	25	75	25	100	55°	No	A	Lambda			b
SnaBI	CutSmart	50	50	10	100	37°	80°	A	T7		CpG	1
SpeI	CutSmart	75	100	25	100	37°	80°	C	Adenovirus-2			
SpeI-HF	CutSmart	25	50	10	100	37°	80°	C	pXba			
SphI	2.1	100	100	50	100	37°	65°	B	Lambda			2
SphI-HF	CutSmart	50	25	10	100	37°	65°	B	Lambda			
SrfI	CutSmart	10	50	0	100	37°	65°	B	pNEB193-SrFI		CpG	
SspI	U	50	100	50	50	37°	65°	C	Lambda			
SspI-HF	CutSmart	25	100	< 10	100	37°	65°	B	Lambda			
StuI	CutSmart	50	100	50	100	37°	No	A	Lambda	dcm		
StyD4I	CutSmart	10	100	100	100	37°	65°	B	Lambda	dcm	CpG	
StyI	3.1	10	25	100	10	37°	65°	A	Lambda			b
StyI-HF	CutSmart	25	100	25	100	37°	65°	A	Lambda			
SwaI	3.1	10	10	100	10	25°	65°	B	pXba			b, d
Taq^I	CutSmart	50	75	100	100	65°	80°	B	Lambda	dam		
TfiI	CutSmart	50	100	100	100	65°	No	C	Lambda		CpG	
TseI	CutSmart	75	100	100	100	65°	No	B	Lambda		CpG	3
Tsp45I	CutSmart	100	50	< 10	100	65°	No	A	Lambda			
TspMI	CutSmart	50*	75*	50*	100	75°	No	B	pUCAdeno		CpG	d
TspRI	CutSmart	25	50	25	100	65°	No	B	Lambda			
Tth111I	CutSmart	25	100	25	100	65°	No	B	pBC4			b
XbaI	CutSmart	< 10	100	75	100	37°	65°	A	Lambda HindIII dam-	dam		
XcmI	2.1	10	100	25	100	37°	65°	C	Lambda			2
XhoI	CutSmart	75	100	100	100	37°	65°	A	Lambda HindIII			b
XmaI	CutSmart	25	50	< 10	100	37°	65°	A	pXba		CpG	3
XmnI	CutSmart	50	75	< 10	100	37°	65°	A	Lambda			b
ZraI	CutSmart	100	25	10	100	37°	80°	B	Lambda		CpG	

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Germany & Austria
New England Biolabs GmbH
Brüningstr. 50, Geb B852
65926 Frankfurt/Main, Germany
Tel: +49/(0)69/305-23140
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

www.neb-online.de

France
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 Evry cedex, France
Tel.: 0800 100 632 (Customer Service)
Tel.: 0800 100 633 (Technical Service)
FAX.: 0800 100 610
info.fr@neb.com

www.neb-online.fr

USA
New England Biolabs, Inc.
Telephone: (978) 927-5054
Toll Free (USA Orders): 1-800-632-5227
Toll Free (USA Tech): 1-800-632-7799
Fax: (978) 921-1350
info@neb.com
www.neb.com

www.neb.com

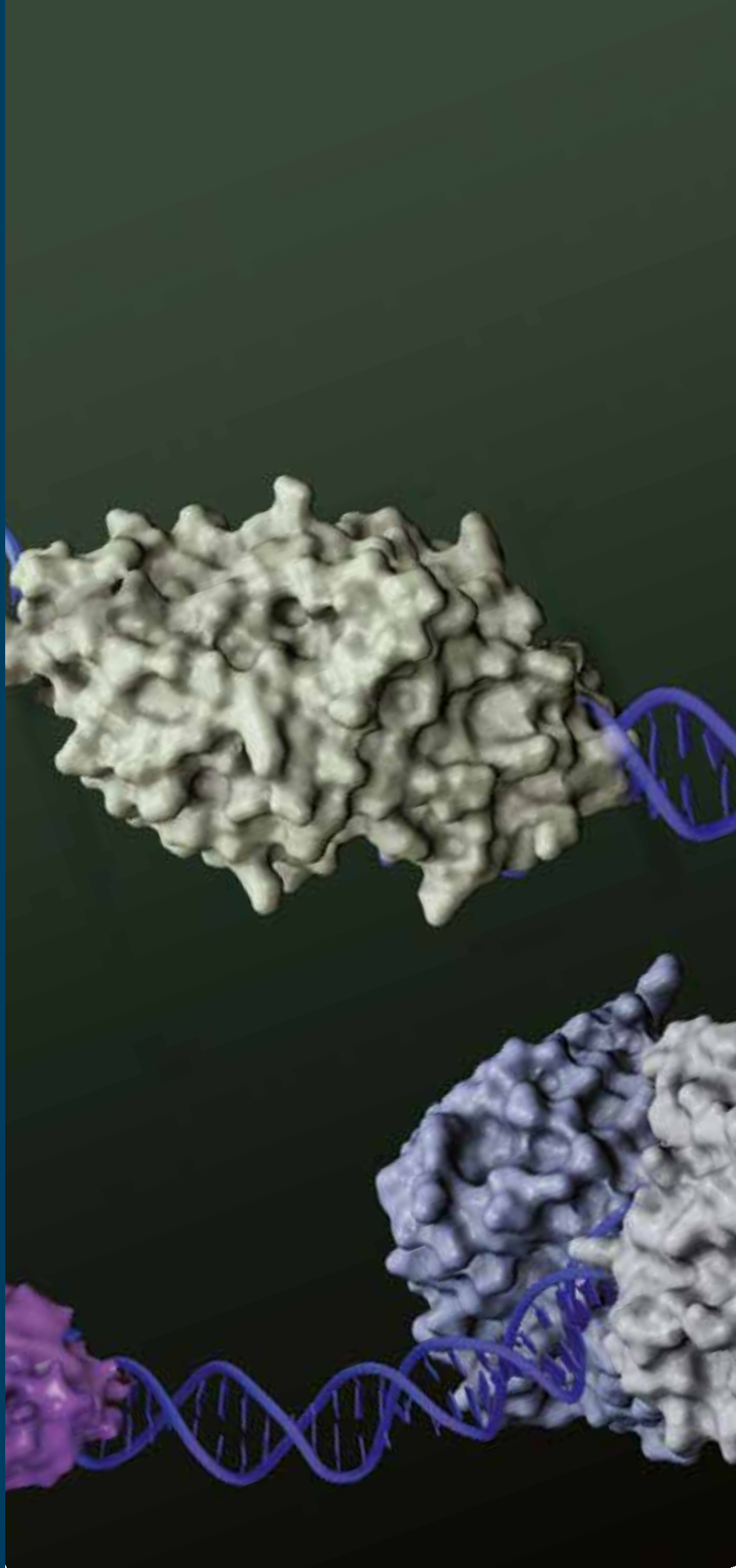
Canada
New England Biolabs, Ltd.
Toll Free: 1-800-387-1095
info.ca@neb.com

China, People's Republic
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
info@neb-china.com

Japan
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info.jp@neb.com

Singapore
New England Biolabs, PTE. Ltd.
Telephone: +65 63859623
sales.sg@neb.com

United Kingdom
New England Biolabs (UK), Ltd.
Call Free: 0800 318486
info.uk@neb.com



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