Reference Appendix

Technical Support – for scientists, by scientists

As a partner to the scientific community, New England Biolabs is committed to providing top quality tools and scientific expertise. This philosophy still stands, and has led to long-standing relationships with many of our fellow scientists. NEB's commitment to scientists is the same regardless of whether or not they purchase product from NEB: their ongoing research is supported by our catalog, website and technical staff.

NEB's technical support model is unique as it utilizes most of the scientists at NEB. Several of our product lines have designated technical support scientists assigned to servicing customers in those application areas. Any questions regarding a product could be dealt with by one of the technical support scientists, the product manager who manufactures it, the product development scientist who optimizes it, or a researcher who uses the product in their daily research. As such, customers are supported by scientists and often experts in the product or its application.

To access technical support:

- Call 1-800-632-7799 (Monday – Friday: 9:00 am - 6:00 pm EST)
- Submit an online form at www.neb.com/techsupport
- Email info@neb.com
- International customers can contact a local NEB subsidiary or distributor. For more information see inside back cover.

Learn more about NEB's tech support program.

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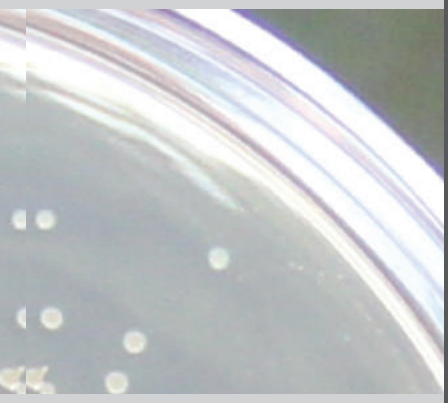
View NEB TV Episode #22 to learn more about our Technical Support program.

Visit the Tools & Resources tab at www.neb.com to find additional online tools, video tech tips and tutorials to help you in your research.



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Online Interactive Tools

Use the Tools & Resources tab at www.neb.com to access our growing selection of interactive technical tools. These tools can also be accessed directly in the footer of every web page.

NEB scientists are often involved in the development of online tools that will aid in their research. We are now making these tools and in some circumstances, the source code, available for you to evaluate. To learn more, visit **www.neb.com/NEBetaTools.**

Online Tools

Competitor Cross-Reference Tool

Use this tool to select another company's product and find out which NEB product is compatible. Choose either the product name or catalog number from the available selections, and this tool will identify the recommended NEB product.

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 nomenclature, and Enzyme Finder will identify the right enzyme for the job.

Glycan Analyzer

Use this tool to interpret ultra or high pressure liquid chromatography (UPLC/HPLC) N-glycan profiles following exoglycosidase digestions.

NEB Golden Gate Assembly Tool

Use this tool to assist with in silico DNA construct design for Golden Gate DNA assembly. It enables the accurate design of primers with appropriate Type IIS restriction sites and overlaps, quick import of sequences in many formats and export of the final assembly, primers and settings.

NEBaseChanger®

NEBaseChanger can be used to design primers specific to the mutagenesis experiment you are performing using the Q5® Site-Directed Mutagenesis Kit. This tool will also calculate a recommended custom annealing temperature based on the sequence of the primers by taking into account any mismatches.

NEBNext® Selector

Use this tool to guide you through the selection of NEBNext reagents for next generation sequencing sample preparation.

NEBcutter® V2.1

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

NEBioCalculator®

Use this tool for your scientific calculations and conversions for DNA and RNA. Options include conversion of mass to moles, ligation amounts, conversion of OD to concentration, dilution and molarity. Additional features include sgRNA template oligo design and qPCR library quantification.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation, transformation and mutagenesis) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBuilder® Assembly Tool

Use this tool to design primers for your DNA assembly reaction, based on the entered fragment sequences and the polymerase being used for amplification.

PCR Fidelity Estimator

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases.

PCR Selector

Use this tool to help select the right DNA polymerase for your PCR setup. Whether your amplicon is long, complex, GC-rich or present in a single copy, the PCR selection tool will identify the perfect DNA polymerase for your reaction.

Tm Calculator

Determine the optimal annealing temperature for your amplicon with our Tm Calculator. Simply input your DNA polymerase, primer concentration and your primer sequence, and the Tm Calculator will guide you to successful reaction conditions.

Thermostable Ligase Reaction Temperature Calculator

This tool will help you estimate an optimal reaction temperature to minimize mismatch for thermostable ligation of two adjacent ssDNA probes annealed to a template.

Online Tools (continued)

Read Coverage Calculator

This tool allows for easy calculation of values associated with read coverage in NGS protocols.

Additional Databases

Polbase®

Polbase is a repository of biochemical, genetic and structural information about DNA Polymerases.

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.

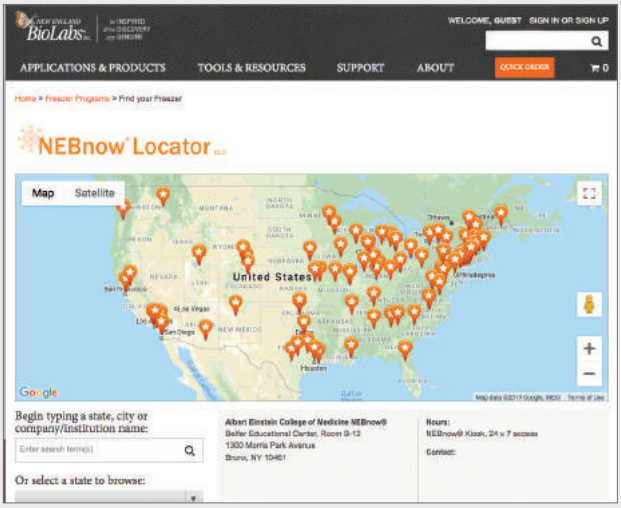
- Use Enzyme Finder to select a restriction enzyme by category or recognition sequence, or search by name to find information on any NEB enzyme. Sort your results so they make sense to you, then email them to your inbox or connect directly to www.neb.com.
- Use Double Digest Finder to determine buffer and reaction conditions for experiments requiring two restriction enzymes.
- Use Tm Calculator to calculate annealing temperatures for your PCR reaction.
- Also included are several popular calculators from the NEBioCalculator web app.

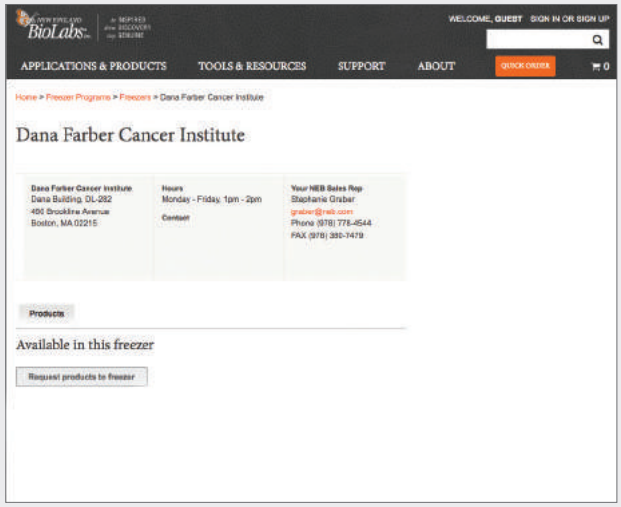
When using either of these tools, look for CutSmart®, HF® and Time-Saver™ enzymes for the ultimate in convenience. NEB Tools enables quick and easy access to the most requested restriction enzyme information, and allows you to plan your experiments from anywhere.

Looking for a Freezer Program?

NEBnow® Locator

NEBnow Freezer Programs are ideally suited for researchers in academics and industry looking for on-site access to the world's finest restriction enzymes and related products. NEB freezers offer you convenience, flexibility and value.





Optimizing Restriction Enzyme Reactions

While standard recommended reaction conditions are a good place to start, in some cases, optimization may be necessary to achieve the best results. Depending on the enzyme(s) being used, variables such as incubation time, number of enzyme units used, and reaction temperature should be tested to find the optimal reaction conditions for your substrate DNA and enzyme(s) of choice.

Protocol: Restriction Enzyme Reactions

	STANDARD PROTOCOL	TIME-SAVER PROTOCOL
DNA	up to 1 µg	up to 1 µg
10X NEBuffer	5 µl (1X)	5 µl (1X)
Restriction Enzymes	10 units*	1 µl
Total Volume	50 µl	50 µl
Incubation Temperature	Enzyme-dependent	Enzyme-dependent
Incubation Time	60 minutes	5–15 minutes**

*Sufficient to digest all types of DNAs.
**Time-Saver qualified enzymes can also be incubated overnight with no star activity.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Tips to avoid star activity
- Restriction Enzyme Performance Chart
- Troubleshooting guide
- Access to NEB's online tools, including: **Enzyme Finder, Double Digest Finder** and **NEBcloner**

TIPS FOR OPTIMIZATION

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 per µg of genomic DNA in a 1 hour digest

Star Activity

- Unwanted cleavage that can occur when enzyme is used under sub-optimal conditions, such as:
  – Too much enzyme present
  – Too long of an incubation time
  – Using a non-recommended buffer
  – Glycerol concentrations above 5%
- Star activity can be reduced by using a High-Fidelity (HF®) enzyme, reducing incubation time, using a Time-Saver™ enzyme or increasing reaction volume

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents and salts. Spin column purification readily accomplishes this; extra washes during purification can also help.
- Methylation of DNA can affect digestion with certain enzymes. For more information about methylation visit **www.neb.com/methylation**

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.
- Restriction enzymes that do not require BSA for optimal activity are not adversely affected if BSA is present in the reaction

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of up to 1 µg of substrate. This helps maintain salt levels introduced by miniprepped DNA low enough that they don't affect enzyme activity.
- 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

	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.

Incubation Time

- Incubation time for the Standard Protocol is 1 hour. Incubation for the Time-Saver Protocol is 5–15 minutes.
- Visit **www.neb.com/timesaver** for list of Time-Saver qualified enzymes
- It is possible, with many enzymes, 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. Visit **www.neb.com** for storage information.
- 10X NEBuffers should be stored at –20°C

Stability

- The expiration date is found on the label
- Long term exposure to temperatures above –20°C should be minimized whenever possible

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 (pages 293–298) rates the percentage activity of each restriction endonuclease in the four standard NEBuffers.

Setting up a Double Digestion

- 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 (see page 290). The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity (see page 300). 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, if it can be heat inactivated, 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 Digestion with a Unique Buffer (designated "U")

- NEB currently supplies three enzymes with unique buffers: EcoRI, SspI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI and SspI have HF versions (NEB #R3101 and NEB #R3132, respectively) which is supplied with CutSmart Buffer.

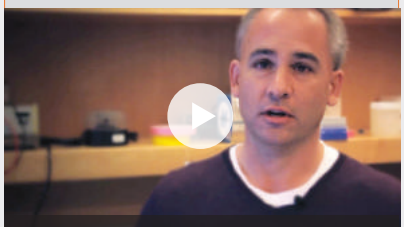
Setting up a Sequential Digestion

- If there is no buffer in which the two enzymes 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** and **NEBcloner®**



TIPS FOR SETTING UP DOUBLE DIGESTS

Types of Restriction Enzymes

Restriction enzymes are traditionally classified into four types on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements. However, amino acid sequencing has uncovered extraordinary variety among restriction enzymes and revealed that at the molecular level there are many more than four different types.

Type I Enzymes are complex, multisubunit, combination restriction-and-modification enzymes that cut DNA at random far from their recognition sequences. Type I enzymes are of considerable biochemical interest, but they have little practical value since they do not produce discrete restriction fragments or distinct gel-banding patterns.

Type II Enzymes cut DNA at defined positions close to or within their recognition sequences. They produce discrete restriction fragments and distinct gel banding patterns, and they are the only class used in the laboratory for DNA analysis and gene cloning. Rather than forming a single family of related proteins, Type II enzymes are a collection of unrelated proteins of many different sorts. Type II enzymes frequently differ so utterly in amino acid sequence from one another, and indeed from every other known protein, that they exemplify the class of rapidly evolving proteins that are often indicative of involvement in host-parasite interactions.

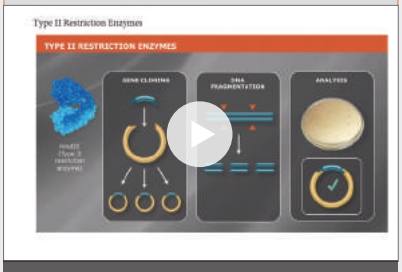
Type III Enzymes are also large combination restriction-and-modification enzymes. They cleave outside of their recognition sequences and require two such sequences in opposite orientations within the same DNA molecule to accomplish cleavage; they rarely yield complete digests.

Type IV Enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of *E. coli*.

TOOLS & RESOURCES

Visit the video library at www.neb.com to find:

- Tutorials on Type I, II and III restriction enzymes



TYPE I, II AND III RESTRICTION ENZYMES

View double digest protocol.

Restriction Enzyme 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 to dissociate the enzyme from the DNA
	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 supercolied DNA. Increase the number of enzyme units in the reaction.
	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. Miniprep 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 to dissociate the enzyme from the substrate
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • 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. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	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

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.

RR Recombinant

e Engineered enzyme for maximum performance

Time-Saver qualified

2*site Indicates that the restriction enzyme requires two or more sites for cleavage

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.

dcm dcm methylation sensitivity

dam dam methylation sensitivity

CpG CpG methylation sensitivity

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.

			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-v2	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	dcm	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	100	100	25	100	37°	80°	B	pXba (Xho digested)		CpG	2, b
RR	⏱		AvaI	CutSmart	< 10	100	25	100	37°	80°	A	Lambda		CpG	
RR	⏱		AvaII	CutSmart	50	75	10	100	37°	80°	A	Lambda	dcm	CpG	
RR	⏱		AvrII	CutSmart	100	50	50	100	37°	No	B	Lambda HindIII			
RR	⏱		BaeI	CutSmart + SAM	50	100	50	100	25°	65°	A	Lambda		CpG	e
RR	⏱		BaeGI	3.1	75	75	100	25	37°	80°	A	Lambda			
RR	⏱		BamHI	3.1	75*	100*	100	100*	37°	No	A	Lambda			3
RR	⏱	e	BamHI-HF	CutSmart	100	50	10	100	37°	No	A	Lambda			
RR			BanI	CutSmart	10	25	< 10	100	37°	65°	A	Lambda	dcm	CpG	1

Performance Chart for Restriction Enzymes (continued)

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)
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			
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		
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
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-HFv2	CutSmart	100	100	100	100	37°	80°	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	
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
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			

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.

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)
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
BsrFI-v2	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	
BssSI-v2	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
BtsI-v2	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
EcoO109I	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
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	
Esp3I	CutSmart	100	100	< 10	100	37°	65°	B	Lambda	CpG	
FatI	2.1	10	100	50	50	55°	80°	A	pUC19		

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.

Performance Chart for Restriction Enzymes (continued)

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)
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		1, 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	CpG	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			1, 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	
MspJI	CutSmart	< 10	< 10	< 10	100	37°	65°	B	pBR322			1, 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	

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.

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)
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		e
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		e
Nt.CviPII	CutSmart	10	100	25	100	37°	65°	A	pUC19	CpG	e
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	b
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	
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		

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.

Performance Chart for Restriction Enzymes (continued)

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)
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	pXba	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$^{\alpha}$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	

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.

Activity of Enzymes at 37°C

Listed below is the percentage of activity exhibited at 37°C for enzymes that have an optimal incubation temperature higher (thermophiles) or lower (25°C) than 37°C.

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
AbaSI	25°	0
ApaI	25°	100*
ApeKI	75°	10
ApoI	50°	50
BaeI	25°	20
BclI	50°	50
BfuAI	50°	50
BsaBI	60°	20
BsaJI	60°	20
BsaWI	60°	20
BsiEI	60°	30
BsiHKAI	65°	5
BsiWI	55°	50
BslI	55°	30
BsmAI	55°	50
BsmBI	55°	20
BsmFI	65°	50
BsmI	65°	20

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
BspCNI	25°	75
BspQI	50°	10
BsrI	65°	20
BsrDI	65°	30
BssHII	50°	75
BstAPI	60°	10
BstBI	65°	10
BstEII	60°	50
BstNI	60°	30
BstUI	60°	20
BstYI	60°	30
BtgZI	60°	75
BtsI-v2	55°	75
BtsCI	50°	50
BtsIMutI	55°	N/A
CviAII	25°	20
CviQI	25°	10
FatI	55°	20

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
FauI	55°	20
MwoI	60°	10
Nb.BsmI	65°	25
Nb.BsrDI	65°	75
Nt.BspQI	50°	80
Nt.BstNBI	55°	10
PI-PspI	65°	5
PspGI	75°	10
SfiI	50°	10
SmaI	25°	50
SmlI	55°	10
SwaI	25°	50
TaqαI	65°	10
TfiI	65°	10
TseI	65°	20
Tsp45I	65°	10
TspMI	75°	20
TspRI	65°	10
Tth111I	65°	10

*ApaI has 100% activity at 37°C, however the half-life of this enzyme at 37°C is only 30 minutes.

Activity of DNA Modifying Enzymes in CutSmart Buffer

A selection of DNA modifying enzymes were assayed in CutSmart Buffer, in lieu of their supplied buffers. Functional activity was compared to the activity in its supplied buffer, plus required supplements. Reactions were set up according to the recommended reaction conditions, with CutSmart Buffer replacing the supplied buffer.

ENZYME	ACTIVITY IN CUTSMART	REQUIRED SUPPLEMENTS
Alkaline Phosphatase (CIP)	+++	
Antarctic Phosphatase	+++	Requires Zn^{2+}
Bst DNA Polymerase	+++	
CpG Methyltransferase (M. SssI)	+++	
DNA Polymerase I	+++	
DNA Polymerase I, Large (Klenow) Fragment	+++	
DNA Polymerase Klenow Exo⁻	+++	
DNase I (RNase-free)	+++	Requires Ca^{2+}
E. coli DNA Ligase	+++	Requires NAD
Endonuclease III (Nth), recombinant	+++	
Endonuclease VIII	+++	
Exonuclease I	+++	
Exonuclease III	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
GpC Methyltransferase (M. CviPI)	+	Requires DTT
Lambda Exonuclease	++	
McrBC	+++	

ENZYME	ACTIVITY IN CUTSMART	REQUIRED SUPPLEMENTS
Micrococcal Nuclease	+++	Requires Ca^{2+}
Nuclease Bal-31	+++	
phi29 DNA Polymerase	+++	
Quick Dephosphorylation Kit	+++	
$RecJ_f$	+++	
Shrimp Alkaline Phosphatase (rSAP)	+++	
T3 DNA Ligase	+++	Requires ATP + PEG
T4 DNA Ligase	+++	Requires ATP
T4 DNA Polymerase	+++	
T4 Phage β-glucosyltransferase (T4-BGT)	+++	
T4 Polynucleotide Kinase	+++	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+++	Requires ATP + DTT
T5 Exonuclease	+++	
T7 DNA Ligase	+++	Requires ATP + PEG
T7 DNA Polymerase (unmodified)	+++	
T7 Exonuclease	+++	
Thermolabile ExoI	+++	
USER Enzyme, recombinant	+++	

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

Tips for Avoiding Star Activity

Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar but not identical to their defined recognition sequence. This altered specificity has been termed "star activity". 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 (4), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (5)]	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 new additions to the HF restriction enzyme product line.

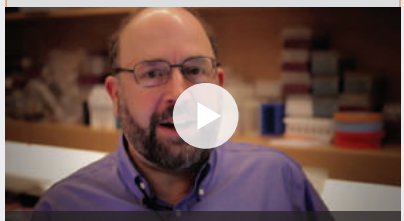
Reference:

(1) Nasri, M. and Thomas, D. (1986) *Nucleic Acids Res.* 14, 811.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Online 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 STAR ACTIVITY

Giron has been with NEB for over 2 years as a Development Scientist. Giron's family lives in Germany and he frequently travels to spend time with them. Giron is also an experienced spiritual teacher and feng shui consultant, as well as a skilled violinist.

Learn about the benefits of HF enzymes.

High-Fidelity (HF) Restriction Enzymes

As part of our ongoing commitment to the study and improvement of restriction enzymes, NEB offers a line of High-Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as the native enzymes, are all active in CutSmart Buffer and have reduced star activity. Star activity, or off-target cleavage, is an intrinsic property of restriction enzymes. Most restriction enzymes will not exhibit star activity when used under recommended reaction conditions. However, for enzymes that have reported star activity, extra caution must be taken to set up reactions according to the recommended conditions to avoid unwanted cleavage.

Many techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and require the use of enzymes under suboptimal conditions. HF enzymes with reduced star activity offer increased flexibility to reaction setup and help maximize results under a wide range of conditions.

In addition to reduced star activity, all of these engineered 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 can also be incubated overnight without degredation of DNA. HF enzymes are available at the same price as the native enzymes and are supplied with purple loading dye.

Visit **www.neb.com/HF** to learn more about HF enzymes.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

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

Reduced Star Activities of HF Enzymes

The following table indicates the number of units of HF enzyme that can be used compared to the native enzyme before any significant star activity is detected. The HF Factor refers to the X-fold increase in fidelity that is achieved by choosing an HF enzyme. This data clearly illustrates the flexibility that is offered by using an HF restriction enzyme.

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	MAXIMUM UNITS WITH NO STAR ACTIVITY*	HF FACTOR
AgeI-HF	#R3552	CutSmart	≥ 250	≥ 8
AgeI	#R0552	1.1	32	
ApoI-HF	#R3566	CutSmart	500	25
ApoI	#R0566	3.1	20	
BamHI-HF	#R3136	CutSmart	≥ 4,000	≥ 125
BamHI	#R0136	3.1	32	
BbsI-HF	#R3539	CutSmart	≥ 500	≥ 4
BbsI	#R0539	2.1	120	
BclI-HF	#R3160	CutSmart	500	16
BclI	#R0160	3.1	32	
BmtI-HF	#R3658	CutSmart	1,000,000	62,500
BmtI	#R0658	3.1	32	
BsaI-HFv2	#R3733	CutSmart	500	16
BsaI	#R0535	CutSmart	32	
BsiWI-HF	#R3553	CutSmart	100	1
BsiWI	#R0553	3.1	100	
BsrGI-HF	#R3575	CutSmart	≥ 2,000	≥ 62
BsrGI	#R0575	2.1	16	
BstEII-HF	#R3162	CutSmart	> 2,000	> 125
BstEII	#R0162	3.1	16	
BstZ17I-HF	#R3594	CutSmart	500	25
BstZ17I**	N/A	CutSmart	20	
DraIII-HF	#R3510	CutSmart	≥ 2,000	≥ 1,000
DraIII**	N/A	3.1	2	
EagI-HF	#R3505	CutSmart	500	2
EagI	#R0505	3.1	250	
EcoRI-HF	#R3101	CutSmart	16,000	64
EcoRI	#R0101	U	250	
EcoRV-HF	#R3195	CutSmart	≥ 64,000	≥ 64
EcoRV	#R0195	3.1	1,000	
HindIII-HF	#R3104	CutSmart	≥ 500,000	≥ 2,000
HindIII	#R0104	2.1	250	
KpnI-HF	#R3142	CutSmart	≥ 1,000,000	≥ 62,500
KpnI	#R0142	1.1	16	
MfeI-HF	#R3589	CutSmart	≥ 500	≥ 16
MfeI	#R0589	CutSmart	32	

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	MAXIMUM UNITS WITH NO STAR ACTIVITY*	HF FACTOR
MluI-HF	#R3198	CutSmart	≥ 4,000	2
MluI	#R0198	3.1	≥ 2,000	
NcoI-HF	#R3193	CutSmart	≥ 64,000	≥ 530
NcoI	#R0193	3.1	120	
NheI-HF	#R3131	CutSmart	≥ 32,000	≥ 266
NheI	#R0131	2.1	120	
NotI-HF	#R3189	CutSmart	≥ 64,000	≥ 16
NotI	#R0189	3.1	4,000	
NruI-HF	#R3192	CutSmart	≥ 32,000	64
NruI	#R0192	3.1	≥ 500	
NsiI-HF	#R3127	CutSmart	≥ 8,000	2
NsiI	#R0127	3.1	≥ 4,000	
PstI-HF	#R3140	CutSmart	4,000	33
PstI	#R0140	3.1	120	
PvuI-HF	#R3150	CutSmart	≥ 16,000	≥ 32
PvuI	#R0150	3.1	500	
PvuII-HF	#R3151	CutSmart	500	32
PvuII	#R0151	3.1	16	
SacI-HF	#R3156	CutSmart	≥ 32,000	≥ 266
SacI	#R0156	1.1	120	
SalI-HF	#R3138	CutSmart	≥ 32,000	≥ 8,000
SalI	#R0138	3.1	4	
SbfI-HF	#R3642	CutSmart	250	32
SbfI	#R0642	CutSmart	8	
ScaI-HF	#R3122	CutSmart	250	62
ScaI**	N/A	3.1	4	
SpeI-HF	#R3133	CutSmart	≥ 8,000	≥ 16
SpeI	#R0133	CutSmart	500	
SphI-HF	#R3182	CutSmart	8,000	250
SphI	#R0182	2.1	32	
SspI-HF	#R3132	CutSmart	500	16
SspI	#R0132	U	32	
StyI-HF	#R3500	CutSmart	4,000	125
StyI	#R0500	3.1	32	

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

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. Over 185 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. Only 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.

TOOLS & RESOURCES

Visit www.neb.com/TimeSaver to find:

- The full list of Time-Saver qualified restriction enzymes available
- Online tutorials on using Time-Saver qualified enzymes to speed up restriction enzyme digests

Chart Legend

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

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
AatII	■	▲	●
AccI	■	▲	▲
Acc65I	●	▲	●
AciI	●	●	●
AclI	●	■	▲
AcuI	■	▲	▲
AflII	●	●	●
AgeI-HF	●	●	●
AhdI	●	●	■
AluI	●	▲	●
AlwNI	●	●	▲
ApaI	●	●	●
ApaLI	●	●	▲
ApeKI	●	■	▲
ApoI	●	●	●
ApoI-HF	■	■	▲
AscI	●	●	NT
AseI	●	●	NT
AvaI	●	▲	▲
AvaII	●	●	●
AvrII	●	NT	NT
BaeI	■	●	▲
BaeGI	●	▲	▲
BamHI	●	●	▲
BamHI-HF	●	●	●
BbsI	■	▲	▲
BbsI-HF	■	▲	▲
BbvI	●	▲	▲
BccI	■	▲	▲
BceAI	■	■	▲
BciVI	●	■	▲
BclI	●	▲	▲
BclI-HF	■	▲	▲
BcoDI	●	●	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
BfuAI	●	●	▲
BfuCI	■	▲	●
BglI	●	●	▲
BglII	●	■	▲
BlpI	●	●	●
BmgBI	●	●	▲
BmrI	■	▲	■
BmtI-HF	●	●	▲
BpuEI	●	●	▲
BsaI	●	●	■
BsaI-HFv2	■	■	▲
BsaAI	●	●	■
BsaHI	■	■	●
BsaWI	■	▲	▲
BsaXI	●	▲	▲
BseRI	●	●	■
BsgI	●	●	▲
BsiEI	●	▲	▲
BsiHKAI	●	●	▲
BsiWI	●	●	▲
BsiWI-HF	■	■	▲
BsmI	●	■	■
BsmAI	●	●	▲
BsmBI	■	▲	▲
BsmFI	●	●	▲
BsoBI	●	■	●
Bsp1286I	●	●	▲
BspCNI	■	▲	▲
BspEI	●	▲	▲
BspHI	■	●	●
BspQI	●	●	▲
BsrI	●	■	▲
BsrBI	●	■	▲
BsrDI	●	■	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
BsrFI-v2	■	▲	▲
BsrGI	■	▲	▲
BsrGI-HF	■	■	▲
BssHII	●	▲	▲
BssSI-v2	■	▲	▲
BstBI	●	●	▲
BstEII	●	●	▲
BstEII-HF	●	●	●
BstNI	●	●	▲
BstUI	●	●	▲
BstXI	●	●	▲
BstYI	■	●	▲
BstZ17I-HF	■	■	▲
Bsu36I	■	▲	■
BtgI	●	●	■
BtsI-v2	■	■	■
BtsCI	●	■	▲
Cac8I	■	▲	▲
ClaI	●	●	▲
CspCI	●	●	▲
CviAII	■	●	●
CviQI	●	●	●
DdeI	●	■	■
DpnI	●	●	▲
DpnII	■	▲	●
DraI	●	●	■
DraIII-HF	●	●	▲
DrdI	■	●	●
EagI	●	▲	▲
EagI-HF	■	■	▲
EarI	■	■	▲
Eco53KI	●	●	■
EcoNI	●	■	●
Eco0109I	●	▲	▲
EcoP15I	■	▲	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
EcoRI	●	●	▲
EcoRI-HF	●	●	●
EcoRV	●	●	▲
EcoRV-HF	●	●	▲
Esp3I	■	■	▲
Fnu4HI	●	■	■
FokI	●	●	●
FseI	●	●	▲
FspI	■	▲	■
HaeII	■	▲	▲
HaeIII	●	●	●
HgaI	■	▲	▲
HhaI	●	■	▲
HincII	■	▲	●
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	■	■	●

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
MslI	●	●	●
MspI	●	●	●
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	●	●	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
SacI-HF	●	●	●
SacII	●	▲	▲
SalI	●	■	▲
SalI-HF	●	●	▲
SapI	■	▲	▲
SbfI	●	●	▲
SbfI-HF	●	●	▲
ScaI-HF	●	●	▲
SfaNI	▲	▲	■
SfiI	●	▲	▲
SfoI	●	●	●
SmaI	●	■	■
SpeI	●	●	●
SpeI-HF	■	■	▲
SphI	●	●	▲
SphI-HF	●	●	▲
SrfI	■	■	▲
SspI	●	●	▲
SspI-HF	●	●	▲
StuI	■	▲	▲
StyI	■	▲	▲
StyI-HF	●	●	▲
StyD4I	■	▲	▲
SwaI	■	▲	▲
Taq$^{\alpha}$I	●	●	▲
TfiI	■	●	▲
TseI	■	▲	▲
TspMI	●	■	▲
TspRI	●	■	▲
Tth111I	■	■	▲
XbaI	●	●	▲
XhoI	●	●	▲
XmaI	■	▲	■
XmnI	●	●	▲

Learn more about Time-Saver qualified enzymes.

Cross Index of Recognition Sequences

Sequences at the top of each column are written 5′ to 3′ according to convention. Open squares at the left of each row are place holders for nucleotides within a restriction enzyme recognition sequence; arrowheads indicate the point of cleavage.

Sequences of complementary strands and their cleavage sites are implied.

blue type = enzymes that recognize only one sequence

black type = enzymes that recognize multiple sequences (degenerate)

Palindromic Tetra- and Hexa-Nucleotide Recognition Sequences

	AATT	ACGT	AGCT	ATAT	CATG	CCGG	CGCG	CTAG	GATC	GCGC	GGCC	GTAC	TATA	TCGA	TGCA	TTAA
▼□□□□	**MluCI**				**FatI**				**BfuCI DpnII MboI Sau3AI**							
□□□□▼		**HpyCH2IV**			**CviAII**	**MspI HpaII**		**BfaI**		**HinP1I**		**Csp6I CviQI**		**TaqI**		**MseI**
□□▼□□			**AluI** CviKI-1				**BstUI**		**DpnI**		**HaeIII PhoI** CviKI-1	**RsaI**			**HpyCH4V**	
□□□▼□									**BstKTI**	**HhaI**						
□□□□▼		**TaiI**			**NlaIII**											
A▼□□□T	ApoI ●		**HindIII** ●		**PciI** AflIII	**AgeI** ● BsrFI BsaWI	**MluI** ● AflIII	**SpeI** ●	**BglII** BstYI			TatI				
A□▼□□T		**AclI**												**ClaI BspDI**		**AseI**
A□□▼□T				**SspI** ●						**AfeI**	**StuI**	**ScaI** ●				
A□□□▼T																
A□□□□▼T					NspI					HaeII					**NsiI**●	
C▼□□□□G	**MfeI** ●				**NcoI** ● StyI ● BtgI BsaJI	**TspMI XmaI** AcoI AvaI BsaJI BsoBI BmeT110I	BsaJI BtgI	**AvrII** BsaJI StyI ●			**EagI** ● EaeI	**BsiWI** ●	SfcI	**PaeR7I TliI XhoI** AvaI BsoBI SmlI BmeT110I	SfcI	**AflII** SmlI
C□▼□□□G				**NdeI**												
C□□▼□□G		**PmlI** BsaAI	**PvuII** ● MspA1I			**SmaI**	MspA1I									
C□□□▼□G							**SacII**		**PvuI** ● BsiEI		BsiEI					
C□□□□▼G															**PstI** ●	
G▼□□□□C	**EcoRI** ● ApoI ●					**NgoMIV** BsrFI	**BssHII**	**NheI** ●	**BamHI** ● BstYI	**KasI** BanI	**PspOMI**	**Acc65I** BanI		**SalI** ●	**ApaLI**	
G□▼□□□C		BsaHI								**NarI** BsaHI			AccI	AccI		
G□□▼□□C		**ZraI**	**Ecl136II Eco53KI**	**EcoRV** ●	Cac8I	**NaeI** Cac8I	Cac8I	Cac8I	NlaIV	**SfoI** NlaIV	NlaIV	NlaIV	**BstZ17I** ● Hpy8I Hpy166II	HincII Hpy8I Hpy166II	Hpy8I Hpy166II	**HpaI** HincII Hpy8I Hpy166II
G□□□▼□C																
G□□□□▼C		**AatII**	**SacI** ● BanII BsiHKAI Bsp1286I		**SphI** ● NspI			**BmtI** ●		**PluTI BbeI** HaeII	**ApaI** BanII BaeGI Bsp1286I	**KpnI** ●			BaeGI Bsp1286I BsiHKAI	
T▼□□□□A					**BspHI**	**BspEI** BsaWI AcoI		**XbaI**	**BclI** ●		EaeI	**BsrGI** ● TatI				
T□▼□□□A					Hpy188III	Hpy188III	Hpy188III	Hpy188III						**BstBI**		
T□□▼□□A		**SnaBI** BsaAI					**NruI** ●			**FspI**	**MscI**		**PsiI**			**DraI**
T□□□▼□A																
T□□□□▼A																

● HF (High-Fidelity) versions of these enzymes are available for simplified reactions and reduced star activity, at no additional cost. See page 301.

Palindromic Penta-Nucleotide Recognition Sequences

	AA □ TT	AC □ GT	AG □ CT	AT □ AT	CA □ TG	CC □ GG	CG □ CG	CT □ AG	GA □ TC	GC □ GC	GG □ CC	GT □ AC	TA □ TA	TC □ GA	TG □ CA	TT □ AA
▼□□ N □□						BssKI StyD4I						MaeIII				
□□ N □□								DdeI	HinfI		Sau96I					
□□▼N □□						ScrFI				Fnu4HI	BmgT120I					
□□ N ▼□□		HpyCH4III												Hpy188I		
□□ N □□▼											FmuI					
▼□□ A T □□						PspGI										
□□▼ A T □□									TfiI	ApeKI TseI	AvaII					
□□▼ A T □□						BstNI										
□□ A T ▼□□																
□□ A T □□▼							Hpy99I									
▼□□ G C □□												Tsp45I				
□□▼ G C □□																
□□▼ G C □□						NciI										
□□ G C ▼□□																
□□ G C ▼□□										TauI BspUI						
□□ G C □□▼																

Single Letter Code:

R = A or G
K = G or T
H = A or C or T
V = A or C or G
N = A or C or G or T
Y = C or T
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W = A or T
B = C or G or T
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In addition, see homing endonucleases on pages 55–56.

Note:

Enzymes marked with a "▲" are available from NEB.

● HF (High-Fidelity) versions of these enzymes are available for simplified reactions and reduced star activity, at no additional cost. See page 301.

Enzymes marked with a "⊗" are not currently commercially available.

SPECIFICITIES GREATER THAN 6 BASES

AarI	CACCTGC(4/8)
Aba6411II⊗	CRRTAAG
AbaCIII⊗	CTATCAV
AbsI	CC/TCGAGG
AcoY31II⊗	TAGCRAB
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AjuI	(7/12)GAANNNNNNNTTGG(11/6)
AloI	(7/12)GAACNNNNNNTCC(12/7)
AlwFI⊗	GAAAYNNNNNRTG
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
▲ AscI	GG/CGCGCC
▲ AsiSI	GCGAT/CGC
Asp103I⊗	CGRAGGC
AspJHL3II⊗	CGCCCAG
AspNIH4III⊗	AAGAACB
Asp114pII⊗	AGCABCC
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
BarI	(7/12)GAAGNNNNNNTAC(12/7)
Bbr57III⊗	GTRAAYG
▲ BbvCI	CCTCAGC(-5/-2)
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
Bsp460III⊗	CGCGCAG
▲ BspQI	GCTCTTC(1/4)
CalB3II⊗	GRTTRAG
Cbo67071IV⊗	GCRGAAG
CcrNAIII⊗	CGACCAG
Cdi81III⊗	GCMGAAG
Cgl13032II⊗	ACGABGG
Cly7489II⊗	AAAAGRG

SPECIFICITIES GREATER THAN 6 BASES (CONT.)

▲ CspCI	(11/13)CAANNNNNGTGG(12/10)
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
▲ FseI	GGCCGG/CC
FspAI	RTGC/GCAY
FspPK15I⊗	GARGAAG
GauT27I⊗	CGCGCAGG
Jma19592II⊗	GRGCRAC
KfiI	GG/GWCCC
Kpn156V⊗	CRTGATT
Lmo370I⊗	AGCGCCG
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
MauBI	CG/CGCGCG
Mcr10I⊗	GAAGNNNNNCTC
MkaDII⊗	GAGAYGT
MreI	CG/CCGGCG
MspSC27II⊗	CCGCGAC
MteI	GCGC/NGCGC
MtuHN878II⊗	CACGCAG
NhaXI⊗	CAAGRAG
▲ NotI ●	GC/GGCCGC
NpeUS61II⊗	GATCGAC
▲ PacI	TTAAT/TAA
Pal408I⊗	CCRTGAG
PasI	CC/CWGGG
PflPt14I⊗	RGCCCAC
PfrJS12V⊗	GGCGGAG
PinP23II⊗	CTRKCAG
PliMI⊗	CGCCGAC
▲ PmeI	GTTT/AAAC
PpiI⊗	(7/12)GAACNNNNNCTC(13/8)
PpiP13II⊗	CGCRGAC
▲ PpuMI	RG/GWCCY
Pse18267I⊗	RCCGAAG
PspOMII⊗	CGCCCAR(20/18)
▲ PspXI	VC/TCGAGB
PsrI	(7/12)GAACNNNNNNTAC(12/7)
Pst145I⊗	CTAMRAG
Pst273I⊗	GATCGAG
Rba2021I⊗	CACGAGH
RceI⊗	CATCGAC(20/18)
RpaI⊗	GTYGGAG(11/9)
RpaBI⊗	CCCGCAG(20/18)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
RspPBTS2III⊗	CTTCGAG

SPECIFICITIES GREATER THAN 6 BASES (CONT.)

▲ RsrII	CG/GWCCG
▲ SapI	GCTCTTC(1/4)
▲ SbfI ●	CCTGCA/GG
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
▲ SexAI	A/CCWGGT
▲ SfiI	GGCCNNNN/NGGCC
▲ SgrAI	CR/CCGGYG
SgrDI	CG/TCGACG
SmaUMH8I⊗	GCGAACB
Sno506I⊗	GGCCGAG
SpoDI⊗	GCGGRAG
▲ SrfI	GCCC/GGGC
Sse8647I⊗	AG/GWCCT
Ssp714II⊗	CGCAGCG
SstE37I⊗	CGAAGAC(20/18)
Sth20745III⊗	GGACGAC
▲ SwaI	ATTT/AAAT
TspARh3I⊗	GRACGAC
UbaF9I⊗	TACNNNNNRTGT
UbaF12I⊗	CTACNNNGTC
UbaF13I⊗	GAGNNNNNNCTGG
Vtu19109I⊗	CACRAYC

INTERRUPTED PALINDROMES

AgsI	TTS/AA
▲ AhdI	GACNNN/NNGTC
▲ AleI	CACNN/NNGTG
AlfI⊗	(10/12)GCANNNNNNTGC(12/10)
▲ AlwNI	CAGNNN/CTG
ApaBI⊗	GCANNNNN/TGC
▲ ApeKI	G/CWGC
▲ AvaII	G/GWCC
BdaI⊗	(10/12)TGANNNNNNTCA(12/10)
▲ BglI	GCCNNNN/NGGC
BisI	GC/NGC
▲ BlpI	GC/TNAGC
BlsI	GCN/GC
BplI	(8/13)GAGNNNNNCTC(13/8)
▲ BsaBI	GATNN/NNATC
▲ BsaJI	C/CNNGG
▲ BsaWI	W/CCGGW
▲ BsiHKAI	GWGCW/C
▲ BsmI	CCNNNNN/NNGG
▲ BstAPI	GCANNNN/NTGC
▲ BstEII ●	G/GTNACC
▲ BstNI	CC/WGG
▲ BstXI	CCANNNNN/NTGG

INTERRUPTED PALINDROMES (CONT.)	
▲ Bsu36I	CC/TNAGG
BthCI⊗	GCNG/C
▲ Cac8I	GCN/NGC
CjuI⊗	CAYNNNNNRTG
▲ DdeI	C/TNAG
Dde51507I⊗	CCWGG
▲ DraIII ●	CACNNN/GTG
▲ DrdI	GACNNNN/NNGTC
EcoHI⊗	/CCSGG
▲ EcoNI	CCTNN/NNNAGG
▲ Eco0109I	RG/GNCCY
FaII	(8/13)AAGNNNNNCTT(13/8)
FmuI⊗	GGNC/C
▲ Fnu4HI	GC/NGC
HaeI⊗	WGG/CCW
HgiEII⊗	ACCNNNNNNGGT
▲ HinfI	G/ANTC
▲ Hpy99I	CGWCG/
▲ Hpy166II	GTN/NAC
▲ Hpy188I	TCN/GA
▲ Hpy188III	TC/NNGA
▲ HpyCH4III	ACN/GT

INTERRUPTED PALINDROMES (CONT.)	
HpyUM032XIII⊗	CYANNNNNNNTRG
HsoII⊗	(8/14)CAYNNNNNRTG(14/8)
KfiI	GG/GWCCC
MaeIII	/GTNAC
MjaIV⊗	GTNNAC
▲ MsiI	CAYNN/NNRTG
MteI	GCGC/NGCGC
▲ MwoI	GCNNNNN/NNGC
▲ NciI	CC/SGG
NhoI⊗	GCWGC
▲ NlaIV	GGN/NCC
PasI	CC/CWGGG
PcsI	WCGNNNN/NNNCGW
Pfl8569I⊗	GCN/NGC
▲ PflFI	GACN/NNGTC
▲ PflMI	CCANNNN/NTGG
PfoI	T/CCNGGA
▲ PpuMI	RG/GWCCY
▲ PshAI	GACNN/NNGTC
Psp03I⊗	GGWC/C
▲ PspGI	/CCWGG
PssI⊗	RGGNC/CY

INTERRUPTED PALINDROMES (CONT.)	
▲ RsrII	CG/GWCCG
▲ Sau96I	G/GNCC
▲ ScrFI	CC/NGG
SetI	ASST/
▲ SexAI	A/CCWGGT
▲ SfiI	GGCCNNNN/NGGCC
Sse8647I⊗	AG/GWCCT
▲ StyI	C/CWWGG
▲ StyD4I	/CCNGG
TatI	W/GTACW
TauI	GCSG/C
▲ TfiI	G/AWTC
▲ TseI	G/CWGC
▲ Tsp45I	/GTSAC
▲ TspRI	CASTGNN/
TssI⊗	GAGNNNCTC
▲ Tth111I	GACN/NNGTC
UnbI⊗	/GGNCC
VpaK11AI⊗	/GGWCC
▲ XcmI	CCANNNNN/NNNNTGG
▲ XmnI	GAANN/NNTTC

Multiple Recognition Sequences

TOOLS & RESOURCES

Visit the Tools & Resources tab at NEB.com to find:

- Access to our online tool **NEBcutter**, for help with restriction enzyme mapping

Single Letter Code:

R = A or G
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N = A or C or G or T
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S = C or G
M = A or C
W = A or T
B = C or G or T
D = A or G or T

Note:

Enzymes marked with a "▲" are available from NEB. Enzymes marked with a "⊗" are not currently commercially available.

MULTIPLE RECOGNITION SEQUENCES	
Aba6411II⊗	CRRTAAG
AbaCIII⊗	CTATCAV
AbaUMB2I⊗	YCCGSS
▲ AccI	GT/MKAC
Aco12261II⊗	CCRGAG
AcoY31II⊗	TAGCRAB
▲ AflIII	A/CRYGT
AgsI	TTS/AA
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AlwFI⊗	GAAAYNNNNNRTG
▲ ApeKI	G/CWGC
▲ ApoI	R/AATTY
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
Asp103I⊗	CGRAGGC
AspBHI⊗	YSCNS(8/12)
AspNIH4III⊗	AAGAACB
Asp114pII⊗	AGCABCC
Asu14238IV⊗	CGTRAC
AteTI⊗	GGGRAG
▲ AvaI	C/YCGRG

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ AvaII	G/GWCC
Awo1030IV⊗	GCCRAG
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
▲ BaeGI	GKGCM/C
▲ BanI	G/GYRCC
▲ BanII	GRGCY/C
BanLI⊗	RTCAGG
Bbr11I⊗	GGRCAG
Bbr57III⊗	GTRAAYG
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
BmgI⊗	GKGCCC
▲ BsaAI	YAC/GTR
▲ BsaHI	GR/CGYC
▲ BsaWI	W/CCGGW
▲ BsiEI	CGRY/CG
▲ BsiHKAI	GWGCW/C
▲ BsoBI	C/YCGRG
▲ Bsp1286I	GDGCH/C
▲ BsrFI	R/CCGGY
▲ BstNI	CC/WGG
▲ BstYI	R/GATCY
▲ BtgI	C/CRYGG
CalB3II⊗	GRTTRAG
Cba16038I⊗	CCTNAYNC
Cbo67071IV⊗	GCRGAAG
CchII⊗	GGARGA(11/9)
Cch467III⊗	GNGAAAY
Cco14983V⊗	GGGTDA
Cco14983VI⊗	GCYGA
Cdi81III⊗	GCMGAAG
Cfupf3II⊗	GARCAG
Cgl13032II⊗	ACGABGG
Cje265V⊗	GKAAGC
Cje54107III⊗	GKAAYC
CjeFV⊗	GGRCA
CjeNIII⊗	GKAAYG(19/17)
CjeNV⊗	CCYGA
CjuI⊗	CAYNNNNNRTG
CjuII⊗	CAYNNNNNCTC
Cly7489II⊗	AAAAGRG

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ CviKI-1	RG/CY
Dde51507I⊗	CCWGG
▲ EaeI	Y/GGCCR
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
EcoBLMcrX⊗	RCSRC(-3/-2)
EcoE1140I⊗	ACCYAC
EcoHI⊗	/CCSGG
Eco57MI⊗	CTGRAG(16/14)
▲ Eco0109I	RG/GNCCY
FaiI	YA/TR
Fco1691IV⊗	GCVGAG
FspAI	RTGC/GCAY
FspPK15I⊗	GARGAAG
GdiII⊗	CGGCCR(-5/-1)
HaeI⊗	WGG/CCW
▲ HaeII	RGCGC/Y
HaeIV⊗	(7/13)GAYNNNNNRTC(14/9)
Hin4I⊗	(8/13)GAYNNNNNVTC(13/8)
▲ HincII	GTY/RAC
▲ Hpy99I	CGWCG/
Hpy99XIV⊗	GGWTAA
Hpy99XIV-mut1⊗	GGWCNA
Hpy99XXII⊗	TCANNNNNNTRG
Hpy300XI⊗	CCTYNA
HpyAXVI-mut1⊗	CRTTAA
HpyAXVI-mut2⊗	CRTCNA
HpyUM032XIII⊗	CYANNNNNNNTRG
HpyUM032XIII-mut1⊗	CYANNNNNNNTTC
HsoII⊗	(8/14)CAYNNNNNRTG(14/8)
Jma19592II⊗	GRGCRAC
Jsp2502II⊗	GRNGAAT
KfiI	GG/GWCCC
Kor51II⊗	RTCGAG
Kpn156V⊗	CRTGATT
KpnNH25III⊗	CTRGAG
KpnNIH50I⊗	GCYAAG
Lba2029III⊗	CYAAANG
LlaG50I⊗	CCGTKA
Lmo911II⊗	TAGRAG

Multiple Recognition Sequences (continued)

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
LpI1004II⊗	AGGRAG
LpnI⊗	RGC/GCY
▲ LpnPI	CCDG(10/14)
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
MkaDII⊗	GAGAYGT
▲ MmeI	TCCRAC(20/18)
▲ MsII	CAYNN/NNRTG
▲ MspA1I	CMG/CKG
MspI7II⊗	ACGRAG
▲ MspJI	CNNR(9/13)
▲ NciI	CC/SGG
NhaXI⊗	CAAGRAG
NhoI+	GCWGC
NIi3877I⊗	CYCGR/G
NmeDI⊗	(12/7)RCCGGY(7/12)
▲ NspI	RCATG/Y
OspHL35III⊗	YAGGAG
PaI408I⊗	CCRTGAG
PasI	CC/CWGGG
PcsI	WCGNNNN/NNNCGW
PflPt14I⊗	RGCCCAC
Pin17FIII⊗	GGYGAB
PinP23II⊗	CTRKCAG
PpiP13II⊗	CGCRGAC

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ PpuMI	RG/GWCCY
Pse18267I⊗	RCCGAAG
Psp03I⊗	GGWC/C
▲ PspGI	/CCWGG
PspOMII⊗	CGCCCAR(20/18)
PspPRI⊗	CCYCAG(15/13)
▲ PspXI	VC/TCGAGB
PssI⊗	RGGNC/CY
Pst145I⊗	CTAMRAG
Pst14472I⊗	CNYACAC
PsuGI⊗	BBCGD
Rba2021I⊗	CACGAGH
RdeGBIII⊗	(9/11)TGRYCA(11/9)
RlaI⊗	VCW
Rmu369III⊗	GGCYAC
RpaI⊗	GTYGGAG(11/9)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
▲ RsrII	CG/GWCCG
Sba460II⊗	GGNGAYG
SdeAI⊗	CAGRAG(21/19)
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
SenSARA26III⊗	ACRCAG
SetI	ASST/
▲ SexAI	A/CCWGGT

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ SfcI	C/TRYAG
▲ SgrAI	CR/CCGGYG
SgrTI⊗	CCDS(10/14)
SmaUMH8I⊗	GCGAACB
▲ SmlI	C/TYRAG
SpoDI⊗	GCGGRAG
Sse8647I⊗	AG/GWCCT
▲ StyI	C/CWWGG
SurP32aII⊗	ACRGAG
TatI	W/GTACW
TauI	GCSG/C
▲ TfiI	G/AWTC
▲ TseI	G/CWGC
TsoI⊗	TARCCA(11/9)
▲ Tsp45I	/GTSAC
TspARh3I⊗	GRACGAC
▲ TspRI	CASTGNN/
Tth111II⊗	CAARCA(11/9)
UbaF9I⊗	TACNNNNNRTGT
Van9116I⊗	CCKAAG
Vdi96II⊗	GNCYTAG
VpaK11AI⊗	/GGWCC
Vtu19109I⊗	CACRAYC
WviI⊗	CACRAG(21/19)

Nonpalindromic Recognition Sequences

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B = C or G or T
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Enzymes marked with a "⊗" are not currently commercially available.

NONPALINDROMIC SEQUENCES	
AarI	CACCTGC(4/8)
Aba6411II⊗	CRRTAAG
AbaB8342IV⊗	CATTAG
AbaCIII⊗	CTATCAV
▲ AbaSI	C(11/9)
AbaUMB2I⊗	YCCGSS
Acc65V⊗	GACGCA
AceIII⊗	CAGCTC(7/11)
AchA6III⊗	AGCCAG
▲ AciI	CCGC(-3/-1)
Aco12261II⊗	CCRGAG
AcoY31II⊗	TAGCRAB
▲ AcuI	CTGAAG(16/14)
Adh6U21I⊗	GAANCAG
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AjuI	(7/12)GAANNNNNNNTTGG(11/6)
AloI	(7/12)GAACNNNNNNTCC(12/7)
▲ AlwI	GGATC(4/5)
AlwFI⊗	GAAAYNNNNNRTG
AmaCSI⊗	GCTCCA(11/9)
ApyPI⊗	ATCGAC(20/18)
AquII⊗	GCCGNAC(20/18)
AquIII⊗	GAGGAG(20/18)
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
Asp103I⊗	CGRAGGC
AspBHI⊗	YSCNS(8/12)
AspDUT2V⊗	GNGCAAC
AspJHL3II⊗	CGCCCAG
AspNIH4III⊗	AAGAACB

NONPALINDROMIC SEQUENCES (CON'T)	
AspSLV7III⊗	GTCTCA
Asp114pII⊗	AGCABCC
Asu14238IV⊗	CGTRAC
AteTI⊗	GGGRAG
Awo1030IV⊗	GCCRAG
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
Bag18758I⊗	CCCGAG
BanLI⊗	RTCAGG
BarI	(7/12)GAAGNNNNNNTAC(12/7)
Bbr11I⊗	GGRCAG
Bbr52II⊗	GGCGAG
Bbr57III⊗	GTRAAYG
▲ BbsI	GAAGAC(2/6)
▲ BbvI	GCAGC(8/12)
▲ BbvCI	CCTCAGC(-5/-2)
▲ BccI	CCATC(4/5)
Bce3081I⊗	TAGGAG
▲ BceAI	ACGGC(12/14)
BceSIV⊗	(7/5)GCAGC(9/11)
BcefI⊗	ACGGC(12/13)
▲ BcgI	(10/12)CGANNNNNNTGC(12/10)
▲ BciVI	GTATCC(6/5)
▲ BcoDI	GTCTC(1/5)
BfaSII⊗	GANGGAG
▲ BfuAI	ACCTGC(4/8)
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
BloAII⊗	GAGGAC
BmeDI⊗	C(2/0)
BmgI⊗	GKGCCC
▲ BmgBI	CACGTC(-3/-3)

NONPALINDROMIC SEQUENCES (CON'T)	
▲ BmrI	ACTGGG(5/4)
▲ BpmI	CTGGAG(16/14)
▲ Bpu10I	CCTNAGC(-5/-2)
▲ BpuEI	CTTGAG(16/14)
▲ BsaI ●	GGTCTC(1/5)
▲ BsaXI	(9/12)ACNNNNNCTCC(10/7)
BsbI⊗	CAACAC(21/19)
BscAI⊗	GCATC(4/6)
BscGI⊗	CCCGT
BseMII	CTCAG(10/8)
▲ BseRI	GAGGAG(10/8)
▲ BseYI	CCCAGC(-5/-1)
▲ BsgI	GTGCAG(16/14)
▲ BsmI	GAATGC(1/-1)
▲ BsmAI	GTCTC(1/5)
▲ BsmBI	CGTCTC(1/5)
▲ BsmFI	GGGAC(10/14)
Bsp24I⊗	(8/13)GACNNNNNNTGG(12/7)
Bsp460III⊗	CGCGCAG
Bsp3004IV⊗	CCGCAT
▲ BspCNI	CTCAG(9/7)
BspD6I⊗	GAGTC(4/6)
BspGI⊗	CTGGAC
▲ BspMI	ACCTGC(4/8)
BspNCI⊗	CCAGA
▲ BspQI	GCTCTTC(1/4)
▲ BsrI	ACTGG(1/-1)
▲ BsrBI	CCGCTC(-3/-3)
▲ BsrDI	GCAATG(2/0)
▲ BssSI	CACGAG(-5/-1)
▲ BtgZI	GCGATG(10/14)

NONPALINDROMIC SEQUENCES (CON'T)	
▲ BtsI	GCAGTG(2/0)
▲ BtsIMutI	CAGTG(2/0)
▲ BtsCI	GGATG(2/0)
Cal14237I⊗	GGTTAG
CalB3II⊗	GRTTRAG
Cau10061II⊗	GTTAAT
Cba13II⊗	AGGAAT
Cba16038I⊗	CCTNAYNC
Cbo67071IV⊗	GCRGAAG
CchII⊗	GGARGA(11/9)
CchIII⊗	CCCAAG(20/18)
Cch467III⊗	GNGAAAY
Cco14983V⊗	GGGTDA
Cco14983VI⊗	GCYGA
CcrNAIII⊗	CGACCAG
CdiI⊗	CATCG(-1/-1)
Cdi81III⊗	GCMGAAG
Cdi11397I⊗	GCGCAG
CdpI⊗	GCGGAG(20/18)
Cdu23823II⊗	GTGAAG
Cfupf3II⊗	GARCAG
Cgl13032I⊗	GGCGCA
Cgl13032II⊗	ACGABGG
CjeI⊗	(8/14)CCANNNNNNGT(15/9)
Cje265V⊗	GKAAGC
Cje54107III⊗	GKAAYC
CjeFIII⊗	GCAAGG
CjeFV⊗	GGRCA
CjeNII⊗	GAGNNNNNGT
CjeNIII⊗	GKAAYG(19/17)
CjeNV⊗	CCYGA
CjePI⊗	(7/13)CCANNNNNNNTC(14/8)
CjeP659IV⊗	CACNNNNNNNGAA
CjuII⊗	CAYNNNNNCTC
Cla11845III⊗	GCGAA
Cly7489II⊗	AAAAGRG
Cma23826I⊗	CGGAAG
Csp2014I⊗	GGAGGC
▲ CspCI	(11/13)CAANNNNNGTGG(12/10)
CstMI⊗	AAGGAG(20/18)
DraRI⊗	CAAGNAC(20/18)
DrdII⊗	GAACCA
▲ EarI	CTCTTC(1/4)
▲ EciI	GGCGGA(11/9)
Ecl234I⊗	CGGNAAG
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
EcoBLMcrX⊗	RCSRC(-3/-2)
EcoE1140I⊗	ACCYAC
Eco57MI⊗	CTGRAG(16/14)
EcoMVII⊗	CANCATC
EcoNIH6II⊗	ATGAAG
Eli8509II⊗	CCGGAG
EsaSSI⊗	GACCAC
▲ Esp3I	CGTCTC(1/5)
Esp3007I⊗	CAGAAG
Exi27195I⊗	GCCGAC
▲ FauI	CCCGC(4/6)
Fco1691IV⊗	GCVGAG
FinI⊗	GGGAC
▲ FokI	GGATG(9/13)
▲ FspEI	CC(12/16)
FspPK15I⊗	GARGAAG
FtnUV⊗	GAAACA
GauT27I⊗	CGCGCAGG
Gba708II⊗	ATGCAC
GdiII⊗	CGGCCR(-5/-1)
GsaI	CCCAGC(-1/-5)
HaeIV⊗	(7/13)GAYNNNNNRTC(14/9)

NONPALINDROMIC SEQUENCES (CON'T)	
HauII⊗	TGGCCANNNNNNNNNN/
HbaII⊗	GCCCAG
HdeNY26I⊗	CGANNNNNNTCC
HdeZA17I⊗	GCANNNNNNTCC
▲ HgaI	GACGC(5/10)
Hin4I⊗	(8/13)GAYNNNNNVTC(13/8)
▲ HphI	GGTGA(8/7)
Hpy99XIII⊗	GCCTA
Hpy99XIV⊗	GGWTAA
Hpy99XIV-mut1⊗	GGWCNA
Hpy99XXII⊗	TCANNNNNNTRG
Hpy300XI⊗	CCTYNA
▲ HpyAV	CCTTC(6/5)
HpyAXIV⊗	GCGTA
HpyAXVI-mut1⊗	CRTTAA
HpyAXVI-mut2⊗	CRTCNA
HpyUM032XIII-mut1⊗	CYANNNNNNNTTC
HpyUM032XIV⊗	GAAAG
HpyUM037X⊗	GTGGNAG, TNGGNAG
Jma19592I⊗	GTATNAC
Jma19592II⊗	GRGCRAC
Jsp2502II⊗	GRNGAAT
Kor51II⊗	RTCGAG
Kpn156V⊗	CRTGATT
KpnNH25III⊗	CTRGAG
KpnNIH30III⊗	GTTCNAC
KpnNIH50I⊗	GCYAAG
Lba2029III⊗	CYAAANG
Lde4408II⊗	ACAAAG
LlaG50I⊗	CCGTKA
LmnI	GCTCC(1/-1)
Lmo370I⊗	AGCGCCG
Lmo911II⊗	TAGRAG
Lpl1004II⊗	AGGRAG
▲ LpnPI	CCDG(10/14)
Lra68I⊗	GTTCNAG
LsaDS4I⊗	TGGAAT
Lsp48III⊗	AGCACC
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
Mba11I⊗	AGGCGA
▲ MboII	GAAGA(8/7)
Mcr10I⊗	GAAGNNNNNCTC
MkaDII⊗	GAGAYGT
▲ MlyI	GAGTC(5/5)
▲ MmeI	TCCRAC(20/18)
▲ MnlI	CCTC(7/6)
MspI7II⊗	ACGRAG
▲ MspJI	CNNR(9/13)
MspSC27II⊗	CCGCGAC
MtuHN878II⊗	CACGCAG
NalI45188II⊗	ACCAGC
Nbr128II⊗	ACCGAC
NgoAVII⊗	GCCGC(7/7)
NgoAVIII⊗	(12/14)GACNNNNNTGA(13/11)
NhaXI⊗	CAAGRAG
NlaCI⊗	CATCAC(19/17)
▲ NmeAIII	GCCGAG(21/19)
NpeUS61II⊗	GATCGAC
OspHL35III⊗	YAGGAG
PacIII⊗	GTAATC
Pac19842II⊗	CCTTGA
Pal408I⊗	CCRTGAG
Pba2294I⊗	GTAAG
PcaII⊗	GACGAG
Pcr308II⊗	CCAAAG
Pdi8503III⊗	CCGGNAG
Pdu1735I⊗	CACCAC
PenI⊗	GCAGT
Pfl1108I⊗	TCGTAG

NONPALINDROMIC SEQUENCES (CON'T)	
PflPt14I⊗	RGCCCAC
PfrJS12IV⊗	TANAAG
PfrJS12V⊗	GGCGGAG
PfrJS15III⊗	CTTCNAC
Pin17FIII⊗	GGYGAB
PinP23II⊗	CTRKCAG
PinP59III⊗	GAAGNAG
PlaDI⊗	CATCAG(21/19)
▲ PleI	GAGTC(4/5)
PliMI⊗	CGCCGAC
PpiI⊗	(7/12)GAACNNNNNCTC(13/8)
PpiP13II⊗	CGCRGAC
Pse18267I⊗	RCCGAAG
Psp0357II⊗	GCGAAG
PspOMII⊗	CGCCCAR(20/18)
PspPRI⊗	CCYCAG(15/13)
PsrI	(7/12)GAACNNNNNNTAC(12/7)
Pst145I⊗	CTAMRAG
Pst273I⊗	GATCGAG
Pst14472I⊗	CNYACAC
PsuGI⊗	BBCGD
Rba2021I⊗	CACGAGH
RceI⊗	CATCGAC(20/18)
RdeGBI⊗	CCGCAG
RdeGBII⊗	ACCCAG(20/18)
RflFIII⊗	CGCCAG
RlaI⊗	VCW
RlaII⊗	ACACAG(20/18)
RleAI⊗	CCCACA(12/9)
Rmu369III⊗	GGCYAC
RpaI⊗	GTYGGAG(11/9)
RpaBI⊗	CCCGCAG(20/18)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
Rsp008IV⊗	ACGCAG
Rsp008V⊗	GCCCAT
RspPBTS2III⊗	CTTCGAG
Rtr1953I⊗	TGANNNNNNTGA
Saf8902III⊗	CAATNAG
▲ SapI	GCTCTTC(1/4)
Sba460II⊗	GGNGAYG
Sbo46I⊗	TGAAC
ScoDS2II⊗	GCTAAT
SdeAI⊗	CAGRAG(21/19)
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
Sen17963III⊗	CCAAAC
SenA1673III⊗	GNGGCAG
SenSARA26III⊗	ACRCAG
SenTFIV⊗	GATCAG
▲ SfaNI	GCATC(5/9)
SgeI	CNNGNNNNNNNNN/
SgrTI⊗	CCDS(10/14)
SimI⊗	GGGTC(-3/0)
SmaUMH5I⊗	CTTGAC
SmaUMH8I⊗	GCGAACB
Sno506I⊗	GGCCGAG
SpnRII⊗	TCGAG
SpoDI⊗	GCGGRAG
Ssp714II⊗	CGCAGCG
Ssp6803IV⊗	GAAGGC
SspD5I⊗	GGTGA(8/8)
SstE37I⊗	CGAAGAC(20/18)
Sth132I⊗	CCCG(4/8)
Sth20745III⊗	GGACGAC
SthSt3II⊗	GAAGT
StsI⊗	GGATG(10/14)
SurP32aII⊗	ACRGAG
TaqII	GACCGA(11/9)
TaqIII⊗	CACCCA(11/9)
TsoI⊗	TARCCA(11/9)

Nonpalindromic Recognition Sequences (continued)

NONPALINDROMIC SEQUENCES (CON'T)	
TspARh3I⊗	GRACGAC
TspDTI	ATGAA(11/9)
TspGWI	ACGGA(11/9)
TstI⊗	(8/13)CACNNNNNNTCC(12/7)
TsuI⊗	GCGAC
Tth111II⊗	CAARCA(11/9)
UbaF9I⊗	TACNNNNNRTGT
UbaF11I⊗	TCGTA
UbaF12I⊗	CTACNNNGTC
UbaF13I⊗	GAGNNNNNNCTGG

NONPALINDROMIC SEQUENCES (CON'T)	
UbaF14I⊗	CCANNNNNTCG
UbaPI⊗	CGAACG
Van9116I⊗	CCKAAG
Vdi96II⊗	GNCYTAG
Vtu19109I⊗	CACRAYC
WviI⊗	CACRAG(21/19)
Xca85IV⊗	TACGAG
YkrI⊗	C(10/9)
Yps3606I⊗	CGGAAG

Alphabetized List of NEB Recognition Sequences

All restriction enzyme recognition specificities and recommended enzymes available from New England Biolabs are listed below. For enzymes that recognize non-palindromic sequences, the complementary sequence of each strand is listed. For example, CCTC(7/6) and (6/7)GAGG both represent an MnlI site. New entries are listed in **bold** type.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleaveage for non-palindromic enzymes. For example, GGTCTC(1/5) indicates cleavage at: 5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

AA/CGTT	AclI
A/AGCTT	HindIII-HF
AAT/ATT	SspI-HF
/AATT	MluCI
A/CATGT	PciI
A/CCGGT	AgeI-HF
ACCTGC(4/8)	BfuAI
ACCTGC(4/8)	BspMI
A/CCWGGT	SexAI
A/CGCGT	MluI-HF
ACGGC(12/14)	BceAI
A/CGT	HpyCH4IV
ACN/GT	HpyCH4III
(10/15)ACNNNNGTAYC(12/7)	BaeI
(9/12)ACNNNNNCTCC(10/7)	BsaXI
A/CRYGT	AflIII
A/CTAGT	SpeI-HF
ACTGG(1/-1)	BsrI
ACTGGG(5/4)	BmrI
A/GATCT	BglII
AGC/GCT	AfeI
AG/CT	AluI
AGG/CCT	StuI
AGT/ACT	ScaI-HF
AT/CGAT	BspDI
AT/CGAT	ClaI
ATGCA/T	NsiI-HF
AT/TAAT	AseI
ATTT/AAAT	SwaI
C(11/9)	AbaSI
(11/13)CAANNNNNGTGG(12/10)	CspCI
C/AATTG	MfeI-HF
CACGAG(-5/-1)	BssSI-v2
CACGTC(-3/-3)	BmgBI
CAC/GTG	PmlI
CACNNN/GTG	DraIII-HF
CACNN/NNGTG	AleI-v2
(0/2)CACTG	BtsIMutI
(0/2)CACTGC	BtsI-v2
CAG/CTG	PvuII-HF
CAGNNN/CTG	AlwNI
CAGTG(2/0)	BtsIMutI
CASTGNN/	TspRI
CA/TATG	NdeI
(0/2)CATCC	BtsCI
(13/9)CATCC	FokI
(14/10)CATCGC	BtgZI
C/ATG	CviAII

/CATG	FatI
CATG/	NlaIII
(0/2)CATTGC	BsrDI
CAYNN/NNRTG	MslI
CC(12/16)	FspEI
(10/12)CCACNNNNNTTG	CspCI
(-1/1)CCAGT	BsrI
CCANNNNN/NNNNTGG	XcmI
CCANNNNN/NTGG	BstXI
CCANNNN/NTGG	PflMI
CCATC(4/5)	BccI
C/CATGG	NcoI-HF
CCCAGC(-5/-1)	BseYI
(4/5)CCCAGT	BmrI
CCCGC(4/6)	FauI
CCC/GGG	SmaI
C/CCGGG	TspMI
C/CCGGG	XmaI
CCDG(10/14)	LpnPI
CCGC(-3/-1)	AciI
CCGC/GG	SacII
CCGCTC(-3/-3)	BsrBI
C/CGG	HpaII
C/CGG	MspI
CC/NGG	ScrFI
/CCNGG	StyD4I
C/CNNGG	BsaJI
CCNNNNN/NNGG	BslI
C/CRYGG	BtgI
CC/SGG	NciI
C/CTAGG	AvrII
CCTC(7/6)	MnlI
CCTCAGC(-5/-2)	BbvCI
CCTGCA/GG	SbfI-HF
CCTNAGC(-5/-2)	Bpu10I
CC/TNAGG	Bsu36I
CCTNN/NNNAGG	EcoNI
CCTTC(6/5)	HpyAV
CC/WGG	BstNI
/CCWGG	PspGI
C/CWWGG	StyI-HF
(10/12)CGANNNNNNTGC(12/10)	BcgI
CGAT/CG	PvuI-HF
CG/CG	BstUI
C/GGCCG	EagI-HF
CG/GWCCG	RsrII
CGRY/CG	BsiEI
C/GTACG	BsiWI

CGTCTC(1/5)	BsmBI
CGTCTC(1/5)	**Esp3I**
CGWCG/	Hpy99I
(14/10)CHGG	LpnPI
CMG/CKG	MspA1I
CNNR(9/13)	MspJI
CR/CCGGYG	SgrAI
C/TAG	BfaI
(14/16)CTCAAG	BpuEI
CTCAG(9/7)	BspCNI
(14/16)CTCCAG	BpmI
(8/10)CTCCTC	BseRI
C/TCGAG	PaeR7I
C/TCGAG	XhoI
(19/21)CTCGGC	NmeAIII
(-1/-5)CTCGTG	BssSI-v2
CTCTTC(1/4)	EarI
CTGAAG(16/14)	AcuI
(7/9)CTGAG	BspCNI
(14/16)CTGCAC	BsgI
CTGCA/G	PstI-HF
CTGGAG(16/14)	BpmI
C/TNAG	DdeI
C/TRYAG	SfcI
C/TTAAG	AflII
(14/16)CTTCAG	AcuI
CTTGAG(16/14)	BpuEI
C/TYRAG	SmlI
C/YCGRG	AvaI
C/YCGRG	BsoBI
(9/11)G	AbaSI
GAAGA(8/7)	MboII
GAAGAC(2/6)	BbsI-HF
(4/1)GAAGAG	EarI
(4/1)GAAGAGC	BspQI
(4/1)GAAGAGC	SapI
(5/6)GAAGG	HpyAV
GAANN/NNTTC	XmnI
GAATGC(1/-1)	BsmI
G/AATTC	EcoRI-HF
GACGC(5/10)	HgaI
GACGT/C	AatII
GAC/GTC	ZraI
(-3/-3)GACGTG	BmgBI
GACN/NNGTC	PflFI
GACN/NNGTC	Tth111I
GACNN/NNGTC	PshAI
GACNNN/NNGTC	AhdI

GACNNNN/NNGTC	DrdI
(5/5)GACTC	MlyI
(5/4)GACTC	PleI
(5/1)GAGAC	BcoDI
(5/1)GAGAC	BsmAI
(5/1)GAGACC	BsaI
(5/1)GAGACG	BsmBI
(5/1)GAGACG	**Esp3I**
(-3/-3)GAGCGG	BsrBI
GAG/CTC	Eco53kI
GAGCT/C	SacI-HF
(6/7)GAGG	MnlI
GAGGAG(10/8)	BseRI
GAGTC(5/5)	MlyI
GAGTC(4/5)	PleI
G/ANTC	HinfI
GAT/ATC	EcoRV-HF
GA/TC	DpnI
/GATC	DpnII
/GATC	MboI
/GATC	Sau3AI
(5/4)GATCC	AlwI
(9/5)GATGC	SfaNI
(5/4)GATGG	BccI
GATNN/NNATC	BsaBI
G/AWTC	TfiI
GCAATG(2/0)	BsrDI
GCAGC(8/12)	BbvI
(8/4)GCAGGT	BfuAI
(8/4)GCAGGT	BspMI
GCAGTG(2/0)	BtsI-v2
(10/12)GCANNNNNNTCG	BcgI
GCANNNN/NTGC	BstAPI
GCATC(5/9)	SfaNI
GCATG/C	SphI-HF
(-1/1)GCATTC	BsmI
GCCC/GGGC	SrfI
GCCGAG(21/19)	NmeAIII
GCC/GGC	NaeI
G/CCGGC	NgoMIV
(14/12)GCCGT	BceAI
GCCNNNN/NGGC	BglI
GCGAT/CGC	AsiSI
GCGATG(10/14)	BtgZI
GCG/C	HhaI
G/CGC	HinP1I
G/CGCGC	BssHII
(-1/-3)GCGG	AciI

GC/GGCCGC	NotI-HF
(6/4)GCGGG	FauI
(10/5)GCGTC	HgaI
GC/NGC	Fnu4HI
GCN/NGC	Cac8I
GCNNNNN/NNGC	MwoI
GCTAG/C	BmtI-HF
G/CTAGC	NheI-HF
GCTCTTC(1/4)	BspQI
GCTCTTC(1/4)	SapI
(-2/-5)GCTGAGG	BbvCI
(12/8)GCTGC	BbvI
(-1/-5)GCTGGG	BseYI
GC/TNAGC	BlpI
(-2/-5)GCTNAGG	Bpu10I
G/CWGC	ApeKI
G/CWGC	TseI
GDGCH/C	Bsp1286I
(16/12)GG	FspEI
(7/10)GGAGNNNNNGT	BsaXI
(5/6)GGATAC	BciVI
GGATC(4/5)	AlwI
G/GATCC	BamHI-HF
GGATG(2/0)	BtsCI
GGATG(9/13)	FokI
GG/CC	HaeIII

GGCCGG/CC	FseI
GGCCNNNN/NGGCC	SfiI
G/GCGCC	KasI
GG/CGCC	NarI
GGCGC/C	PluTI
GGC/GCC	SfoI
GG/CGCGCC	AscI
GGCGGA(11/9)	EciI
GGGAC(10/14)	BsmFI
GGGCC/C	ApaI
G/GGCCC	PspOMI
G/GNCC	Sau96I
GGN/NCC	NlaIV
G/GTACC	Acc65I
GGTAC/C	KpnI-HF
GGTCTC(1/5)	BsaI-HFv2
GGTGA(8/7)	HphI
G/GTNACC	BstEII-HF
G/GWCC	AvaII
G/GYRCC	BanI
GKGCM/C	BaeGI
GR/CGYC	BsaHI
GRGCY/C	BanII
(7/12)GRTACNNNNGT	BaeI
G/TAC	CviQI
GT/AC	RsaI

GTA/TAC	BstZ17I
GTATCC(6/5)	BciVI
(14/10)GTCCC	BsmFI
G/TCGAC	SalI-HF
GTCTC(1/5)	BcoDI
GTCTC(1/5)	BsmAI
(6/2)GTCTTC	BbsI-HF
G/TGCAC	ApaLI
GTGCAG(16/14)	BsgI
GT/MKAC	AccI
GTN/NAC	Hpy166II
/GTSAC	Tsp45I
GTT/AAC	HpaI
GTTT/AAAC	PmeI
(18/20)GTYGGA	MmeI
GTY/RAC	HincII
GWGCW/C	BsiHKAI
R/AATTY	ApoI
RCATG/Y	NspI
R/CCGGY	BsrFI-v2
R/GATCY	BstYI
RGCGC/Y	HaeII
RG/CY	CviKI-1
RG/GNCCY	Eco0109I
RG/GWCCY	PpuMI
TAC/GTA	SnaBI

(7/8)TCACC	HphI
T/CATGA	BspHI
(9/11)TCCGCC	EciI
T/CCGGA	BspEI
TCCRAC(20/18)	MmeI
T/CGA	Taq$^{\alpha}$I
TCG/CGA	NruI-HF
TCN/GA	Hpy188I
TC/NNGA	Hpy188III
T/CTAGA	XbaI
(7/8)TCTTC	MboII
T/GATCA	BclI
TG/CA	HpyCH4V
TGC/GCA	FspI
TGG/CCA	MscI
T/GTACA	BsrGI-HF
T/TAA	MseI
TTAAT/TAA	PacI
TTA/TAA	PsiI
TT/CGAA	BstBI
TTT/AAA	DraI
VC/TGAGB	PspXI
W/CCGGW	BsaWI
YAC/GTR	BsaAI
Y/GGCCR	EaeI
(13/9)YNNG	MspJI

NEB is the primary sponsor of the **LabCentral Learning Lab** located in Kendall Square in Cambridge, Massachusetts. We use this space to provide hands-on training for our sales team members, as well as our customers in the Boston area. We also share the space with BioBuilder®, a STEM education program focused on experiential learning. Pictured here are several NEB members participating in hands-on training.

Isoschizomers

Restriction enzymes that recognize the same sequence are isoschizomers. The first example discovered is called a prototype, and all subsequent enzymes that recognize the same sequence are isoschizomers of the prototype. The list below contains isoschizomers for commercially-available restriction endonucleases. It also specifies which isoschizomer is available from New England Biolabs.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleavage for non-palindromic enzymes. For example, GGTCTC(1/5) indicates cleavage at: 5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

Isoschizomers with alternative cleavage sites (neoschizomers) are indicated with a "^". Enzymes that are not currently commercially available are indicated with a "⊗". For more information on isoschizomers, visit **REBASE.neb.com**

Neoschizomers are a subset of isoschizomers that recognize the same sequence, but cleave at different positions from the prototype. Thus, AatII (recognition sequence: GACGT↓C) and ZraI (recognition sequence: GAC↓GTC) are neoschizomers of one another, while HpaII (recognition sequence: C↓CGG) and MspI (recognition sequence: C↓CGG) are isoschizomers. Analogous designations are not appropriate for methyltransferases, where the differences between enzymes are not so easily defined and usually have not been well characterized.

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
AanI	TTA/TAA	PsiI	R0657	TTA/TAA	PsiI
AarI	CACCTGC(4/8)				
AasI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	DrdI, DseDI
AatII	GACGT/C	AatII	R0117	GACGT/C	ZraI^
		ZraI^	R0659	GAC/GTC	
Aba6411II ⊗	CRRTAAG				
AbaB8342IV ⊗	CATTAG				
AbaCIII ⊗	CTATCAV				
AbaSI	C(11/9)	AbaSI	R0665	C(11/9)	
AbaUMB2I ⊗	YCCGSS				
AbsI	CC/TCGAGG				
AccI	GT/MKAC	AccI	R0161	GT/MKAC	FbII, XmiI
AccII	CG/CG	BstUI	R0518	CG/CG	Bsh1236I, BspFNI, BstFNI, BstUI, MvnI
AccIII	T/CCGGA	BspEI	R0540	T/CCGGA	Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I, MroI
Acc16I	TGC/GCA	FspI	R0135	TGC/GCA	FspI, NsbI
Acc36I	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	BfuAI, BspMI, BveI
		BspMI	R0502	ACCTGC(4/8)	
Acc65I	G/GTACC	Acc65I	R0599	G/GTACC	Asp718I, KpnI^, KpnI-HF^
		KpnI-HF^	R3142	GGTAC/C	
Acc65V ⊗	GACGCA				
AccB1I	G/GYRCC	BanI	R0118	G/GYRCC	BanI, BshNI, BspT107I
AccB7I	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	PflMI, Van91I
AccBSI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	BsrBI, MbiI
AceIII ⊗	CAGCTC(7/11)				
AchA6III ⊗	AGCCAG				
AciI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	BspACI, SsiI
AclI	AA/CGTT	AclI	R0598	AA/CGTT	Psp1406I
AclWI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AlwI, BspPI
AcoI	Y/GGCCR	EaeI	R0508	Y/GGCCR	EaeI
Aco12261II ⊗	CCRGAG				
AcoY31II ⊗	TAGCRAB				
AcsI	R/AATTY	ApoI-HF	R3566	R/AATTY	ApoI, ApoI-HF, XapI,
AcuI	CTGAAG(16/14)	AcuI	R0641	CTGAAG(16/14)	Eco57I
AcvI	CAC/GTG	PmlI	R0532	CAC/GTG	BbrPI, Eco72I, PmaCI, PmlI, PspCI
AcyI	GR/CGYC	BsaHI	R0556	GR/CGYC	BsaHI, BssNI, BstACI, Hin1I, Hsp92I
AdeI	CACNNN/GTG	DraIII-HF	R3510	CACNNN/GTG	DraIII, DraIII-HF
Adh6U21I ⊗	GAANCAG				
AfaI	GT/AC	CviQI^	R0639	G/TAC	Csp6I^, CviQI^, RsaI, RsaNI^
		RsaI	R0167	GT/AC	
AfeI	AGC/GCT	AfeI	R0652	AGC/GCT	Aor51HI, Eco47III
AfiI	CCNNNNN/NNGG	BsiI	R0555	CCNNNNN/NNGG	Bsc4I, BseLI, BsiI
AflII	C/TTAAG	AflII	R0520	C/TTAAG	BfrI, BspTI, BstAFI, MspCI, Vha464I
AflIII	A/CRYGT	AflIII	R0541	A/CRYGT	
AgeI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, CspAI, PinAI
AgsI	TTS/AA				
AhaIII ⊗	TTT/AAA	DraI	R0129	TTT/AAA	DraI
AhdI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	BmeRI, DriI, Eam1105I
AhlI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	BcuI, SpeI, SpeI-HF
AhyRBAHI ⊗	GCYYGAC				
AhyYL17I ⊗	YAAMGAG				
AjiI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	BmgBI, BtrI
AjnI	/CCWGG	BstNI^	R0168	CC/WGG	BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, Psp6I, PspGI
		PspGI	R0611	/CCWGG	
AjuI	(7/12)GAANNNNNNNTTGG(11/6)				
AleI	CACNN/NNGTG	AleI	R0634	CACNN/NNGTG	OliI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
AlfI ⊗	(10/12)GCANNNNNNTGC(12/10)				
AloI	(7/12)GAACNNNNNNTCC(12/7)				
AluI	AG/CT	AluI	R0137	AG/CT	AluBI
AluBI	AG/CT	AluI	R0137	AG/CT	AluI
AlwI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, BspPI
Alw21I	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Bbv12I, BsiHKAI
Alw26I	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	BcoDI, BsmAI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
Alw44I	G/TGCAC	ApaLI	R0507	G/TGCAC	ApaLI, VneI
AlwFI ⊗	GAAAYNNNNNRTG				
AlwNI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	CaiI, PstNI
Ama87I	C/YCGRG	AvaI	R0152	C/YCGRG	AvaI, BmeT110I, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
AmaCSI ⊗	GCTCCA(11/9)				
Aor13HI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, BseAI, Bsp13I, BspEI, Kpn2I, MroI
Aor51HI	AGC/GCT	AfeI	R0652	AGC/GCT	AfeI, Eco47III
AoxI	/GGCC				
ApaI	GGGCC/C	ApaI	R0114	GGGCC/C	Bsp120I^, PspOMI^
		PspOMI^	R0653	G/GGCCC	
ApaBI ⊗	GCANNNNN/TGC	BstAPI^	R0654	GCANNNN/NTGC	BstAPI^
ApaLI	G/TGCAC	ApaLI	R0507	G/TGCAC	Alw44I, VneI
ApeKI	G/CWGC	ApeKI	R0643	G/CWGC	TseI
		TseI	R0591	G/CWGC	
ApoI	R/AATTY	ApoI-HF^	R3566	R/AATTY	AcsI, XapI, ApoI, ApoI-HF^
ApyPI ⊗	ATCGAC(20/18)				
AquII ⊗	GCCGNAC(20/18)				
AquIV ⊗	GRGGAAG(19/17)				
ArsI	(8/13)GACNNNNNNTTYG(11/6)				
AscI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	PalAI, SgsI
AseI	AT/TAAT	AseI	R0526	AT/TAAT	PshBI, VspI
AsiGI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, BshTI, CspAI, PinAI
AsiSI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	RgaI, SfaAI, SgfI
Asp103I ⊗	CGRAGGC				
Asp700I	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	MroXI, PdmI, XmnI
Asp718I	G/GTACC	Acc65I	R0599	G/GTACC	Acc65I, KpnI^, KpnI-HF^
		KpnI-HF^	R3142	GGTAC/C	
AspA2I	C/CTAGG	AvrII	R0174	C/CTAGG	AvrII, BlnI, XmaJI
AspBHI ⊗	YSCNS(8/12)				
AspDUT2V ⊗	GNGCAAC				
AspJHL3II ⊗	CGCCCAG				
AspLEI	GCG/C	HhaI	R0139	GCG/C	BstHHI, CfoI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
AspNIH4III ⊗	AAGAACB				
AspS9I	G/GNCC	Sau96I	R0165	G/GNCC	BmgT120I, Cfr13I, PspPI, Sau96I
AspSLV7III ⊗	GTCTCA				
Asp114pII ⊗	AGCABCC				
AsuI ⊗	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, PspPI, Sau96I
AsuII	TT/CGAA	BstBI	R0519	TT/CGAA	Bpu14I, Bsp119I, BspT104I, BstBI, NspV, SfuI
Asu14238IV ⊗	CGTRAC				
AsuC2I	CC/SGG	NciI	R0196	CC/SGG	BcnI, BpuMI, NciI
AsuHPI	GGTGA(8/7)	HphI	R0158	GGTGA(8/7)	HphI
AsuNHI	G/CTAGC	BmtI-HF^	R3658	GCTAG/C	BmtI^, BmtI-HF^, BspOI^, NheI, NheI-HF
		NheI-HF	R3131	G/CTAGC	
AteTI ⊗	GGGRAG				
AvaI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, BmeT110I, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
AvaII	G/GWCC	AvaII	R0153	G/GWCC	Bme18I, Eco47I, SinI, VpaK11BI
AvaIII ⊗	ATGCAT	NsiI-HF	R3127	ATGCA/T	EcoT22I, Mph1103I, NsiI, NsiI-HF, Zsp2I
AvrII	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, BlnI, XmaJI
Awo1030IV ⊗	GCCRAG				
AxyI	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	Bse21I, Bsu36I, Eco81I
B					
BaeI	(10/15)ACNNNNGTAYC(12/7)	BaeI	R0613	(10/15)ACNNNNGTAYC(12/7)	
BaeGI	GKGCM/C	BaeGI	R0708	GKGCM/C	BseSI, BstSLI
Bag18758I ⊗	CCCGAG				

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BalI	TGG/CCA	MscI	R0534	TGG/CCA	MlsI, MluNI, Mox20I, MscI, Msp20I
BamHI	G/GATCC	BamHI-HF	R3136	G/GATCC	BamHI, BamHI-HF
BanI	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BshNI, BspT107I
BanII	GRGCY/C	BanII	R0119	GRGCY/C	Eco24I, EcoT38I, FriOI
BanLI ⊗	RTCAGG				
BarI	(7/12)GAAGNNNNNNTAC(12/7)				
BauI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BssSI, BssSI-v2, Bst2BI
Bbr11I ⊗	GGRCAG				
Bbr52II ⊗	GGCGAG				
Bbr57III ⊗	GTRAAYG				
BbrPI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, Eco72I, PmaCI, PmlI, PspCI
BbsI	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI, BstV2I
BbvI	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BseXI, BstV1I, Lsp1109I
BbvII ⊗	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI, BstV2I
Bbv12I	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, BsiHKAI
BbvCI	CCTCAGC(-5/-2)	BbvCI	R0601	CCTCAGC(-5/-2)	
BccI	CCATC(4/5)	BccI	R0704	CCATC(4/5)	
Bce83I ⊗	CTTGAG(16/14)	BpuEI	R0633	CTTGAG(16/14)	BpuEI
Bce3081I ⊗	TAGGAG				
BceAI	ACGGC(12/14)	BceAI	R0623	ACGGC(12/14)	
BcefI ⊗	ACGGC(12/13)	BceAI^	R0623	ACGGC(12/14)	BceAI^
BcgI	(10/12)CGANNNNNNTGC(12/10)	BcgI	R0545	(10/12)CGANNNNNNTGC(12/10)	
BciT130I	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BseBI, BstNI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BciVI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BfuI, BsuI
BclI	T/GATCA	BclI-HF	R3160	T/GATCA	FbaI, Ksp22I, BclI, BclI-HF
BcnI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BpuMI, NciI
BcoDI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	Alw26I, BsmAI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
BcuI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	AhlI, SpeI, SpeI-HF
BdaI ⊗	(10/12)TGANNNNNNTCA(12/10)				
BetI ⊗	W/CCGGW	BsaWI	R0567	W/CCGGW	BsaWI
BfaI	C/TAG	BfaI	R0568	C/TAG	FspBI, MaeI, SspMI, XspI
BfaSII ⊗	GANGGAG				
BfiI ⊗	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmrI, BmuI
BfmI	C/TRYAG	SfcI	R0561	C/TRYAG	BstSFI, SfcI
BfoI	RGCGC/Y	HaeII	R0107	RGCGC/Y	BstH2I, HaeII
BfrI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BspTI, BstAFI, MspCI, Vha464I
BfuI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BciVI, BsuI
BfuAI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BspMI, BveI
		BspMI	R0502	ACCTGC(4/8)	
BglI	GCCNNNN/NGGC	BglI	R0143	GCCNNNN/NGGC	
BglII	A/GATCT	BglII	R0144	A/GATCT	
BinI ⊗	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, AlwI, BspPI
BisI	GC/NGC				BisI^, GluI^, PkrI^
BkrAM31DI ⊗	RTTAAATM				
Ble402II ⊗	GRAGCAG				
BlnI	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, AvrII, XmaJI
BloAII ⊗	GAGGAC				
BlpI	GC/TNAGC	BlpI	R0585	GC/TNAGC	Bpu1102I, Bsp1720I
BlsI	GCN/GC				BisI^, GluI^, PkrI
BmcAI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	ScaI, ScaI-HF, ZrmI
Bme18I	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Eco47I, SinI, VpaK11BI
Bme1390I	CC/NGG	ScrFI	R0110	CC/NGG	BmrFI, BstSCI^, MspR9I, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
BmeRI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, DriI, Eam1105I
BmeT110I	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
BmgI ⊗	GKGCCC				
BmgBI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	AjiI, BtrI
BmgT120I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, Cfr13I, PspPI, Sau96I
BmiI	GGN/NCC	NlaIV	R0126	GGN/NCC	BspLI, NlaIV, PspN4I
BmrI	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmuI
BmrFI	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BstSCI^, MspR9I, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
BmsI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	LweI, SfaNI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BmtI	GCTAG/C	BmtI-HF^	R3658	GCTAG/C	BmtI, BmtI-HF^, AsuNHI^, BspOI, NheI^, NheI-HF^
		NheI-HF^	R3131	G/CTAGC	
BmuI	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmrI
BoxI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BstPAI, PshAI
BpiI	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BstV2I
BplI	(8/13)GAGNNNNNCTC(13/8)				
BpmI	CTGGAG(16/14)	BpmI	R0565	CTGGAG(16/14)	GsuI
Bpu10I	CCTNAGC(-5/-2)	Bpu10I	R0649	CCTNAGC(-5/-2)	
Bpu14I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bsp119I, BspT104I, BstBI, NspV, SfuI
Bpu1102I	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bsp1720I
BpuEI	CTTGAG(16/14)	BpuEI	R0633	CTTGAG(16/14)	
BpuMI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, NciI
BsaI	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, BspTNI, Eco31I
Bsa29I	AT/CGAT	BspDI	R0557	AT/CGAT	BseCI, BshVI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BsaAI	YAC/GTR	BsaAI	R0531	YAC/GTR	BstBAI, Ppu21I
BsaBI	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	Bse8I, BseJI
BsaHI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BssNI, BstACI, Hin1I, Hsp92I
BsaJI	C/CNNGG	BsaJI	R0536	C/CNNGG	BseDI, BssECI
BsaWI	W/CCGGW	BsaWI	R0567	W/CCGGW	
BsaXI	(9/12)ACNNNNNCTCC(10/7)	BsaXI	R0609	(9/12)ACNNNNNCTCC(10/7)	
BsbI ⊗	CAACAC(21/19)				
Bsc4I	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	AfiI, BseLI, BslI
BscGI ⊗	CCCGT				
Bse1I	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	BseNI, BsrI
Bse8I	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	BsaBI, BseJI
Bse21I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bsu36I, Eco81I
Bse118I	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	BsrFI-v2, BssAI, Cfr10I
BseAI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, Bsp13I, BspEI, Kpn2I, MroI
BseBI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BstNI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BseCI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BshVI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BseDI	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BssECI
Bse3DI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	BseMI, BsrDI
BseGI	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BstF5I, BtsCI, FokI^
		FokI^	R0109	GGATG(9/13)	
BseJI	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	BsaBI, Bse8I
BseLI	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BslI
BseMI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	Bse3DI, BsrDI
BseMII	CTCAG(10/8)	BspCNI^	R0624	CTCAG(9/7)	BspCNI^
BseNI	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	Bse1I, BsrI
BsePI	G/CGCGC	BssHII	R0199	G/CGCGC	BssHII, PauI, PteI
BseRI	GAGGAG(10/8)	BseRI	R0581	GAGGAG(10/8)	
BseSI	GKGCM/C	BaeGI	R0708	GKGCM/C	BaeGI, BstSLI
BseXI	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BstV1I, Lsp1109I
BseX3I	C/GGCCG	EagI-HF	R3505	C/GGCCG	BstZI, EagI, EagI-HF, EclXI, Eco52I
BseYI	CCCAGC(-5/-1)	BseYI	R0635	CCCAGC(-5/-1)	GsaI^, PspFI
BsgI	GTGCAG(16/14)	BsgI	R0559	GTGCAG(16/14)	
Bsh1236I	CG/CG	BstUI	R0518	CG/CG	AccII, BspFNI, BstFNI, BstUI, MvnI
Bsh1285I	CGRY/CG	BsiEI	R0554	CGRY/CG	BsiEI, BstMCI
BshFI	GG/CC	HaeIII	R0108	GG/CC	BsnI, BspANI, BsuRI, HaeIII
BshNI	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BspT107I
BshTI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, CspAI, PinAI
BshVI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BsiI ⊗	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI-v2, Bst2BI
BsiEI	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BstMCI
BsiHKAI	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, Bbv12I
BsiHKCI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
BsiSI	C/CGG	HpaII	R0171	C/CGG	HapII, HpaII, MspI
		MspI	R0106	C/CGG	
BsiWI	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II, PspLI
BsiYI ⊗	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BseLI, BslI

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BslI	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BseLI
BslFI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BsmFI, FaqI
BsmI	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	Mva1269I, PctI
BsmAI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	Alw26I, BcoDI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
BsmBI	CGTCTC(1/5)	BsmBI	R0580	CGTCTC(1/5)	Esp3I
		Esp3I	R0734	CGTCTC(1/5)	
BsmFI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BslFI, FaqI
BsnI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BspANI, BsuRI, HaeIII
Bso31I	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, BspTNI, Eco31I
BsoBI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsiHKCI, Eco88I
		BsoBI	R0586	C/YCGRG	
Bsp13I	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, BspEI, Kpn2I, MroI
Bsp19I	C/CATGG	NcoI-HF	R3193	C/CATGG	NcoI, NcoI-HF
Bsp24I ⊗	(8/13)GACNNNNNNTGG(12/7)				
Bsp68I	TCG/CGA	NruI-HF	R3192	TCG/CGA	BtuMI, NruI, NruI-HF, RruI
Bsp119I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, BspT104I, BstBI, NspV, SfuI
Bsp120I	G/GGCCC	ApaI^	R0114	GGGCC/C	ApaI^, PspOMI
		PspOMI	R0653	G/GGCCC	
Bsp143I	/GATC	DpnII	R0543	/GATC	BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
Bsp460III ⊗	CGCGCAG				
Bsp1286I	GDGCH/C	Bsp1286I	R0120	GDGCH/C	MhlI, SduI
Bsp1407I	T/GTACA	BsrGI-HF	R3575	T/GTACA	BsrGI, BsrGI-HF, BstAUI
Bsp1720I	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bpu1102I
Bsp3004IV ⊗	CCGCAT				
BspACI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	AciI, SsiI
BspANI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BsuRI, HaeIII
BspCNI	CTCAG(9/7)	BspCNI	R0624	CTCAG(9/7)	BseMII^
BspDI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BspEI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, Kpn2I, MroI
BspFNI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BstFNI, BstUI, MvnI
BspGI ⊗	CTGGAC				
BspHI	T/CATGA	BspHI	R0517	T/CATGA	CciI, PagI
BspLI	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, NlaIV, PspN4I
BspLU11I ⊗	A/CATGT	PciI	R0655	A/CATGT	PciI, PscI
BspMI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BfuAI, BveI
		BspMI	R0502	ACCTGC(4/8)	
BspMII ⊗	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I, MroI
BspMAI	CTGCA/G	PstI-HF	R3140	CTGCA/G	PstI, PstI-HF
BspNCI ⊗	CCAGA				
BspOI	GCTAG/C	BmtI-HF	R3658	GCTAG/C	AsuNHI^, BmtI, BmtI-HF, NheI^, NheI-HF^
		NheI-HF^	R3131	G/CTAGC	
BspPI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, AlwI
BspQI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	LguI, PciSI, SapI
		SapI	R0569	GCTCTTC(1/4)	
BspTI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BstAFI, MspCI, Vha464I
BspT104I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BstBI, NspV, SfuI
BspT107I	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BshNI
BspTNI	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, Eco31I
BsrI	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	Bse1I, BseNI
BsrBI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	AccBSI, MbiI
BsrDI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	Bse3DI, BseMI
BsrFI	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, BssAI, Cfr10I
BsrGI	T/GTACA	BsrGI-HF	R3575	T/GTACA	BsrGI, BsrGI-HF, Bsp1407I, BstAUI
BssAI	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, Cfr10I
BssECI	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BseDI
BssHII	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, PauI, PteI
BssMI	/GATC	DpnII	R0543	/GATC	Bsp143I, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
BssNI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BstACI, Hin1I, Hsp92I
BssNAI	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	Bst1107I, BstZ17I, BstZ17I-HF
BssSI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI-v2, Bst2BI
BssT1I	C/CWWGG	StyI-HF	R3500	C/CWWGG	Eco130I, EcoT14I, ErhI, StyI, StyI-HF

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
Bst6I	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Eam1104I, EarI
Bst1107I	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, BstZ17I, BstZ17I-HF
BstACI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, Hin1I, Hsp92I
BstAFI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, MspCI, Vha464I
BstAPI	GCANNNN/NTGC	BstAPI	R0654	GCANNNN/NTGC	
BstAUI	T/GTACA	BsrGI-HF	R3575	T/GTACA	Bsp1407I, BsrGI, BsrGI-HF
BstBI	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, NspV, SfuI
Bst2BI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI, BssSI-v2
BstBAI	YAC/GTR	BsaAI	R0531	YAC/GTR	BsaAI, Ppu21I
Bst4CI	ACN/GT	HpyCH4III	R0618	ACN/GT	HpyCH4III, TaaI
BstC8I	GCN/NGC	Cac8I	R0579	GCN/NGC	Cac8I
BstDEI	C/TNAG	DdeI	R0175	C/TNAG	DdeI, HpyF3I
BstDSI	C/CRYGG	BtgI	R0608	C/CRYGG	BtgI
BstEII	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, EcoO65I, PspEI
BstENI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	EcoNI, XagI
BstF5I	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BseGI, BtsCI, FokI^
		FokI^	R0109	GGATG(9/13)	
BstFNI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstUI, MvnI
BstH2I	RGCGC/Y	HaeII	R0107	RGCGC/Y	BfoI, HaeII
BstHHI	GCG/C	HhaI	R0139	GCG/C	AspLEI, CfoI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
BstKTI	GAT/C	DpnII^	R0543	/GATC	Bsp143I^, BssMI^, BstMBI^, DpnII^, Kzo9I^, MboI^, NdeII^, Sau3AI^
		MboI^	R0147	/GATC	
		Sau3AI^	R0169	/GATC	
BstMAI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	AIw26I, BcoDI, BsmAI
		BsmAI	R0529	GTCTC(1/5)	
BstMBI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
BstMCI	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BsiEI
BstMWI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	HpyF10VI, MwoI
BstNI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BstNSI	RCATG/Y	NspI	R0602	RCATG/Y	NspI, XceI
BstPI	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, Eco91I, EcoO65I, PspEI
BstPAI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BoxI, PshAI
BstSCI	/CCNGG	ScrFI^	R0110	CC/NGG	Bme1390I^, BmrFI^, MspR9I^, ScrFI^, StyD4I
		StyD4I	R0638	/CCNGG	
BstSFI	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, SfcI
BstSLI	GKGCM/C	BaeGI	R0708	GKGCM/C	BaeGI, BseSI
BstSNI	TAC/GTA	SnaBI	R0130	TAC/GTA	Eco105I, SnaBI
BstUI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, MvnI
Bst2UI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, BstNI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BstV1I	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BseXI, Lsp1109I
BstV2I	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI
BstXI	CCANNNNN/NTGG	BstXI	R0113	CCANNNNN/NTGG	
BstX2I	R/GATCY	BstYI	R0523	R/GATCY	BstYI, MfiI, PsuI
BstYI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, MfiI, PsuI
BstZI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, EagI, EagI-HF, EclXI, Eco52I
BstZ17I	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, Bst1107I, BstZ17I, BstZ17I-HF
BsuI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BciVI, BfuI
Bsu15I	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
Bsu36I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Eco81I
BsuRI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BspANI, HaeIII
BsuTUI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, Bsu15I, ClaI
		ClaI	R0197	AT/CGAT	
BtgI	C/CRYGG	BtgI	R0608	C/CRYGG	BstDSI
BtgZI	GCGATG(10/14)	BtgZI	R0703	GCGATG(10/14)	
BtrI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	AjiI, BmgBI
BtsI	GCAGTG(2/0)	BtsI-v2	R0667	GCAGTG(2/0)	
BtsIMutI	CAGTG(2/0)	BtsIMutI	R0664	CAGTG(2/0)	
BtsCI	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BseGI, BstF5I, FokI^
		FokI^	R0109	GGATG(9/13)	

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ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BtuMI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, NruI, NruI-HF, RruI
BveI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BfuAI, BspMI
		BspMI	R0502	ACCTGC(4/8)	
C					
Cac8I	GCN/NGC	Cac8I	R0579	GCN/NGC	BstC8I
CaiI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	AlwNI, PstNI
Cal14237I ⊗	GGTTAG				
CalB3II ⊗	GRTTRAG				
CauII ⊗	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, BpuMI, NciI
Cau10061II ⊗	GTTAAT				
Cba13II ⊗	AGGAAT				
Cba16038I ⊗	CCTNAYNC				
Cbo67071IV ⊗	GCRGAAG				
CchII ⊗	GGARGA(11/9)				
CchIII ⊗	CCCAAG(20/18)				
Cch467III ⊗	GNGAAAY				
CciI	T/CATGA	BspHI	R0517	T/CATGA	BspHI, PagI
CciNI	GC/GGCCGC	NotI-HF	R3189	GC/GGCCGC	NotI, NotI-HF
Cco14983V ⊗	GGGTDA				
Cco14983VI ⊗	GCYGA				
CcrNAIII ⊗	CGACCAG				
CdiI ⊗	CATCG(-1/-1)				
Cdi81III ⊗	GCMGAAG				
Cdi11397I ⊗	GCGCAG				
CdpI ⊗	GCGGAG(20/18)				
Cdu23823II ⊗	GTGAAG				
CfoI	GCG/C	HhaI	R0139	GCG/C	AspLEI, BstHHI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
CfrI ⊗	Y/GGCCR	EaeI	R0508	Y/GGCCR	AcoI, EaeI
Cfr9I	C/CCGGG	SmaI^	R0141	CCC/GGG	SmaI^, TspMI, XmaI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
Cfr10I	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, BssAI
Cfr13I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, PspPI, Sau96I
Cfr42I	CCGC/GG	SacII	R0157	CCGC/GG	KspI, SacII, Sfr303I, SgrBI
Cfupf3II ⊗	GARCAG				
Cgl13032I ⊗	GGCGCA				
Cgl13032II ⊗	ACGABGG				
CjeI ⊗	(8/14)CCANNNNNNGT(15/9)				
Cje265V ⊗	GKAAGC				
Cje54107III ⊗	GKAAYC				
CjeFIII ⊗	GCAAGG				
CjeFV ⊗	GGRCA				
CjeNII ⊗	GAGNNNNNGT				
CjeNIII ⊗	GKAAYG(19/17)				
CjeNV ⊗	CCYGA				
CjePI ⊗	(7/13)CCANNNNNNNTC(14/8)				
CjeP659IV ⊗	CACNNNNNNNGAA				
CjuI ⊗	CAYNNNNNRTG				
CjuII ⊗	CAYNNNNNCTC				
ClaI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, Bsu15I, BsuTUI
		ClaI	R0197	AT/CGAT	
Cla11845III ⊗	GCGAA				
Cly7489II ⊗	AAAAGRG				
Cma23826I ⊗	CGGAAG				
CpoI	CG/GWCCG	RsrII	R0501	CG/GWCCG	CspI, RsrII, Rsr2I
CseI	GACGC(5/10)	HgaI	R0154	GACGC(5/10)	HgaI
CsiI	A/CCWGGT	SexAI	R0605	A/CCWGGT	MabI, SexAI
CspI	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, RsrII, Rsr2I
Csp6I	G/TAC	CviQI	R0639	G/TAC	AfaI^, CviQI, RsaI^, RsaNI
		RsaI^	R0167	GT/AC	
Csp2014I ⊗	GGAGGC				
CspAI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, PinAI
CspCI	(11/13)CAANNNNNGTGG(12/10)	CspCI	R0645	(11/13)CAANNNNNGTGG(12/10)	
CstMI ⊗	AAGGAG(20/18)				

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
CviAII	C/ATG	CviAII	R0640	C/ATG	FaeI^, FatI^^, Hin1II^, Hsp92II^, NlaIII^
		FatI^^	R0650	/CATG	
		NlaIII^	R0125	CATG/	
CviJI	RG/CY	CviKI-1	R0710	RG/CY	CviKI-1
CviKI-1	RG/CY	CviKI-1	R0710	RG/CY	CviJI
CviQI	G/TAC	CviQI	R0639	G/TAC	AfaI^, Csp6I, RsaI^, RsaNI
		RsaI^	R0167	GT/AC	
CviRI ⊗	TG/CA	HpyCH4V	R0620	TG/CA	HpyCH4V
D					
DdeI	C/TNAG	DdeI	R0175	C/TNAG	BstDEI, HpyF3I
Dde51507I ⊗	CCWGG				
DinI	GGC/GCC	KasI^	R0544	G/GCGCC	EgeI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
DpnI	GA/TC	DpnI	R0176	GA/TC	MalI
DpnII	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
DraI	TTT/AAA	DraI	R0129	TTT/AAA	
DraII ⊗	RG/GNCCY	Eco0109I	R0503	RG/GNCCY	Eco0109I
DraIII	CACNNN/GTG	DraIII-HF	R3510	CACNNN/GTG	AdeI, DraIII, DraIII-HF
DraRI ⊗	CAAGNAC(20/18)				
DrdI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	AasI, DseDI
DrdII ⊗	GAACCA				
DriI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, BmeRI, Eam1105I
DsaI ⊗	C/CRYGG	BtgI	R0608	C/CRYGG	BstDSI, BtgI
DseDI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	AasI, DrdI
E					
EaeI	Y/GGCCR	EaeI	R0508	Y/GGCCR	AcoI
EagI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI, Eco52I
Eam1104I	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, EarI
Eam1105I	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, BmeRI, DriI
EarI	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, Eam1104I
EciI	GGCGGA(11/9)	EciI	R0590	GGCGGA(11/9)	
Ecl136II	GAG/CTC	Eco53kI	R0116	GAG/CTC	EcoICRI, Eco53kI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Ecl234I ⊗	CGGNAAG				
Ecl35734I ⊗	GAAAYTC				
EclXI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, Eco52I
Eco24I	GRGCY/C	BanII	R0119	GRGCY/C	BanII, EcoT38I, FriOI
Eco31I	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, BspTNI
Eco32I	GAT/ATC	EcoRV-HF	R3195	GAT/ATC	EcoRV, EcoRV-HF
Eco47I	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, SinI, VpaK11BI
Eco47III	AGC/GCT	AfeI	R0652	AGC/GCT	AfeI, Aor51HI
Eco52I	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI
Eco53KI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, EcoICRI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Eco57I	CTGAAG(16/14)	AcuI	R0641	CTGAAG(16/14)	AcuI
Eco72I	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, PmaCI, PmlI, PspCI
Eco81I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Bsu36I
Eco88I	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsiHKCI, BsoBI
		BsoBI	R0586	C/YCGRG	
Eco91I	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco065I, PspEI
Eco105I	TAC/GTA	SnaBI	R0130	TAC/GTA	BstSNI, SnaBI
Eco130I	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, EcoT14I, ErhI, StyI, StyI-HF
Eco147I	AGG/CCT	StuI	R0187	AGG/CCT	PceI, SseBI, StuI
Eco4465II ⊗	GAAABCC				
Eco43896II ⊗	CRARCAG				
EcoBLMcrX ⊗	RCSRC(-3/-2)				
EcoE1140I ⊗	ACCYAC				
EcoICRI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, Eco53kI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Eco57MI ⊗	CTGRAG(16/14)				
EcoMVII ⊗	CANCATC				

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
EcoNI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	BstENI, XagI
EcoNIH6II ⊗	ATGAAG				
EcoO65I	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, PspEI
EcoO109I	RG/GNCCY	EcoO109I	R0503	RG/GNCCY	
EcoRI	G/AATTC	EcoRI-HF	R3101	G/AATTC	EcoRI, EcoRI-HF
EcoRII	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, MvaI^, Psp6I, PspGI
		PspGI	R0611	/CCWGG	
EcoRV	GAT/ATC	EcoRV-HF	R3195	GAT/ATC	Eco32I, EcoRV, EcoRV-HF
EcoT14I	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, ErhI, StyI, StyI-HF
EcoT22I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	Mph1103I, NsiI, NsiI-HF, Zsp2I
EcoT38I	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, FriOI
Eco53kI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, EcoICRI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
EgeI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
EheI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EgeI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
Eli8509II ⊗	CCGGAG				
ErhI	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, EcoT14I, StyI, StyI-HF
EsaSSI ⊗	GACCAC				
EspI ⊗	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bpu1102I, Bsp1720I
Esp3I	CGTCTC(1/5)	BsmBI	R0580	CGTCTC(1/5)	BsmBI
		Esp3I	R0734	CGTCTC(1/5)	
Esp3007I ⊗	CAGAAG				
Exi27195I ⊗	GCCGAC				
F					
FaeI	CATG/	CviAII^	R0640	C/ATG	CviAII^, FatI^^, Hin1II, Hsp92II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
FaiI	YA/TR				
FalI	(8/13)AAGNNNNNCTT(13/8)				
FaqI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BslFI, BsmFI
FatI	/CATG	CviAII^	R0640	C/ATG	CviAII^, FaeI^^, Hin1II^^, Hsp92II^^, NlaIII^^
		FatI	R0650	/CATG	
		NlaIII^^	R0125	CATG/	
FauI	CCCGC(4/6)	FauI	R0651	CCCGC(4/6)	
FauNDI	CA/TATG	NdeI	R0111	CA/TATG	NdeI
FbaI	T/GATCA	BclI-HF	R3160	T/GATCA	BclI, BclI-HF, Ksp22I
FbII	GT/MKAC	AccI	R0161	GT/MKAC	AccI, XmiI
Fco1691IV ⊗	GCVGAG				
FinI ⊗	GGGAC	BsmFI	R0572	GGGAC(10/14)	BslFI, BsmFI, FaqI
FnuDII ⊗	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, BstUI, MvnI
Fnu4HI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fsp4HI, SatI
FokI	GGATG(9/13)	BtsCI^	R0647	GGATG(2/0)	BseGI^, BstF5I^, BtsCI^
		FokI	R0109	GGATG(9/13)	
FriOI	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, EcoT38I
FseI	GGCCGG/CC	FseI	R0588	GGCCGG/CC	RigI
FspI	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, NsbI
FspAI	RTGC/GCAY				
FspBI	C/TAG	BfaI	R0568	C/TAG	BfaI, MaeI, SspMI, XspI
FspEI	CC(12/16)	FspEI	R0662	CC(12/16)	
Fsp4HI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fnu4HI, SatI
FspPK15I ⊗	GARGAAG				
FtnUV ⊗	GAAACA				
G					
GauT27I ⊗	CGCGCAGG				
Gba708II ⊗	ATGCAC				
GdiII ⊗	CGGCCR(-5/-1)				
GlaI	GC/GC				
GluI	GC/NGC				BisI, BlsI^, PkrI^
GsaI	CCCAGC(-1/-5)	BseYI^	R0635	CCCAGC(-5/-1)	BseYI^, PspFI^
GsuI	CTGGAG(16/14)	BpmI	R0565	CTGGAG(16/14)	BpmI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
H					
HaeI ⊗	WGG/CCW				
HaeII	RGCGC/Y	HaeII	R0107	RGCGC/Y	BfoI, BstH2I
HaeIII	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BspANI, BsuRI
HaeIV ⊗	(7/13)GAYNNNNNRTC(14/9)				
HapII	C/CGG	HpaII	R0171	C/CGG	BsiSI, HpaII, MspI
		MspI	R0106	C/CGG	
HbaII ⊗	GCCCAG				
HdeNY26I ⊗	CGANNNNNNTCC				
HdeZA17I ⊗	GCANNNNNNTCC				
HgaI	GACGC(5/10)	HgaI	R0154	GACGC(5/10)	CseI
HgiAI ⊗	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, Bbv12I, BsiHKAI
HgiCI ⊗	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BshNI, BspT107I
HgiEII ⊗	ACCNNNNNNGGT				
HgiJII ⊗	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, EcoT38I, FriOI
HhaI	GCG/C	HhaI	R0139	GCG/C	AspLEI, BstHHI, CfoI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
Hin1I	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, BstACI, Hsp92I
Hin1II	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hsp92II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
Hin4I ⊗	(8/13)GAYNNNNNVTC(13/8)				
Hin4II ⊗	CCTTC(6/5)	HpyAV	R0621	CCTTC(6/5)	HpyAV
Hin6I	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, HinP1I, HspAI
		HinP1I	R0124	G/CGC	
HinP1I	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, Hin6I, HspAI
		HinP1I	R0124	G/CGC	
HincII	GTY/RAC	HincII	R0103	GTY/RAC	HindII
HindII	GTY/RAC	HincII	R0103	GTY/RAC	HincII
HindIII	A/AGCTT	HindIII-HF	R3104	A/AGCTT	HindIII, HindIII-HF
HinfI	G/ANTC	HinfI	R0155	G/ANTC	
HpaI	GTT/AAC	HpaI	R0105	GTT/AAC	KspAI
HpaII	C/CGG	HpaII	R0171	C/CGG	BsiSI, HapII, MspI
		MspI	R0106	C/CGG	
HphI	GGTGA(8/7)	HphI	R0158	GGTGA(8/7)	AsuHPI
Hpy8I	GTN/NAC	Hpy166II	R0616	GTN/NAC	Hpy166II
Hpy99I	CGWCG/	Hpy99I	R0615	CGWCG/	
Hpy99XIII ⊗	GCCTA				
Hpy99XIV ⊗	GGWTAA				
Hpy99XIV-mut1 ⊗	GGWCNA				
Hpy99XXII ⊗	TCANNNNNNTRG				
Hpy166II	GTN/NAC	Hpy166II	R0616	GTN/NAC	Hpy8I
Hpy178III ⊗	TC/NNGA	Hpy188III	R0622	TC/NNGA	Hpy188III
Hpy188I	TCN/GA	Hpy188I	R0617	TCN/GA	
Hpy188III	TC/NNGA	Hpy188III	R0622	TC/NNGA	
Hpy300XI ⊗	CCTYNA				
HpyAV	CCTTC(6/5)	HpyAV	R0621	CCTTC(6/5)	
HpyAXIV ⊗	GCGTA				
HpyAXVI-mut1 ⊗	CRTTAA				
HpyAXVI-mut2 ⊗	CRTCNA				
HpyCH4III	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, TaaI
HpyCH4IV	A/CGT	HpyCH4IV	R0619	A/CGT	HpySE526I, MaeII, TaiI^
HpyCH4V	TG/CA	HpyCH4V	R0620	TG/CA	
HpyF3I	C/TNAG	DdeI	R0175	C/TNAG	BstDEI, DdeI
HpyF10VI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	BstMWI, MwoI
HpySE526I	A/CGT	HpyCH4IV	R0619	A/CGT	HpyCH4IV, MaeII, TaiI^
HpyUM032XIII ⊗	CYANNNNNNNTRG				
HpyUM032XIII-mut1 ⊗	CYANNNNNNNTTC				
HpyUM032XIV ⊗	GAAAG				
HpyUM037X ⊗	GTGGNAG, TNGGNAG				
Hsp92I	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, BstACI, Hin1I
Hsp92II	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hin1II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	

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ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
HspAI	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, Hin6I, HinP1I
		HinP1I	R0124	G/CGC	
J					
Jma19592I ⊗	GTATNAC				
Jma19592II ⊗	GRGCRAC				
Jsp2502II ⊗	GRNGAAT				
K					
KasI	G/GCGCC	KasI	R0544	G/GCGCC	DinI^, EgeI^, EheI^, MIy113I^^, NarI^^, PluTI^^^, SfoI^, SspDI
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^	R0606	GGC/GCC	
KflI	GG/GWCCC				
Kor51II ⊗	RTCGAG				
KpnI	GGTAC/C	Acc65I^	R0599	G/GTACC	Acc65I^, Asp718I^, KpnI, KpnI-HF
		KpnI-HF	R3142	GGTAC/C	
Kpn2I	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, MroI
Kpn156V ⊗	CRTGATT				
KpnNH25III ⊗	CTRGAG				
KpnNIH30III ⊗	GTTCNAC				
KpnNIH50I ⊗	GCYAAG				
KroI	G/CCGGC				
KspI	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, SacII, Sfr303I, SgrBI
Ksp22I	T/GATCA	BclI-HF	R3160	T/GATCA	BclI, BclI-HF, FbaI
Ksp632I ⊗	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, Eam1104I, EarI
KspAI	GTT/AAC	HpaI	R0105	GTT/AAC	HpaI
Kzo9I	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
L					
Lba2029III ⊗	CYAAANG				
Lde4408II ⊗	ACAAAG				
LguI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, PciSI, SapI
		SapI	R0569	GCTCTTC(1/4)	
LlaG50I ⊗	CCGTKA				
LmnI	GCTCC(1/-1)				
Lmo370I ⊗	AGCGCCG				
Lmo911II ⊗	TAGRAG				
Lpl1004II ⊗	AGGRAG				
LpnPI	CCDG(10/14)	LpnPI	R0663	CCDG(10/14)	
Lra68I ⊗	GTTCNAG				
LsaDS4I ⊗	TGGAAT				
Lsp48III ⊗	AGCACC				
Lsp1109I	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BseXI, BstV1I
Lsp6406VI ⊗	CRAGCAC				
LweI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	BmsI, SfaNI
M					
MabI	A/CCWGGT	SexAI	R0605	A/CCWGGT	CsiI, SexAI
MaeI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, SspMI, XspI
MaeII	A/CGT	HpyCH4IV	R0619	A/CGT	HpyCH4IV, HpySE526I, TaiI^
MaeIII	/GTNAC				
MalI	GA/TC	DpnI	R0176	GA/TC	DpnI
MaqI ⊗	CRTTGAC(21/19)				
MauBI	CG/CGCGCG				
Mba11I ⊗	AGGCGA				
MbiI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	AccBSI, BsrBI
MboI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
MboII	GAAGA(8/7)	MboII	R0148	GAAGA(8/7)	
McrI ⊗	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BsiEI, BstMCI
Mcr10I ⊗	GAAGNNNNNCTC				
MfeI	C/AATTG	MfeI-HF	R3589	C/AATTG	MunI, MfeI, MfeI-HF
MflI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, PsuI
MhlI	GDGCH/C	Bsp1286I	R0120	GDGCH/C	Bsp1286I, SduI
MjaIV ⊗	GTNNAC	Hpy166II	R0616	GTN/NAC	Hpy8I, Hpy166II
MkaDII ⊗	GAGAYGT				
MlsI	TGG/CCA	MscI	R0534	TGG/CCA	BaII, MluNI, Mox20I, MscI, Msp20I

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
MluI	A/CGCGT	MluI-HF	R3198	A/CGCGT	MluI, MluI-HF
MluCI	/AATT	MluCI	R0538	/AATT	Sse9I, TasI
MluNI	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, Mox20I, MscI, Msp20I
MlyI	GAGTC(5/5)	MlyI	R0610	GAGTC(5/5)	PleI^, PpsI^, SchI
		PleI^	R0515	GAGTC(4/5)	
Mly113I	GG/CGCC	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, NarI, PluTI^^^, SfoI^^, SspDI^
		NarI	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
MmeI	TCCRAC(20/18)	MmeI	R0637	TCCRAC(20/18)	
MnlI	CCTC(7/6)	MnlI	R0163	CCTC(7/6)	
Mox20I	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, MscI, Msp20I
Mph1103I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, NsiI, NsiI-HF, Zsp2I
MreI	CG/CCGGCG				
MroI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I
MroNI	G/CCGGC	NaeI^	R0190	GCC/GGC	NaeI^, NgoMIV, PdiI^
		NgoMIV	R0564	G/CCGGC	
MroXI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, PdmI, XmnI
MscI	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, Mox20I, Msp20I
MseI	T/TAA	MseI	R0525	T/TAA	SaqAI, Tru1I, Tru9I
MslI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	RseI, SmiMI
MspI	C/CGG	HpaII	R0171	C/CGG	BsiSI, HapII, HpaII
		MspI	R0106	C/CGG	
Msp20I	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, Mox20I, MscI
MspA1I	CMG/CKG	MspA1I	R0577	CMG/CKG	
MspCI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, BstAFI, Vha464I
MspI7II ⊗	ACGRAG				
MspJI	CNNR(9/13)	MspJI	R0661	CNNR(9/13)	
MspR9I	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BmrFI, BstSCI^, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
MspSC27II ⊗	CCGCGAC				
MssI	GTTT/AAAC	PmeI	R0560	GTTT/AAAC	PmeI
MstI ⊗	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, FspI, NsbI
MteI	GCGC/NGCGC				
MtuHN878II ⊗	CACGCAG				
MunI	C/AATTG	MfeI-HF	R3589	C/AATTG	MfeI, MfeI-HF
MvaI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, BstNI, Bst2UI, EcoRII^, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
Mva1269I	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	BsmI, PctI
MvnI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, BstUI
MwoI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	BstMWI, HpyF10VI
N					
NaeI	GCC/GGC	NaeI	R0190	GCC/GGC	MroNI^, NgoMIV^, PdiI
		NgoMIV^	R0564	G/CCGGC	
NaI45188II ⊗	ACCAGC				
NarI	GG/CGCC	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, Mly113I, PluTI^^^, SfoI^^, SspDI^
		NarI	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
Nbr128II ⊗	ACCGAC				
NciI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, BpuMI
NcoI	C/CATGG	NcoI-HF	R3193	C/CATGG	Bsp19I, NcoI, NcoI-HF
NdeI	CA/TATG	NdeI	R0111	CA/TATG	FauNDI
NdeII	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
NgoAVII ⊗	GCCGC(7/7)				
NgoAVIII ⊗	(12/14)GACNNNNNTGA(13/11)				
NgoMIV	G/CCGGC	NaeI^	R0190	GCC/GGC	MroNI, NaeI^, PdiI^
		NgoMIV	R0564	G/CCGGC	
NhaXI ⊗	CAAGRAG				
NheI	G/CTAGC	BmtI-HF^	R3658	GCTAG/C	AsuNHI, BmtI^, BmtI-HF^, BspOI^, NheI, NheI-HF
		NheI-HF	R3131	G/CTAGC	
NhoI ⊗	GCWGC				

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ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
NlaIII	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hin1II, Hsp92II
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
NlaIV	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, BspLI, PspN4I
NlaCI ⊗	CATCAC(19/17)				
NmeAIII	GCCGAG(21/19)	NmeAIII	R0711	GCCGAG(21/19)	
NmuCI	/GTSAC	Tsp45I	R0583	/GTSAC	TseFI, Tsp45I
NotI	GC/GGCCGC	NotI-HF	R3189	GC/GGCCGC	CciNI, NotI, NotI-HF
NpeUS61II ⊗	GATCGAC				
NruI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, BtuMI, NruI, NruI-HF, RruI
NsbI	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, FspI
NsiI	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, NsiI, NsiI-HF, Mph1103I, Zsp2I
NspI	RCATG/Y	NspI	R0602	RCATG/Y	BstNSI, XceI
NspV	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, BstBI, SfuI
NspBII ⊗	CMG/CKG	MspA1I	R0577	CMG/CKG	MspA1I
O					
OliI	CACNN/NNGTG	AleI-v2	R0685	CACNN/NNGTG	AleI, AleI-v2
OspHL35III ⊗	YAGGAG				
P					
PacI	TTAAT/TAA	PacI	R0547	TTAAT/TAA	
PacIII ⊗	GTAATC				
Pac19842II ⊗	CCTTGA				
PaeI	GCATG/C	SphI-HF	R3182	GCATG/C	SphI, SphI-HF
PaeR7I	C/TCGAG	PaeR7I	R0177	C/TCGAG	Sfr274I, SlaI, XhoI
		XhoI	R0146	C/TCGAG	
PagI	T/CATGA	BspHI	R0517	T/CATGA	BspHI, CciI
Pal408I ⊗	CCRTGAG				
PalAI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	AscI, SgsI
PasI	CC/CWGGG				
PauI	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, BssHII, PteI
Pba2294I ⊗	GTAAG				
PcaII ⊗	GACGAG				
PceI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, SseBI, StuI
PciI	A/CATGT	PciI	R0655	A/CATGT	PscI
PciSI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, LguI, SapI
		SapI	R0569	GCTCTTC(1/4)	
Pcr308II ⊗	CCAAAG				
PcsI	WCGNNNN/NNNCGW				
PctI	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	BsmI, Mva1269I
PdiI	GCC/GGC	NaeI	R0190	GCC/GGC	MroNI^, NaeI, NgoMIV^
		NgoMIV^	R0564	G/CCGGC	
Pdi8503III ⊗	CCGGNAG				
PdmI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, MroXI, XmnI
Pdu1735I ⊗	CACCAC				
PenI ⊗	GCAGT				
PfeI	G/AWTC	TfiI	R0546	G/AWTC	TfiI
Pfl23II	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, PspLI
Pfl1108I ⊗	TCGTAG				
Pfl8569I ⊗	GCN/NGC				
PflFI	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PsyI, Tth111I
		Tth111I	R0185	GACN/NNGTC	
PflMI	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	AccB7I, Van91I
PflPt14I ⊗	RGCCCAC				
PfoI	T/CCNGGA				
PfrJS12IV ⊗	TANAAG				
PfrJS12V ⊗	GGCGGAG				
PfrJS15III ⊗	CTTCNAC				
PinAI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, CspAI
Pin17FIII ⊗	GGYGAB				
PinP23II ⊗	CTRKCAG				
PinP59III ⊗	GAAGNAG				
PkrI	GCN/GC				BisI^, BlsI, GluI^
PleI	GAGTC(4/5)	MlyI^	R0610	GAGTC(5/5)	MlyI^, PpsI, SchI^
		PleI	R0515	GAGTC(4/5)	
Ple19I	CGAT/CG	PvuI-HF	R3150	CGAT/CG	PvuI, PvuI-HF
PliMI ⊗	CGCCGAC				

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
PluTI	GGCGC/C	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, MlyI13I^^^, NarI^^^, SfoI^^, SspDI^
		NarI^^^	R0191	GG/CGCC	
		PluTI	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
PmaCI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmlI, PspCI
PmeI	GTTT/AAAC	PmeI	R0560	GTTT/AAAC	MssI
PmlI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmaCI, PspCI
PpiI ⊗	(7/12)GAACNNNNNCTC(13/8)				
PpiP13II ⊗	CGCRGAC				
PpsI	GAGTC(4/5)	MlyI^	R0610	GAGTC(5/5)	MlyI^, PleI, SchI^
		PleI	R0515	GAGTC(4/5)	
Ppu21I	YAC/GTR	BsaAI	R0531	YAC/GTR	BsaAI, BstBAI
PpuMI	RG/GWCCY	PpuMI	R0506	RG/GWCCY	Psp5II, PspPPI
PscI	A/CATGT	PciI	R0655	A/CATGT	PciI
Pse18267I ⊗	RCCGAAG				
PshAI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BoxI, BstPAI
PshBI	AT/TAAT	AseI	R0526	AT/TAAT	AseI, VspI
PsiI	TTA/TAA	PsiI	R0657	TTA/TAA	AanI
Psp5II	RG/GWCCY	PpuMI	R0506	RG/GWCCY	PpuMI, PspPPI
Psp6I	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, PspGI
		PspGI	R0611	/CCWGG	
Psp0357II ⊗	GCGAAG				
Psp1406I	AA/CGTT	AclI	R0598	AA/CGTT	AclI
Psp124BI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	EcI136II^, EcoICRI^, Eco53kI^, SacI, SacI-HF, SstI
		SacI-HF	R3156	GAGCT/C	
PspCI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmaCI, PmlI
PspEI	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, EcoO65I
PspFI	CCCAGC(-5/-1)	BseYI	R0635	CCCAGC(-5/-1)	BseYI, GsaI^
PspGI	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, Psp6I
		PspGI	R0611	/CCWGG	
PspLI	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II
PspN4I	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, BspLI, NlaIV
PspOMI	G/GGCCC	ApaI^	R0114	GGGCC/C	ApaI^, Bsp120I
		PspOMI	R0653	G/GGCCC	
PspOMII ⊗	CGCCCAR(20/18)				
PspPI	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, Sau96I
PspPPI	RG/GWCCY	PpuMI	R0506	RG/GWCCY	PpuMI, Psp5II
PspPRI ⊗	CCYCAG(15/13)				
PspXI	VC/TCGAGB	PspXI	R0656	VC/TCGAGB	
PsrI	(7/12)GAACNNNNNNTAC(12/7)				
PstI	CTGCA/G	PstI-HF	R3140	CTGCA/G	BspMAI, PstI, PstI-HF
Pst145I ⊗	CTAMRAG				
Pst273I ⊗	GATCGAG				
Pst14472I ⊗	CNYACAC				
PstNI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	AlwNI, CaiI
PsuI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, MflI
PsuGI ⊗	BBCGD				
PsyI	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PflFI, Tth111I
		Tth111I	R0185	GACN/NNGTC	
PteI	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, BssHII, PauI
PvuI	CGAT/CG	PvuI-HF	R3150	CGAT/CG	Ple19I, PvuI, PvuI-HF
PvuII	CAG/CTG	PvuII-HF	R3151	CAG/CTG	PvuII, PvuII-HF
R					
Rba2021I ⊗	CACGAGH				
RceI ⊗	CATCGAC(20/18)				
RdeGBI ⊗	CCGCAG				
RdeGBII ⊗	ACCCAG(20/18)				
RdeGBIII ⊗	(9/11)TGRYCA(11/9)				
RflFIII ⊗	CGCCAG				
RgaI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, SfaAI, SgfI
RigI	GGCCGG/CC	FseI	R0588	GGCCGG/CC	FseI
RlaI ⊗	VCW				
RlaII ⊗	ACACAG(20/18)				
RleAI ⊗	CCCACA(12/9)				
Rmu369III ⊗	GGCYAC				

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V = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
RpaI ⊗	GTYGGAG(11/9)				
RpaBI ⊗	CCCGCAG(20/18)				
RpaB5I ⊗	CGRGGAC(20/18)				
RpaTI ⊗	GRTGGAG				
RruI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, BtuMI, NruI, NruI-HF
RsaI	GT/AC	CviQI^	R0639	G/TAC	AfaI, Csp6I^, CviQI^, RsaNI^
		RsaI	R0167	GT/AC	
RsaNI	G/TAC	CviQI	R0639	G/TAC	AfaI^, Csp6I, CviQI, RsaI^
		RsaI^	R0167	GT/AC	
RseI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	MslI, SmiMI
Rsp008IV ⊗	ACGCAG				
Rsp008V ⊗	GCCCAT				
RspPBTS2III ⊗	CTTCGAG				
RsrII	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, CspI, Rsr2I
Rsr2I	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, CspI, RsrII
Rtr1953I ⊗	TGANNNNNNTGA				
S					
SacI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	Ecl136II^, EcoICRI^, Eco53kI^, Psp124BI, SacI, SacI-HF, SstI
		SacI-HF	R3156	GAGCT/C	
SacII	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, Sfr303I, SgrBI
Saf8902III ⊗	CAATNAG				
SalI	G/TCGAC	SalI-HF	R3138	G/TCGAC	SalI, SalI-HF
SanDI ⊗	GG/GWCCC				KfiI
SapI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, LguI, PciSI
		SapI	R0569	GCTCTTC(1/4)	
SaqAI	T/TAA	MseI	R0525	T/TAA	MseI, Tru1I, Tru9I
SatI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fnu4HI, Fsp4HI
SauI ⊗	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Bsu36I, Eco81I
Sau96I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, PspPI
Sau3AI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
Sba460II ⊗	GGNGAYG				
SbfI	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, SdaI, Sse8387I
Sbo46I ⊗	TGAAC				
ScaI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	BmcAI, ScaI, ScaI-HF, ZrmI
SchI	GAGTC(5/5)	MlyI	R0610	GAGTC(5/5)	MlyI, PleI^, PpsI^
		PleI^	R0515	GAGTC(4/5)	
ScoDS2II ⊗	GCTAAT				
ScrFI	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BmrFI, BstSCI^, MspR9I, StyD4I^
		StyD4I^	R0638	/CCNGG	
SdaI	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, Sse8387I
SdeAI ⊗	CAGRAG(21/19)				
SdeOSI ⊗	(11/13)GACNNNNRTGA(12/10)				
SduI	GDGCH/C	Bsp1286I	R0120	GDGCH/C	Bsp1286I, MhlI
SecI ⊗	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BseDI, BssECI
Sen17963III ⊗	CCAAAC				
SenA1673III ⊗	GNGGCAG				
SenSARA26III ⊗	ACRCAG				
SenTFIV ⊗	GATCAG				
SetI	ASST/				
SexAI	A/CCWGGT	SexAI	R0605	A/CCWGGT	CsiI, MabI
SfaAI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, RgaI, SgfI
SfaNI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	BmsI, LweI
SfcI	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, BstSFI
SfeI ⊗	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, BstSFI, SfcI
SfiI	GGCCNNNN/NGGCC	SfiI	R0123	GGCCNNNN/NGGCC	
SfoI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EgeI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
Sfr274I	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, SlaI, XhoI
		XhoI	R0146	C/TCGAG	
Sfr303I	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, SacII, SgrBI
SfuI	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, BstBI, NspV
SgeI	CNNGNNNNNNNNN/				
SgfI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, RgaI, SfaAI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
SgrAI	CR/CCGGYG	SgrAI	R0603	CR/CCGGYG	
SgrBI	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, SacII, Sfr303I
SgrDI	CG/TCGACG				
SgrTI ⊗	CCDS(10/14)				
SgsI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	AscI, PalAI
SimI ⊗	GGGTC(-3/0)				
SinI	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, Eco47I, VpaK11BI
SlaI	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, Sfr274I, XhoI
		XhoI	R0146	C/TCGAG	
SmaI	CCC/GGG	SmaI	R0141	CCC/GGG	Cfr9I^, TspMI^, XmaI^
		TspMI^	R0709	C/CCGGG	
		XmaI^	R0180	C/CCGGG	
SmaUMH5I ⊗	CTTGAC				
SmaUMH8I ⊗	GCGAACB				
SmiI	ATTT/AAAT	SwaI	R0604	ATTT/AAAT	SwaI
SmiMI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	MslI, RseI
SmlI	C/TYRAG	SmlI	R0597	C/TYRAG	SmoI
SmoI	C/TYRAG	SmlI	R0597	C/TYRAG	SmlI
SnaI ⊗	GTATAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, Bst1107I, BstZ17I, BstZ17I-HF
SnaBI	TAC/GTA	SnaBI	R0130	TAC/GTA	BstSNI, Eco105I
Sno506I ⊗	GGCCGAG				
SpeI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	AhlI, BcuI, SpeI, SpeI-HF
SphI	GCATG/C	SphI-HF	R3182	GCATG/C	PaeI, SphI, SphI-HF
SplI ⊗	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II, PspLI
SpnRII ⊗	TCGAG				
SpoDI ⊗	GCGGRAG				
SrfI	GCCC/GGGC	SrfI	R0629	GCCC/GGGC	
Sse9I	/AATT	MluCI	R0538	/AATT	MluCI, TasI
Sse232I ⊗	CG/CCGGCG				MreI
Sse8387I	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, SdaI
Sse8647I ⊗	AG/GWCCT				
SseBI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, PceI, StuI
SsiI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	AciI, BspACI
SspI	AAT/ATT	SspI-HF	R3132	AAT/ATT	SspI, SspI-HF
Ssp714II ⊗	CGCAGCG				
Ssp6803IV ⊗	GAAGGC				
SspDI	G/GCGCC	KasI	R0544	G/GCGCC	DinI^, EgeI^, EheI^, KasI, Mly113I^^, NarI^^, PluTI^^^, SfoI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^	R0606	GGC/GCC	
SspMI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, MaeI, XspI
SstI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	Ecl136II^, EcoICRI^, Eco53kI^, Psp124BI, SacI, SacI-HF
		SacI-HF	R3156	GAGCT/C	
SstE37I ⊗	CGAAGAC(20/18)				
Sth132I ⊗	CCCG(4/8)				
Sth20745III ⊗	GGACGAC				
SthSt3II ⊗	GAAGT				
StuI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, PceI, SseBI
StyI	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, EcoT14I, ErhI, StyI, StyI-HF
StyD4I	/CCNGG	ScrFI^	R0110	CC/NGG	Bme1390I^, BmrFI^, BstSCI, MspR9I^, ScrFI^
		StyD4I	R0638	/CCNGG	
SurP32aII ⊗	ACRGAG				
SwaI	ATTT/AAAT	SwaI	R0604	ATTT/AAAT	SmiI
T					
TaaI	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, HpyCH4III
TaiI	ACGT/	HpyCH4IV^	R0619	A/CGT	HpyCH4IV^, HpySE526I^, MaeII^
TaqI	T/CGA	TaqI	R0149	T/CGA	
TaqII	GACCGA(11/9)				
TaqIII ⊗	CACCCA(11/9)				
TasI	/AATT	MluCI	R0538	/AATT	MluCI, Sse9I
TatI	W/GTACW				
TauI	GCSG/C				
TfiI	G/AWTC	TfiI	R0546	G/AWTC	PfeI
Tru1I	T/TAA	MseI	R0525	T/TAA	MseI, SaqAI, Tru9I
Tru9I	T/TAA	MseI	R0525	T/TAA	MseI, SaqAI, Tru1I

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
TscAI	CASTGNN/	TspRI	R0582	CASTGNN/	TspRI
TseI	G/CWGC	ApeKI	R0643	G/CWGC	ApeKI
		TseI	R0591	G/CWGC	
TseFI	/GTSAC	Tsp45I	R0583	/GTSAC	NmuCI, Tsp45I
TsoI ⊗	TARCCA(11/9)				
Tsp45I	/GTSAC	Tsp45I	R0583	/GTSAC	NmuCI, TseFI
TspARh3I ⊗	GRACGAC				
Tsp4CI ⊗	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, HpyCH4III, TaaI
TspDTI	ATGAA(11/9)				
TspEI ⊗	/AATT	MluCI	R0538	/AATT	MluCI, Sse9I, TasI
TspGWI	ACGGA(11/9)				
TspMI	C/CCGGG	SmaI^	R0141	CCC/GGG	Cfr9I, SmaI^, XmaI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
TspRI	CASTGNN/	TspRI	R0582	CASTGNN/	TscAI
TssI ⊗	GAGNNNCTC				
TstI ⊗	(8/13)CACNNNNNNTCC(12/7)				
TsuI ⊗	GCGAC				
Tth111I	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PflFI, PsyI
		Tth111I	R0185	GACN/NNGTC	
Tth111II ⊗	CAARCA(11/9)				
U					
UbaF9I ⊗	TACNNNNNRTGT				
UbaF11I ⊗	TCGTA				
UbaF12I ⊗	CTACNNNGTC				
UbaF13I ⊗	GAGNNNNNNCTGG				
UbaF14I ⊗	CCANNNNNTCG				
UbaPI ⊗	CGAACG				
V					
Van91I	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	AccB7I, PflMI
Van9116I ⊗	CCKAAG				
Vdi96II ⊗	GNCYTAG				
Vha464I	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, BstAFI, MspCI
VneI	G/TGCAC	ApaLI	R0507	G/TGCAC	Alw44I, ApaLI
VpaK11BI	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, Eco47I, SinI
VspI	AT/TAAT	AseI	R0526	AT/TAAT	AseI, PshBI
Vtu19109I ⊗	CACRAYC				
W					
WviI ⊗	CACRAG(21/19)				
X					
XagI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	BstENI, EcoNI
XapI	R/AATTY	ApoI-HF	R3566	R/AATTY	AcsI, ApoI, ApoI-HF
XbaI	T/CTAGA	XbaI	R0145	T/CTAGA	
Xca85IV ⊗	TACGAG				
XceI	RCATG/Y	NspI	R0602	RCATG/Y	BstNSI, NspI
XcmI	CCANNNNN/NNNNTGG	XcmI	R0533	CCANNNNN/NNNNTGG	
XhoI	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, Sfr274I, SlaI
		XhoI	R0146	C/TCGAG	
XhoII ⊗	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, MflI, PsuI
XmaI	C/CCGGG	SmaI^	R0141	CCC/GGG	Cfr9I, SmaI^, TspMI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
XmaIII ⊗	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI, Eco52I
XmaJI	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, AvrII, BlnI
XmiI	GT/MKAC	AccI	R0161	GT/MKAC	AccI, FbII
XmnI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, MroXI, PdmI
XspI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, MaeI, SspMI
Y					
Yps3606I ⊗	CGGAAG				
Z					
ZraI	GAC/GTC	AatII^	R0117	GACGT/C	AatII^
		ZraI	R0659	GAC/GTC	
ZrmI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	BmcAI, ScaI, ScaI-HF
Zsp2I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, Mph1103I, NsiI, NsiI-HF

Survival in a Reaction

Restriction enzymes vary with respect to their ability to maintain activity in a reaction over an extended period of time.

+ + + Enzyme is active > 8 hours
+ + Enzyme is active 4–8 hours
+ Enzyme is active 2–4 hours
– No benefit from digesting over 1 hour
N/A Not Available

While most routine restriction digests are incubated for one hour or less at 37°C, there are certain applications that require the addition of less than 1 unit/µg of DNA and increasing the reaction time beyond one hour. The table below can be used as a guide when low levels of enzyme and extended reaction times are needed.

For example, 1 unit of AatII can be used to digest 8 µg of DNA in a 16 hour digest (+ + +).

Extended activity was determined by performing the restriction endonuclease unit assay, using a 16 hour incubation in place of the standard 1 hour digestion. After the 16 hour digestion, extended activity enzymes (+ + +) required only 0.13 units to completely digest 1 µg of DNA. Intermediate activity enzymes required either 0.25 (+ +) or 0.50 (+) units for complete digestion over this extended incubation time. Finally, enzymes marked (–) required 1.0 unit for complete digestion, the same amount of enzyme required for a 1 hour digestion.

Note: Reaction temperature is 37°C, unless otherwise noted.

ENZYME	SURVIVAL
AatII	+ + +
AbaSI @25°C	N/A
AccI	+ + +
Acc65I	+
AciI	–
AclI	+
AcuI	–
AfeI	+ +
AflII	+ + +
AflIII	+ + +
AgeI	+
AgeI-HF	+ +
AhdI	+ + +
AleI-v2	N/A
AluI	+ + +
AlwI	+ + +
AlwNI	+ + +
ApaI @25°C	+ + +
ApaLI	+ + +
ApeKI @75°C	–
ApoI @50°C	+ + +
ApoI-HF	+ + +
AscI	–
AseI	+ + +
AsiSI	+ + +
AvaI	+
AvaII	+ + +
AvrII	+ + +
BaeI @25°C	+
BaeGI	+ + +
BamHI	+
BamHI-HF	+
BanI	+ + +
BanII	+ + +
BbsI	+ +
BbsI-HF	–
BbvI	+ +
BbvCI	+ + +
BccI	+
BceAI	+ + +
BcgI	+ +
BciVI	+ +
BclI @50°C	+
BclI-HF	N/A
BcoDI	+ + +
BfaI	+
BfuAI @50°C	+ +
BglI	+ + +
BglII	+ +
BlpI	+
BmgBI	–
BmrI	+ +
BmtI	+ + +
BmtI-HF	+ + +
BpmI	–

ENZYME	SURVIVAL
Bpu10I	+
BpuEI @25°C	+
BsaI	–
BsaI-HFv2	+ +
BsaAI	+ +
BsaBI @60°C	+ + +
BsaHI	+ + +
BsaJI @60°C	+ + +
BsaWI @60°C	+ + +
BsaXI	+ +
BseRI	+
BseYI	+
BsgI	+
BsiEI @60°C	+ +
BsiHKAI @65°C	–
BsiWI @55°C	–
BsiWI-HF	+ + +
BsII @55°C	+
BsmI @65°C	+ + +
BsmAI @55°C	+ + +
BsmBI @55°C	+
BsmFI @65°C	+ + +
BsoBI	+ + +
Bsp1286I	+
BspCNI @25°C	–
BspDI	–
BspEI	+ + +
BspHI	+ +
BspMI	+ + +
BspQI @50°C	+ + +
BsrI @65°C	+ +
BsrBI	+
BsrDI @65°C	+
BsrFI-v2	+ + +
BsrGI	+ + +
BsrGI-HF	+ + +
BssHII @50°C	+ +
BssSI-v2	+ + +
BstAPI @60°C	+ +
BstBI @65°C	+ + +
BstEII @60°C	–
BstEII-HF	–
BstNI @60°C	–
BstUI @60°C	+ + +
BstXI @55°C	+
BstYI @60°C	+ +
BstZ17I-HF	+ +
Bsu36I	+ + +
BtgI	+
BtgZI @60°C	–
BtsI-v2 @55°C	+ + +
BtsMutI @55°C	N/A
BtsCI @50°C	+
Cac8I	+ + +
ClaI	+

ENZYME	SURVIVAL
CspCI	+ + +
CviAII @25°C	–
CviKI-1	+ +
CviQI @25°C	+ +
DdeI	+ + +
DpnI	+ + +
DpnII	+ + +
DraI	+
DraIII-HF	+ + +
DrdI	+ + +
EaeI	+ + +
EagI	+ + +
EagI-HF	+ + +
EarI	+ +
EciI	–
Eco53kI	+ +
EcoNI	+ + +
EcoO109I	+ + +
EcoP15I	–
EcoRI	+ + +
EcoRI-HF	+ +
EcoRV	+
EcoRV-HF	+ + +
Esp3I	N/A
FatI @55°C	–
FauI @55°C	–
Fnu4HI	+ + +
FokI	+ +
FseI	–
FspI	+ + +
FspEI	+ + +
HaeII	–
HaeIII	+ + +
HgaI	–
HhaI	+ +
HincII	+ + +
HindIII	+ + +
HindIII-HF	+ + +
HinfI	+ + +
HinP1I	+ + +
HpaI	+ +
HpaII	+ + +
HphI	+ + +
Hpy99I	–
Hpy166II	+
Hpy188I	+ + +
Hpy188III	+ +
HpyAV	–
HpyCH4III	+ + +
HpyCH4IV	+ + +
HpyCH4V	+ + +
I-CeuI	+ +
I-SceI	+ +
KasI	–
KpnI	+ +

ENZYME	SURVIVAL
KpnI-HF	+
LpnPI	–
MboI	+ + +
MboII	+
MfeI	+ +
MfeI-HF	+ +
MluI	+ + +
MluI-HF	+ + +
MluCI	–
MlyI	–
MmeI	–
MnlI	+ +
MscI	+ + +
MseI	+ + +
MslI	+ +
MspI	+
MspA1I	+ +
MspJI	+ + +
MwoI @60°C	+ + +
NaeI	–
NarI	+ + +
Nb.BbvCI	+ + +
Nb.BsmI @65°C	+ +
Nb.BsrDI @65°C	+ +
Nb.BssSI	+ + +
Nb.BtsI	+ +
NciI	+
NcoI	+ + +
NcoI-HF	+ + +
NdeI	+ +
NgoMIV	+ +
NheI	+ +
NheI-HF	+ + +
NlaIII	+
NlaIV	+
NmeAIII	–
NotI	+ +
NotI-HF	+ + +
NruI	+ + +
NruI-HF	+ + +
NsiI	+ + +
NsiI-HF	+ + +
NspI	+ +
Nt.AlwI	+ + +
Nt.BbvCI	+ + +
Nt.BsmAI	+ + +
Nt.BspQI @50°C	+ +
Nt.BstNBI @55°C	+
Nt.CviPII	–
PacI	+ + +
PaeR7I	+ + +
PciI	+ +
PflFI	+ + +
PflMI	+
PI-PspI @65°C	+ + +

Survival in a Reaction (continued)

ENZYME	SURVIVAL
PI-SceI	+++
PhoI @75°C	–
PleI	+++
PluTI	N/A
PmeI	–
PmlI	+
PpuMI	+++
PshAI	–
PsiI	++
PspGI @75°C	++
PspOMI	+++
PspXI	+++
PstI	+
PstI-HF	+++
PvuI	+++
PvuI-HF	+++
PvuII	+++

ENZYME	SURVIVAL
PvuII-HF	+++
RsaI	++
RsrII	+++
SacI	+++
SacI-HF	+++
SacII	+++
SalI	+++
SalI-HF	+++
SapI	+++
Sau3AI	+
Sau96I	+++
SbfI	+
SbfI-HF	+++
ScaI-HF	++
ScrFI	+++
SexAI	++
SfaNI	++

ENZYME	SURVIVAL
SfcI	–
SfiI @50°C	++
SfoI	–
SgrAI	+++
SmaI @25°C	–
SmlI @55°C	++
SnaBI	+
SpeI	++
SpeI-HF	N/A
SphI	+
SphI-HF	+
SrfI	+++
SspI	++
SspI-HF	+++
StuI	+++
StyI	++
StyI-HF	+++

ENZYME	SURVIVAL
StyD4I	+
SwaI @25°C	++
Taq$^{\alpha}$I @65°C	+
TfiI @65°C	++
TliI @16°C	++
TseI @65°C	+
Tsp45I @65°C	+
TspMI @75°C	+
TspRI @65°C	+++
Tth111I @65°C	++
XbaI	+++
XcmI	+++
XhoI	+++
XmaI	+++
XmnI	++
ZraI	–

Cleavage of Supercoiled DNA

Restriction enzymes cleave different DNA substrates with varying efficiency. Restriction enzymes were tested for their ability to cleave various plasmids (pBR322, pUC19 and pLITMUS) under standard reaction conditions. Single sites were tested on each of these plasmids, depending on availability, and average values were taken when there was more than one data point available. Lambda DNA was used as the standard (1 unit to cleave in all cases).

ENZYME	UNITS TO CLEAVE PLASMID
AatII	3
AflIII	1
AhdI	1
AccI	4
Acc65I	1
AflII	2
AgeI	1
AlwNI	2
ApaI	1
ApoI	1
AseI	0.3
AvaI	10
AvrII	1
BaeI	3
BamHI	3
BanII	1
BglII	8
BpmI	1
BsaI	2
BsaAI	20
BsaXI	2
BsiWI	3
BsgI	1
BsmI	1
BspDI	1
BspEI	1
BspMI	**
BspQI	3
BsrFI	2

ENZYME	UNITS TO CLEAVE PLASMID
BsrGI	1
BssHI	4
BtgI	5
ClaI	5
EagI	10
Eco0109I	8
EcoNI	3
EcoRI	3
EcoRV	1
HincII	4
HindIII	5
KasI	4
KpnI	2
MluI	2
NarI	10
NcoI	1
NdeI	3
NgoMIV	2
NheI	5
NruI	1
NsiI	1
PciI	3
PsiI	3
PstI	1
PvuI	2
PvuII	2
SacI	5
SalI	10
SapI	1

ENZYME	UNITS TO CLEAVE PLASMID
ScaI	20
SmaI	1
SnaBI	1
SpeI	1
SphI	3
SspI	4
StuI	3
StyI	4
TliI	2
TspMI	1
Tth111I	2
XbaI	2
XhoI	10
XmnI	5

** Requires two copies of its recognition sequence for cleavage to occur.

Generating New Cleavage Sites

New restriction sites can be generated by ligation of DNA fragments with compatible ends. These ends may be generated by:

1. Cleavage followed by fill-in of 5´ overhangs to generate blunt ends.
2. Cleavage with two restriction enzymes that produce blunt ends.
3. Cleavage with two restriction enzymes that produce compatible overhangs.

Compatible ends, generated by each of the above methods, can be ligated to produce DNA sequences that often contain useful restriction endonuclease sites. Generation of these sites is detailed in the following tables.

Recleavable Filled-in 5´ Overhangs

The table below lists a selection of restriction enzymes that generate 5´ overhangs which, if filled-in and ligated, result in new restriction sites. The combinations listed were identified by computer analysis, and have not necessarily been confirmed by experimentation. For a more complete listing visit our website, www.neb.com.

Restriction enzymes that have degenerate recognition specificities (e.g., recognize more than one sequence) have been excluded from this list. Where isoschizomers exist, only one member of each set is listed. Only commercially available enzymes have been listed.

Example:

EcoRI Fragments
5´...G AATTC...3´
3´...CTTAA G...5´

Fill-in and Ligate →

XmnI and AseI Sites
5´...GAATTAATTC...3´
3´...CTTAATTAAG...5´

ENZYME	CLEAVAGE SITE	AFTER FILL-IN/ LIGATION	RECLEAVED BY
Acc65I	G/GTACC	GGTACGTACC	BsaAI, HpyCH4IV, RsaI, **SnaBI**[6]
AciI	C/CGC	CCGCGC	(AciI), BstUI, HhaI
AclI	AA/CGTT	AACGCGTT	AflIII, BstUI, **MluI**[6]
AflII	C/TTAAG	CTTAATTAAG	MseI[2], **PacI**[8], MluCI
AgeI	A/CCGGT	ACCGGCCGGT	BsiEI, (BsrFI)[2], EaeI, **EagI**[6], HaeIII, HpaII
ApaLI	G/TGCAC	GTGCATGCAC	Cac8I, NlaIII, NspI, **SphI**[6]
AscI	GG/CGCGCC	GGCGCGCGCGCC	(BssHII)[2], BstUI, Cac8I, HhaI
AvrII	C/CTAGG	CCTAGCTAGG	AluI, (BfaI)[2]
BamHI	G/GATCC	GGATCGATCC	AlwI, **ClaI**[6], (DpnII[2]), TaqI
BclI	T/GATCA	TGATCGATCA	**ClaI**[6], (DpnII[2]), TaqI
BfaI	C/TAG	CTATAG	SfcI
BglII	A/GATCT	AGATCGATCT	**ClaI**[6], DpnII[2], TaqI
BsiWI	C/GTACG	CGTACGTACG	BsaAI, (BsiWI), HpyCH4IV, RsaI, **SnaBI**[6]
BspDI/ClaI	AT/CGAT	ATCGCGAT	BstUI, **NruI**[6]
BspEI	T/CCGGA	TCCGGCCGGA	BsiEI, EaeI, **EagI**[6], HaeIII, (HpaII)[2]
BspHI	T/CATGA	TCATGCATGA	(NlaIII)[2], **NsiI**[6]
BsrGI	T/GTACA	TGTACGTACA	BsaAI, (RsaI)[2], **SnaBI**[6]
BssHII	G/CGCGC	GCGCGCGCGC	(BssHII), BstUI, Cac8I, HhaI
BstBI	TT/CGAA	TTCGCGAA	BstUI, **NruI**[6]
DpnII/MboI/Sau3AI	/GATC	GATCGATC	**ClaI**[6], (DpnII), TaqI
EagI	C/GGCCG	CGGCCGGCCG	BsiEI, BsrFI, Cac8I, EaeI[2], (EagI[2]), **FseI**[8], HaeIII, HpaII, NaeI
EcoRI	G/AATTC	GAATTAATTC	**AseI**[6], MseI, MluCI, **XmnI**[6]
FatI	/CATG	CATGCATG	**BfrBI**[6], HpyCH4V , (FatI[2])
HinP1I	G/CGC	GCGCGC	**BssHII**[6], BstUI, Cac8I, (HhaI)
HindIII	A/AGCTT	AAGCTAGCTT	AluI, BfaI, Cac8I, **NheI**[6]
HpaII/MspI	C/CGG	CCGCGG	AciI, BsaJI, BstUI, BtgI, MspA1I, **SacII**[6]
HpyCH4IV	A/CGT	ACGCGT	AflIII, BstUI, **MluI**[6]
KasI	G/GCGCC	GGCGCGCGCC	**(BssHII**[6]**)**[2], (BstUI)[2], Cac8I, (HhaI)[2]
MfeI	C/AATTG	CAATTAATTG	**AseI**[6], MluCI[2]
MluI	A/CGCGT	ACGCGCGCGT	**BssHII**[6], BstUI, Cac8I, (HhaI)[2]
MluCI	/AATT	AATTAATT	**AseI**[6], MseI, (MluCI[2])
NarI	GG/CGCC	GGCGCGCC	**AscI**[8], BssHII, BstUI, Cac8I, HhaI
NcoI	C/CATGG	CCATGCATGG	NlaIII, **NsiI**[6]
NgoMIV	G/CCGGC	GCCGGCCGGC	BsiEI, BsrFI, Cac8I, EaeI, **EagI**[6], HaeIII, HpaII, (NgoMIV[2])
NheI	G/CTAGC	GCTAGCTAGC	AluI, BfaI, Cac8I, (NheI)
NotI	GC/GGCCGC	GCGGCCGGCCGC	AciI, BsiEI, BsrFI, Cac8I, EaeI, **(EagI**[6]**)**[2], Fnu4HI, **FseI**[8], HaeIII, HpaII, **NaeI**[6]
PaeR7I/XhoI	C/TCGAG	CTCGATCGAG	BsiEI, DpnII, **PvuI**[6], (TaqI[2])
PciI	A/CATGT	ACATGCATGT	HpyCH4V , (NlaIII[2]), **NsiI**[6]
PspOMI	G/GGCCC	GGGCCGGCCC	BsrFI, Cac8I, **FseI**[8], HaeIII, HpaII, NaeI, Sau96I
PspXI	VC/TCGAGB	VCTCGATCGAGB	**PvuI**[6], (TaqI[2])
SalI	G/TCGAC	GTCGATCGAC	BsiEI, DpnII, **PvuI**[6], TaqI
SpeI	A/CTAGT	ACTAGCTAGT	AluI, (BfaI)[2]
TaqI	T/CGA	TCGCGA	BstUI, **NruI**[6]
XbaI	T/CTAGA	TCTAGCTAGA	AluI, BfaI
XmaI	C/CCGGG	CCCGGCCGGG	BsiEI, EaeI, **EagI**[6], HaeIII, HpaII, NciI, ScrFI

Table Notes

Enzymes in **bold** have recognition sequences of 6 or 8 bases. Sequence length is indicated by superscript (e.g., **AscI**[8] = 8-base cutter).

Enzymes in parentheses indicate that the new sequence is still a substrate for the original enzyme.

A superscript 2 indicates that two identical sites have been generated within the filled-in/ligated sequence. For example, fill-in/ligation of AflII generates the sequence CTTAATTAAG which contains two MseI sites (TTAA).

Recleavable Blunt Ends

The table below lists a selection of blunt-end cutters that produce recleavable ligation products. The combinations listed were identified by computer analysis, and although we have tried to ensure their accuracy, they have not necessarily been confirmed by experimentation. For a more complete listing visit our website, **www.neb.com**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed. Where isoschizomers exist, only one member of each set is listed. Only commercially available enzymes are shown.

ENZYME	LIGATED TO	RECLEAVED BY
AfeI (AGC/GCT)	BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI FspI NaeI SmaI	HpaII AluI SfaNI HaeII, HhaI HhaI AciI, Fnu4HI AciI
AluI (AG/CT)	BsrBI, MspA1I (CMG/CGG) BstZ17I MspA1I (CMG/CTG), PvuII EcoRV SfoI	AciI RsaI AluI MboI HaeIII
BsaAI (YAC/GTR) (CAC/GTR) (TAC/GTR) (TAC/GTR)	BsrBI, MspA1I (CMG/CGG) PmlI PmlI, SnaBI SnaBI	HpaII BsaAI, PmlI BsaAI BsaAI, SnaBI
BstUI (CG/CG)	BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI NruI	AciI, BstUI RsaI MboI HaeIII BstUI
BstZ17I (GTA/TAC)	AluI, BsrBI, BstUI, HaeIII, MscI, MspA1I (CMG/CKG), NruI, PvuII, StuI AfeI HincII (GTY/GAC) SspI	RsaI AluI AccI MluCI
DraI (TTT/AAA)	HincII (GTY/AAC), HpaI NruI PmeI, SwaI	MseI TaqI DraI, MseI
EcoRV (GAT/ATC)	AluI, BsrBI, BstUI, MspA1I (CMG/CKG), PvuII HaeIII, MscI, StuI AfeI, SfoI, FspI NruI	MboI AlwI, MboI SfaNI MboI, TaqI
FspI (TGC/GCA)	BsrBI, MspA1I (CMG/CGG) AfeI, SfoI EcoRV NaeI SmaI	HpaII HhaI SfaNI AciI, Fnu4HI AciI
HaeIII/PhoI (GG/CC)	BsrBI, MspA1I (CMG/CGG) BstZ17I MscI, StuI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
HpaI (GTT/AAC)	DraI, PmeI HincII (GTY/AAC) HincII (GTY/GAC) NruI	MseI HincII, HpaI, MseI HincII TaqI
HincII (GTC/RAC) (GTC/RAC) (GTC/RAC) (GTT/RAC) (GTC/RAC) (GTT/RAC) (GTT/RAC) (GTC/RAC)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, MscI, StuI DraI, PmeI HpaI HpaI NruI RsaI, ScaI	HpaII AccI BsmFI MseI HincII HincII, HpaI, MseI TaqI Tsp45I
MscI (TGG/CCA)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, StuI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
MspA1I (CAG/CKG) (CCG/CKG) (CCG/CKG) (CAG/CKG) (CCG/CKG) (CMG/CKG) (CMG/CKG) (CCG/CKG) (CCG/CKG) (CAG/CKG) (CCG/CKG)	AluI AluI, HaeIII, MscI, StuI BsaAI, FspI, HincII (GTY/GAC), PmlI, SnaBI BsrBI, PvuII BsrBI BstZ17I BstUI, NruI AfeI EcoRV SfoI SfoI NaeI PvuII SmaI	AluI AciI HpaII AciI, MspA1I AciI, BsaJI, BstUI, MspA1I, Sac II RsaI AciI, BstUI HpaII MboI HaeIII HaeIII, HpaII HpaII, NciI, ScrFI AluI, MspA1I, PvuII BsaJI, HpaII, NciI, ScrFI
NaeI (GCC/GGC)	BsrBI, MspA1I (CMG/CGG), SmaI AfeI, SfoI, FspI	HpaII, NciI, ScrFI AciI, Fnu4HI
NruI (TCG/CGA)	BsrBI, MspA1I (CMG/CGG) BstZ17I BstUI DraI, HincII (GTY/AAC), HpaI, PmeI, RsaI, ScaI, SspI EcoRV SfoI	AciI, BstUI RsaI BstUI TaqI MboI, TaqI HaeIII
PmeI (GTTT/AAAC)	DraI, SwaI HincII (GTY/AAC), HpaI NruI	DraI, MseI MseI TaqI
PmlI (CAC/GTG)	BsaAI (YAC/GTA), SnaBI BsaAI (YAC/GTG) BsrBI, MspA1I (CMG/CGG) BmgBI	BsaAI BsaAI, PmlI HpaII PmlI
PvuII (CAG/CTG)	AluI BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI MspA1I (CMG/CTG)	AluI AciI, MspA1I RsaI MboI HaeIII AluI, MspA1I, PvuII
RsaI (GT/AC)	HincII (GTY/GAC) NruI ScaI	Tsp45I TaqI RsaI
ScaI (AGT/ACT)	HincII (GTY/GAC) NruI RsaI	Tsp45I TaqI RsaI
SfoI (GGC/GCC)	AluI, BstUI, MspA1I (CMG/CKG), NruI, PvuII BsrBI, MspA1I (CMG/CGG) HaeIII, MscI, StuI AfeI EcoRV FspI NaeI SmaI	HaeIII HaeIII, HpaII HaeIII, Sau96I HaeII, HhaI SfaNI HhaI AciI, Fnu4HI AciI
SmaI (CCC/GGG)	BsrBI, MspA1I (CMG/CGG) AfeI, SfoI, FspI NaeI	BsaJI, HpaII, NciI, ScrFI AciI HpaII, NciI, ScrFI
SnaBI (TAC/GTA)	BsaAI (YAC/GTA) BsaAI (YAC/GTG), PmlI BsrBI, MspA1I (CMG/CGG)	BsaAI, SnaBI BsaAI HpaII
SspI (AAT/ATT)	BstZ17I NruI	MluCI TaqI
StuI (AGG/CCT)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, MscI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
SwaI (ATTT/AAAT)	DraI, PmeI	DraI, MseI

Compatible Cohesive Ends

Restriction enzymes that produce compatible cohesive ends often produce recleavable ligation products. The combinations listed were identified by computer analysis, and have not necessarily been confirmed by experimentation.

Where isoschizomers exist, only one member of each set is listed. A selection of enzymes available from New England Biolabs has been listed. For a more complete listing visit our website, **www.neb.com**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed. A "–" denotes a ligation product that cannot be recleaved.

ENZYME	LIGATED TO	RECLEAVED BY
AccI (GT/CGAC) (GT/CGAC)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI ClaI, BstBI, TaqI	— TaqI
AciI (C/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI BsaHI (GR/CGCC), HinP1I, NarI HpaII	— AciI HpaII
AclI (AA/CGTT)	AccI (GT/CGAC), AciI, ClaI, BstBI, HinP1I, HpaII, NarI, TaqI	—
AgeI (A/CCGGT)	AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (A/CCGGT), SgrAI (CA/CCGGTG) NgoMIV	HpaII, NciI, ScrFI BsaWI, HpaII AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII
ApaI (GGGCC/C)	BanII (GGGCC/C), Bsp1286I (GGGCC/C)	ApaI, BanII, Bsp120I, Bsp1286I, HaeIII, NlaIV, Sau96I
ApaLI (G/TGCAC)	SfcI (C/TGCAG)	BsgI
ApoI (A/AATTY) (G/AATTY) (R/AATTY)	EcoRI EcoRI MfeI, MluCI	ApoI, MluCI ApoI, EcoRI, MluCI MluCI
AscI (GG/CGCGCC)	MluI BssHII	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
AseI (AT/TAAT)	BfaI, Csp6I, NdeI MseI	— MseI
AsiSI (GCGAT/CGC)	BsiEI (CGAT/CG) PacI PvuI	DpnII, PvuI MseI DpnII, PvuI
AvaI (C/CCGGG) (C/TCGAG) (C/TCGAG) (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI (R/CCGGY), NgoMIV, SgrAI (CR/CCGGYG) XhoI SalI XmaI	HpaII, NciI, ScrFI AvaI, TaqI, XhoI TaqI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI
AvaII (G/GWCC)	PpuMI (RG/GACCY) RsrII PpuMI (RG/GTCCY)	AvaII, NlaIV, Sau96I AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I
AvrII (C/CTAGG)	NheI, SpeI, XbaI StyI (C/CTAGG)	BfaI AvrII, BfaI, BsaJI, StyI
BamHI (G/GATCC)	BclI, DpnII BglII, BstYI (R/GATCY) BstYI (G/GATCC)	AlwI, DpnII AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV
BanI (G/GTACC) (G/GCGCC) (G/GTACC)	Acc65I KasI BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV RsaI
BanII (GGGCC/C) (GAGCT/C)	ApaI, Bsp1286I (GGGCC/C) Bsp1286I (GAGCT/C), SacI	ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I AluI, BanII, BsiHKAI, Bsp1286I, SacI
BclI (T/GATCA)	BamHI, BstYI (R/GATCY) BglII, MboI	AlwI, DpnII DpnII

ENZYME	LIGATED TO	RECLEAVED BY
BfaI (C/TAG)	AseI, Csp6I, MseI, NdeI	—
BglII (A/GATCT)	BamHI, BstYI (R/GATCY) BclI, DpnII	AlwI, BstYI, DpnII DpnII
BsaHI (GR/CGYC) (GA/CGYC) (GG/CGYC) (GG/CGYC) (GA/CGYC) (GG/CGYC)	AccI (GT/CGAC), ClaI, BstBI, TaqI AciI, HinP1I AciI, HinP1I HpaII NarI NarI	— HgaI HhaI AciI BsaHI, HgaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV
BsaWI (W/CCGGW)	AgeI, BsrFI (R/CCGGY), SgrAI (CR/ CCGGYG) AvaI (C/CCGGG), XmaI BspEI BsrFI (R/CCGGY), NgoMIV NgoMIV	AgeI, BsaWI, BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII BsrFI, HpaII HpaII
BsiEI (CGAT/CG) (CGAT/CG) (CGGC/CG)	PacI PvuI SacII	MseI BsiEI, DpnII, PvuI AciI
BsiHKAI (GTGCA/C)	Bsp1286I (GTGCA/C) Bsp1286I (GAGCA/C) Bsp1286I (GAGCT/C), SacI NsiI PstI, SbfI	BsiHKAI, Bsp1286I BsiHKAI, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI — BsgI
BsiWI (C/GTACG)	Acc65I, BanI (G/GTACC), BsrGI	RsaI
Bsp1286I (GGGCC/C) (GTGCA/C) (GGGCC/C) (GAGCT/C) (GWGCW/C) (GTGCA/C) (GTGCA/C)	ApaI, BanII (GGGCC/C) BsiHKAI BanII (GGGCC/C) BanII (GAGCT/C), BsiHKAI, SacI BsiHKAI NsiI PstI, SbfI	ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I ApaLI, BsiHKAI, Bsp1286I BanII, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI BsiHKAI, Bsp1286I — BsgI
BspEI (T/CCGGA)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI (CR/CCGGYG) AvaI (C/CCGGG), XmaI BsaWI BsrFI (R/CCGGY), NgoMIV	BsaWI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII HpaII
BspHI (T/CATGA)	FatI, NcoI, PciI	FatI, NlaIII
BsrFI (A/CCGGY) (G/CCGGY) (R/CCGGY) (A/CCGGY) (R/CCGGY) (G/CCGGY) (CR/CCGGYG)	AgeI, BsaWI AgeI, BsaWI, NgoMIV AvaI (C/CCGGG), XmaI BsaWI, BspEI BsaWI, BspEI NgoMIV SgrAI	AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, HpaII HpaII BsrFI, Cac8I, HpaII, NaeI BsrFI, HpaII
BsrGI (T/GTACA)	Acc65I, BanI (G/GTACC), BsiWI	RsaI
BssHII (G/CGCGC)	MluI AscI	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
BstBI (TT/CGAA)	AccI (GT/CGAC), ClaI, TaqI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —

ENZYME	LIGATED TO	RECLEAVED BY
BstYI		
(A/GATCY)	BamHI, BglII	AlwI, BstYI, DpnII
(G/GATCY)	BamHI	AlwI, BamHI, BstYI, DpnII, NlaIV
(R/GATCY)	BclI, DpnII	DpnII
(G/GATCY)	BclI, DpnII	AlwI, DpnII
(A/GATCY)	BglII	BglII, BstYI, DpnII
ClaI	AccI (GT/CGAC), BstBI, TaqI	TaqI
(AT/CGAT)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	—
DpnII/MboI/	BamHI, BstYI (R/GATCC)	AlwI, DpnII
Sau3AI (/GATC)	BclI, BglII, BstYI (R/GATCY)	DpnII
EaeI		
(Y/GGCCR)	PspOMI	HaeIII, Sau96I
(C/GGCCR)	EagI	BsiEI, EaeI, EagI, HaeIII
(T/GGCCR)	EagI	EaeI, HaeIII
(C/GGCCR)	NotI	AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
(T/GGCCR)	NotI	AciI, EaeI, Fnu4HI, HaeIII
EagI	PspOMI	HaeIII, Sau96I
(C/GGCCG)	EaeI (Y/GGCCR)	EaeI, HaeIII
	EaeI (C/GGCCG)	BsiEI, EaeI, EagI, HaeIII
	NotI	AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
EcoRI	ApoI (G/AATTC)	ApoI, EcoRI, MluCI
(G/AATTC)	ApoI (R/AATTY)	ApoI, MluCI
	MfeI, MluCI	MluCI
FatI		
(/CATG)	BspHI, NcoI, PciI	FatI, NlaIII
HinP1I	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI	—
(G/CGC)	AciI, BsaHI (GR/CGCC), NarI	HhaI
	BsaHI (GR/CGTC)	HgaI
	HpaII	AciI
HpaII/MspI	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI	—
(C/CGG)	AciI, BsaHI (GR/CGCC), HinP1I, NarI	AciI
KasI	BanI (G/GCGCC)	BanI, BsaHI, HaeII, HhaI,
(G/GCGCC)		KasI, NarI, NlaIV
MfeI		
(C/AATTG)	ApoI (R/ATTTY), EcoRI, MluCI	MluCI
MluI		
(A/CGCGT)	AscI, BssHII	BstUI, HhaI
MluCI		
(/AATT)	ApoI (R/AATTY), EcoRI, MfeI	MluCI
MseI	AseI	MseI
(T/TAA)	BfaI, Csp6I, NdeI	—
NarI	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI	—
(GG/CGCC)	AciI, HinP1I	HhaI
	BsaHI (GR/CGCC)	BanI, BsaHI, HaeII, HhaI, NarI, NlaIV
	BsaHI (GR/CGTC)	BsaHI, HgaI
	HpaII	AciI
NcoI		
(C/CATGG)	BspHI, FatI, PciI	FatI, NlaIII
NdeI		
(CA/TATG)	AseI, BfaI, Csp6I, MseI	—
NgoMIV	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI	BsrFI, HpaII
(G/CCGGC)	AvaI (C/CCGGG), XmaI	HpaII, NciI, ScrFI
	BsaWI, BspEI	HpaII
	BsrFI (R/CCGGC), SgrAI	BsrFI, Cac8I, HpaII, NaeI
NheI		
(G/CTAGC)	AvrII, SpeI, StyI (C/CTAGG), XbaI	BfaI
NlaIII		
(CATG/)	SphI, NspI	NlaIII
NotI	PspOMI	AciI, EaeI, Fnu4HI, HaeIII
(GC/GGCCGC)	EagI	AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
	EaeI (Y/GGCCR)	AciI, BsiEI, EaeI, Fnu4HI, HaeIII

ENZYME	LIGATED TO	RECLEAVED BY
NsiI	BsiHKAI (GTGCA/C), Bsp1286I (GTGCA/C),	
(ATGCA/T)	PstI, SbfI	—
NspI		
(RCATG/Y)	NlaIII, SphI	NlaIII
PacI	AsiSI	
(TTAAT/TAA)	BsiEI (CGAT/CG), PvuI	MseI
PciI		
(A/CATGT)	BspHI, FatI, NcoI	FatI, NlaIII
PluTI		
(GGCGC/C)	HaeII	HaeII
PpuMI		
(RG/GWCCY)	AvaII, RsrII	AvaII, Sau96I
(GG/GTCCY)	AvaII, RsrII	AvaII, BsmFI, NlaIV, Sau96I
(GG/GACCY)	AvaII, RsrII	AvaII, NlaIV, Sau96I
PspOMI	EaeI (Y/GGCCR), EagI	HaeIII, Sau96I
(G/GGCCC)	NotI	AciI, Fnu4HI, HaeIII, Sau96I
PspXI	XhoI, TliI	XhoI, TliI
(VC/TCGAGB)	SalI	TaqI
PstI	BsiHKAI, Bsp1286I (GTGCA/C)	BsgI
(CTGCA/G)	NsiI	—
	SbfI	PstI
PvuI	AsiSI	DpnII, PvuI
(CGAT/CG)	PacI	MseI
	BsiEI (CGAT/CG)	BsiEI, DpnII, PvuI
RsrII	AvaII, PpuMI (RG/GACCY)	AvaII, Sau96I
(CG/GWCCG)	PpuMI (RG/GACCY)	AvaII, NlaIV, Sau96I
	PpuMI (RG/GTCCY)	AvaII, BsmFI, NlaIV, Sau96I
SacI	BanII (GAGCT/C), BsiHKAI, Bsp1286I (GAGCT/C)	AluI, BanII, BsiHKAI,
(GAGCT/C)		Bsp1286I, SacI
SacII		
(CCGC/GG)	BsiEI (CGGC/CG)	AciI
SalI		
(G/TCGAC)	PspXI, XhoI	TaqI
SbfI	BsiHKAI, Bsp1286I (GTGCA/C)	BsgI
(CCTGCA/GG)	NsiI	—
	PstI	PstI
SfcI		
(C/TGCAG)	ApaLI	BsgI
SgrAI		
(CR/CCGGYG)	See BsrFI	
SpeI		
(A/CTAGT)	AvrII, NheI, StyI (C/CTAGG), XbaI	BfaI
SphI		
(GCATG/C)	NlaIII, NspI	NlaIII
StyI	AvrII	AvrII, BfaI, BsaJI, StyI
(C/CTAGG)	NheI, SpeI, XbaI	BfaI
(C/CATGG)	BspHI	NlaIII
	NcoI	BsaJI, NcoI, NlaIII, StyI
TaqI	AccI (GT/CGAC), ClaI, BstBI	TaqI
(T/CGA)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	—
XbaI		
(T/CTAGA)	AvrII, NheI, SpeI, StyI (C/CTAGG)	BfaI
XhoI (TliI)	PspXI	XhoI, TliI
(C/TCGAG)	SalI	TaqI
XmaI	AgeI, BsaWI, BspEI, BsrFI, NgoMIV, SgrAI	HpaII, NciI, ScrFI
(C/CCGGG)	AvaI (C/CCGGG)	AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI, XmaI

Dam (G^mATC), Dcm (C^mCWGG) and CpG (^mCG) Methylation

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.**

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.

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

ENZYME	SEQUENCE	Dam	Dcm	CpG
AatII	GACGT/C	●	●	■
AbaSI	^{m}C(11/9)	●	●	●
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-v2	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

ENZYME	SEQUENCE	Dam	Dcm	CpG
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(-5/-2)	●	●	◇ 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
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

ENZYME	SEQUENCE	Dam	Dcm	CpG
BsaI-HFv2	GGTCTC(1/5)	●	◇ scol	□ 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	●	●	■
BslI	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)	●	●	●
BsrFI-v2	R/CCGGY	●	●	■
BsrGI	T/GTACA	●	●	●
BsrGI-HF	T/GTACA	●	●	●
BssHII	G/CGCGC	●	●	■
BssSI-v2	CACGAG(-5/-1)	●	●	●
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)	●	●	◆
BtsI-v2	GCAGTG(2/0)	●	●	●
BtsCI	GGATG(2/0)	●	●	●
BtsIMutI	CAGTG(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

ENZYME	SEQUENCE	Dam	Dcm	CpG
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
Esp3I	CGCCTC(1/5)	●	●	■
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	●	●	■
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	●	●	■

Dam (G^mATC), Dcm (C^mCWGG) and CpG (^mCG) Methylation (continued)

ENZYME	SEQUENCE	Dam	Dcm	CpG
Nb.BbvCI	CCTCAGC(none/-2)	●	●	●
Nb.BsmI	GAATGC(none/-1)	●	●	●
Nb.BsrDI	GCAATG(none/0)	●	●	●
Nb.BssSI	CACGAG(none/-1)	●	●	●
Nb.BtsI	GCAGTG(none/0)	●	●	●
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/none)	■	●	●
Nt.BbvCI	CCTCAGC(-5/none)	●	●	□ scol
Nt.BsmAI	GTCTCN(1/none)	●	●	■
Nt.BspQI	GCTCTTC(1/none)	●	●	●
Nt.BstNBI	GAGTC(4/none)	●	●	●
Nt.CviPII	CCD(-3/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	●	●	●

ENZYME	SEQUENCE	Dam	Dcm	CpG
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	●	●
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	●	●	■

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

SETUP GUIDELINES

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20–30 nucleotides in length, with 40–60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (**TmCalculator.neb.com**)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5´ to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. *Please note that Q5® and Phusion®* annealing temperature recommendations are unique.*
- Final concentration of each primer should be 0.05–1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One*Taq*® Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Magnesium Concentration

- Optimal Mg^{2+} concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg^{2+} at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg^{2+} can be added for applications that require complete control over Mg^{2+} concentration
- Further optimization of Mg^{2+} concentration can be done in 0.2-1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg^{2+} in the reaction.
- Excess Mg^{2+} may lead to spurious amplification; Insufficient Mg^{2+} concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 µM of each, however, some enzymes may require as much as 400 µM each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg^{2+} and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use One*Taq* or *Taq* DNA Polymerases for these applications.

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase. Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).

CYCLING GUIDELINES

Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (**TmCalculator.neb.com**)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures are usually set at 2°–5°C below the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures should be set at 0°–3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed.

Extension

- Extension temperature recommendations range from 65°–72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Additional tips for optimizing reactions can be found in the technical reference section of our website, **www.neb.com.**

PROBLEM	POSSIBLE CAUSE	SOLUTION
Sequence errors	Low fidelity polymerase	• Choose a higher fidelity polymerase such as Q5 High-Fidelity (NEB #M0491) or Phusion (NEB #M0530)* DNA Polymerases
	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time
	Unbalanced nucleotide concentrations	• Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
Incorrect product size	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (**TmCalculator.neb.com**)
	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{2+} concentration	• Adjust Mg^{2+} concentration in 0.2–1 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
No product	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (**TmCalculator.neb.com**) • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	• Repeat reaction setup
	Suboptimal reaction conditions	• Optimize Mg^{2+} concentration by testing 0.2–1 mM increments • Thoroughly mix Mg^{2+} solution and buffer prior to adding to the reaction • Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{2+} • Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent thermocycler block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents
	Complex template	• Use Q5 High-Fidelity (NEB #M0491) or One*Taq* DNA Polymerase (NEB #M0482) • For GC-rich templates, use One*Taq* DNA Polymerase (NEB #M0480) with One*Taq* GC Reaction Buffer (plus One*Taq* High GC Enhancer, if necessary) or Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer • For longer templates, we recommend LongAmp® *Taq* DNA Polymerase (NEB #M0323), Q5 or Q5 Hot Start High Fidelity DNA Polymerase (NEB #M0493)
Multiple or non-specific products	Premature replication	• Use a hot start polymerase, such as Q5 Hot Start High-Fidelity (NEB #M0493) or One*Taq* Hot Start (NEB #M0481) DNA Polymerases • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	• Recalculate primer Tm values using the NEB Tm Calculator (**TmCalculator.neb.com**) • Increase annealing temperature
	Incorrect Mg^{2+} concentration	• Adjust Mg^{2+} in 0.2–1 mM increments
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction • For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Optimization Tips for Luna® qPCR

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize qPCR. For RT-qPCR guidelines, please see page 340.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible

DNA Template

- Use high quality, purified DNA templates whenever possible. Luna qPCR is compatible with DNA samples prepared through typical nucleic acid purification methods.
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Generally, useful concentrations of standard and unknown material will be in the range of 10^6 copies to 1 copy. For gDNA samples from large genomes, (e.g., human, mouse) a range of 50–1 pg of gDNA is typical. For small genomes, adjust as necessary using 10^6–1 copy input as an approximate range. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution.
- To generate cDNA, use of the LunaScript® RT SuperMix Kit (NEB #E3010) is recommended. Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction.
- cDNA does not need to be purified before addition to the Luna reaction but should be diluted at least 1:20 before addition to qPCR

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator (**TmCalculator.neb.com**) using the Hot Start *Taq* setting
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- Optimal primer concentration for dye-based experiments (250 nM) is lower than for probe-based experiments (400 nM). If necessary, the primer concentration can be optimized between 100–500 nM for dye-based qPCR or 200–900 nM for probe-based experiments.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification.
- For cDNA targets, it is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA
- Primers designed to target intronic regions can ensure amplification exclusively from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the hot start nature of the polymerase, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio®)
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Optimization Tips for Luna One-Step RT-qPCR

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize your one-step RT-qPCR. For qPCR guidelines (DNA/cDNA starting material), please see page 339.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA Template

- Use high quality, purified RNA templates whenever possible. Luna qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator. (**TmCalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Reverse Transcription

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template.
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Luna qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Reagent omitted from qPCR assay Reagent added improperly to qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect cycling protocol	• Refer to the proper qPCR cycling protocol in product manual
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	DNA template or reagents are contaminated or degraded	• Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in this user manual • Rerun the qPCR assay with fresh reagents • Confirm template input amount
Inconsistent qPCR traces for triplicate data	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. • Exclude problematic trace(s) from data analysis.
	Poor mixing of reagents during qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
DNA standard curve has a poor correlation coefficient/efficiency of the DNA standard curve falls outside the 90–110% range	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification, NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software

Mark has been with NEB for over 35 years and currently serves as our Senior Network Engineer, keeping our communications running smoothly.

Luna One-Step RT-qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Incorrect RT step temperature or RT step omitted	• For typical use, a 55°C RT step temperature is optimal for the Luna WarmStart Reverse Transcriptase.
	Incorrect cycling protocol	• Refer to the proper RT-qPCR cycling protocol in product manual
	Reagent omitted from RT-qPCR assay Reagent added improperly to RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	RNA template or reagents are contaminated or degraded	• Prepare high quality RNA without RNase/DNase contamination • Confirm template input amount • Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in product manual • Rerun the RT-qPCR assay with fresh reagents
Inconsistent qPCR traces for triplicate data	Improper pipetting during RT-qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
Standard curve has a poor correlation coefficient/efficiency of the standard curve falls outside the 90–110% range	Cycling protocol is incorrect	• Refer to the proper RT-qPCR cycling protocol in product manual • Use a 55°C RT step temperature • For ABI instruments, use a 1 minute 60°C annealing/extension step
	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during RT-qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification/NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (Melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (Melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software
Amplification in No-RT control	RNA is contaminated with genomic DNA	• Treat sample with DNase I • Redesign amplicon to span exon-exon junction

Cleavage Close to the End 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.

Chart Legend

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

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
AciI	–	+	+	++	+++
AgeI	+++	+++	+++	+++	+++
AgeI-HF	++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BbsI-HF	+++	+++	+++	+++	+++
BclI-HF	–	–	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI	+++	+++	+++	+++	+++
BmtI-HF	+++	+++	+++	+++	+++
BsaI	+++	+++	+++	+++	+++
BsaI-HFv2	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsiWI-HF	+++	+++	+++	+++	+++
BsmBI	+++	+++	+++	+++	+++
BsrGI	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
BstZ17I-HF	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII-HF	+++	+++	+++	+++	+++
EagI	++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
Esp3I	+++	+++	+++	+++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI	+	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI	+	++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI	+	++	+++	+++	+++
MseI	+++	+++	+++	+++	+++

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
NcoI	–	++	+++	+++	+++
NcoI-HF	+	++	+++	+++	+++
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	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI	++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI	+	++	++	++	++
SpeI-HF	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI	+	+++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI	+	++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Activity of Restriction Enzymes in PCR Buffers

Frequently, a PCR product must be digested with restriction enzymes. For convenience, digestion can be performed directly in the PCR mix without any purification of the DNA. This table summarizes the activity of restriction enzymes on the DNA in *Taq*, Phusion*, One*Taq* and LongAmp *Taq* PCR mixes. 50 µl reactions containing 5 units of restriction enzyme were incubated at the appropriate temperature for 1 hour in a PCR mix containing the following: 1 µg DNA, 1 unit of DNA Polymerase and 1X ThermoPol Reaction Buffer, Standard *Taq* Reaction Buffer, Phusion HF Buffer, One*Taq* Standard Reaction Buffer or LongAmp *Taq* Reaction Buffer. Reactions were supplemented with 200 µM dNTPs. Enzyme activity was analyzed by gel electrophoresis.

Notes: The polymerase is still active and can alter the ends of DNA fragments after cleavage, affecting subsequent ligation. Primers containing the restriction enzyme recognition site can act as competitive inhibitors in the cleavage reaction. The use of restriction enzymes under non-optimal conditions may increase the likelihood of star activity. If any problems are encountered, the DNA should be purified by spin column or phenol/chloroform extraction followed by alcohol precipitation.

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Chart Legend

Cleavage in extension mix with 5 units of enzyme:

+++	complete cleavage	++	~ 50% cleavage
+	~ 25% cleavage	–	no cleavage

** It has been shown that the addition of 1X Restriction Enzyme Buffer may help to improve the ability of some enzymes to cleave.

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
AatII	<+++	<+	+	++	+
AccI	<+++	<+	<+	+++	+++
Acc65I	+++	<+	<+	<+	+
AciI	++	++	+++	+++	+++
AclI	+++	<+	<+	+++	+++
AcuI	+++	<+	++	+++	+++
AfeI	+++	<+	++	+++	+++
AflII	+	<+	<+	+	<+
AflIII	<+	+++	+	<+	<+
AgeI	+++	+	+++	+++	<+
AgeI-HF	+++	<+	++	+++	+++
AhdI	<+	–	–	<+	<+
AleI-v2	–	–	–	+	+
AluI	+++	+	+++	+++	+++
AlwI	–	<+	<+	<+	<+
AlwNI	<+++	+	+++	<+	+
ApaI	+++	<+	<+	<+	–
ApaLI	+++	<+	<+	+++	+++
ApeKI	<+++	++	+++	<+	+
ApoI	+++	++	+++	++	+++
ApoI-HF	+++	+	++	+++	+++
AscI	+++	<+	<+	<+	–
AseI	+++	<+	+	++	++
AsiSI	+++	<+	++	+++	+++
AvaI	+++	<+	+++	+++	+
AvaII	+++	<+	++	+++	+++
AvrII	+++	<+	<+	+++	+++
BaeGI	+++	<+	+++	+++	+++
BaeI	–	<+	++	<+	<+
BamHI	+++	<+	+++	+++	+++
BamHI-HF	+++	<+	–	<+	++
BanI	+++	<+	+++	+++	+++
BanII	+++	<+	+++	+++	+++
BbsI	+++	<+	<+	+++	+++
BbsI-HF	+	–	–	–	+
BbvCI	+++	–	–	<+	<+
BbvI	+++	<+	++	+++	+++
BccI	<+	<+	<+	<+	<+
BceAI	<+	<+	++	+	<+
BcgI	<+	<+	+	++	++
BciVI	–	–	–	<+	–
BclI	+++	++	+++	+++	+++
BclI-HF	+++	–	–	+	+
BcoDI	<+	<+	+	+	<+
BfaI	–	<+	–	–	–
BfuAI	<+++	–	+	<+	–
BglI	<+	++	+	<+	<+
BglII	<+	+	++	<+	<+
BlpI	<+++	<+	<+	<+	–
BmgBI	–	++	+	<+	<+
BmrI	<+++	<+	+++	+++	+++
BmtI	+++	<+	++	+++	+++
BmtI-HF	++	<+	+	++	+++
BpmI	<+	<+	+++	<+++	<+++
BpuEI	+++	–	++	<+++	<+++
Bpu10I	<+	<+	+++	++	+++
BsaAI	+++	++	+++	+++	+++
BsaBI	+	<+	++	++	+++

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
BsaHI	+++	+	+++	+++	+++
BsaI-HFv2	+	<+	+	+	++
BsaJI	+++	<+	++	+++	+++
BsaWI	<+++	<+	++	+	+
BsaXI	<+++	<+	<+	<+	<+
BseRI	+++	<+	++	++	+
BseYI	+++	++	++	+++	+++
BsgI	<+	<+	+	<+	<+
BsiEI	+++	<+	++	++	++
BsiHKAI	–	++	+	–	–
BsiWI	+++	<+	+++	+++	+++
BsiWI-HF	–	–	–	–	–
BslI	+++	++	+++	+++	+++
BsmAI	+++	++	+++	<+	<+
BsmBI	<+++	+	++	<+	<+
BsmFI	<+	+++	++	+	+
BsmI	+++	+	<+	+++	+
BsoBI	+++	+++	+++	++	+++
BspCNI	<+	<+	+	–	–
BspDI	<+++	<+	++	+++	+++
BspEI	–	<+	<+	–	–
BspHI	+++	<+	+++	+++	+++
Bsp1286I	<+	<+	<+	<+	<+
BspMI	+++	<+	++	<+	<+
BspQI	+	++	+++	+++	+++
BsrBI	+++	<+	+	+++	+++
BsrDI	<+	<+	+	<+	<+
BsrFI-v2	<+	–	–	–	–
BsrGI	<+	+	+++	<+	+++
BsrI	+++	<+	+++	++	+++
BssHII	+++	<+	+	+++	+++
BssSI-v2	+++	–	+	+++	+++
BstAPI	+++	<+	++	+++	+++
BstBI	+++	++	+++	+++	+++
BstEII	+++	<+	<+	+++	+++
BstEII-HF	+++	<+	<+	++	++
BstNI	+++	<+	<+	<+	<+
BstUI	+++	<+	<+	+++	+
BstXI	<+++	+	+	+	<+
BstYI	+++	<+	<+	++	+
BstZ17I-HF	+++	–	+	+++	+++
Bsu36I	<+	<+	<+	<+	+
BtgI	+++	<+	+	<+	<+
BtgZI	+++	+	++	++	++
BtsI-v2	+++	–	+	+++	+++
BtsCI	+++	<+	<+	+++	+++
Cac8I	+++	<+	<+	+++	++
ClaI	++	<+	<+	<+	++
CspCI	<+	–	+	<+	<+
CviAII	+++	<+	+	+++	+++
CviKI-1	+++	<+	++	+++	+++
CviQI	+++	+	+++	++	+++
DdeI	+++	++	+	+++	+++
DpnI	+++	++	+++	++	++
DpnII	+++	++	+++	+++	++
DraI	+++	<+	+++	+++	+++
DraIII-HF	++	++	+++	++	++
DrdI	+++	<+	+++	+++	+++

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
EaeI	+++	<+	–	<+	<+
EagI	<+	+++	+++	+++	+++
EagI-HF	+	<+	+	++	++
EarI	+++	<+	+++	+	<+
EciI	<+	++	+++	<++	<++
Eco53kI	+++	<+	<+	+++	+++
EcoNI	+++	<+	+	+++	+++
EcoO109I	+++	<+	–	<+	+
EcoP15I	<+	<+	+	<+	+
EcoRI	+	<+	+++	–	–
EcoRI-HF	+++	<+	+	+++	+++
EcoRV	<+	<+	+	–	<+
EcoRV-HF	+	<+	<+	+	++
Esp3I	+++	-	+++	+	+++
FatI	++	<+	+++	<+	+++
FauI	+	<+	++	+++	++
Fnu4HI	+++	<+	<+	++	+
FokI	+++	+	+	+++	+++
FseI	+	<+	++	+++	–
FspI	<++	<+	+	+	+
HaeII	+++	<+	+++	+++	+++
HaeIII	+++	<+	+++	+++	+++
HgaI	<+	<+	+	<++	<++
HhaI	+++	<+	+++	+++	+++
HinclI	+++	<+	<+	+++	+++
HindIII	+++	<+	+	++	+++
HindIII-HF	+++	<+	<+	+++	+++
HinfI	+++	+++	+++	+	+++
HinP1I	+++	+	+++	+++	+++
HpaI	+++	<+	+++	+++	+++
HpaII	+++	<+	<+	<+	<+
HphI	<++	<+	<+	<+	<+
HpyAV	+++	–	++	+	++
HpyCH4III	<++	<+	+	<++	<++
HpyCH4IV	+++	<+	<+	+++	+++
HpyCH4V	+++	<+	<+	+++	+++
Hpy99I	+++	–	+	<+	<+
Hpy188I	+++	<+	+	++	++
Hpy166II	+++	+	++	+++	+++
Hpy188III	+	<+	<+	+	<+
KasI	+++	<+	+++	+++	–
KpnI	+++	++	+	++	<+
KpnI-HF	++	–	++	<+	<+
MboI	+++	<+	+++	+++	+++
MboII	+++	+	++	+	+
MfeI	+++	<+	<+	+++	+
MfeI-HF	+	–	–	+++	<+
MluCI	+	<+	<+	++	+
MluI	+++	++	++	++	++
MluI-HF	++	–	++	++	++
MlyI	+++	+	++	<+	+
MmeI	<+	–	++	<+	<+
MnlI	+++	+	+	+	+
MscI	<+	<+	+	<+	<+
MseI	<+	<+	<+	<+	<+
MslI	+++	<+	+	+++	++
MspA1I	+++	<+	+++	++	+++
MspI	+++	<+	+++	++	+++
MwoI	+++	+++	+++	++	+++
NaeI	<+	<+	+	<+	<+
NarI	–	<+	++	+++	+++
NciI	+++	<+	<+	+	<+
NcoI	+++	<+	+	++	++
NcoI-HF	+++	<+	–	++	+
NdeI	<++	++	+++	++	<+
NgoMIV	–	<+	+	<+	<+
NheI	+++	<+	<+	+++	+++
NheI-HF	+++	<+	–	++	++
NlaIII	<+	<+	+	++	<+
NlaIV	+++	<+	+++	+++	+++
NmeAIII	<+	–	+++	<+	<+
NotI	++	<+	+	<+	<+
NotI-HF	+++	<+	<+	<+	+

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
NruI	++	+	+	++	++
NruI-HF	++	–	–	+	–
NsiI	+++	+	+++	++	+
NsiI-HF	+++	++	+++	+++	+++
NspI	+++	<+	<+	+++	++
PacI	+++	<+	<+	++	+++
PaeR7I	+++	<+	<+	+++	+++
PciI	<+	<+	–	–	–
PflFI	+++	<+	<+	<+	+
PflMI	+	<+	+++	++	+++
PleI	+++	<+	<+	<+	<+
PluTI	+++	<+	+	+++	+++
PmeI	+++	<+	<+	+++	+++
PmlI	–	–	–	+	<+
PpuMI	+++	<+	+++	+++	+++
PshAI	+++	<+	<+	<+	<+
PsiI	+++	<+	<+	<+	+++
PspGI	+++	+++	+++	+++	+++
PspOMI	+++	<+	+	+++	+++
PspXI	+++	<+	++	+++	+++
PstI	++	+	+	<+	<+
PstI-HF	+++	<+	++	++	+
PvuI	<+	<+	+++	–	<+
PvuI-HF	+++	<+	+++	++	+++
PvuII	+++	<+	+	+++	+++
PvuII-HF	+	–	–	<+	<+
RsaI	+++	<+	++	+++	+++
RsrII	<++	–	–	<+	<+
SacI	+++	<+	+	++	++
SacI-HF	+++	<+	<+	<+	++
SacII	+++	<+	+++	++	+
SalI	<+	+	++	–	–
SalI-HF	+	<+	+++	+	+++
SapI	<++	<+	++	++	++
Sau3AI	+++	<+	<+	<+	<+
Sau96I	<++	+	+	+++	+++
SbfI	<++	<+	+	<+	+++
SbfI-HF	+	–	–	<+	<+
ScaI-HF	+	<+	<+	–	–
ScrFI	+++	+++	+++	+++	+++
SexAI	+++	<+	+++	+++	+++
SfaNI	–	<+	++	<++	<++
SfcI	+++	<+	<+	+	+
SfiI	+++	–	–	+++	+++
SfoI	+++	<+	+++	+	+++
SgrAI	<++	<+	++	+	+++
SmaI	+++	<+	++	+++	+++
SmlI	<+	<+	+	+	+
SnaBI	<+	<+	<+	+++	+++
SpeI	+++	+	<+	+++	+++
SpeI-HF	+++	–	<+	+++	+++
SphI	+++	+	++	<+	<+
SphI-HF	+++	<+	+	+++	+++
SrfI	<+	<+	+++	+	++
SspI-HF	++	<+	+	+++	+++
StuI	+++	<+	<+	+++	+++
StyD4I	<++	<+	+	<+	<+
StyI	<+	+	<+	<+	<+
StyI-HF	+	<+	<+	++	+++
SwaI	<+	<+	<+	<+	+++
Taq^αI	+++	<+	+	+++	+++
TfiI	<++	<+	<+	++	++
TseI	+++	+++	+++	+++	+++
Tsp45I	+++	–	–	+	<+
TspMI	+++	<+	+	+++	+++
TspRI	+	<+	<+	+++	+++
Tth111I	+++	<+	++	<+	+
XbaI	+++	–	<+	++	++
XcmI	+++	<+	+	+++	+++
XhoI	<+	<+	+++	++	+++
XmaI	+++	<+	+	–	–
XmnI	+++	<+	<+	++	+++
ZraI	+++	<+	<+	++	+

Getting Started with Molecular Cloning

Molecular cloning has traditionally used restriction enzymes to excise a fragment from source DNA, and to linearize a plasmid vector, while creating compatible ends. After purification, insert and vector are ligated to form a recombinant vector, which is transformed into an *E. coli* host. Alternatively, PCR can been used to generate both the vector and insert, which can be joined using a variety of techniques, such as standard DNA ligation, enzymatic joining using a recombinase or topoisomerase, or homologous recombination.

Regardless of the method chosen, the process can be made more efficient and successful by following good practices in the lab. The following tips will help improve the success of your cloning experiments.

1. Take the time to plan your experiments

Pay attention to the junction sequences and the effect on reading frames of any translated sequences. Check both the vector and insert for internal restriction sites (we recommend NEBcutter at **NEBcutter.neb.com**) prior to designing PCR primers that contain similar sites to those used for cloning. Verify that the antibiotic selective marker in the vector is compatible with the chosen host strain.

2. Start with clean DNA at the right concentration

Ensure that your source DNA is free of contaminants, including nucleases and unwanted enzymatic activities. Use commercially-available spin columns to purify starting DNA, (e.g., Monarch Plasmid Miniprep Kit, NEB #T1010 for DNA plasmids, Monarch PCR & DNA Cleanup Kit, NEB #T1030 for DNA Fragments). Completely remove solvents, such as phenol, chloroform and ethanol, prior to manipulation of the DNA. Elute DNA from the spin columns with salt-free buffer to prevent inhibition of the downstream steps, either restriction digestion or PCR amplification. Use a sufficient amount of DNA for the technique being used. Preparative restriction digests often require between 0.2 –2.0 µg, while single nanogram amounts are usually sufficient for DNA being used as a PCR template.

3. Perform your restriction digests carefully

The reaction volume should be compatible with the downstream step (e.g., smaller than the volume of the well of an agarose gel used to resolve the fragments). For a typical cloning reaction, this is often between 20–50 µl. The volume of restriction enzyme(s) added should be no more than 10% of the total reaction volume, to ensure that the glycerol concentration stays below 5%; this is an important consideration to minimize star activity (unwanted cleavage).

4. Mind your ends

DNA ends prepared for cloning by restriction digest are ready for ligation without further modification, assuming the ends to be joined are compatible. If the ends are non-compatible, they can be modified using blunting reagents, phosphatases, etc.

DNA ends prepared by PCR for cloning may have a 3´ addition of a single adenine (A) residue following amplification using *Taq* DNA Polymerase (NEB #M0273). High-fidelity DNA polymerases, such as Q5 (NEB #M0491), leave blunt ends. PCR using standard commercial primers produces non-phosphorylated fragments, unless the primers were 5´ phosphorylated. The PCR product may need to be kinase treated to add a 5´ phosphate prior to ligation with a dephosphorylated vector.

5. Clean up your DNA prior to vector:insert joining

This can be done with gel electrophoresis or column purification (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). Isolating the desired DNA from unwanted parent vectors and/or other DNA fragments can dramatically improve your cloning results.

Confirm digested DNA on an agarose gel prior to ligation. For a single product, run a small amount of the digest, and then column purify to capture the remainder (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). When multiple fragments are produced and only one is to be used, resolve the fragments on a gel and excise the desired fragment under UV light. Using longwave (365 nm) UV light will minimize any radiation-induced DNA damage to the fragment. Recover the DNA fragment from the agarose slice using a gel extraction kit (e.g., Monarch DNA Gel Extraction Kit, NEB #T1020) or β-Agarase I (NEB #M0392).

6. Quantitate your isolated material

Simple quantitation methods, such as gel electrophoresis with mass standards or spectroscopic quantitation on low-input spectrophotometers (such as a NanoSpec®), ensure that the proper amount of material is used for the downstream joining reaction.

7. Follow the manufacturer's guidelines for the joining reaction

For traditional cloning, follow the guidelines specified by the ligase supplier. If a 3:1 molar ratio of insert to vector is recommended, try this first for best results. Using a 3:1 mass ratio is not the same thing (unless the insert and vector have the same mass). Ligation usually proceeds quickly and, unless your cloning project requires the generation of a high-complexity library that benefits from the absolute capture of every possible ligation product, long incubation times are not necessary.

Follow the manufacturer's guidelines for the joining reactions in PCR cloning and seamless cloning. If you are performing a cloning protocol for the first time, adhere to the recommended protocol for optimal results.

8. Use competent cells that are suited to your needs

While some labs prepare their own competent cells "from scratch" for transformations, the levels of competence achieved rarely matches the high levels attained with commercially-available competent cells. Commercially-available competent cells save time and resources, and make cloning more reproducible.

Find an overview of traditional cloning.

Traditional Cloning Quick Guide

PREPARATION OF INSERT AND VECTORS

Insert From a Plasmid Source

- Digest plasmid with the appropriate restriction enzymes to produce a DNA fragment that can be cloned directly into a vector. Unidirectional cloning is achieved with restriction enzymes that produce non-compatible ends.

Insert From a PCR Product

- Design primers with appropriate restriction sites to clone unidirectionally into a vector
- Addition of 6 bases upstream of the restriction site is sufficient for digestion with most enzymes
- If fidelity is a concern, choose a proofreading polymerase such as Q5 High-Fidelity DNA Polymerase (NEB #M0491)
- Visit **www.NEBPCRPolymerases.com** for additional guidelines for PCR optimization
- Purify PCR product by running the DNA on an agarose gel and excising the band or by using a spin column (e.g., Monarch® DNA Gel Extraction Kit, NEB #T1020, Monarch PCR & DNA Cleanup Kit, NEB #T1030)
- Digest with the appropriate restriction enzyme

Standard Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	10 units is sufficient, generally 1 µl is used
Nuclease-free Water	To 50 µl
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

** Can be decreased by using a Time-Saver qualified enzyme*

Time-Saver Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	1 µl
Nuclease-free Water	To 50 µl
Incubation Time	5–15 minutes*
Incubation Temperature	Enzyme dependent

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

Insert from Annealed Oligos

- Annealed oligos can be used to introduce a fragment (e.g., promoter, polylinker, etc.)
- Anneal two complementary oligos that leave protruding 5´ or 3´ overhangs for ligation into a vector cut with appropriate enzymes
- Non-phosphorylated oligos can be phosphorylated using T4 Polynucleotide Kinase (NEB #M0201)

Typical Annealing Reaction

Primer	1 µg
10X T4 Ligase Buffer	5 µl
Nuclease-free Water	To 50 µl
Incubation	85°C for 10 minutes, cool slowly (30-60 min.)

Vector

- Digest vector with appropriate restriction enzymes. Enzymes that leave non-compatible ends are ideal as they prevent vector self-ligation

DEPHOSPHORYLATION

- Dephosphorylation is sometimes necessary to prevent self-ligation. NEB offers four products for dephosphorylation of DNA:
- The Quick Dephosphorylation Kit (NEB #M0508), Shrimp Alkaline Phosphatase (rSAP) (NEB #M0371) and Antarctic Phosphatase (AP) (NEB #M0289) are heat-inactivatable phosphatases. They work in all NEBuffers, but AP requires supplementation with Zn^{2+}
- Calf Intestinal Phosphatase (CIP) (NEB #M0290) will function under many different conditions and in most NEBuffers. However, CIP cannot be heat inactivated and requires a purification step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) before ligation.

Dephosphorylation of 5´ ends of DNA Using the Quick Dephosphorylation Kit

DNA	1 pmol of DNA ends
10X CutSmart Buffer	2 µl
Quick CIP	1 µl
Nuclease-free Water	To 20 µl
Incubation	37°C for 10 minutes
Heat Inactivation	80°C for 2 minutes

Note: Scale larger reaction volumes proportionally.

BLUNTING

- In some instances, the ends of the insert or vector require blunting
- PCR with a proofreading polymerase will leave a predominantly blunt end
- T4 DNA Polymerase (NEB #M0203) or Klenow (NEB #M0210) will fill in a 5´ overhang and chew back a 3´ overhang
- The Quick Blunting Kit (NEB #E1201) is optimized to blunt and phosphorylate DNA ends for cloning in less than 30 minutes
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

Blunting with the Quick Blunting Kit

DNA	Up to 5 µg
10X Blunting Buffer	2.5 µl
dNTP Mix (1 mM)	2.5 µl
Blunt Enzyme Mix	1 µl
Nuclease-free Water	To 25 µl
Incubation	room temperature; 15 min for RE-digested DNA; 30 min for sheared/nebulized DNA or PCR products*
Heat Inactivation	70°C for 10 minutes

** PCR-generated DNA must be purified before blunting using a purification kit (NEB #T1030), phenol extraction/ethanol precipitation, or gel extraction (NEB #T1020).*

PHOSPHORYLATION

- For ligation to occur, at least one of the DNA ends (insert or vector) should contain a 5´ phosphate
- Primers are usually supplied non-phosphorylated; therefore, the PCR product will not contain a 5´ phosphate
- Digestion of DNA with a restriction enzyme will always produce a 5´ phosphate
- A DNA fragment can be phosphorylated by incubation with T4 Polynucleotide Kinase (NEB #M0201)

Phosphorylation With T4 PNK

DNA (20 mer)	1–2 µg
10X T4 PNK Buffer	5 µl
10 mM ATP	5 µl (1 mM final conc.)
T4 PNK	1 µl (10 units)
Nuclease-free Water	To 50 µl
Incubation	37°C for 30 minutes

PURIFICATION OF VECTOR AND INSERT

- Purify the vector and insert by either running the DNA on an agarose gel and excising the appropriate bands or by using a spin column, such as Monarch DNA Gel Extraction Kit or PCR & DNA Cleanup Kit (NEB #T1020 or T1030)
- DNA can also be purified using β-Agarase I (NEB #M0392) with low melt agarose, or an appropriate spin column or resin
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

LIGATION OF VECTOR AND INSERT

- Use a molar ratio of 1:3 vector to insert. Use NEBioCalculator to calculate molar ratios.
- If using T4 DNA Ligase (NEB #M0202) or the Quick Ligation Kit (NEB #M2200), thaw and resuspend the Ligase Buffer at room temp. If using Ligase Master Mixes, no thawing is necessary.
- The Quick Ligation Kit (NEB #M2200) is optimized for ligation of both sticky and blunt ends
- Instant Sticky-end Ligase Master Mix (NEB #M0370) is optimized for instant ligation of sticky/cohesive ends
- Blunt/TA Ligase Master Mix (NEB #M0367) is optimized for ligation of blunt or single base overhangs, which are the more challenging type of ends for T4 DNA Ligase
- Following ligation, chill on ice and transform
- DO NOT heat inactivate when using the Quick Ligation Buffer or Ligase Master Mixes, as this will inhibit transformation
- Electroligase (NEB #M0369) is optimized for ligation of both sticky and blunt ends and is compatible with electroporation (i.e., no cleanup step required)
- Improved Golden Gate Assembly can be achieved by selecting high fidelity overhangs [Potapov, V. et al. (2018) *ACS Synth. Biol.* 7(11), 2665–2674.

Ligation with the Quick Ligation Kit

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	To 50 ng
2X Quick Ligation Buffer	10 µl
Quick T4 DNA Ligase	1 µl
Nuclease-free Water	20 µl (mix well)
Incubation	Room temperature for 5 minutes

Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	None

Ligation with Blunt/TA Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	Room temperature for 15 minutes

TRANSFORMATION

- To obtain tranformants in 8 hrs., use NEB Turbo Competent *E. coli* (NEB #C2984)
- If recombination is a concern, then use the *recA*⁻ strains NEB 5-alpha Competent *E. coli* (NEB #C2987), NEB-10 beta Competent *E. coli* (NEB #C3019) or NEB Stable Competent *E. coli* (NEB #C3040)
- NEB-10 beta Competent *E. coli* works well for constructs larger than 5 kb
- NEB Stable Competent *E. coli* (NEB #C3040) can be used for constructs with repetitive sequences such as lentiviral constructs
- If electroporation is required, use NEB 5-alpha (NEB #C2989) or NEB 10-beta (NEB #C3020) Electrocompetent *E. coli*
- Use pre-warmed selection plates
- Perform several 10-fold serial dilutions in SOC for plating

Transformation with NEB 5-alpha Competent *E. coli*

DNA	1–5 µl containing 1 pg – 100 ng of plasmid DNA
Competent *E. coli*	50 µl
Incubation	On ice for 30 minutes
Heat Shock	Exactly 42°C for exactly 30 seconds
Incubation	On ice for 5 minutes Add 950 µl room temperature SOC 37°C for 60 minutes, with shaking

Troubleshooting Guide for Cloning

We strongly recommend running the following controls during transformations. These controls may assist in identifying which step(s) in the cloning workflow has failed.

- Transform 100 pg – 1 ng of uncut vector to check cell viability, calculate transformation efficiency and verify the antibiotic resistance of the plasmid.
- Transform the cut vector to determine the amount of background due to undigested plasmid. The number of colonies in this control should be < 1% of the number of colonies in the uncut plasmid control transformation (from control #1).
- Transform a vector only ligation reaction. The ends of the vector should not be able to re-ligate because either they are incompatible (e.g., digested with two restriction enzymes that do not generate compatible ends) or the 5´ phosphate group has been removed in a dephosphorylation reaction (e.g., blunt ends treated with rSAP). This control transformation should yield the same number of colonies as control #2.
- Digest vector DNA with a single restriction enzyme, re-ligate and transform. The ends of the vector DNA should be compatible and easily joined during the ligation reaction, resulting in approximately the same number of colonies as control #1.

The cloning workflow often benefits from an accurate quantitation of the amount of DNAs that are being worked with. We recommend quantification of DNAs whenever possible.

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Cells are not viable	• Transform an uncut plasmid (e.g., pUC19) and calculate the transformation efficiency of the competent cells. If the transformation efficiency is low (< 10^4) re-make the competent cells or consider using commercially available high efficiency competent cells.
	Incorrect antibiotic or antibiotic concentration	• Confirm antibiotic and antibiotic concentration
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C). • Transformation may need to be carried out using a strain that exerts tighter transcriptional control over the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli* (NEB #C2992))
	If using chemically competent cells, the wrong heat-shock protocol was used	• Follow the manufacturer's specific transformation protocol (Note: going above the recommended temperature during the heat shock can result in competent cell death)
	If using electrocompetent cells, PEG is present in the ligation mix	• Clean up DNA by drop dialysis prior to transformation with Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Try NEB's ElectroLigase (NEB #M0369)
	If using electrocompetent cells, arcing was observed or no voltage was registered	• Clean up the DNA prior to the ligation step • Tap the cuvette to get rid of any trapped air bubbles • Be sure to follow the manufacturer's specified electroporation parameters
	Construct is too large	• Select a competent cell strain that can be transformed efficiently with large DNA constructs (≥ 10 kb, we recommend trying NEB 10-beta Competent *E. coli* (NEB #C3019)) • For very large constructs (> 10 kb), consider using electroporation
	Construct may be susceptible to recombination	• Select a *recA*- strain such as NEB 5-alpha (NEB #C2987), NEB 10-beta (NEB #C3019) or NEB Stable (NEB #C3040) Competent *E. coli*
	The insert comes directly from mammalian or plant DNA and contains methylated cytosines, which are degraded by many *E. coli* strains	• Use a strain that is deficient in McrA, McrBC and Mrr, such as NEB 10-beta Competent *E. coli*
	Too much ligation mixture was used	• Use < 5 µl of the ligation reaction for the transformation
	Inefficient ligation	• Make sure that at least one fragment being ligated contains a 5´ phosphate moiety • Vary the molar ratio of vector to insert from 1:1 to 1:10. Use NEBiocalculator to calculate molar ratios • Purify the DNA to remove contaminants such as salt and EDTA with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N0312)
	Inefficient phosphorylation	• Purify the DNA prior to phosphorylation with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). Excess salt, phosphate or ammonium ions may inhibit the kinase. • If the ends are blunt or 5´ recessed, heat the substrate/buffer mixture for 10 minutes at 70°C. Rapidly chill on ice before adding the ATP and enzyme, then incubate at 37°C. • ATP was not added. Supplement the reaction with 1 mM ATP, as it is required by T4 Polynucleotide Kinase (NEB #M0201) • Alternatively, use 1X T4 DNA Ligase Buffer (contains 1 mM ATP) instead of the 1X T4 PNK Buffer

Troubleshooting Guide for Cloning (continued)

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Inefficient blunting	• Heat inactivate or remove the restriction enzymes prior to blunting • Clean up the PCR fragment prior to blunting with Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Sonicated gDNA should be blunted for at least 30 minutes • Do not use > 1 unit of enzyme/µg of DNA • Do not incubate for > 15 minutes • Do not incubate at temperatures > 12°C (for T4 DNA Polymerase, NEB #M0203) or > 24°C (for Klenow, NEB #M0210) • Make sure to add a sufficient amount of dNTPs to the reaction (33 µM each dNTP for DNA Polymerase I, Large (Klenow) Fragment, NEB #M0210 and 100 µM each dNTP for T4 DNA Polymerase, NEB #M0203). • When using Mung Bean Nuclease (NEB #M0250), incubate the reaction at room temperature. Do not use > 1 unit of enzyme/µg DNA or incubate the reaction > 30 minutes.
	Inefficient A-Tailing	• Clean up the PCR prior to A-tailing. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). High-fidelity enzymes will remove any non-templated nucleotides.
	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. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
Colonies don't contain a plasmid	Antibiotic level used was too low	• Increase the antibiotic level on plates to the recommended amount • Use fresh plates with fresh antibiotics
	Satellite colonies were selected	• Choose large, well-established colonies for analysis
Colonies contain the wrong construct	Recombination of the plasmid has occurred	• Use a *recA*⁻ strain such NEB 5-alpha, NEB 10-beta or NEB Stable Competent *E. coli*
	Incorrect PCR amplicon was used during cloning	• Optimize the PCR conditions • Gel purify the correct PCR fragment. NEB recommends the Monarch DNA Gel Extraction Kit (NEB #T1020).
	Internal recognition site was present	• Use NEBcutter to analyze insert sequence for presence of an internal recognition site
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C) • Transformation may need to be carried out using a strain that exerts tighter transcriptional control of the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli*)
	Mutations are present in the sequence	• Use a high-fidelity polymerase (e.g., Q5 High-Fidelity DNA Polymerase, NEB #M0491) • Re-run sequencing reactions
Too much background	Inefficient dephosphorylation	• Heat inactivate or remove the restriction enzymes prior to dephosphorylation
	Kinase is present/active	• Heat inactivate the kinase after the phosphorylation step. Active kinase will re-phosphorylate the dephosphorylated vector.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the restriction enzyme(s) to be sure it is not inhibited by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove contaminants. (e.g., too much salt). NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	Antibiotic level is too low	• Confirm the correct antibiotic concentration
Ran the ligation on a gel and saw no ligated product	Inefficient ligation	• Make sure at least one DNA fragment being ligated contains a 5´ phosphate • Vary the molar ratios of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios. • Purify the DNA to remove contaminants such as salt and EDTA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix, Quick Ligation Kit or concentrated T4 DNA Ligase • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA
The ligated DNA ran as a smear on an agarose gel	The ligase is bound to the substrate DNA	• Treat the ligation reaction with Proteinase K (NEB #P8107) prior to running on a gel
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 to dissociate the enzyme from the DNA
	Nuclease contamination	• Use fresh, clean running buffer • Use a fresh agarose gel • Clean up the DNA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).

PROBLEM	CAUSE	SOLUTION
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. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. 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. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	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 supercolied DNA. Increase the number of enzyme units in the reaction.
	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. For more information, visit the table "Restriction Enzymes Requiring Multi-sites" on neb.com.
	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. Miniprep DNA is particularly susceptible to contaminants. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Clean DNA with a spin column, with Monarch PCR & DNA Cleanup Kit (NEB #T1030), 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 to dissociate the enzyme from the substrate
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • 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. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	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. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume • Clean-up the PCR fragment prior to restriction digest. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per µg of DNA • Digest the DNA for 1–2 hours
No PCR fragment amplified	Used the wrong primer sequence	• Double check the primer sequence
	Incorrect annealing temperature	• Use the NEB Tm calculator to determine the correct annealing temperature (**www.neb.com/TmCalculator**)
	Incorrect extension temperature	• Each polymerase type has a different extension temperature requirement. Follow the manufacturer's recommendations.
	Too few units of polymerase	• Use the recommended number of polymerase units based on the reaction volume
	Incorrect primer concentration	• Each polymerase has a different primer concentration requirement. Make sure to follow the manufacturer's recommendations.
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Follow the manufacturer's recommendations.
	Difficult template	• With difficult templates, try different polymerases and/or buffer combinations
The PCR reaction is a smear on a gel	If bands are larger than expected it may indicate binding of the enzyme(s) to the DNA	• Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
Extra bands in PCR reaction	Annealing temperature is too low	• Use the NEB Tm calculator to determine the annealing temperature of the primers
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Make sure to follow the manufacturer's recommendations.
	Additional priming sites are present	• Double check the primer sequence and confirm it does not bind elsewhere in the DNA template
	Formation of primer dimers	• Primer sequence may not be optimal. Additional primers may need to be tested in the reaction.
	Incorrect polymerase choice	• Try different polymerases and/or buffer combinations

Optimization Tips for Your Cloning Reactions

New England Biolabs offers a wide selection of reagents for your cloning experiments. For more information, visit **ClonewithNEB.com**. The following tips can be used to help optimize each step in your cloning workflow. Tips for restriction enzyme digestion and amplification can be found on pages 290 and 337, respectively.

cDNA SYNTHESIS

Starting Material

- Intact RNA of high purity is essential for generating cDNA for cloning applications
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for cDNA synthesis reactions. However, if desired, mRNA can be easily obtained using a PolyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for cDNA cloning depends on the abundance of the transcript-of-interest. In general, 1 ng to 1 µg total RNA or 0.1–100 ng mRNA are recommended.

Product Selection

- Streamline your reaction setup by using the ProtoScript II First Strand cDNA Synthesis Kit (NEB #E6560). This kit combines ProtoScript II Reverse Transcriptase (NEB #M0360), a thermostable M-MuLV (RNase H^-) Reverse Transcriptase, and recombinant RNase Inhibitor in an enzyme Master Mix, along with a separate Reaction Mix containing dNTPs. Additionally, the kit contains two optimized reverse transcription primer mixes.

Yield

- ProtoScript II Reverse Transcriptase is capable of generating cDNA of more than 10 kb up to 48°C. We recommend 42°C for routine reverse transcription.
- You can increase the yield of a long cDNA product by doubling the amount of enzyme and dNTPs

Additives

- For most RT-PCR reactions, RNase H treatment is not required. But for some difficult amplicons or sensitive assays, add 2 units of *E. coli* RNase H to the reaction and incubate at 37°C for 20 minutes

PHOSPHORYLATION

Enzyme

- T4 Polynucleotide Kinase (NEB #M0201) and T4 DNA Ligase (NEB #M0202) can be used together in the T4 DNA Ligase Buffer
- T4 Polynucleotide Kinase is inhibited by high levels of salt (50% inhibition by 150 mM NaCl), phosphate (50% inhibition by 7 mM phosphate) and ammonium ions (75% inhibited by 7 mM $(NH_4)_2SO_4$)
- If using T4 Polynucleotide Kinase and working with 5´-recessed ends, heat the reaction mixture for 10 min at 70°C, chill rapidly on ice before adding the ATP (or Ligase Buffer containing ATP) and enzyme, then incubate at 37°C

Additives

- The addition of PEG 8000 (up to 5%) can improve results

DEPHOSPHORYLATION

Enzyme

- When dephosphorylating a fragment following a restriction enzyme digest, a DNA clean up step is required if the restriction enzyme(s) used is NOT heat inactivatable. We recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
- When working with the Quick Dephosphorylation Kit (NEB #M0508), rSAP (NEB #M0371) or AP (NEB #M0289), which are heat-inactivatable enzymes, a DNA clean-up step after dephosphorylation is not necessary prior to the ligation step. However, when using CIP (NEB #M0290), a clean-up step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) prior to ligation is necessary.

Additives

- AP requires the presence of Zn^{2+} in the reaction, so don't forget to supplement the reaction with 1X Antarctic Phosphatase Reaction Buffer when using other NEBuffers

BLUNTING/END REPAIR

Enzyme

- Make sure that you choose the correct enzyme to blunt your fragment. The Quick Blunting Kit (NEB #E1201), T4 DNA Polymerase (NEB #M0203) and DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) will fill 5´ overhangs and degrade 3´ overhangs. Mung Bean Nuclease (NEB #M0250) degrades 5´ overhangs.
- T4 DNA Polymerase and DNA Polymerase I, Large (Klenow) Fragment are active in all NEBuffers. Please remember to add dNTPs.

Clean-up

- When trying to blunt a fragment after a restriction enzyme digestion, if the restriction enzyme(s) used are heat inactivable, then a clean-up step prior to blunting is not needed. Alternatively, if the restriction enzyme(s) used are not heat inactivable, a DNA clean-up step is recommended prior to blunting.
- When trying to blunt a fragment amplified by PCR, a DNA clean-up step is necessary prior to the blunting step to remove the nucleotides and polymerase
- When trying to dephosphorylate a fragment after the blunting step, you will need to add a DNA clean-up step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) after the blunting and before the addition of the phosphatase

Temperature

- When trying to blunt a fragment with Mung Bean Nuclease, the recommended temperature of incubation is room temperature, since higher temperatures may cause sufficient breathing of the dsDNA ends that the enzyme may degrade some of the dsDNA sequence. The number of units to be used and time of incubation may be determined empirically to obtain best results.

Heat Inactivation

- Mung Bean Nuclease reactions should not be heat inactivated. Although Mung Bean Nuclease can be inactivated by heat, this is not recommended because the DNA begins to "breathe" before the Mung Bean Nuclease is inactivated and undesirable degradation occurs at breathing sections. Purify DNA by phenol/chloroform extraction and ethanol precipitation or spin column purification [e.g., Monarch PCR & DNA Cleanup Kit (NEB #T1030)].

A-TAILING

- If the fragment to be tailed has been amplified with a high-fidelity polymerase, the DNA needs to be purified prior to the tailing reaction. For this we recommend the Monarch PCR & DNA Cleanup Kit (NEB T1030). Otherwise, any high-fidelity polymerase present in the reaction will be able to remove any non-templated nucleotides added to the end of the fragments.

DNA LIGATION

Reaction Buffers

- T4 DNA Ligase Buffer (NEB #B0202) should be thawed on the bench or in the palm of your hand, and not at 37°C (to prevent breakdown of ATP)
- Once thawed, T4 DNA Ligase Buffer should be placed on ice
- Ligations can be performed in any of the four standard restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer (NEB #B0201) supplemented with 1 mM ATP
- When supplementing with ATP, use ribo-ATP (NEB #P0756). Deoxyribo-ATP will inhibit ligation.
- Before ligation, completely inactivate restriction enzyme by heat inactivation, spin column (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) or Phenol/EtOH purification

DNA

- Either heat inactivate (AP, SAP, Quick Dephosphorylation Kit) or remove phosphatase (CIP) before ligation
- Keep total DNA concentration between 1–10 µg/ml
- Vector:Insert molar ratios between 1:1 and 1:10 are optimal for single insertions. Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate molar ratios.
- For cloning more than one insert, we recommend the NEBuilder® HiFi DNA Assembly Master Mix (NEB #E2621) or Cloning Kit (NEB #E5520)
- If you are unsure of your DNA concentration, perform multiple ligations with varying ratios

Ligase

- For cohesive-end ligations, standard T4 DNA Ligase. Instant Sticky-end Ligase Master Mix or the Quick Ligation Kit are recommended.
- For blunt and single-base overhangs the Blunt/TA Ligase Master Mix is recommended.
- For ligations that are compatible with electroporation, Electroligase is recommended
- Standard T4 DNA Ligase can be heat inactivated at 65°C for 20 minutes
- Do not heat inactivate the Quick Ligation Kit or the ligase master mixes

Transformation

- Add between 1–5 µl of ligation mixture to competent cells for transformation
- Extended ligation with PEG causes a drop off in transformation efficiency
- Electroporation is recommended for larger constructs (> 10,000 bp). Dialyze samples or use a spin column first if you have used the Quick Ligation Kit or ligase master mixes
- For ligations that are compatible with electroporation, Electroligase is recommended.

TRANSFORMATION

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

DNA

- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency

Incubation & Heat Shock

- Incubate on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.
- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended, except when using BL21 (NEB #C2530) which requires exactly 10 seconds.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened.
- SOC and NEB 10-beta/Stable Outgrowth Medium give 2-fold higher transformation efficiency than LB medium
- Incubation with shaking or rotation results in 2-fold higher transformation efficiency

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on transformation efficiency
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA Contaminants to Avoid

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify (e.g., Monarch PCR & DNA Cleanup Kit) or phenol/chloroform extract and ethanol precipitate

Troubleshooting Guide for DNA Cleanup & Plasmid Purification using Monarch® Kits

PROBLEM	PRODUCT	POSSIBLE CAUSE	SOLUTION
No DNA purified	Monarch Plasmid Miniprep Kit (NEB #T1010)	Buffers added incorrectly	• Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence • Ensure ethanol was added to Plasmid Wash Buffer 2
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Ethanol not added to wash buffer	• Ensure the proper amount of ethanol was added to Monarch DNA Wash Buffer
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)		
Low DNA yield	Monarch Plasmid Miniprep Kit (NEB #T1010)	Incomplete lysis	• Pellet must be completely resuspended before addition of Plasmid Lysis Buffer (B2) – color should change from light to dark pink • Avoid using too many cells; this can overload the column. If culture volume is larger than recommended, scale up buffers B1-B3.
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
		Low-copy plasmid selected	• Increase amount of cells processed and scale buffers accordingly
		Lysis of cells during growth	• Harvest culture during transition from logarithmic growth to stationary phase (~12-16 hours)
		Incomplete neutralization	• Invert tube several times until color changes to yellow
		Incomplete elution	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of plasmids > 10 kb, heat the DNA Elution Buffer to 50°C and extend incubation time to 5 minutes
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Buffers added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Gel slice not fully dissolved	• Undissolved agarose may clog the column and interfere with binding. Incubate in Monarch Gel Dissolving Buffer for proper time and temperature.
		Gel dissolved above 60°C	• Dissolve gel slice in specified range (37-55°C). Higher temperatures can denature DNA
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Buffers added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
Low DNA quality	Monarch Plasmid Miniprep Kit (NEB #T1010)	Plasmid degradation	• Be cautious of strains with high levels of endogenous endonuclease (e.g., HB101 and JM 100 series)
		Plasmid is denatured	• Limit incubation with Plasmid Lysis Buffer (B2) to two minutes, as NaOH in the buffer can denature the plasmid
		gDNA contamination	• Use careful inversion mixing after cell lysis to avoid shearing of host cell chromosomal DNA. Do not vortex.
		RNA contamination	• Incubate sample in neutralization buffer for the full 2 minutes. For cell culture volumes > 3 ml, increase the spin after neutralization to 5 minutes.
		Improper storage	• Elute DNA in DNA Elution Buffer or nuclease-free water, and store at -20°C. Do not store in solutions containing magnesium.
Low DNA performance	Monarch Plasmid Miniprep Kit (NEB #T1010)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Excessive salt in sample	• Use both plasmid wash buffers and do not skip wash steps
		Excessive carbohydrate has been carried over	• Avoid strains with high amounts of endogenous carbohydrate (e.g., HB101 and JM 100 series). Be sure to follow protocol and include Plasmid Wash Buffer 1 step.
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Gel slice not fully dissolved	• Undissolved agarose may leach salts into the eluted DNA
		Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube for elution
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube

Guidelines for Choosing Sample Input Amounts When Using the Monarch Genomic Purification Kit

Genomic DNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and DIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Genomic DNA Purification Kit. It is very important not to overload the column and the buffer system when extracting and purifying gDNA, as DNA yields, purity, integrity, and length may suffer.

SAMPLE TYPE	RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
TISSUE*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
Spleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
BLOOD**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
CELLS				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
BACTERIA				
E. coli (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (Gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
ARCHAEA				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
YEAST				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
SALIVA/BUCCAL CELLS***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

*** Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Troubleshooting Guide for Genomic DNA Purification using the Monarch Kit

PROBLEM	CAUSE	SOLUTION
LOW YIELD		
Cells	Frozen cell pellet was thawed and/or resuspended too abruptly	• Thaw cell pellets slowly on ice and flick tube several times to release the pellet from bottom of tube. Use cold PBS, and resuspend gently by pipetting up and down 5–10 times until pellet is dissolved
	Cell Lysis Buffer was added concurrently with enzymes	• Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer
Blood	Blood was thawed, allowing for DNase activity	• Keep blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples
	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
Tissue	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
	Sample was not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Genomic DNA was degraded (common in DNase-rich tissues)	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
	Column is overloaded with DNA	• Some organ tissues (e.g., spleen, kidney, liver) are extremely rich in genomic DNA. Using inputs larger than recommended will result in the formation of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material.
	Incorrect amount of Proteinase K added	• Most samples are digested with 10 µl Proteinase K, but for brain, kidney and ear clips, use 3 µl.
DNA DEGRADATION		
Tissue	Tissue samples were not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before Proteinase K can lyse the tissue.
	High DNase content of soft organ tissue	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
Blood	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Blood was thawed, allowing for DNase activity	• Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples
SALT CONTAMINATION		
	Guanidine Thiocyanate salt from the binding buffer was carried over into the eluate	• When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip and always pipet carefully onto the silica membrane. • Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the spin column. • Close the caps gently to avoid splashing the mixture into the upper column area and move the samples with care in and out of the centrifuge. • If salt contamination is a concern, invert the columns a few times (or vortex briefly) with gDNA Wash Buffer as indicated in the protocol.
PROTEIN CONTAMINATION		
Tissue	Incomplete digestion	• Cut samples to the smallest possible pieces. Incubate sample in the lysis buffer for an extra 30 minutes to 3 hours to degrade any remaining protein complexes.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small, indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Blood	High hemoglobin content	• Some blood samples (e.g., horse) are rich in hemoglobin, evidenced by their dark red color. Extend lysis time by 3–5 minutes for best purity results.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis from 5 to 3 minutes.
RNA CONTAMINATION		
Tissue	Too much input material	• DNA-rich tissues (e.g., spleen, liver and kidney) will become very viscous during lysis and may inhibit RNase A activity. Do not use more than the recommended input amount.
	Lysis time was insufficient	• Extend lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved
TISSUE DIGESTION TAKES TOO LONG		
	Tissue pieces too large	• Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis
	Tissue pieces are stuck to bottom of tube	• Vortex to release pieces from the tube bottom, and immediately after adding Proteinase K and Tissue Lysis Buffer
	Too much starting material	• Use recommended input amount
TISSUE LYSATE APPEARS TURBID		
	Formation of indigestible fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
RATIO $A_{260}/A_{230} > 2.5$		
	Slight variations in EDTA concentration in eluates	• EDTA in elution buffer may complex with cations like Mg^{2+} and Ca^{2+} samples present in genomic DNA, which may lead to higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Guidelines for Choosing Sample Input Amounts When Using the Monarch Total RNA Miniprep Kit

RNA yield, purity, and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Total RNA Miniprep Kit. It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

SAMPLE TYPE [1]		INPUT	AVERAGE YIELD (µg)	OBSERVED RIN	MAXIMUM STARTING MATERIAL
CULTURED CELLS					
HeLa		1 x 10^6 cells	12–15	9-10	1 x 10^7 cells
HEK 293		1 x 10^6 cells	12–14	9-10	1 x 10^7 cells
NIH3T3		1 x 10^6 cells	8–12	9-10	1 x 10^7 cells
MAMMALIAN BLOOD [2]					
Human	Fresh	200 µl	0.5–1.0	7-8	3 ml
	Frozen	200 µl	0.5–1.0	7-8	3 ml
	Stabilized	200 µl	0.5–1.0	7-8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml*
BLOOD CELLS					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 x 10^7 cells
TISSUE					
Rat liver	Frozen pulverized	10 mg	25	8–9	20 mg
	Stabilized solid	10 mg	50–60	8–9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40–50	9	20 mg
Rat kidney (frozen pulverized)		10 mg	7–10	9	50 mg
Rat brain	Frozen pulverized	10 mg	2–3	8–9	50 mg
	Stabilized solid	10 mg	0.5–1.5	8–9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5–8	8–9	50 mg
Rat muscle (frozen pulverized)		10 mg	2–3	8–9	50 mg
Mouse muscle	Frozen pulverized	10 mg	3	8–9	50 mg
	Powder with bead homogenizer	10 mg	5	7–8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8–10	9	50 mg
Mouse heart (stabilized solid w/bead homogenizer)		10 mg	5–6	8–9	50 mg
YEAST					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	50	9–10**	5 x 10^7 cells
	Fresh with Zymolyase®	1 x 10^7 cells	60	9**	5 x 10^7 cells
BACTERIA					
E. coli	Frozen	1 x 10^9 cells	5	10	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^9 cells	10	10	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	70	10	1 x 10^9 cells
B. cereus	Frozen with lysozyme	1 x 10^8 cells	20–30	9	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^8 cells	8	9–10	1 x 10^9 cells
PLANT					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg

[1] RNA for other blood samples, including drosophila, zebrafish embryos/larvae, plasma, serum, saliva, buccal swabs and nucleated blood have been successfully purified with this kit; protocols are available in the product manual.

[2] A protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Mouse blood also has a maximum input of 1 ml.

** *S.cerevisiae* total RNA was run on an Agilent® Nano 600 Chip using plant assay.

Find tips for RNA purification using Monarch.

Troubleshooting Guide for Total RNA Extraction & Purification Using Monarch Kits

PROBLEM	CAUSE	SOLUTION
Clogged column	Insufficient sample disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Protection Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample-specific protocols in the product manual.
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
Low RNA yield	Incomplete elution	• After addition of Nuclease-free Water (NEB #B1500) to column matrix, incubate 5-10 min at room temperature and then centrifuge to elute • Perform a second elution (note: this will dilute sample)
	Sample is degraded	• Store input sample at -80°C prior to use • Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage
	Insufficient disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample specific protocol in the product manual. • For Proteinase K treated samples, doubling Proteinase K (from 5% to 10%) may lead to an increase in RNA yield
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
RNA degradation	Starting material not handled/stored properly	• Store input sample at -80°C prior to use. Degradation of RNA may occur if sample is not flash frozen or protected by a preservation reagent. Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage.
	Deviation from the stated protocol may expose RNA to unwanted RNase activities	• Refer to the General Guidelines for working with RNA in the product manual
	RNase contamination of eluted materials or kit buffers may have occurred	• See General Guidelines for working with RNA in the product manual for advice on reducing risks of contamination
Low OD ratios	Low $A_{260/280}$ values indicate residual protein in the purified sample	• Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA Purification Column.
	Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution	• Ensure wash steps are carried out prior to eluting sample. Use care to ensure the tip of the column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
DNA contamination	Genomic DNA not removed by column	• Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample • Perform in-tube/off-column DNase I treatment to remove gDNA
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the tip of the RNA Purification Column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the RNA Purification Column for 2 minutes following the final wash with RNA Wash Buffer • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer • Add additional wash step and/or extend spin time for final wash
Unusual spectrophotometric readings	RNA concentration is too low for spectrophotometric analysis	• For more concentrated RNA, elute with 30 µl of nuclease-free water • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Sample Input Amounts on page 357.
	Silica fines in eluate	• Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the $A_{260/230}$ is unaffected by possible elution of silica particles

Genetic Markers

A *genotype* indicates the genetic state of the DNA in an organism. It is a theoretical construct describing a genetic situation that explains the observed properties (phenotype, see below) of a strain. *E. coli* genotypes list only genes that are defective (1). If a gene is not mentioned, then it is not known to be mutated*, **. Prophages and plasmids that were present in the original K-12 strain (F, λ, e14, rac) are normally listed only if absent. However, for simplicity, we have not listed λ except when it is present, and we have listed F and its variants in all cases. Parentheses or brackets surround a prophage or plasmid when listed. Genes are given three-letter, lower-case, italicized names (e.g., *dam*) that are intended to be mnemonics suggesting the function of the gene (here, **D**NA **a**denine **m**ethylase). If the same function is affected by several genes, the different genes are distinguished with uppercase italic letters (e.g., *recA, recB, recC, recD* all affect **rec**ombination). Proper notation omits superscript + or – in a genotype, but these are sometimes used redundantly for clarity, as with F´ *lac*$^-$ *proA*$^+$*B*$^+$. Deletion mutations are noted as Δ, followed by the names of deleted genes in parentheses, [e.g., Δ(*lac-pro*)]. All genes between the named genes are also deleted. Specific mutations are given allele numbers that are usually italic arabic numerals (e.g., *hsdR17*) and may be characterized as *am*=amber (UAG) mutation or *ts*=inactive at high temperature, as appropriate. Some common alleles [e.g., Δ*(lac-pro)X111*] break the rules. If two strains' genotypes list a gene with the same allele number, they should carry exactly the same mutation.

The *phenotype* of a strain is an observable behavior, e.g., Lac$^-$ fails to grow on lactose as a sole carbon source. Phenotypes are capitalized and in Roman type, and the letters are always followed by superscript + or – (or sometimes r, resistant, or s, sensitive). Although phenotypes do not, strictly speaking, belong in a genotype, they are sometimes included following the genotype designation when the former is not obvious from the latter [e.g., *rpsL104* (Str^r)–gene name from **r**ibosomal **p**rotein, **s**mall subunit, S12, confers resistance to streptomycin].

Some common genes of interest are described below and on the next page; a catalogue of genetically defined genes can be found in reference 2 and on the very useful internet site maintained by the *E. coli* Genetic Stock Center (CGSC) at Yale University <http://cgsc.biology.yale.edu/>. Additional information from CGSC can be obtained from curator Mary Berlyn by e-mail <cgsc@yale.edu>.

* Most *E. coli* laboratory strains have been heavily mutagenized over forty years of study, and different lines may carry different, so far undiscovered, mutations that may or may not affect your situation. For this reason, it is sometimes useful to try more than one line or strain background in your experiments.

** *E. coli* B and its derivatives are naturally Lon$^-$ and Dcm$^-$. We have listed this in brackets even though it is the wild type state for these strains.

References

(1) Demerec et al. (1966) *Genetics,* 54, 61–76.
(2) Berlyn, M.K.B. (1996). In F. C. Niedhardt et al. (Ed.), *Escherichia coli and Salmonella: cellular and molecular biology,* (2nd ed.), Vol. 2, (pp. 1715–1902). ASM Press.
(3) Raleigh, E.A. et al. (1991) *J. Bacteriol.,* 173, 2707–2709.

dam Endogenous adenine methylation at GATC sequences is abolished. *dam* strains have a high recombination frequency, express DNA repair functions constitutively, and are poorly transformed by Dam-modified plasmids. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., BclI).

dcm Endogenous cytosine methylation at CCWGG sequences is abolished. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., AvaII).

dnaJ One of several "chaperonins" is inactive. This defect has been shown to stabilize certain mutant proteins expressed in *E. coli*.

dut dUTPase activity is abolished. This mutation, in combination with *ung*, allows incorporation of uracil into DNA. Used for oligonucleotide mutagenesis.

endA Activity of nonspecific Endonuclease I is abolished. DNA preparations are thought to be of higher quality when prepared from *endA* strains.

e14 An excisable prophage-like element, present in K-12 but missing from many derivatives. e14 carries the *mcrA* gene among others, therefore e14$^-$ strains are McrA$^-$.

F A low-copy number self-transmissible plasmid. F´ factors carry portions of the *E. coli* chromosome, most notably the *lac* operon and *proAB* on F´ *lac-proA*$^+$*B*$^+$.

fhuA An iron uptake receptor is mutated. This mutation confers resistance to phage T1 (ferric hydroxamate uptake). Former name is *tonA*.

gal The ability to metabolize galactose is abolished.

glnV See ***supE***.

gyrA A point mutation in DNA gyrase, subunit A. This mutation confers resistance to the antibiotic nalidixic acid.

hflA This mutation results in high frequency lysogenization by λ.

hsdR, DNA that does not contain methylation

hsdS of certain sequences is recognized as foreign by EcoKI or EcoBI and restricted (degraded). These enzymes recognize different sequences and are encoded by different alleles of *hsdRMS*. *hsdR* mutations abolish restriction but not protective methylation (r$^-$m$^+$), while *hsdS* mutations abolish both (r$^-$m$^-$). DNA made in the latter will be restricted when introduced into a wild-type strain.

lacIq The *lac* repressor is overproduced, turning off expression from P*lac* more completely.

lacZ β-galactosidase activity is abolished.

lacZ::T7gene 1 The phage T7 RNA polymerase (= gene 1) is inserted into the lacZ gene.

lacY Lactose permease activity is abolished.

Δ(*lac*) = deletion; there are four common deletions involving *lac*:

Δ(*lacZ*)M15 expresses a fragment that complements the *lac* α-fragment encoded by many vectors. These vectors will yield blue color on X-Gal only if the host carries ΔM15.

ΔU169, ΔX111, and ΔX74 all delete the entire *lac* operon from the chromosome, in addition to varying amounts of flanking DNA. ΔX111 deletes *proAB* as well, so that the cell requires proline for growth on minimal medium, unless it also carries F´ *lac proA*$^+$*B*$^+$.

lon Activity of a protease responsible for degrading aberrant proteins is abolished. Some eukaryotic proteins are stabilized in *lon* strains. *E. coli* B naturally lacks Lon.

Genetic Markers (continued)

lysY The lysozyme gene from the T7 bacteriophage is mutated. The mutation K128Y eliminates lysozyme activity, but the mutant protein still binds to and inhibits T7 RNA polymerase.

malB The *malB* region encompasses the genes *malEFG* and *malK lamB malM*. Δ(*malB*) deletes most or all of this region and eliminates expression of Maltose Binding Protein (MalE).

mcrA, mcrBC A restriction system that requires methyl cytosine is abolished. DNA containing methylcytosine in some sequences is restricted by Mcr$^+$. *dcm*-modified DNA is not restricted by Mcr$^+$. Δ(*mcrC-mrr*) deletes six genes: *mcrC-mcrB-hsdS-hsdM-hsdR-mrr*; *mcrA* is lost with e14.

mrr A restriction system that requires cytosine or adenine methylation is abolished; however, *dam*$^-$, *dcm*$^-$ or EcoKI-modified DNA is not restricted by Mrr$^+$. The methylcytosine-dependent activity is also known as McrF (3).

mtl The ability to metabolize the sugar alcohol mannitol is abolished.

ompT Activity of outer membrane protease (protease VII) is abolished.

phoA Activity of alkaline phosphatase is abolished.

prc See ***tsp***.

recA Homologous recombination is abolished; particularly desirable when working with sequences containing direct repeats > 50 bp.

recB, recC Exonuclease and recombination activity of Exonuclease V is abolished. Homologous recombination is much reduced in *recB recC* strains that are not also *sbcB* or *sbcA*. Stability of inverted repeat sequences is enhanced in *recB recC* strains, especially if they are also *sbcB sbcC*. Plasmid replication may be aberrant.

recD Exonuclease activity of ExoV is abolished, but recombination activity is elevated. Inverted repeat sequences in λ can be propagated in *recD* strains. Plasmid replication is aberrant.

recF Plasmid-by-plasmid homologous recombination is abolished.

recJ Plasmid-by-plasmid homologous recombination is abolished.

relA1 Lacks ppGpp synthesis during the stringent response to amino acid starvation; activity of ATP:GTP 3´-pyrophosphotransferase (EC2.7.6.5) is abolished.

rfbD Lacks functional TDP-rhamnose synthetase, and thus does not synthesize the cell surface O-antigen.

rpoH (also known as *htpR)* Lack of this heat-shock transcription factor abolishes expression of some stress-induced protease activities in addition to *lon*. Some cloned proteins are more stable in *rpoH*am *sup*Cts strains at high temperature.

sbcB Exo I activity is abolished. Strains carrying *recB recC* and *sbcB* are usually also *sbcC*. These quadruple mutant strains are recombination-proficient and propagate inverted repeats in λ, but plasmid replication is aberrant.

sbcC Usually found with *recB recC sbcB*. However, strains carrying *sbcC* alone are recombination-proficient and stably propagate inverted repeats both in λ and in plasmids.

sulA Mutations in this gene allows cells to divide and recover from DNA damage in a lon mutant background (suppressor of Lon).

supC(ts) A thermosensitive tyrosine-inserting ochre (UAA) and amber (UAG) suppressor tRNA. Nonsense mutations in the same strain are suppressed only at low temperatures. Now called ***tyrT***.

supE A glutamine-inserting amber (UAG) suppressor tRNA; required for growth of some phage vectors. Now called ***glnV***.

supF A tyrosine-inserting amber (UAG) suppressor tRNA; required for lytic growth of S7 or S100 λ phage, such as λgt11. Now called ***tyrT***.

thi-1 The ability to synthesize thiamine is abolished (vitamin B1).

traD The self-transmissibility of the F factor is severely reduced.

tsp A periplasmic protease that may degrade secreted or cytoplasmically overexpressed proteins after lysis is abolished. Now called ***prc***.

tsx Confers resistance to bacteriophage T6.

tyrT See ***supC, supF***.

ung Uracil N-glycosylase activity is abolished. Uracil incorporated into DNA is removed by Ung$^+$, leaving baseless site. See *dut*.

xyl The ability to metabolize the sugar xylose is abolished.

(P1) The cell carries a P1 prophage. Such strains express the P1 restriction system.

(P2) The cell carries a P2 prophage. This allows selection against Red$^+$ Gam$^+$ λ (Spi$^-$ selection).

(ϕ80) The cell carries the lambdoid prophage ϕ80. A defective ϕ80 prophage carrying the *lac* M15 deletion is present in some strains.

(Mu) Mu prophage; Mud means the phage is defective.

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocol

1. Thaw cells on ice for 10 minutes
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 30 minutes
4. Heat shock at 42°C for 10–30 seconds or according to recommendations. For BL21, use exactly 10 seconds.
5. Place on ice for 5 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle® strains) or according to recommendations

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 2 minutes
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37-42°C. (30°C for SHuffle strains) NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

Transformation Tips

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA

- DNA should be purified and resuspended in water or TE Buffer
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA Contaminants to Avoid

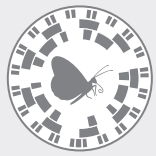
CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/ chloroform extract and ethanol precipitate

Electroporation Tips

NEB Turbo (NEB #C2986), NEB 5-alpha (NEB #C2989) and NEB 10-beta (NEB #C3020) Competent *E. coli* Strains are available as electrocompetent cells. The following tips will help maximize transformation efficiencies.

- Pre-chill electroporation cuvettes and microcentrifuge tubes on ice
- Thaw cells on ice and suspended well by carefully flicking the tubes
- Once DNA is added, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.
- DNA should be purified and suspended in water or TE. Transformation efficiency is > 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1mm gap are recommended (e.g., BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage is required for cuvettes with 2 mm gap.
- Arcing may occur due to high concentration of salts or air bubbles
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

Find tips for successful transformation.



T7 Protein Expression

1. Transform expression plasmid into a T7 expression strain. Plate out on antibiotic selection plates and incubate overnight at 37°C (24 hours at 30°C for SHuffle strains).
2. Resuspend a single colony in 10 ml liquid culture with antibiotic
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6
4. Induce with 40 µl of a 100 mM stock of IPTG (final conc. = 0.4 mM) and induce for 2 hours at 37°C (4 hours at 30°C or 16°C overnight for SHuffle strains)
5. Check expression by Coomassie stained protein gel, Western Blot or activity assay. Check expression in the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C (30°C for SHuffle strains) until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight (4 hours at 30°C or 16°C overnight for SHuffle strains).

Troubleshooting Tips

No Colonies or No Growth in Liquid Culture

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In *I*^{q} strains over-expression of the *LacI*^{q} repressor reduces basal expression of the T7 RNA polymerase
  - In *lysY* strains, mutant T7 lysozyme is produced which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues
- Check antibiotic concentration (test with control plasmid)

No Protein Visible on Gel or No Activity

- Check for toxicity - the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test *I*^{q} and/or *lysY* strains to reduce basal expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced Protein is Insoluble

T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:

- Induce at lower temperatures (12–15°C overnight)
- Reduce IPTG concentration to 0.01 mM – 0.1 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

Lori is the Senior Executive Assistant to Jim Ellard, NEB's CEO, and Donald Comb, NEB's Founder. Lori is very involved in NEB's Educational Course Support Program, providing reagents to college and high school teaching labs at no charge.

DNA/RNA Input Guidelines for NGS Library Prep

DNA SAMPLE INPUT GUIDELINES

Integrity of DNA

- Start with as high quality DNA as possible. The quality of the input material directly affects the quality of the library. Absorbance measurements can be used as an indication of DNA purity. Ideally, the ratio of the absorbance at 260 nm to 280 nm should be between 1.8–2.0. However, measurements can be affected by the presence of RNA or small nucleic acid fragments. A DNA Integrity Number can be determined using the Agilent TapeStation® and qPCR-based methods can also provide a measurement of DNA integrity.

Quantitation of DNA

- It is important to quantify accurately the DNA sample prior to library construction. Fluorescence-based detection which utilizes dsDNA-specific dyes, such as the Qubit® from Life Technologies, is more accurate than UV spectrometer-based measurements, as the presence of RNA or other contaminants can result in overestimation of the amount of the DNA sample.

RNA SAMPLE INPUT GUIDELINES

Integrity of RNA

- It is important to start with high quality RNA. The use of degraded RNA can result in low yield or failure to generate libraries. We recommend determining RNA quality using the RNA Integrity Number (RIN) estimated by the Agilent® Bioanalyzer® or similar instrumentation. Ideally, the RNA sample should have a RIN value higher than 7, enabling use of poly(A) mRNA or rRNA depletion protocols. Degraded RNA with RIN values as low as 1-2 can be used if specific protocols are followed.
- RNA should be completely free of DNA. DNase digestion of the purified RNA with RNase-free DNase is recommended.

Quantitation of RNA

- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer on a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. However, free nucleotides or other organic compounds routinely used to extract RNA will also absorb UV light near 260 nm and will result in an over-estimation of the RNA concentration.

BEAD-BASED CLEAN-UPS AND SIZE SELECTION

Integrity of DNA

- Be careful not to disturb the bead pellet when transferring material
- Be sure to vortex the beads just before use – they should be a uniform suspension
- Do not over-dry the beads. This can make resuspension difficult and reduce yield.
- Bead-based clean-ups and size-selection are explained in animations and videos available on our website
- Use a magnet that is strong enough to separate the beads completely and quickly

INDICES

- Open only one index primer vial at a time, to minimize the risk of contamination
- When you are using a subset of the indices supplied in a kit, or using indices from more than one kit, it is important to optimize the combination of indices used, to ensure balanced sequencing reads.

We provide recommendations for NEBNext index combinations at **NEBNext.com**.

Labeling with SNAP-tag® Technology-Troubleshooting Guide

APPLICATION	PROBLEM	CAUSE	SOLUTION
Cellular Labeling	No labeling	Fusion protein not expressed	• Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with Vista Green label
	Weak labeling	Poor expression and/or insufficient exposure of fusion protein to substrate	• Increase substrate concentration • Increase incubation time
		Rapid turnover of fusion protein	• Analyze samples immediately or fix cells directly after labeling • Label at lower temperature (4°C or 16°C)
	High background	Non-specific binding of substrates	• Reduce substrate concentration and/or incubation time • Allow final wash step to proceed for up to 2 hours • Include fetal calf serum or BSA during labeling
	Signal strongly reduced after short time	Instability of fusion protein	• Fix cells • Switch tag from N-terminus to C-terminus or vice versa
		Photobleaching	• Add commercially available anti-fade reagent • Reduce illumination time and/or intensity
Labeling in Solution	Precipitation	Insoluble fusion	• Test from pH 5.0 to 10.0 • Optimize salt concentration [50 to 250 mM] • Add 0.05 to 0.1% Tween 20
	Weak or no labeling	Exhaustive labeling has not been achieved	• Increase incubation time to 2 hrs at 25°C or 24 hrs at 4°C • Reduce the volume of protein solution labeled • Check expression of fusion protein via SDS-PAGE with Vista Green label
	Loss of activity	Instability of fusion protein	• Reduce labeling time • Decrease labeling temperature (4°C or 16°C)

Cellular Imaging & Analysis FAQs

Q. *How does SNAP-tag® labeling differ from using GFP fusion proteins?*

A. GFP and SNAP-tag are both valuable technologies used to visualize proteins in live cells. GFP is an intrinsically fluorescent protein derived from *Aequorea victoria* while SNAP-tag is derived from hAGT, a human DNA repair protein. In contrast to GFP, the fluorescence of SNAP-tag fusions can be readily turned on with the addition of a variety of fluorescent probes added directly to the culture media. Substituting different fluorophores or other functionalities (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins.

Q. *What is the difference between SNAP- and CLIP-tag™?*

A. SNAP-tag and CLIP-tag are both derived from O^6 -alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O^6-labeled benzylguanine substrates while CLIP-tag recognizes O^2-labeled benzylcytosine substrates. Each tag transfers the label from the substrate to itself, resulting in specific covalent labeling. In creating the tags, hAGT has been engineered to no longer interact with DNA, but rather with derivatives of the free benzylguanine or benzylcytosine substrates. The tags exhibit no cross-reactivity with one another, enabling researchers to simultaneously label fusion proteins containing SNAP- and CLIP-tags with different fluorophores in live cells.

Q. *Can I clone my protein as a fusion to the N- or C-terminus of the tags?*

A. Yes. SNAP- and CLIP-tags can be fused to either the N- or C-terminus of a protein of interest. However, to label surface proteins on the outside of cells, the SNAP-tag or CLIP-tag must be cloned so that it is oriented to the extracellular surface of the plasma membrane. In this orientation, the tag is accessible to its fluorophore conjugated substrate.

Q. *Are the substrates toxic to cells?*

A. No toxicity has been noted by proliferation or viability assays when using up to 20 µM substrate for 2 hours. Most of the substrates can be incubated with cells for 24 hours up to a concentration of 20 µM without significant toxicity.

Q. *How stable is the labeled protein in mammalian cells?*

A. The stability of the tagged protein in the cell is dependent upon the stability of protein of interest. Labeled SNAP-tag fusion protein has been detected for up to 2 days in mammalian cells.

Q. *Are SNAP-tag substrates stable to fixation?*

A. Yes. SNAP-tag substrates are derived from organic fluorophores which are stable to fixation. Fluorescently-labeled SNAP-tag fusion proteins do not lose signal intensity in contrast to some GFP spectral variants. After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, ethanol, methanol, methanol/acetone etc. without loss of signal.

Q. *What conditions are recommended for SNAP-tag labeling in vitro?*

A. The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 5.0 and 10.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM and at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided.

Frequencies of Restriction Sites in Sequenced DNAs

The table below summarizes the frequencies with which restriction enzyme sites occur in eleven commonly used DNA molecules. Detailed restriction maps can be found on subsequent pages. The sites listed in these tables were identified by computer analyses of published sequences. Although we have tried to ensure their accuracy, the sites have not necessarily been confirmed by experimentation. When the same specificity is displayed by several enzymes, the site is listed by the name of the enzyme that is available from New England Biolabs. Other enzymes with the same specificity are listed in the table of isoschizomers on page 311–327. Enzymes not available from NEB are listed with an (x). If NEB offers an HF version of that enzyme, it is indicated by a red dot (•). Recognition sequences are written 5´ to 3´.

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	pSNAP$_F$	pTXB1	pTYB21	pUC19	T7
AarI (x)	CACCTGC	9	12	0	0	0	0	1	0	0	0	5
AatII	GACGTC	3	10	0	1	0	0	5	1	0	1	1
AccI	GTMKAC	17	9	1	2	5	1	2	5	3	1	33
Acc65I	GGTACC	8	2	1	0	0	0	2	0	1	1	5
AciI	CCGC	582	516	42	67	81	81	75	102	102	34	199
AclI	AACGTT	3	7	2	4	2	5	3	12	13	2	19
AcuI	CTGAAG	23	40	0	2	8	4	5	2	2	2	1
AfeI	AGCGCT	13	2	1	4	0	2	0	1	1	0	0
AflII	CTTAAG	4	3	0	0	0	0	0	0	0	0	19
AflIII	ACRYGT	25	20	3	1	4	2	5	3	4	1	23
AgeI (•)	ACCGGT	5	13	0	0	1	0	1	1	0	0	2
AhdI	GACNNNNNGTC	9	9	0	1	1	2	1	2	2	1	14
AleI	CACNNNNGTG	10	20	1	0	1	0	1	0	2	0	8
AluI	AGCT	158	143	27	17	38	28	27	30	31	16	140
AlwI	GGATC	35	58	3	12	18	12	20	15	17	10	1
AlwNI	CAGNNNCTG	25	41	1	1	5	2	4	1	2	1	15
ApaI	GGGCCC	12	1	0	0	0	1	1	1	1	0	0
ApaLI	GTGCAC	7	4	0	3	3	6	4	4	4	3	1
ApeKI	GCWGC	179	199	10	21	27	25	20	27	26	12	116
ApoI (•)	RAATTY	29	58	11	0	19	5	3	5	6	1	13
AscI	GGCGCGCC	2	2	0	0	0	0	1	0	0	0	0
AseI	ATTAAT	3	17	7	1	5	4	7	10	10	3	12
AsiSI	GCGATCGC	1	0	0	0	0	0	0	0	0	0	0
AvaI	CYCGRG	40	8	2	1	2	1	2	3	1	1	4
AvaII	GGWCC	73	35	1	8	7	9	6	6	7	2	54
AvrII	CCTAGG	2	2	0	0	0	0	1	0	0	0	3
BaeGI	GKGCMC	45	10	1	3	8	8	8	6	5	3	16
BaeI	ACNNNNGTAYC	5	10	3	0	1	0	0	0	1	0	3
BamHI (•)	GGATCC	3	5	1	1	1	1	1	1	1	1	0
BanI	GGYRCC	57	25	7	9	7	4	8	8	7	4	33
BanII	GRGCYC	57	7	2	2	5	2	5	3	4	1	1
BbsI (•)	GAAGAC	27	24	0	3	3	3	2	4	5	0	38
BbvCI	CCTCAGC	9	7	2	0	0	0	0	0	0	0	10
BbvI	GCAGC	179	199	10	21	27	25	20	27	26	12	116
BccI	CCATC	62	145	14	9	22	16	8	14	20	3	121
BceAI	ACGGC	80	115	7	3	13	11	8	13	12	2	47
BcgI	CGANNNNNNTGC	10	28	0	3	6	4	1	4	6	1	19
BciVI	GTATCC	9	26	0	2	3	4	3	4	4	2	23
BclI (•)	TGATCA	5	8	0	0	2	2	1	1	2	0	1
BcoDI	GTCTC	60	37	5	3	11	8	4	8	9	4	95
BfaI	CTAG	54	13	5	5	19	3	14	8	8	4	60
BfuAI	ACCTGC	39	41	3	1	5	4	4	2	3	1	18
BfuCI (x)	GATC	87	116	6	22	35	23	31	24	27	15	6
BglI	GCCNNNNNGGC	20	29	1	3	3	1	7	2	2	2	2
BglII	AGATCT	11	6	1	0	1	1	2	0	1	0	1
BlpI	GCTNAGC	8	6	0	0	0	1	0	1	1	0	20
BmgBI	CACGTC	15	17	0	0	1	1	2	0	0	0	8
BmrI	ACTGGG	22	4	1	5	2	5	6	11	11	2	6
BmtI (•)	GCTAGC	4	1	0	1	4	0	1	1	1	0	1
BpmI	CTGGAG	32	25	2	4	3	4	1	5	6	1	23
Bpu10I	CCTNAGC	23	19	4	1	0	1	2	0	2	0	39
BpuEI	CTTGAG	19	13	4	6	7	5	9	7	9	4	56
BsaI (•)	GGTCTC	18	2	0	1	3	2	2	2	1	1	29
BsaAI	YACGTR	22	14	5	1	4	0	3	2	4	0	35
BsaBI	GATNNNNATC	2	21	2	1	2	2	1	1	2	0	7
BsaHI	GRCGYC	44	40	1	6	6	5	8	12	8	3	8
BsaJI	CCNNGG	234	105	9	8	18	10	17	15	16	5	85
BsaWI	WCCGGW	28	81	6	5	8	7	5	8	6	3	32

Frequencies of Restriction Sites (continued)

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
BsaXI	ACNNNNNCTCC	29	19	4	0	3	1	1	2	3	1	12
BseRI	GAGGAG	63	19	1	0	2	0	3	0	0	0	13
BseYI	GCTGGG	31	32	3	2	3	4	5	4	4	1	29
BsgI	GTGCAG	34	41	0	1	4	6	3	5	4	0	21
BsiEI	CGRYCG	50	22	3	7	11	8	6	9	7	5	17
BsiHKAI	GWGCWC	38	28	3	8	8	9	9	7	7	5	24
BsiWI (•)	CGTACG	4	1	0	0	0	1	0	1	0	0	0
BslI	CCNNNNNNNGG	216	176	17	20	18	16	26	31	27	6	90
BsmI	GAATGC	10	46	1	1	3	1	5	1	0	0	15
BsmBI	CGTCTC	21	14	1	1	2	2	0	2	2	2	16
BsmFI	GGGAC	59	38	2	4	4	1	5	4	4	0	46
BsoBI	CYCGRG	40	8	2	1	2	1	2	3	1	1	4
Bsp1286I	GDGCHC	105	38	5	10	16	11	15	11	10	5	40
BspCNI	CTCAG	75	80	24	7	10	10	9	20	23	5	142
BspDI	ATCGAT	2	15	2	1	2	0	0	0	0	0	3
BspEI	TCCGGA	8	24	0	1	1	2	0	1	1	0	0
BspHI	TCATGA	3	8	1	4	2	1	2	2	2	3	13
BspMI	ACCTGC	39	41	3	1	5	4	4	2	3	1	18
BspQI	GCTCTTC	7	10	0	1	2	1	3	1	1	1	4
BspUI(x)	GCSGC	232	181	7	21	25	18	27	22	23	7	40
BsrI	ACTGG	86	110	19	18	23	26	19	32	30	11	118
BsrBI	CCGCTC	28	17	4	2	6	4	6	9	9	3	17
BsrDI	GCAATG	14	44	3	2	7	4	4	4	4	2	18
BsrFI	RCCGGY	40	61	1	7	9	2	6	11	7	1	3
BsrGI (•)	TGTACA	5	5	1	0	1	0	1	1	2	0	13
BssHII	GCGCGC	52	6	0	0	1	2	2	1	1	0	1
BssKI (x)	CCNGG	233	185	11	16	25	27	28	46	42	12	11
BssSI	CACGAG	11	8	0	3	5	3	4	2	4	3	31
BstAPI	GCANNNNNTGC	20	34	0	2	3	2	0	3	2	1	12
BstBI	TTCGAA	1	7	0	0	2	0	0	0	1	0	7
BstEII (•)	GGTNACC	10	13	0	0	1	1	0	1	1	0	1
BstNI	CCWGG	136	71	7	6	15	14	19	19	19	5	2
BstUI	CGCG	303	157	17	23	26	31	19	41	35	10	65
BstXI	CCANNNNNNTGG	10	13	0	0	2	4	1	4	3	0	11
BstYI	RGATCY	22	21	2	8	12	9	11	10	12	7	2
BstZ17I	GTATAC	3	3	0	1	3	0	1	1	1	0	8
Bsu36I	CCTNAGG	7	2	1	0	1	1	1	0	0	0	30
BtgI	CCRYGG	82	46	2	2	4	3	6	1	3	0	26
BtgZI	GCGATG	23	45	4	3	4	6	6	4	3	0	24
BtsI	GCAGTG	22	34	1	2	7	5	5	4	4	3	20
BtsCI	GGATG	78	150	4	12	20	17	7	12	12	5	97
Cac8I	GCNNGC	285	238	28	31	33	32	41	49	45	14	104
ClaI	ATCGAT	2	15	2	1	2	0	0	0	0	0	3
CspCI	CAANNNNNGTGG	6	7	1	0	1	0	1	0	0	0	9
CviAII	CATG	183	181	14	26	38	23	21	23	29	11	148
CviKI-1	RGCY	680	692	103	73	131	86	119	112	116	45	562
CviQI	GTAC	83	113	19	3	15	7	14	6	10	3	168
DdeI	CTNAG	97	104	30	8	17	11	11	20	26	6	282
DpnI	GATC	87	116	6	22	35	23	31	24	27	15	6
DpnII	GATC	87	116	6	22	35	23	31	24	27	15	6
DraI	TTTAAA	12	13	5	3	5	1	6	3	3	3	9
DraIII (•)	CACNNNGTG	10	10	1	0	2	0	3	1	1	0	16
DrdI	GACNNNNNNGTC	6	3	1	2	5	2	2	4	4	2	11
EaeI	YGGCCR	70	39	3	6	10	5	15	4	5	3	2
EagI	CGGCCG	19	2	0	1	4	1	2	2	2	0	0
EarI	CTCTTC	29	34	2	2	11	6	4	3	4	3	46
EciI	GGCGGA	29	32	2	4	6	6	8	9	11	3	2
Eco53KI	GAGCTC	16	2	1	0	2	1	2	0	1	1	0
EcoNI	CCTNNNNNAGG	10	9	0	1	3	0	0	2	1	0	1
EcoO109I	RGGNCCY	44	3	0	4	1	2	5	1	2	1	22
EcoP15I	CAGCAG	50	72	4	7	7	10	6	6	5	3	36
EcoRI (•)	GAATTC	5	5	1	1	1	1	1	1	1	1	0
EcoRV (•)	GATATC	9	21	0	1	1	1	1	1	1	0	0
Esp3I	CGTCTC	21	14	1	1	2	2	0	2	2	2	16
FatI	CATG	183	181	14	26	38	23	21	23	29	11	148

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
FauI	CCCGC	147	90	10	10	14	17	11	28	28	5	24
Fnu4HI	GCNGC	411	380	17	42	52	43	47	49	49	19	156
FokI	GGATG	78	150	4	12	20	17	7	12	12	5	97
FseI	GGCCGGCC	3	0	0	0	0	0	0	0	0	0	0
FspI	TGCGCA	17	15	1	4	3	2	2	1	1	2	7
HaeII	RGCGCY	76	48	6	11	6	9	3	7	7	3	26
HaeIII	GGCC	216	149	15	22	31	23	36	34	36	11	68
HgaI	GACGC	87	102	7	11	10	12	7	20	18	4	70
HhaI	GCGC	375	215	26	31	36	39	27	41	39	17	103
HinP1I	GCGC	375	215	26	31	36	39	27	41	39	17	103
HincII	GTYRAC	25	35	1	2	9	7	4	7	6	1	61
HindIII (•)	AAGCTT	12	6	1	1	1	1	4	0	1	1	0
HinfI	GANTC	72	148	26	10	31	9	11	16	20	6	218
HpaI	GTTAAC	6	14	0	0	3	1	1	1	2	0	18
HpaII	CCGG	171	328	18	26	32	25	24	50	40	13	58
HphI	GGTGA	99	168	18	12	15	19	14	21	21	7	102
Hpy99I	CGWCG	61	102	8	9	14	9	13	18	14	5	29
Hpy166II	GTNNAC	116	125	10	8	29	20	13	27	28	5	199
Hpy188I	TCNGA	80	170	31	15	24	19	17	19	26	10	153
Hpy188III	TCNNGA	103	185	28	19	32	22	25	27	29	13	173
HpyAV	CCTTC	84	106	14	10	24	14	11	16	18	6	110
HpyCH4III	ACNGT	122	187	31	14	25	20	15	18	17	8	174
HpyCH4IV	ACGT	83	143	22	10	21	10	19	23	26	5	170
HpyCH4V	TGCA	207	273	18	21	39	28	30	26	25	13	116
KasI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
KpnI (•)	GGTACC	8	2	1	0	0	0	2	0	1	1	5
MboI	GATC	87	116	6	22	35	23	31	24	27	15	6
MboII	GAAGA	113	130	10	11	38	15	14	14	17	8	140
MfeI (•)	CAATTG	4	8	0	0	2	1	2	1	1	0	8
MluI (•)	ACGCGT	5	7	0	0	0	1	2	2	1	0	1
MluCI	AATT	87	189	62	8	43	22	19	31	44	7	79
MlyI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
MmeI	TCCRAC	25	18	3	4	8	3	5	4	4	2	33
MnlI	CCTC	397	262	62	26	56	24	41	35	39	13	342
MscI	TGGCCA	17	18	1	1	0	1	2	0	1	0	2
MseI	TTAA	115	195	63	15	41	24	23	32	41	13	207
MslI	CAYNNNNRTG	35	62	3	7	10	10	6	7	9	3	38
MspA1I	CMGCKG	95	75	4	6	14	11	8	10	11	6	35
MspI	CCGG	171	328	18	26	32	25	24	50	40	13	58
MwoI	GCNNNNNNNGC	391	347	19	34	33	30	42	41	35	13	170
NaeI	GCCGGC	13	1	1	4	3	0	2	5	5	0	0
NarI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
NciI	CCSGG	97	114	4	10	10	13	9	27	23	7	9
NcoI (•)	CCATGG	20	4	0	0	1	1	3	0	1	0	1
NdeI	CATATG	2	7	3	1	1	1	1	1	1	1	7
NgoMIV	GCCGGC	13	1	1	4	3	0	2	5	5	0	0
NheI (•)	GCTAGC	4	1	0	1	4	0	1	1	1	0	1
NlaIII	CATG	183	181	14	26	38	23	21	23	29	11	148
NlaIV	GGNNCC	178	82	18	24	22	14	20	22	24	11	99
NmeAIII	GCCGAG	17	8	0	3	2	3	2	2	3	1	14
NotI (•)	GCGGCCGC	7	0	0	0	1	1	1	1	1	0	0
NruI (•)	TCGCGA	5	5	0	1	1	0	1	1	0	0	3
NsiI (•)	ATGCAT	9	14	0	0	6	0	1	0	0	0	8
NspI	RCATGY	41	32	6	4	9	3	5	5	6	3	24
Nt.BstNBI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
Nt.CviPII	CCD	4148	4641	570	457	806	570	609	716	743	251	3575
PacI	TTAATTAA	1	0	1	0	0	0	1	0	0	0	1
PaeR7I	CTCGAG	6	1	0	0	1	0	1	1	0	0	0
PciI	ACATGT	9	2	3	1	3	1	2	1	1	1	6
PflFI	GACNNNGTC	12	2	0	1	1	1	1	1	2	0	1
PflMI	CCANNNNNTGG	18	14	0	2	3	1	5	2	3	0	8
PhoI (x)	GGCC	216	149	15	22	31	23	36	34	36	11	68
PleI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
PluTI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
PmeI	GTTTAAAC	1	2	0	0	0	0	1	1	1	0	2
PmlI	CACGTG	10	3	0	0	0	0	1	0	1	0	1

Frequencies of Restriction Sites (continued)

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
PpuMI	RGGWCCY	23	3	0	2	1	2	1	0	0	0	12
PshAI	GACNNNNGTC	2	7	0	1	1	0	0	1	2	0	6
PsiI	TTATAA	4	12	2	0	2	1	1	1	1	0	5
PspGI	CCWGG	136	71	7	6	15	14	19	19	19	5	2
PspOMI	GGGCCC	12	1	0	0	0	1	1	1	1	0	0
PspXI	VCTCGAGB	3	1	0	0	0	0	1	1	0	0	0
PstI	CTGCAG	30	28	1	1	3	1	4	1	1	1	0
PvuI (•)	CGATCG	7	3	1	1	3	2	1	1	1	2	0
PvuII (•)	CAGCTG	24	15	3	1	3	3	3	3	3	2	3
RsaI	GTAC	83	113	19	3	15	7	14	6	10	3	168
RsrII	CGGWCCG	2	5	0	0	0	1	1	0	0	0	1
SacI (•)	GAGCTC	16	2	1	0	2	1	2	0	1	1	0
SacII	CCGCGG	33	4	0	0	2	0	1	1	1	0	0
SalI (•)	GTCGAC	3	2	1	1	1	1	1	4	1	1	0
SapI	GCTCTTC	7	10	0	1	2	1	3	1	1	1	4
Sau3AI	GATC	87	116	6	22	35	23	31	24	27	15	6
Sau96I	GGNCC	164	74	4	15	14	20	21	26	28	6	79
SbfI (•)	CCTGCAGG	3	5	1	0	1	1	1	0	1	1	0
ScaI (•)	AGTACT	5	5	0	1	2	1	2	1	2	1	4
ScrFI	CCNGG	233	185	11	16	25	27	28	46	42	12	11
SexAI	ACCWGGT	9	5	0	0	3	0	0	0	0	0	0
SfaNI	GCATC	85	169	7	22	18	20	17	23	19	8	96
SfcI	CTRYAG	47	38	7	4	9	4	10	6	7	4	48
SfiI	GGCCNNNNNGGCC	3	0	0	0	0	0	1	0	0	0	1
SfoI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
SgrAI	CRCCGGYG	6	6	0	1	0	0	0	1	0	0	0
SmaI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
SmlI	CTYRAG	29	17	4	6	8	5	10	8	9	4	75
SnaBI	TACGTA	0	1	1	0	1	0	1	0	0	0	13
SpeI (•)	ACTAGT	3	0	0	0	0	0	1	1	1	0	2
SphI (•)	GCATGC	8	6	1	1	2	0	2	2	2	1	0
SspI (•)	AATATT	5	20	6	1	6	2	1	3	5	1	6
StuI	AGGCCT	11	6	0	0	1	0	0	1	1	0	1
StyI (•)	CCWWGG	44	10	0	1	4	1	4	2	4	0	36
StyD4I	CCNGG	233	185	11	16	25	27	28	46	42	12	11
SwaI	ATTTAAAT	1	0	1	0	0	0	1	1	1	0	1
TaqI	TCGA	50	121	12	7	32	16	15	22	20	4	111
TatI(x)	WGTACW	19	24	5	2	5	1	8	3	6	2	37
TfiI	GAWTC	32	87	18	6	14	4	5	5	10	2	103
TseI	GCWGC	179	199	10	21	27	25	20	27	26	12	116
Tsp45I	GTSAC	73	81	9	9	9	7	5	12	11	4	108
TspMI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
TspRI	CASTG	83	119	9	11	22	14	16	16	14	10	94
Tth111I	GACNNNGTC	12	2	0	1	1	1	1	1	2	0	1
XbaI	TCTAGA	5	1	1	0	1	0	1	1	1	1	3
XcmI	CCANNNNNNNNTGG	14	12	0	0	1	3	0	3	4	0	8
XhoI	CTCGAG	6	1	0	0	1	0	1	1	0	0	0
XmaI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
XmnI	GAANNNNTTC	5	24	2	2	3	1	3	7	8	1	12
ZraI	GACGTC	3	10	0	1	0	0	5	1	0	1	1

Lambda

48,502 base pairs
GenBank Accession #: NC_001416
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AsiSI, FseI, I-CeuI, I-SceI, NotI, PI-PspI, PI-SceI, PacI, SfiI, SpeI, SrfI(x), SwaI

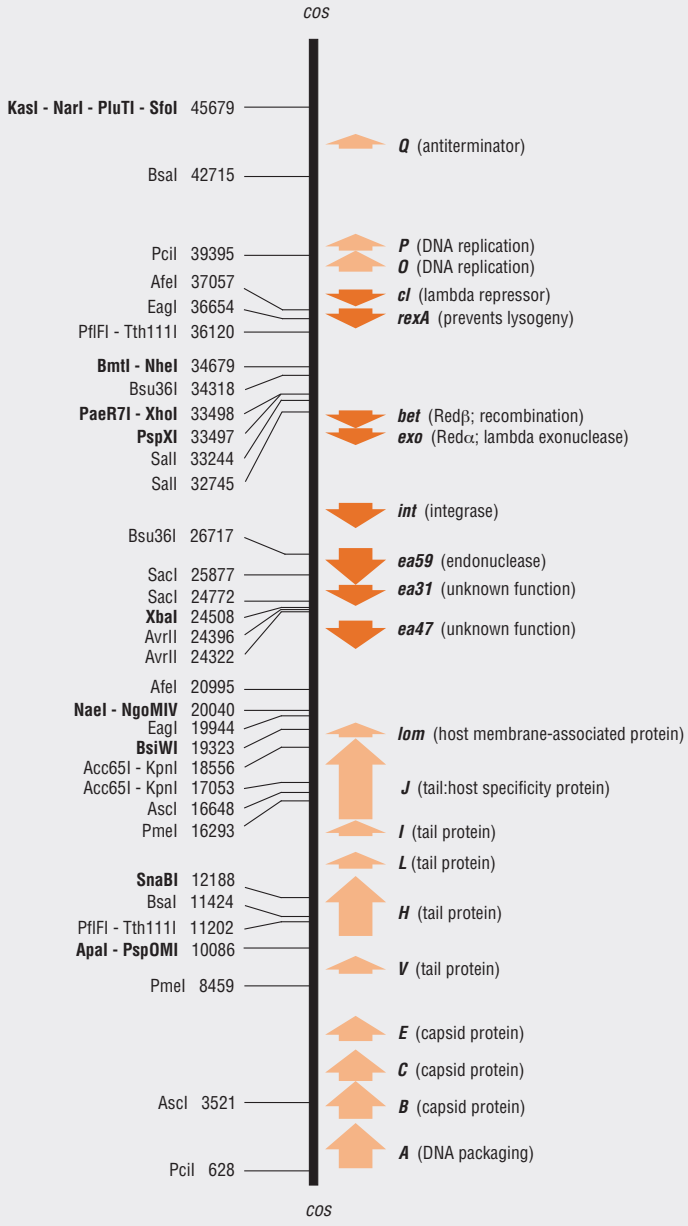
(x) = enzyme not available from NEB

Lambda (λ) is a large, temperate *E. coli* bacteriophage with a linear, largely double-stranded DNA genome (1-5). At each end, the 5´ strand overhangs the 3´ strand by 12 bases. These single-stranded overhangs are complementary and anneal to form a *cos* site following entry into a host cell. Once annealed, the genome is a circular, completely double-stranded molecule which serves as a template for rolling-circle replication.

Many laboratory strains of lambda are derivatives of the strain λ *c*I857*ind*1 *Sam*7, which contains four point mutations relative to the wild type strain. The *ind*1 mutation in the *c*I gene creates a new HindIII site at 37584 not present in the wild type. All lambda products sold by NEB are λ *c*I857*ind*1 *Sam*7.

Numbering of the genome sequence begins at the first (5´-most) base of the left end (bottom of diagram below) and continues rightward from late genes *nu1* and *A* towards the early genes. The map below shows the positions of all known ORFs larger than 200 codons.

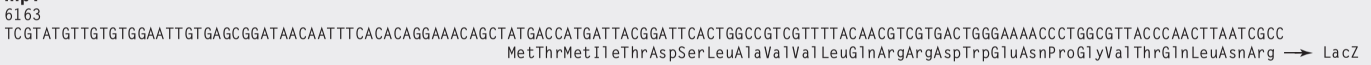
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.



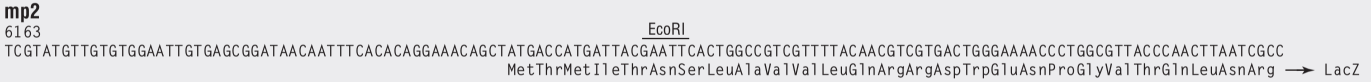
References

(1) Echols, H. and Murialdo, H. (1978) *Microbiol. Rev.,* 42, 577–591.
(2) Szybalski, E.H. and Szybalski, W. (1979) *Gene,* 7, 217–270
(3) Daniels, D.L., de Wet, J.R. and Blattner, F.R. (1980) *J. Virol.,* 33, 390–400.
(4) Sanger, F., Coulson, A.R., Hong, G.F., Hill, D.F. and Petersen, G.B. (1982) *J. Mol. Biol.,* 162, 729–773.
(5) Daniels, D.L. et al. (1983). In R.W. Hendrix, J.W. Roberts, F.W. Stahl and R.A. Weisberg (Eds.), *Lambda II: Appendix,* New York: Cold Spring Harbor Press.

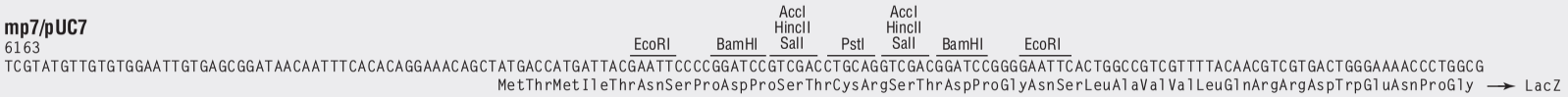
mp1



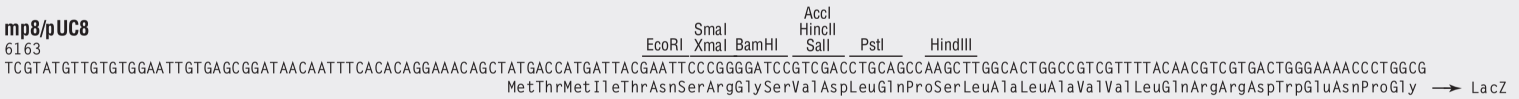
mp2



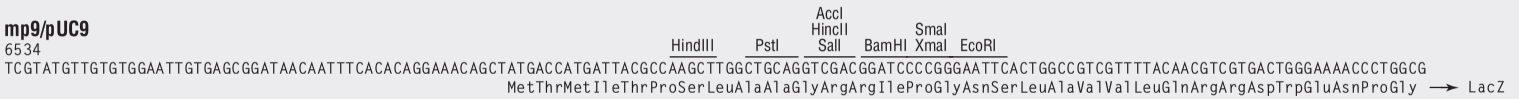
mp7/pUC7



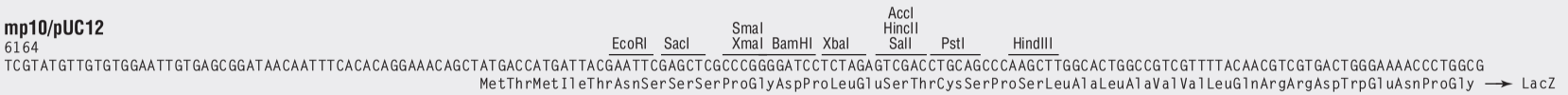
mp8/pUC8



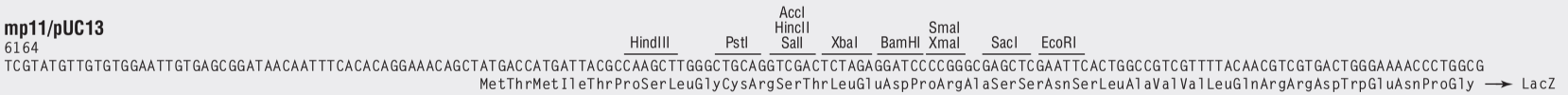
mp9/pUC9



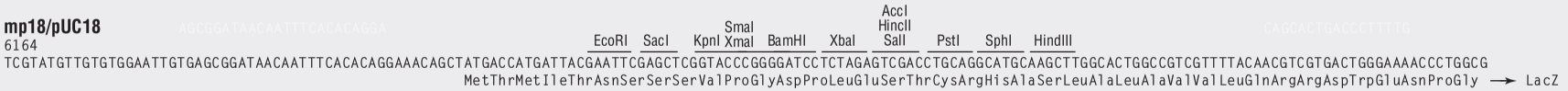
mp10/pUC12



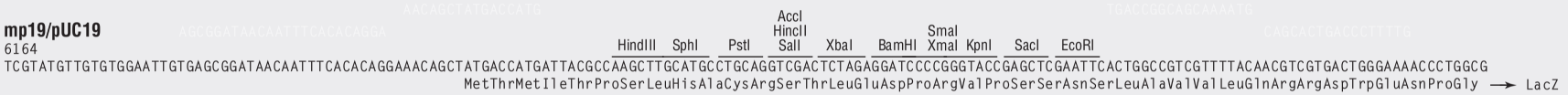
mp11/pUC13



mp18/pUC18



mp19/pUC19



M13mp18

GenBank Accession #: X02513
Revised sequence file available at www.neb.com.
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), AatII, AcuI, AflII, AgeI, AhdI, ApaI, ApaLI, AscI, AsiSI, AvrII, BbsI, BcgI, BciVI, BclI, BlpI, BmgBI, BmtI, BsaI, BsgI, BsiWI, BspEI, BspQI, BssHII, BssSI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, EagI, EcoNI, EcoO109I, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, NcoI, NheI, NmeAIII, NotI, NruI, NsiI, PI-PspI, PI-SceI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SapI, ScaI, SexAI, SfiI, SgrAI, SpeI, SrfI(x), StuI, StyI, Tth111I, XcmI, XhoI, ZraI

(x) = enzyme not available from NEB

M13 is a filamentous *E. coli* bacteriophage specific for male (F factor-containing) cells. Its genome is a circular, single-stranded DNA molecule 6407 bases in length, and contains 10 genes. A double-stranded form (RF) arises as an intermediate during DNA replication.

The M13mp phage vectors, derived from M13, contain the *lacZ*α gene and differ from each other by the cloning sites embedded within it. The location of cloning sites inside this gene allows screening for insertions using α-complementation. The map of M13mp18, whose multiple cloning site (MCS) was later employed to construct the plasmid pUC19, is shown below; sequences of the MCS region from other M13mp vectors are shown on the previous page. M13mp19 is identical to M13mp18 except that the MCS region (6231-6288) is inverted.

The complete nucleotide sequences of M13mp18 and M13mp19 have recently been determined at New England Biolabs (1), resulting in several nucleotide changes relative to the previous sequence data (2,3).

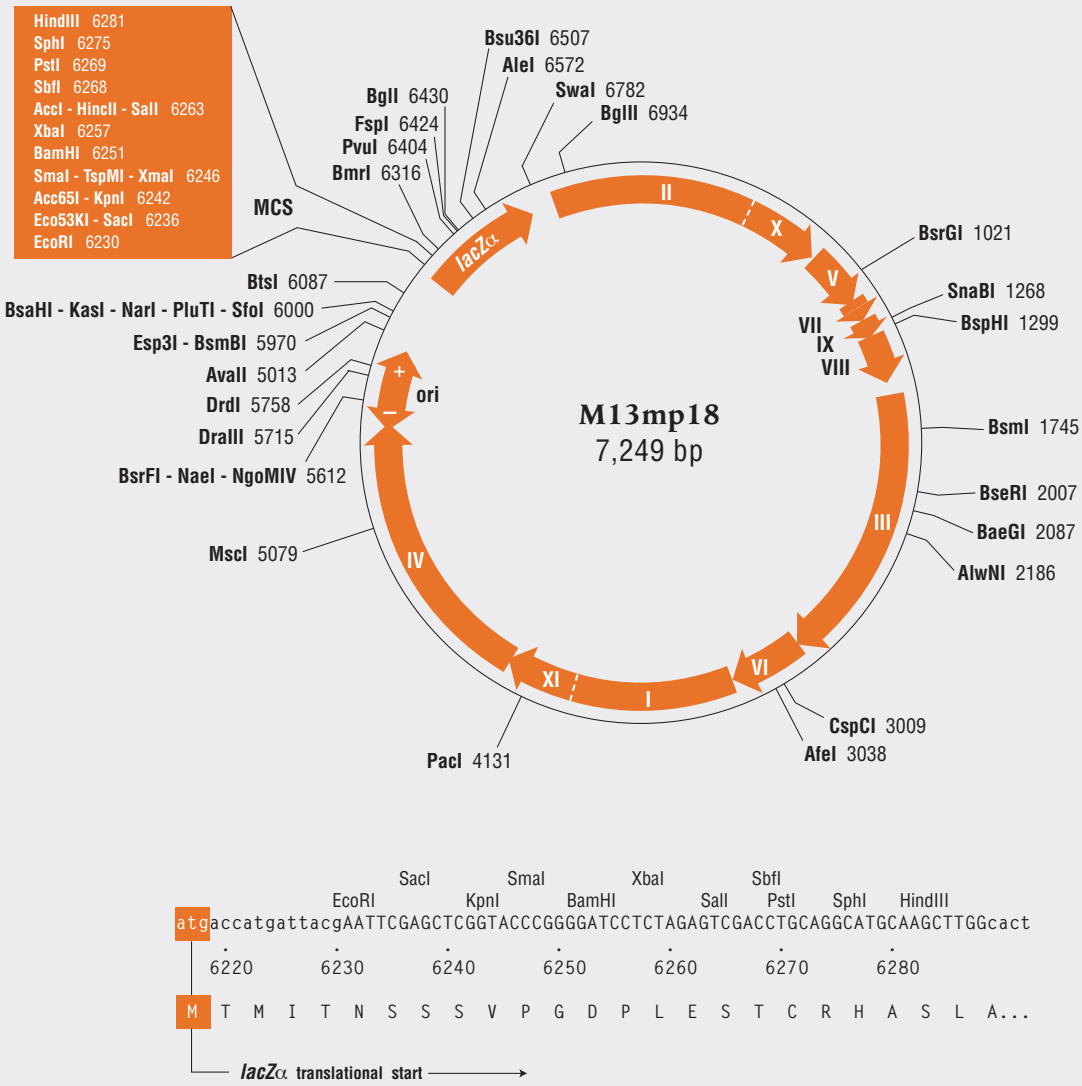
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

M13 origin of replication arrows indicate the direction of synthesis of both the (+) and (–) strands.

Feature	Description	Coordinates
gene II	replication	6848-831 (cw)
gene X	replication	496-831
gene V	replication	843-1106
gene VII	minor coat protein	1108-1209
gene IX	minor coat protein	1206-1304
gene VIII	major coat protein	1301-1522
gene III	minor coat protein	1578-2852
gene VI	minor coat protein	2855-3193
gene I	phage assembly	3195-4241
gene XI (I*)	phage assembly	3915-4241
gene IV	phage assembly	4219-5499
ori	M13 origin (+) of replication	5487-5867
*lacZ*α	for α-complementation	6216-6722
MCS	multiple cloning site	6230-6286

(cw) = clockwise




```
                                  SacI        SmaI        XbaI        SbfI
                          EcoRI       KpnI        BamHI       SalI   PstI   SphI   HindIII
atgaccatgattacgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcact
  .       .       .       .       .       .       .       .       .       .       .
6220    6230    6240    6250    6260    6270    6280

M  T  M  I  T  N  S  S  S  V  P  G  D  P  L  E  S  T  C  R  H  A  S  L  A...
```

*lacZ*α translational start →

References

(1) Stewart, F.J. (2002) unpublished observations.
(2) Messing, J. et al. (1977) *Proc. Natl. Acad. Sci.* USA, 74, 3652–3646.
(3) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene,* 33, 103–119.

pBR322

GenBank Accession #: J01749
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AflII, AgeI, AleI, ApaI, ApuI, AscI, AsiSI, AvrII, BaeI, BbvCI, BclI, BglII, BlpI, BmgBI, BsaXI, BseRI, BsiWI, BsrGI, BssHII, BstBI, BstEII, BstXI, Bsu36I, CspCI, DraIII, Eco53KI, FseI, HpaI, I-CeuI, I-SceI, KpnI, MfeI, MluI, NcoI, NotI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PsiI, PspOMI, PspXI, RsrII, SacI, SacII, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SpeI, SrfI(x), StuI, SwaI, TspMI, XbaI, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–3). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 20 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

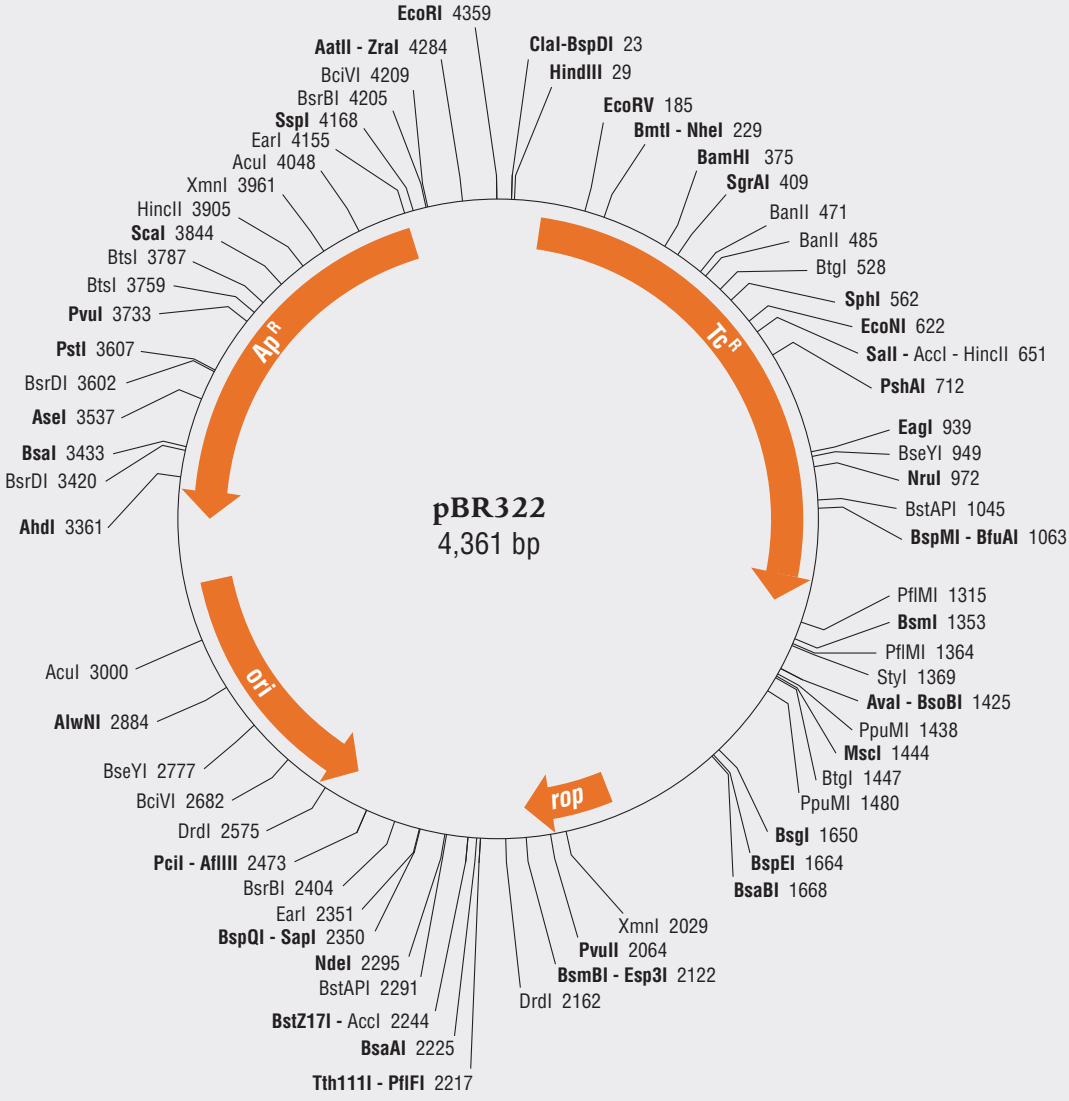
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
tet (Tc^R)	86-1276	pSC101
bla (Ap^R)	4153-3293	*Tn3*
rop	1915-2106	pMB1
origin	3122-2534	pMB1

ori = origin of replication
Ap = ampicillin, Tc = tetracycline



References

(1) Bolivar, F. et al. (1977) *Gene,* 2, 95–113.
(2) Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.,* 43, 77–90.
(3) Watson, N. (1988) *Gene,* 70, 399–403.
(4) Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

pKLAC2

GenBank Accession #: EU196354
See page 215 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AfeI, AflII, ApaI, AscI, AsiSI, AvrII, BbvCI, BlpI, Bpu10I, BsiWI, FseI, FspAI(x), I-CeuI, I-SceI, KpnI, MluI, MscI, PacI, PI-PspI, PI-SceI, PmeI, PmlI, PspOMI, PspXI, RsrII, SanDI(x), SfiI, SgrAI, SpeI, SrfI(x), SwaI, ZraI

(x) = enzyme not available from NEB

pKLAC2 is an expression vector capable both of replication in *E. coli* and stable integration into the genome of the yeast *Kluyveromyces lactis* (1). It is designed for high-level expression of recombinant protein in *K. lactis* using the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. Upon transformation of *K. lactis* GG799 competent cells (NEB #C1001), SacII- or BstXI-linearized pKLAC2 integrates into the *K. lactis* chromosome at the *LAC4* locus. Yeast transformants can be selected using the acetamidase selectable marker (*amdS*), which is expressed from the yeast *ADH1* promoter. Acetamidase expressed from pKLAC2 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium (2).

The multiple cloning site (MCS) is positioned to allow translational fusion of the *K. lactis* α-mating factor secretion domain (α-MF) to the N-terminus of the recombinant target protein. This directs the fusion protein to the general secretory pathway, but the α-MF domain is cleaved off in the Golgi apparatus by the Kex protease, resulting in secretion of the recombinant protein alone.

Expression of the recombinant fusion protein is driven by the *K. lactis LAC4* promoter, which has been modified to be transcriptionally silent in *E. coli* (1). This facilitates the cloning of proteins that are toxic to *E. coli*. This promoter is split such that when pKLAC2 is cleaved with SacII or BstXI, the recombinant protein and selectable marker are flanked by the two halves of the promoter. When these ends recombine with the *LAC4* promoter in the *K. lactis* chromosome, the result is integration of the recombinant fusion protein (driven by the *LAC4* promoter) and *amdS* upstream of the *LAC4* gene (driven by a duplicate copy of the *LAC4* promoter) (2).

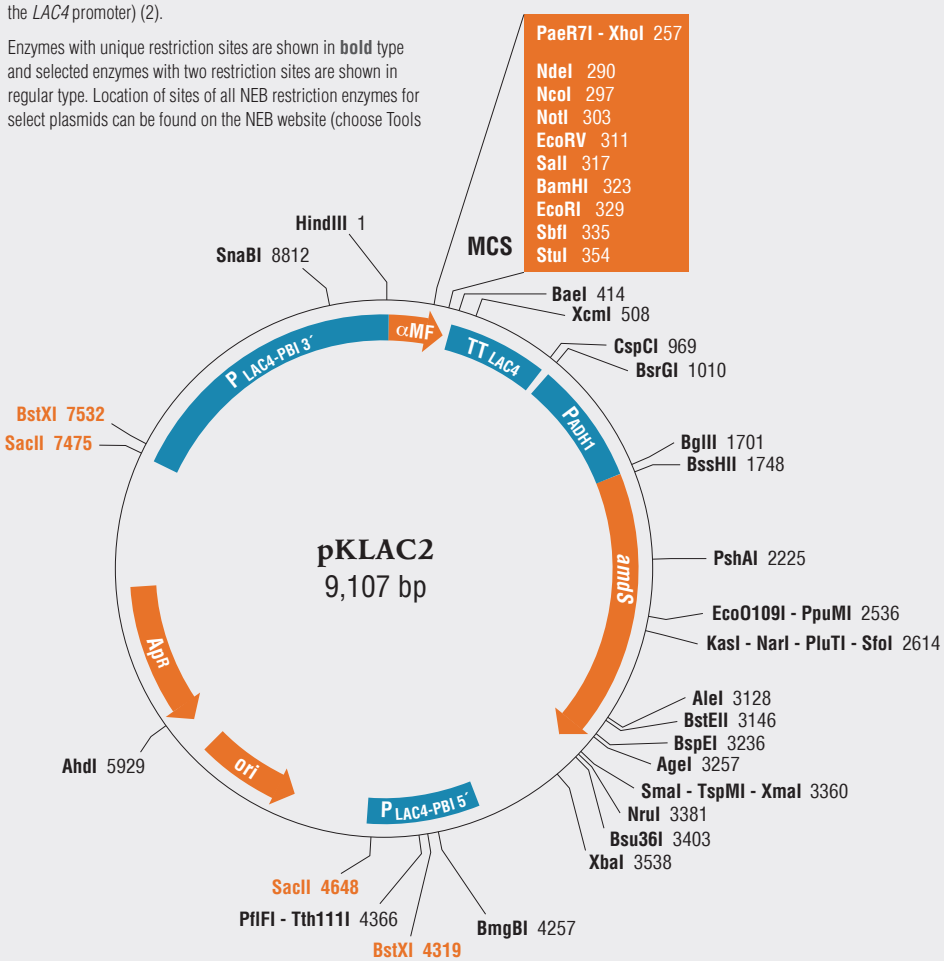
Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Components of coordinated regions are indented below the region itself.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Promoter and transcription terminator coordinates represent cloned regions and not necessarily the precise functional elements.

Feature	Coordinates	Source
expression region:		
α-mating factor		
leader sequence	14-349	*K. lactis*
MCS	257-354	–
LAC4 TT region	371-953	*K. lactis*
AdH1 promoter region	1010-1712	*S. cerevisiae*
amdS	1713-3359	*A. nidulans*
LAC4 promoter		
region (5´ end)	4068-4648	*K. lactis*
origin	5102-5690	pMB1
bla (Ap^R)	6721-5861	Tn3
LAC4 promoter		
region (3´ end)	7475-9107	*K. lactis* (modified)

ori = origin of replication
Ap = ampicillin
TT = transcription terminator



References
(1) Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
(2) van Ooyen, A.J. et al. (2006) *FEMS Yeast Res.*, 6, 381–392.

pMAL-p5X

Sequence file available at www.neb.com.
See page 213 for ordering information.

Feature	Coordinates	Source
lacI^q	81-1163	*E. coli*
P_{tac}	1406-1433	–
expression ORF	1528-2832	–
malE	1528-2703	*E. coli*
MCS	2709-2832	–
bla (Ap^R)	3162-4022	*Tn3*
origin	4110-4698	pMB1
rop	5068-5259	pMB1

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AflII, AgeI, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BmtI, BsaAI, BseRI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, FseI, I-CeuI, I-SceI, KpnI, NaeI, NgoMIV, NheI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PshAI, PspXI, SacII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, SwaI, TspMI, XbaI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pMAL-p5X is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the pMAL Protein Fusion and Purification System (NEB #E8200) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the *malE* gene) to the N-terminus of the cloned target protein. The pMAL-p5 and -c5 series of vectors differs from the -p4 and -c4 series in that they contain a universal multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In addition, *lacZ*α and the M13 origin have been removed. In these vectors, MBP has been engineered for tighter binding to amylose. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using Factor Xa protease (3).

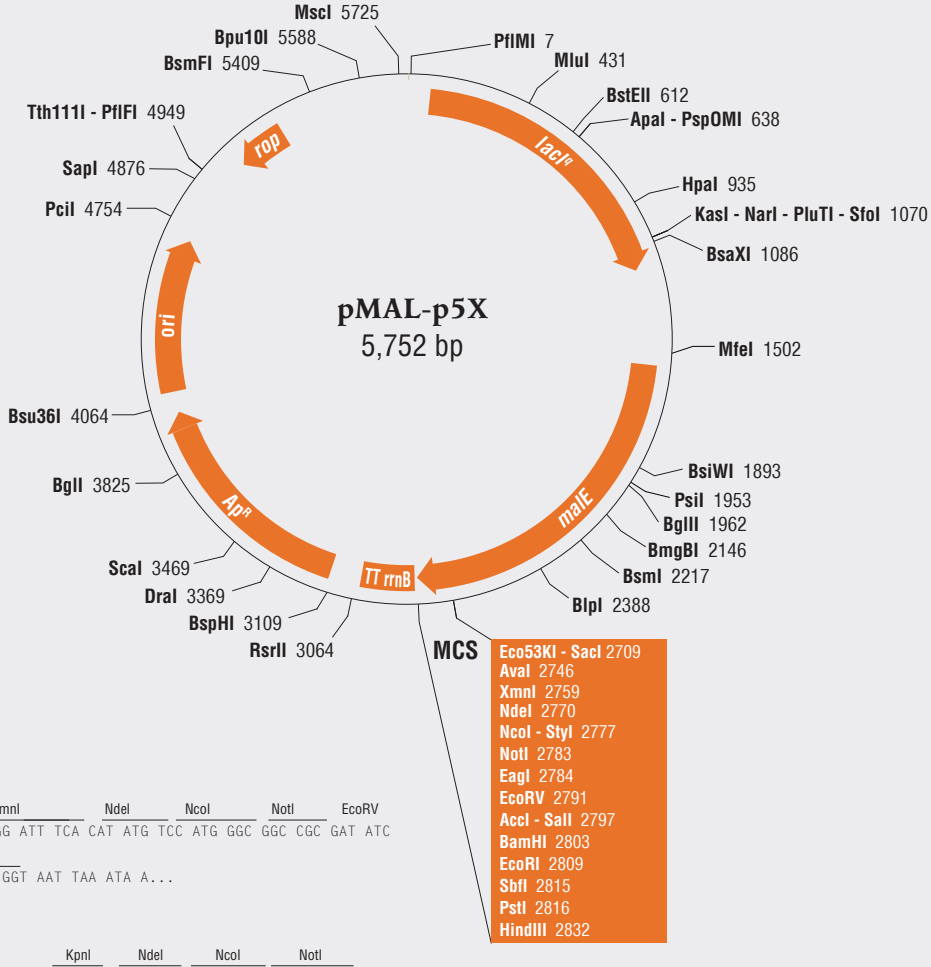
Transcription of the gene fusion is controlled by the inducible "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the *lac* operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

pMAL-c5-series vectors are identical to the pMAL-p5-series vectors above except for a deletion of the *malE* signal sequence (nt 1531-1605) (1).

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point (labeled "ori") and the rop gene, which controls expression of the RNAII transcript. *bla* (Ap^R) gene coordinates include the signal sequence.



pMAL-p5X Polylinker:

```
                 AvaI                    XmnI          NdeI      NcoI      NotI      EcoRV
5´ malE...CTC GGG ATC GAG GGA AGG ATT TCA CAT ATG TCC ATG GGC GGC CGC GAT ATC
   SalI    BamHI   EcoRI     SbfI
GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...
```

pMAL-p5E Polylinker:

```
                 AvaI                                  KpnI      NdeI      NcoI      NotI
5´ malE...CTC GGG GAT GAC GAT GAC AAG GTA CCG CAT ATG TCC ATG GGC GGC CGC
   EcoRV    SalI    BamHI   EcoRI     SbfI
GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...
```

References

(1) Guan, C. et al. (1987) *Gene*, 67, 21–30.
(2) Maina, C.V. et al. (1988) *Gene*, 74, 365–373.
(3) Riggs, P.D. (1992). In F.M. Ausubel, et al. (Eds.), *Current Prot. in Molecular Biol.* New York: John Wiley & Sons, Inc.

pMiniT 2.0

Sequence available at www.neb.com
See page 91 for more information.

Feature	Coordinates	Source
Constitutive promoter	1-214	pNK2138
SP6 promoter	479-496	SP6
Toxic minigene	541-549	–
Synthetic T7 promoter	619-602	T7
bla (Ap^R)	733-1593	*Tn3*
origin	1764-2352	pUC19

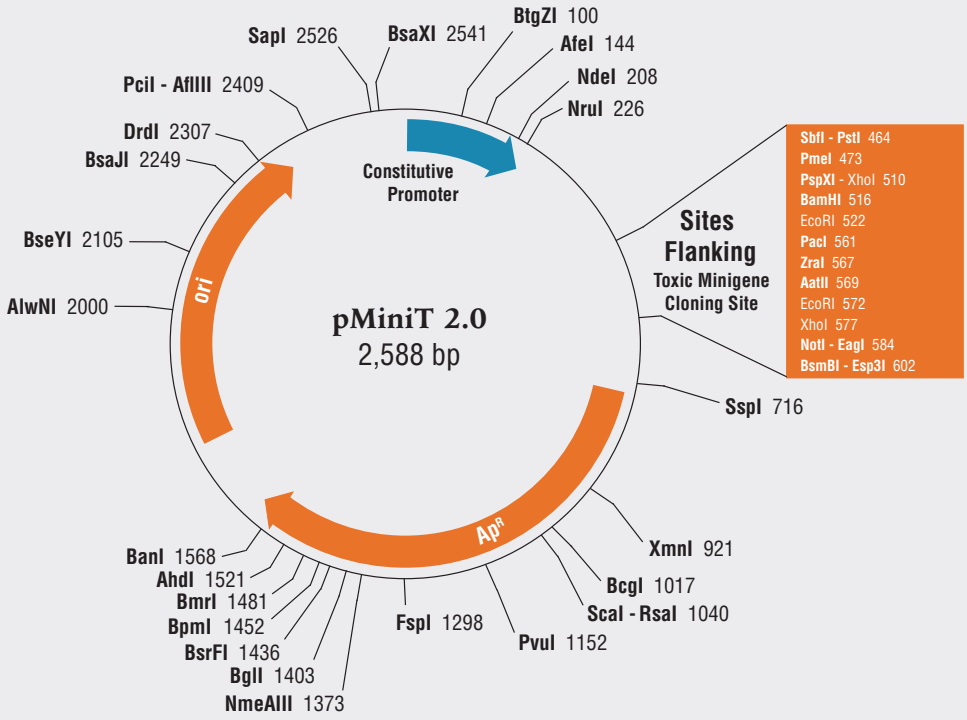
pMiniT 2.0 is an *E. coli* plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT2.0 also enables *in vitro* transcription using SP6 and T7 promoters. It is compatible with Golden Gate Assembly as the BsaI site has been removed from the Ampicillin resistance gene.

In *E. coli*, it replicates using the pMB1 origin of replication from pUC19 and carries the *bla* (ApR) marker for selection with ampicillin. pMiniT2.0 contains a toxic minigene that is under the control of a constitutive promoter. If the pMiniT 2.0 vector recircularizes without an insert, the toxic minigene it will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 Vector carries an insert, a colony will grow.

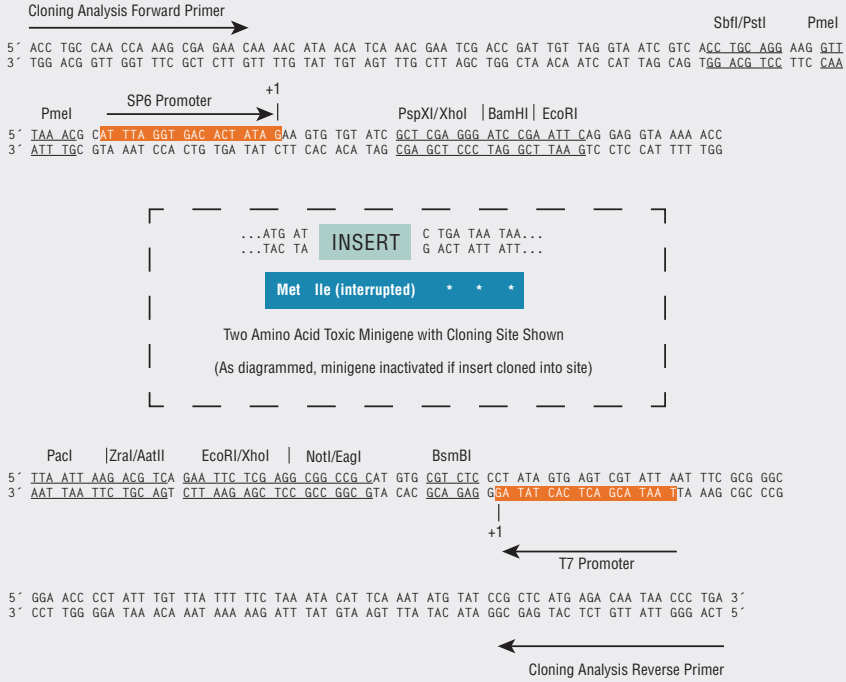
The map shown below displays the construct formed if no insert is present. Unique restriction sites are shown in **bold**. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for *in vitro* transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene.

There are no restriction sites for the following enzymes: AbsI(x), Acc65I, AccI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BanII, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI(x), BmgBI, BmtI, BpiI(x), Bpu10I, BsaI, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, CspCI, DraIII, Eco53kI, EcoNI, EcoO109I, EcoRV, FaiI(x), FseI, FspAI(x), HincII, HindIII, HpaI, KasI, KfiI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NarI, NcoI, NgoMIV, NheI, NsiI, PasI(x), PflFI, PflMI, PfoI(x), PluTI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PsrI(x), PvuII, RsrII, SacI, SacII, SalI, SexAI, SfiI, SfoI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, StyI, SwaI, TspMI, Tth111I, XbaI, XcmI, XmaI

(x) = enzyme not available from NEB



Features within Sequence Flanking the Toxic Minigene/Cloning Site:



pNEB206A

Sequence file available at www.neb.com.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AccI, AfeI, AflII, AgeI, AleI, ApaI, AsiSI, AvaI, AvrII, BaeI, BbsI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, BsaAI, BsaBI, BsgI, BsiWI, BsmFI, BsmI, BsoBI, BspDI, BspEI, BspMI, BsrGI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HincII, HpaI, I-CeuI, I-SceI, KpnI, MfeI, MluI, MscI, NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PaeR7I, PflFI, PflMI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SalI, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, StyI, SwaI, TspMI, Tth111I, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

pNEB206A is an *E. coli* plasmid vector designed for fast and efficient cloning of PCR products to be used in conjunction with USER Enzyme (NEB #M5505; 1). It is derived from pNEB193 containing the high-copy pUC19 origin of replication and *lacZ*α gene for screening of insertions at the cloning site using α-complementation (2).

The plasmid is supplied in a linearized form 2,706 bp in length (with bp 438-453 excised from the circular form), flanked by two noncomplementary 8-base 3´ overhangs at the intended cloning site. Amplification with deoxyuridine-containing primers and subsequent treatment (as defined in the protocol "Cloning with USER Enzyme" found on our website), results in PCR products with 5´ overhangs complementary to those in pNEB206A. These products can be directionally cloned into pNEB206A at high efficiency without the use of restriction enzymes or DNA ligase, forming recombinant circular molecules.

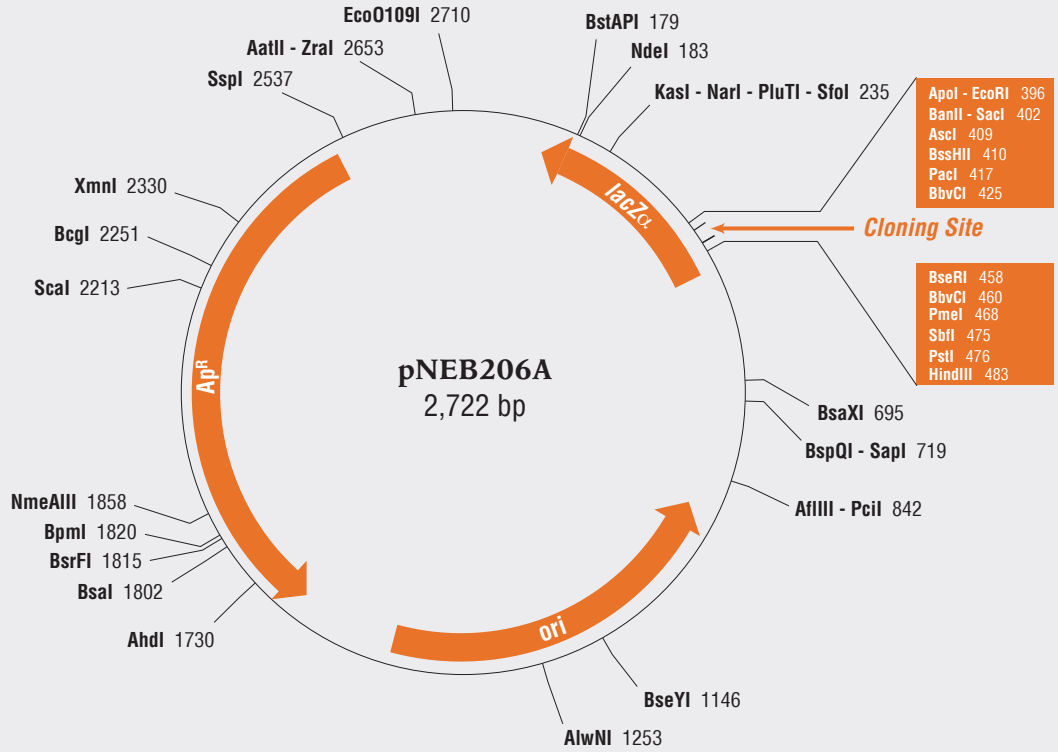
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence. Coordinates on the map and in the tables refer to the 2,722 bp circular plasmid prior to linearization and can be used to calculate relative distances.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence. Cloning site coordinates include those bases in the circular form that are single-stranded in or missing from the supplied linear form.

Feature	Coordinates	Source
*lacZ*α	505-146	–
cloning site	430-461	–
origin	1491-903	pUC19
bla (Ap^R)	2522-1662	*Tn3*

ori = origin of replication
Ap = ampicillin



pNEB206A (linearized form) cloning site:

```
          Eco53KI
            SacI        BssHII
    EcoRI           AscI        PacI        BbvCI
agtgAATTCGAGCTCAGGCGCGCCTTAATTAAGCTGAGGGAAAGT
tcacTTAAGCTCGAGTCCGCGCGGAATTAATTCGACT
      .           .           .           .
    400         410         420         430
...S  N  S  S  L  R  A  K  I  L  S  L  S  L
```

```
                                    SbfI
          BbvCI    PmeI       PstI     HindIII
            TCAGCGTTTAAACCCTGCAGGAAGCTTGGcgtaatcatggtcat
TACAGAGGAGTCGCAAATTTGGGACGTCCTTCGAACCgcattagtaccagta
      .           .           .           .           .
    460         470         480         490         500
  T  E  E  A  N  L  G  Q  L  F  S  P  T  T  I  M  T  M
```

*lacZ*α translational start

References

(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene,* 33, 103–119.

pSNAP$_f$

Sequence file available at www.neb.com.
See page 282 for ordering information.

There are no restriction sites for the following enzymes: AbsI(x), AfeI, AflII, AjuI(x), AlfI(x), AloI(x), AsiSI, BaeI, BarI(x), BbvCI, BlpI, BplI(x), BsiWI, BsmBI, BspDI, BspEI, BstAPI, BstBI, BstEII, ClaI, EcoNI, Esp3I, FseI, FspAI(x), KflI(x), MauBI(x), MreI(x), PasI(x), PfoI(x), PshAI, PsrI(x), SexAI, SgrAI, SrfI(x), StuI, XcmI

(x) = enzyme not available from NEB

pSNAP$_f$ Vector is a mammalian expression plasmid intended for the cloning and stable or transient expression of SNAP-tag® protein fusions in mammalian cells. This plasmid encodes SNAP$_f$, a SNAP-tag protein, which is expressed under control of the CMV promoter. SNAP$_f$ is an improved version of the SNAP-tag which exhibits faster labeling kinetics. The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a small protein based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. Use of this system involves two steps: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Further details are provided with the SNAP-Cell Starter Kit (NEB #E9100) and SNAP-Surface Starter Kit (NEB #E9120).

Codon usage of the gene is optimized for expression in mammalian cells. pSNAP$_f$ contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the N- or C-terminus of the SNAP-tag. The expression vector has an Internal Ribosome Entry Site (IRES) and a neomycin resistance gene downstream of the SNAP-tag for the efficient selection of stable transfectants.

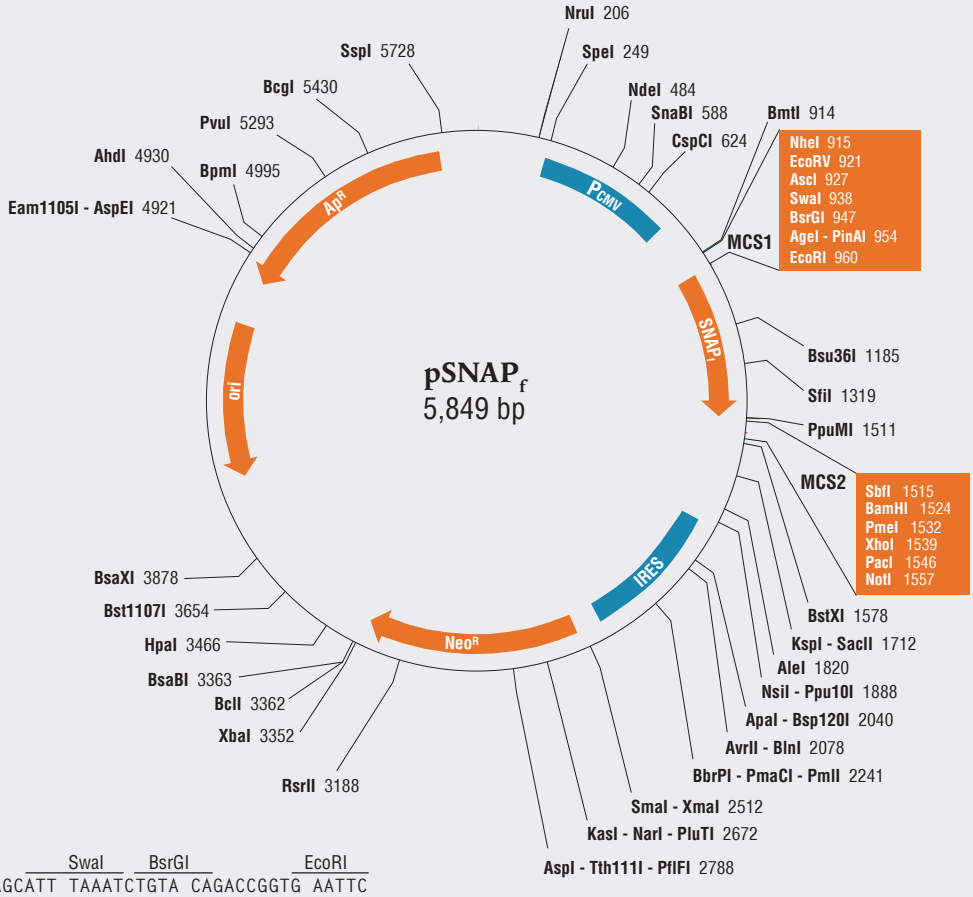
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmid can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
CMV promoter	251-818	–
expression region	915-1564	–
MCS1	915-965	–
SNAP$_f$	969-1514	–
MCS2	1515-1564	–
IRES	1910-2500	*ECMV*
Neo^R	2536-3339	*Tn5*
origin	4094-4682	pUC19
bla (Ap^R)	4853-5713	*Tn3*

ori = origin of replication
Ap = ampicillin
Neo = neomycin
IRES = internal ribosomal entry site



MCS1

```
    NheI        AscI        SwaI     BsrGI        EcoRI
...GCTAGC GATATCGGCG CGCCAGCATT TAAATCTGTA CAGACCGGTG AATTC
   CGATCG CTATAGCCGC GCGGTCGTAA ATTTAGACAT GTCTGGCCAC TTAAG...
```

MCS2

```
      SbfI      BamHI      PmeI      XhoI       PacI       NotI
...CCTGCA GGCGGATCCG CGTTTAAACT CGAGGTTAAT TAATGAGCGG CCGC
   GGACGT CCGCCTAGGC GCAAATTTGA GCTCCAATTA ATTACTCGCC GGCG...
```

pTXB1

Sequence file available at www.neb.com.
See page 214 for ordering information.

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-6558	–
MCS	5722-5775	–
Mxe GyrA intein	5776-6369	*M. xenopi*
CBD	6400-6558	*B. circulans*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AflII, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BmgBI, Bpu10I, BseRI, BspDI, BstBI, Bsu36I, ClaI, CspCI, Eco53KI, FseI, FspAI(x), HindIII, I-CeuI, I-SceI, KpnI, MscI, NcoI, NsiI, PI-PspI, PI-SceI, PacI, PmlI, PpuMI, RsrII, SacI, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SrfI(x), TspMI, XmaI

(x) = enzyme not available from NEB

pTXB1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, purification, and ligation using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTXB1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Mxe* GyrA intein tag to the C-terminus of the cloned target protein (2,3). The chitin binding domain (CBD) from *B. circulans*, fused to the C-terminus of the intein, facilitates purification of the intein-target protein precursor.

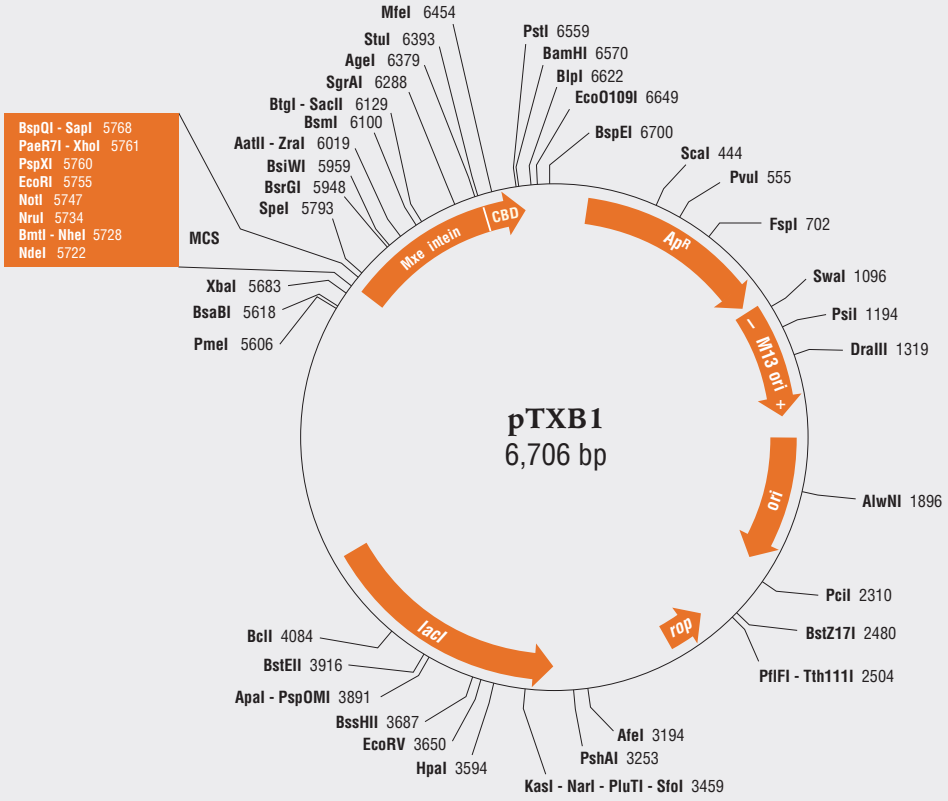
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (4). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

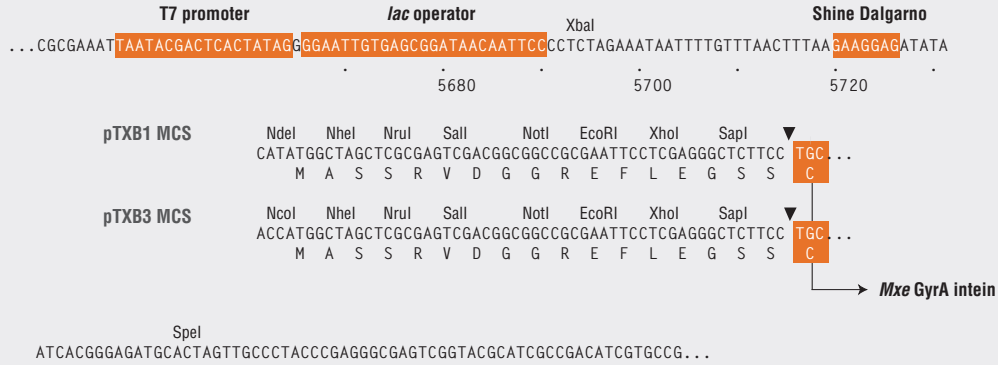
pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys$_1$") of the intein is shaded.

Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.





References

(1) Chong, S. et al. (1997) *Gene,* 192, 271–281.
(2) Evans, T.C., Benner and Xu, M.-Q. (1998) *Protein Sci.*, 7, 2256–2264.
(3) Southworth, M.W. et al. (1999) *Biotechniques,* 27, 110–120.
(4) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.,* 219, 45–59.

pTYB21

Sequence file available at www.neb.com.
See page 214 for ordering information.

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7368	–
MCS	7301-7361	–
Sce VMA intein	5770-7299	*S. cerevisiae*
CBD	6595-6747	*B. circulans*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AatII, AflII, AgeI, AscI, AsiSI, AvrII, BbvCI, BmgBI, BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FseI, FspAI(x), I-CeuI, I-SceI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PpuMI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI(x), TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTYB21 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Sce* VMA intein tag to the N-terminus of the cloned target protein (2). The chitin binding domain (CBD) from *B. circulans*, facilitates purification of the intein-target protein precursor.

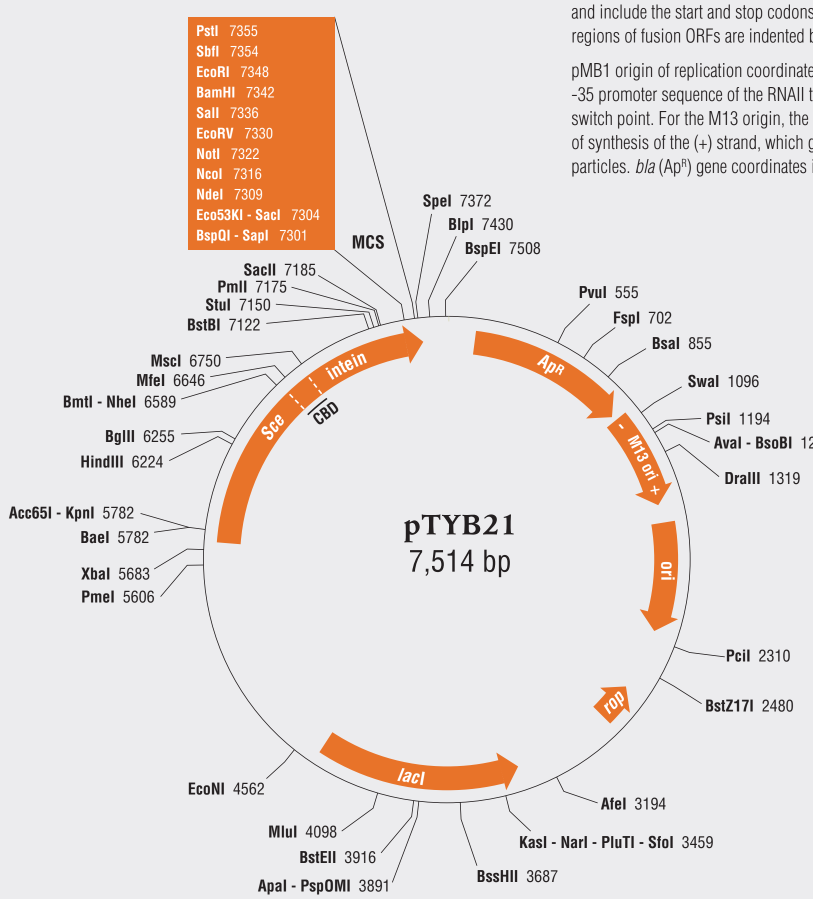
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

pTYB21 contains a SapI site which allows for cloning of a target gene without any extra amino acids. pTYB22 is identical to pTYB21 except for the MCS regions (see below). pTYB22 contains an NdeI site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB11 in that it contains a universal MCS that is compatible with all NEB expression systems.

Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.




```
                                                 Sce VMA Intein     ...    S  D  H  Q  F  L  L  G  S  Q
...TAATACGACTCACTATAGGGGAATTGTG...GAAGACGATTATTATGGGATTACTTTATCTGATGATTCTGATCATCAGTTTTTGCTTGGATCTCAG
      .         .         .         .         .         .         .         .         .         ,
               5650                7220                7240                7260                7280
```

pTYB21 MCS

```
Sce VMA Intein ──┐       SacI                                                       SbfI
                 ↓     SapI      NdeI     NcoI   NotI             SalI     BamHI  EcoRI     PstI
GTTGTTGTACAGAAC   GGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...
 V  V  V  Q  N     G  R  A  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
```

pTYB22 MCS

```
Sce VMA Intein ──┐                                                               SbfI
           BsmI  ↓      NdeI     NcoI   NotI             SalI     BamHI  EcoRI     PstI
GTTGTTGTACAGAAT   GCTGGTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...
 V  V  V  Q  N     A  G  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
```

References

(1) Chong et al. (1996) *J. Biol. Chem.*, 271, 22159–22168
(2) Chong et al. (1998) *NAR*, 26, 5109–5115.
(3) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

APPENDIX

pUC19

GenBank Accession #: L09137
See page 118 for ordering information.

Feature	Coordinates	Source
*lacZ*α	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap^R)	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AfeI, AflII, AgeI, AleI, ApaI, AscI, AsiSI, AvrII, BaeI, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, MscI, NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SexAI, SfiI, SgrAI, SnaBI, SpeI, SrfI(x), StuI, StyI, SwaI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

pUC19 is a small, high-copy number *E. coli* plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and > 200 per cell at 42°C (2,3). The multiple cloning site (MCS) is in frame with the *lacZ*α gene, allowing screening for insertions using α-complementation.

pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

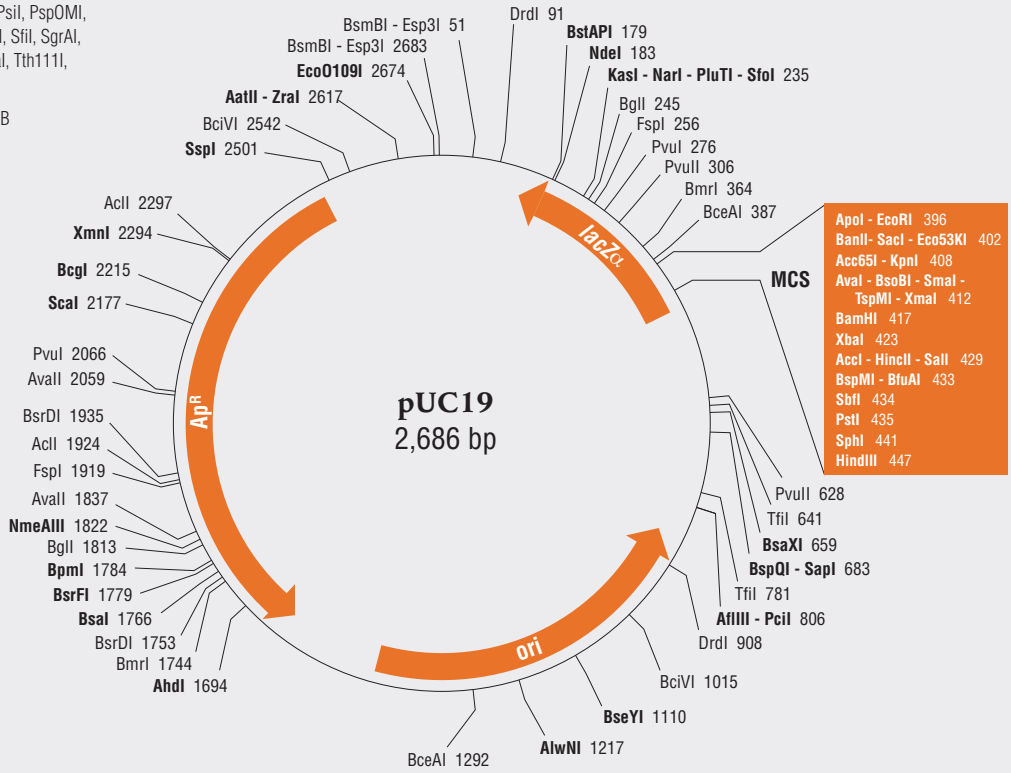
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene,* 33, 103–119.
(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.,* 6, 3385–3393
(3) Miki, T. et al. (1987) *Protein Eng.,* 1, 327–332.



pUC19 MCS

```
          SacI       SmaI       XbaI       SbfI
    EcoRI      KpnI       BamHI      SalI  PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      .          .          .          .          .          .          .
    400        410        420        430        440        450        460
...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
```

*lacZ*α translational start

pNEB193 MCS

```
          SacI       SmaI  BssHII          PacI                          SbfI
    EcoRI      KpnI       AscI       BamHI          XbaI  SalI       PmeI       PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      .          .          .          .          .          .          .          .          .          .          .
    400        410        420        430        440        450        460        470        480        490
...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
```

*lacZ*α translational start

T7

39,937 base pairs
GenBank Accession #: NC_001604
Not currently available from NEB.

There are no restriction sites for the following enzymes: AfeI, ApaI, AscI, AsiSI, BamHI, BsiWI, BspEI, EagI, Eco53KI, EcoRI, EcoRV, FseI, HindIII, I-CeuI, I-SceI, NaeI, NgoMIV, NotI, PI-PspI, PI-SceI, PaeR7I, PspOMI, PspXI, PstI, PvuI, SacI, SacII, SalI, SbfI, SexAI, SgrAI, SmaI, SphI, SrfI(x), TspMI, XhoI, XmaI

(x) = enzyme not available from NEB

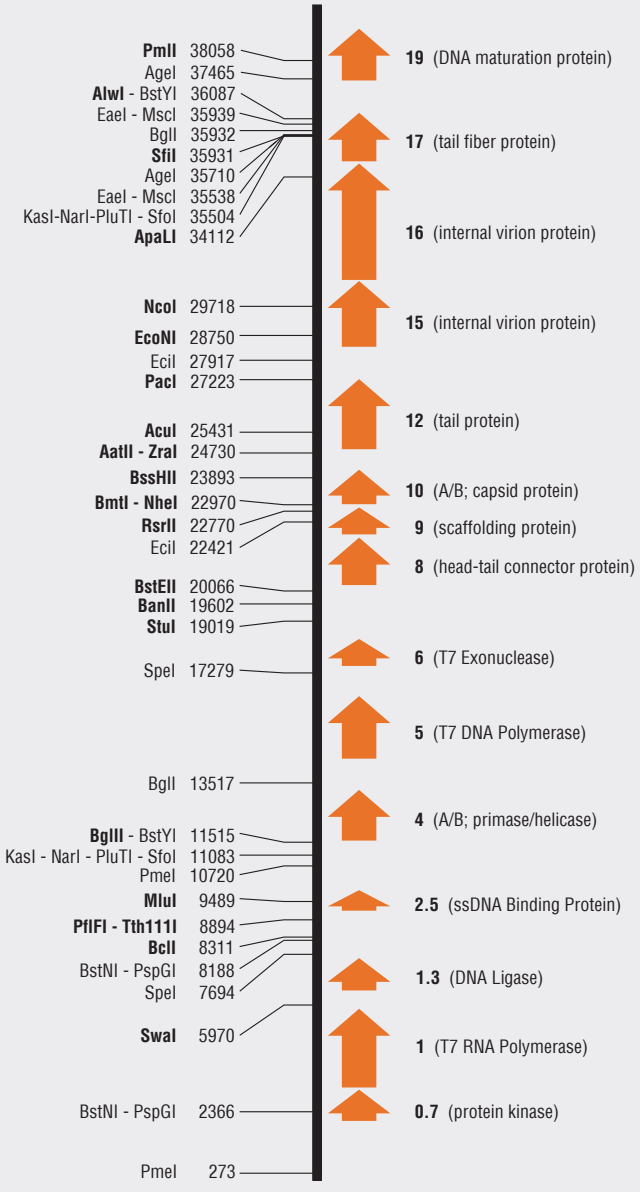
T7 is a lytic *E. coli* bacteriophage with a linear, double-stranded DNA genome containing 56 genes (1-4). Genes are classified as early or late based on the order of transcription in the infected host and their dependence on host or phage RNA polymerase.

Numbering of the sequence begins at the first (5´-most) base of the left end (bottom of the diagram below) and continues rightward (upward) in the direction of early to late genes. The map below shows the positions of all known ORFs larger than 200 codons.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

References

(1) Oakley, J.L. and Coleman, J.E. (1977) *Proc. Natl. Acad. Sci.* USA, 74, 4266–4270.
(2) Stahl, S.J. and Zinn, K. (1981) *J. Mol. Biol.,* 148, 481–485.
(3) Dunn, J.J. and Studier, F.W. (1981) *J. Mol. Biol.,* 148, 303–330.
(4) Dunn, J.J. and Studier, F.W. (1983) *J. Mol. Biol.,* 166, 477–535.



The Genetic Code

	A Ala	R Arg	N Asn	D Asp	C Cys	Q Gln	E Glu	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	W Trp	Y Tyr	V Val
5′	GCA	CGA	AAC	GAC	UGC	CAA	GAA	GGA	CAC	AUA	CUA	AAA	AUG	UUC	CCA	UCA	ACA	UGG	UAC	GUA 3′
	C	C	U	U	U	G	G	C	U	C	C	G		U	C	C	C		U	C
	G	G						G		U	G				G	G	G			G
	U	U						U			U				U	U	U			U
		or									or					or				
		AGA									UUA					AGC				
		G									G					U				

Second Position

First Position (5′ end)	U	C	A	G	Third Position (3′ end)
U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA Ser UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU His CAC CAA Gln CAG	CGU CGC CGA Arg CGG	U C A G
A	AUU AUC AUA Ile AUG Met	ACU ACC ACA Thr ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC GUA Val GUG	GCU GCC GCA Ala GCG	GAU Asp GAC GAA Glu GAG	GGU GGC GGA Gly GGG	U C A G

Termination Signals

UAA (Ochre)
UAG (Amber)
UGA (Opal)

Single Letter Code

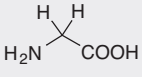
A = adenosine
C = cytidine
G = guanosine
T = thymidine
U = uridine

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

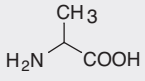
Amino Acid Structures

Each amino acid is accompanied by its three- and one-letter code, residue molecular weight (actual molecular weight minus water) and side-chain pK_a where appropriate.

SMALL

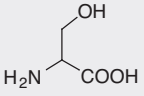


Glycine (Gly, G)
MW: 75.07

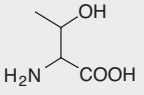


Alanine (Ala, A)
MW: 89.09

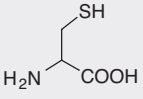
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, pK_a ~ 16

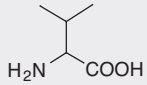


Threonine (Thr, T)
MW: 119.1, pK_a ~ 16

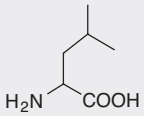


Cysteine (Cys, C)
MW: 121.2, pK_a = 8.18

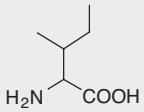
HYDROPHOBIC



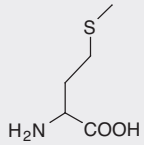
Valine (Val, V)
MW: 117.1



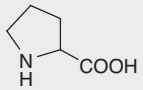
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

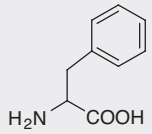


Methionine (Met, M)
MW: 149.2

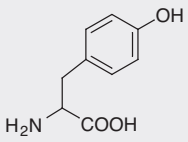


Proline (Pro, P)
MW: 115.1

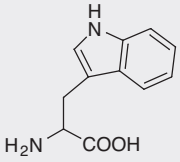
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2

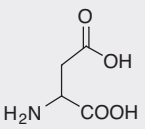


Tyrosine (Tyr, Y)
MW: 181.2, pK_a = 10.46

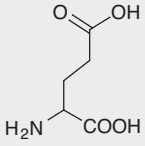


Tryptophan (Trp, W)
MW: 204.2

ACIDIC

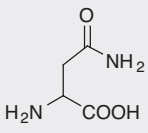


Aspartic Acid (Asp, D)
MW: 133.1, pK_a = 3.9

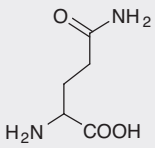


Glutamic Acid (Glu, E)
MW: 147.1, pK_a = 4.07

AMIDE

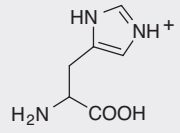


Asparagine (Asn, N)
MW: 132.1

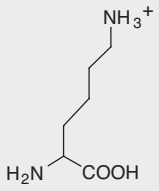


Glutamine (Gln, Q)
MW: 146.1

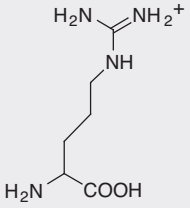
BASIC



Histidine (His, H)
MW: 155.2, pK_a = 6.04



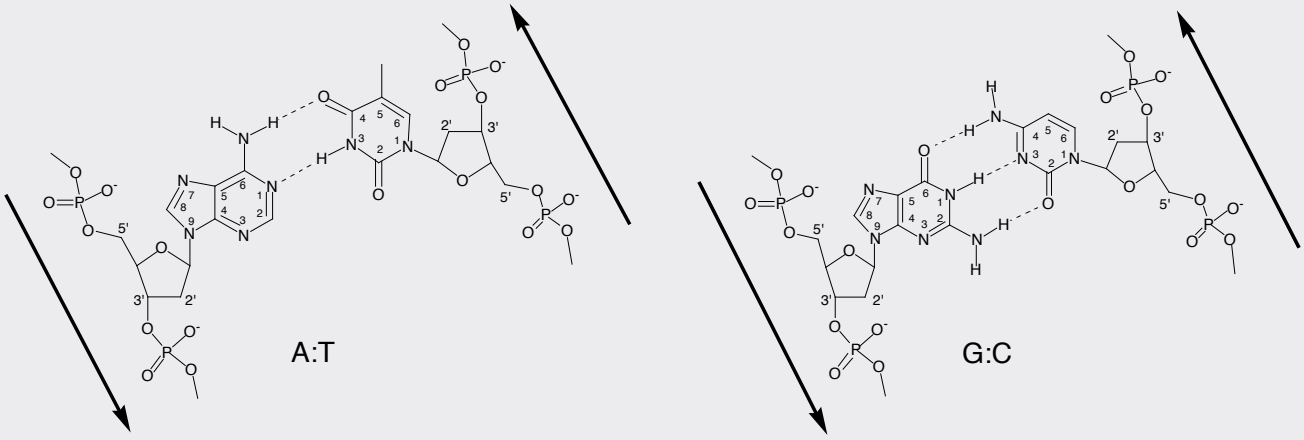
Lysine (Lys, K)
MW: 146.2, pK_a = 10.79



Arginine (Arg, R)
MW: 174.2, pK_a = 12.48

DNA Base Pairs

The structures of the adenosine:thymidine and guanosine:cytidine base pairs are shown in the context of the ribose phosphodiester backbones. The numbering schemes of the ribose and nucleotide moieties are indicated. Arrows indicate the polarity of each strand from 5´ to 3´.



Nucleic Acid Data

Average weight of a DNA basepair (sodium salt) = 650 daltons

1.0 A_{260} unit ds DNA = 50 µg/ml = 0.15 mM (in nucleotides)
1.0 A_{260} unit ss DNA = 33 µg/ml = 0.10 mM (in nucleotides)
1.0 A_{260} unit ss RNA = 40 µg/ml = 0.11 mM (in nucleotides)

MW of a double-stranded DNA molecule = (# of base pairs) x (650 daltons/base pair)
Moles of ends of a double-stranded DNA molecule = 2 x (grams of DNA) / (MW in daltons)
Moles of ends generated by restriction endonuclease cleavage:
a) circular DNA molecule: 2 x (moles of DNA) x (number of sites)
b) linear DNA molecule: 2 x (moles of DNA) x (number of sites) + 2 x (moles of DNA)

1 µg of 1000 bp DNA = 1.52 pmol = 9.1×10^{11} molecules
1 µg of pUC18/19 DNA (2686 bp) = 0.57 pmol = 3.4×10^{11} molecules
1 µg of pBR322 DNA (4361 bp) = 0.35 pmol = 2.1×10^{11} molecules
1 µg of M13mp18/19 DNA (7249 bp) = 0.21 pmol = 1.3×10^{11} molecules
1 µg of λ DNA (48502 bp) = 0.03 pmol = 1.8×10^{10} molecules

1 pmol of 1000 bp DNA = 0.66 µg
1 pmol of pUC18/19 DNA (2686 bp) = 1.77 µg
1 pmol of pBR322 DNA (4361 bp) = 2.88 µg
1 pmol of M13mp18/19 DNA (7249 bp) = 4.78 µg
1 pmol of λ DNA (48502 bp) = 32.01 µg

1.0 kb DNA = coding capacity for 333 amino acids ≈ 37,000 dalton protein
10,000 dalton protein ≈ 270 bp DNA
50,000 dalton protein ≈ 1.35 kb DNA

Isotope Data

Isotope	Particle Emitted	Half Life				
^{14}C	β	5,730 years	1 Ci	=	1,000 mCi
^{3}H	β	12.3 years	1 mCi	=	1,000 µCi
^{125}I	γ	60 days	1 µCi	=	2.2×10^{6} disintegrations/minute
^{32}P	β	14.3 days	1 Becquerel	=	1 disintegration/second
^{33}P	β	25 days	1 µCi	=	3.7×10^{4} Becquerels
^{35}S	β	87.4 days	1 Becquerel	=	2.7×10^{-5} µCi

Acids and Bases

Compound	Formula	Molecular Weight	Specific Gravity	% by Weight	Conc Reagent Molarity
Acetic acid, glacial	CH_3COOH	60.0	1.05	99.5	17.4
Formic acid	$HCOOH$	46.0	1.20	90	23.4
Hydrochloric acid	HCl	36.5	1.18	36	11.6
Nitric acid	HNO_3	63.0	1.42	71	16.0
Perchloric acid	$HClO_4$	100.5	1.67	70	11.6
Phosphoric acid	H_3PO_4	98.0	1.70	85	18.1
Sulfuric acid	H_2SO_4	98.1	1.84	96	18.0
Ammonium hydroxide	NH_4OH	35.0	0.90	28	14.8
Potassium hydroxide	KOH	56.1	1.52	50	13.5
Sodium hydroxide	$NaOH$	40.0	1.53	50	19.1
β-mercaptoethanol	$HSCH_2CH_2OH$	78.1	1.11	100	14.3

Protein Data

Bacterial Cells: *E. coli* or *Salmonella typhimurium*

	Cell Data	per cell	per liter at 10^9 cells per ml
Theoretical maximum yield for a 1 liter culture	Wet Weight	9.5×10^{-13} g	0.95 g
(10^9 cells /ml) if protein of interest is:	Dry Weight	2.8×10^{-13} g	0.28 g
0.1% of total protein: 150 µg/liter	Total Protein	1.55×10^{-13} g	0.15 g
2.0% of total protein: 3 mg/liter	Volume	1.15 μm^3 = 1 femtoliter	
50.0% of total protein: 75 mg/liter	Protein Conc. in the cell: 135 mg/ml		

Common Plasmid Gene Products

Gene	Gene Product # of Residues	Molecular Weight (daltons)
tet (pBR322)	401	43,267
amp (pBR322, bla)	286	31,515
kan (pACYC177, nptI)	264	29,047
cam (pACYC184, cat)	219	25,663
*lacZ*α (pUC19)	107	12,232
lacZ	1,023	116,351

Nucleotide Physical Properties

Compound	Molecular Weight	λ max (pH 7.0)	Absorbance at λ max 1 M solution (pH 7.0)
ATP	507.2	259	15,400
CTP	483.2	271	9,000
GTP	523.2	253	13,700
UTP	484.2	262	10,000
dATP	491.2	259	15,200
dCTP	467.2	271	9,300
dGTP	507.2	253	13,700
dTTP	482.2	267	9,600

pH vs Temperature for Tris Buffer

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40
8.37	7.80	7.52
8.48	7.90	7.62
8.58	8.00	7.71
8.68	8.10	7.80
8.78	8.20	7.91
8.88	8.30	8.01
8.98	8.40	8.10
9.09	8.50	8.22
9.18	8.60	8.31
9.28	8.70	8.42

Agarose Gel Resolution

% Gel	Optimum Resolution for Linear DNA (kb)
0.5	30 to 1.0
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5	3 to 0.2

Common Buffer Chart

The following chart lists some of the common buffers used in biology. The useful buffer range is the pK_a ± 0.5–1 pH unit.
The buffering capacity decreases beyond this range.

COMMON NAME	pK_a AT 25°C	MOLECULAR WEIGHT	CHEMICAL FORMULA	CHEMICAL NAME
Phosphate	2.12	98.00	$H_3PO_4^-$	–
Acetate	4.76	60.00	CH_3CO_2H or $C_2H_4O_2$	–
MES	6.15	195.20	$C_6H_{13}NO_4S$	2-(*N*-morpholino)ethanesulfonic acid
PIPES	6.76	302.40	$C_8H_{18}N_2O_6S_2$	piperazine-N,N´-bis(2-ethanesulfonic acid)
Imidazole	6.95	68.08	$C_3H_4N_2$	1,3-Diaza-2,4-cyclopentadiene
MOPS	7.20	209.30	$C_7H_{15}NO_4S$	3-(*N*-morpholino)propanesulfonic acid
Phosphate	7.21	97.00	$H_2PO_4^-$	–
TES	7.40	229.20	$C_6H_{15}NO_6S$	N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
HEPES	7.48	238.30	$C_8H_{18}N_2O_4S$	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Tricine	8.05	179.20	$C_6H_{13}NO_5$	N-(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine
Tris	8.06	121.14	$C_4H_{11}NO_3$	Tris(hydroxymethyl)methylamine
Bicine	8.35	163.20	$C_6H_{13}NO_4$	N,N-bis(2-hydroxyethyl)glycine
TAPS	8.43	243.30	$C_7H_{17}NO_6S$	N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid
Phosphate	12.67	96.00	HPO_4^{2-}	–

NEB expanded its manufacturing footprint by opening a facility in Rowley, MA for production of GMP-grade materials. Pictured here are several of the team members.