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NEB expressions

a scientific update from New England Biolabs

Welcome to the summer edition of NEB Expressions. In addition to new product announcements and technical tips, we highlight data detailing how NEB restriction enzymes can deliver more flexibility to your experimental design. Extensive testing shows that over 120 of our restriction enzymes are Time-Saver qualified and will digest to completion in only 5 minutes. Please refer to page 3 for a complete list of our Time-Saver qualified enzymes.

As always, we invite your feedback on our products, services and corporate philosophy.

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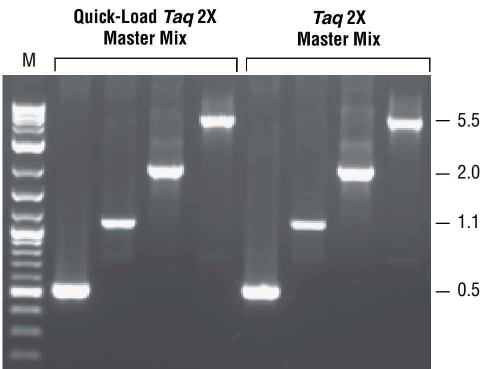
Taq DNA Polymerase

high yield, robust and reliable PCR reactions

For high yield PCR reactions, choose recombinant *Taq* DNA Polymerase from NEB. Known for robust and reliable reactions, this enzyme is the industry standard for routine PCR. NEB provides high quality, recombinant *Taq* at exceptional value in terms of cost per unit. For your convenience, this versatile enzyme is now available in a variety of formats.

- **Value**
  Industry standard for routine PCR at a low price
- **Versatility**
  Tolerates a wide range of templates with minimal optimization
- **Flexibility**
  Able to incorporate dUTP, dITP and fluorescently-labeled nucleotides
- **Choice**
  Reaction buffers accommodate a variety of PCR applications with no sacrifice in amplification performance



Versatility and yield with the Taq 2X Master Mixes. *40 ng human genomic DNA (hDNA) or 0.01 ng lambda DNA (λ DNA) was amplified in the presence of 200 nM primers in a 25 µl volume. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200). The 0.5, 1.1, and 2.0 kb fragments are amplified from hDNA, while the 5.5 kb fragment is from λ DNA.*

Taq PCR Kit (R)
Everything you need to perform 200 PCR reactions
#E5000S

Taq PCR Kit with Controls (R)
Also includes 30 control reactions
#E5100S

Taq 2X Master Mix (R)
Just add template and primers

#M0270S	100 reactions
#M0270L	500 reactions

Quick-Load™ *Taq* 2X Master Mix (R)
Also includes dyes for tracking

#M0271S	100 reactions
#M0271L	500 reactions

Taq DNA Polymerase with ThermoPol Buffer (R)
Buffer is uniquely formulated to promote high product yields

#M0267S	400 units
#M0267L	2,000 units

Taq DNA Polymerase with Standard Taq Buffer (R)
Detergent free buffer for high throughput applications

#M0273S	400 units
#M0273L	2,000 units

(R) = Recombinant

Some applications in which these products can be used may be covered by patents issued and applicable in the United States and certain other countries. Because purchase of these products does not include a license to perform any patented application, users of these products may be required to obtain a patent license depending upon the particular application in which the product is used. The PCR process is the subject of European Patent Nos. 201,184 and 200,262 owned by Hoffman-LaRoche, which expired on March 28, 2006. The corresponding PCR process patents in the United States expired on March 29, 2005.

Enhancing Transformation Efficiencies

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by transforming 1 µg of plasmid into a given volume of competent cells. The term is somewhat misleading in that 1 µg of plasmid is rarely actually transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. The equation for calculating Transformation Efficiency (TE) is: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. We have listed our recommended protocols and tips 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 30 seconds.
5. Place on ice for 5 minutes.
6. Add 250 µl of room temperature SOC.
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.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate at 37°C or according to recommendations.

5 Minute Transformation Protocol

Results in only 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.
5. Place on ice for 2 minutes.
6. Add 250 µl of room temperature SOC. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37–42°C. 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 you shorten this step.

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 you shorten this step.
- SOC gives 2-fold higher TE than LB medium.
- Incubation with shaking or rotating the tube gives 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 for transformation 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.
- To maximize transformants, purification by either a spin column or phenol/chloroform extraction and ethanol precipitation should be performed.
- 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

Competent Cells Available from NEB

*Through September 30th, take advantage of our introductory offer**

Characteristics	Strain	NEB #
Rapid Colony Growth	NEB Turbo Competent *E. coli*	C2984H
Versatile Cloning Strain	NEB 5-alpha Competent *E. coli*	C2991H
Protein Expression Strain	T7 Express Competent *E. coli*†	C2566H
Tight Control of Protein Expression	T7 Express I^q Competent *E. coli*†	C2883H
dam/dcm Methyltransferase Free Plasmid Growth	dam^-/dcm^- Competent *E. coli*	C2925H

* No other discounts apply.

† Notice to Buyer/User: The Buyer and User have a non exclusive license to use this system or any component thereof for RESEARCH PURPOSES ONLY. See Assurance Letter and Statement on www.neb.com for details on terms of the license granted hereunder.

Time-Saver Qualified Restriction Enzymes from NEB

Powerful enough for a 5 minute digest, pure enough for overnight incubation

Over 120 of our enzymes are **Time-Saver** qualified and will digest 1 µg of DNA in 5 minutes.

Look for the **Time-Saver** icon on our website.

At NEB, enzyme production is linked to basic research in the cloning and overexpression of restriction-modification systems. This focus allows us to provide extremely pure enzymes at concentrations that deliver more flexibility for your experimental design.

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. These enzymes will digest 1 µg of DNA in 5 minutes using 1 µl of enzyme under recommended reaction conditions. 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 minutes. In fact, 59% of our enzymes will digest 1 µg of DNA in 5 minutes, while 83% will fully digest in 15 minutes (see table). That means >180 of our restriction enzymes have the power to get the job done fast.

Also, since all of our enzymes are rigorously tested for nuclease contamination, you can safely set up digests for long periods of time without any degradation of your sample. Only NEB can offer you enzymes with power and purity – the power to digest in 5 minutes and the purity to withstand overnight digestions with no loss of sample.

5 15 30 60 O/N M

Minutes

Power and purity of Time-Saver qualified enzymes:
1 µl of SalI digests 1 µg of DNA in 5 minutes with no indication of nuclease contamination in longer digests or overnight (O/N) samples. Marker (M) is the 1 kb DNA Ladder (NEB #N3232).

Enzyme		5 Minutes	15 Minutes
AatII	R		•
Acc65I		•	
AccI	R		•
AciI		•	
AclI	R	•	
AcuI	R		•
AflII	R	•	
AgeI	R	•	
AhdI	R	•	
AluI	R	•	
AlwI	R	•	
AlwNI		•	
ApaI	R	•	
ApaLI	R	•	
ApeKI	R	•	
ApoI	R	•	
AscI	R	•	
AseI	R	•	
AsiSI	R	•	
AvaI	R	•	
AvaII	R	•	
AvrII	R	•	
BaeI			•
BamHI	R	•	
BanII		•	
BbsI			•
BbvI	R	•	
BbvCI	R	•	
BccI	R		•
BceAI			•
BciVI		•	
BclI	R	•	
BfaI			•
BfuAI		•	
BfuCI			•
BglI	R	•	
BglII	R	•	
BlpI	R	•	
Bme1580I			•
BmgBI		•	
BmrI	R		•
BpmI			•
BsaAI	R	•	
BsaBI		•	
BsaHI			•
BsaWI			•
BsaXI		•	
BseRI	R	•	
BsgI		•	
BsiEI		•	
BsiHKAI		•	
BsiWI	R	•	
BslI	R	•	
BsmAI		•	
BsmBI	R		•
BsmFI		•	
BsmI	R	•	
BsoBI	R	•	
Bsp1286I		•	
BspCNI			•
BspEI	R	•	

Enzyme		5 Minutes	15 Minutes
BspHI	R		•
BsrBI		•	
BsrDI		•	
BsrFI	R	•	
BsrGI			•
BssHII	R	•	
BsrI		•	
BssKI			•
BstBI		•	
BstEII	R	•	
BstNI		•	
BstUI		•	
BstXI			•
BstYI	R		•
BstZ17I		•	
Bsu36I			•
BtgI		•	
BtsCI	R	•	
Cac8I			•
ClaI	R	•	
CspCI	R	•	
CviAII	R		•
CviKI-1	R		•
DdeI	R	•	
DpnI	R	•	
DpnII	R		•
DraI	R	•	
DraIII	R	•	
DrdI			•
EagI	R	•	
EarI	R		•
EcoNI		•	
EcoO109I	R	•	
EcoP15I	R		•
EcoRI	R	•	
EcoRV	R	•	
Fnu4HI	R	•	
FokI		•	
FseI	R	•	
FspI	R		•
HaeII	R		•
HaeIII	R	•	
HgaI	R		•
HhaI	R	•	
HincII	R		•
HindIII	R	•	
HinfI	R	•	
HinP1I	R	•	
HpaI	R		•
HpaII	R	•	
HphI	R	•	
Hpy188I	R		•
HpyCH4IV	R	•	
HpyCH4V	R	•	
KpnI	R	•	
MboI	R	•	
MboII	R	•	
MfeI	R	•	
MluI	R	•	
MlyI	R	•	
MmeI	R	•	

Enzyme		5 Minutes	15 Minutes
MnlI	R	•	
MseI	R		•
MslI		•	
MspI	R	•	
MspA1I	R	•	
MwoI	R		•
NciI	R	•	
NcoI	R	•	
NdeI	R	•	
NgoMIV	R		•
NheI	R		•
NlaIII	R		•
NotI	R	•	
NruI		•	
NsiI	R	•	
NspI	R	•	
PacI	R	•	
PaeR7I	R	•	
PflFI		•	
PflMI	R	•	
PmeI	R	•	
PmlI		•	
PpuMI	R	•	
PshAI	R		•
PstI	R	•	
PvuI		•	
PvuII	R	•	
RsaI	R		•
SacI	R	•	
SacII	R	•	
SalI	R	•	
SapI	R		•
SbfI	R	•	
ScaI	R	•	
ScrFI		•	
SfiI	R	•	
SfoI	R	•	
SmaI	R	•	
SnaBI	R	•	
SpeI	R	•	
SphI	R	•	
SspI	R	•	
StuI			•
StyI	R		•
StyD4I	R		•
SwaI	R		•
TaqI	R	•	
TfiI	R		•
TseI			•
Tsp509I	R	•	
TspMI		•	
TspRI	R	•	
Tth111I	R		•
XbaI	R	•	
XcmI	R		•
XhoI	R	•	
XmaI	R		•
XmnI	R	•	

R = Recombinant
Green indicates frequently used enzymes.

Nicking Endonucleases: The Discovery and Engineering of Restriction Enzyme Variants

Siu-hong Chan, Ph.D. and Shuang-yong Xu, Ph.D., New England Biolabs, Inc.

Restriction endonucleases (REases) recognize specific nucleotide sequences in double-stranded DNA and generally cleave both strands. Some sequence-specific endonucleases, however, cleave only one of the strands. These endonucleases are known as nicking endonucleases (NEases). At NEB, we have been developing nicking endonucleases through the discovery of naturally occurring enzymes, as well as genetic engineering of existing restriction enzymes.

Double-stranded cleavage usually results from binding of the two half sites of a palindromic sequence by a homodimeric REase (e.g. Type IIP REases). Within the homodimer, each monomer makes a cut on one of the strands such that both strands of the DNA are cleaved. Strand-specific nicking, however, is achievable only when the recognition sequences are asymmetric. In addition, some of the REases that recognize asymmetric sequences are heterodimeric.

Thus, one can envision that manipulating the catalytic activity of individual monomers or the dimerization state of restriction endonucleases that recognize asymmetric sequences can result in nicking endonucleases. That is how NEB scientists developed the strand-specific NEases Nb.BbvCI and Nt.AlwI.

BbvCI is a heterodimeric Type IIS REase. It recognizes the 7 base-pair asymmetric sequence CCTCAGC and cleaves the DNA at (CC↓TCAGC† and CCTCA↑GC) [CCTCAGC (-5/-2)]. It was discovered that each of the two subunits (R1 and R2) contains its own catalytic site. Each of these subunits cleaves the bottom and the top strands of the target sequence, respectively (1). To utilize this property, cleavage-deficient mutants of each subunit were engineered. Heterodimers of functional R1 and cleavage-deficient R2 reconstitute a nicking endonuclease that cleaves only the bottom strand (Nb.BbvCI), whereas functional R2 and cleavage-deficient R1 reconstitute Nt.BbvCI, which cleaves the top strand only (2). The nicking enzyme Nt.AlwI (GGATCNNNN↓) was also successfully engineered to cleave only the top strand of the AlwI target sequence [GGATC(4/5)] (3). This NEase was created by swapping the dimerization domain of AlwI with a non-functional dimerization domain of the natural NEase, Nt.BstNBI, such that the resulting chimeric enzyme, Nt.AlwI, is rendered monomeric.

Other nicking enzyme engineering projects are less straightforward. Screening libraries of random mutants has enabled us to isolate variants of restriction endonucleases that nick one of the strands specifically (4,5). The engineered enzymes obtained are the bottom-strand specific Nb.BsmI (GAATG↑C) from BsmI [GAATGC(1/-1)] and top-strand specific Nt.SapI (GCTCTTCN↓) from SapI [GCTCTTC(1/4)] (4). Nicking variants have also been generated from BsaI [GGTCTC(1/5)], BsmBI [CGTCTC(1/5)], and BsmAI [GTCTC(1/5)] (5).

In addition to protein engineering, we are also developing products from natural nicking endonucleases. Nt.BstNBI (GAGTCNNNN↓) is a naturally occurring thermostable NEase cloned from *Bacillus stereothermophilus* (6). Nt.CviPII (↓CCD), originally identified in a Chlorella virus isolate as a frequent DNA nickase that recognizes 3-base target sequences (7), is also under development at NEB.

Some nicking endonucleases were discovered quite unexpectedly. Nb.BsrDI (GCAATG↑) is the large subunit of BsrDI [GCAATG(2/0)], a thermostable heterodimeric enzyme identified in *Bacillus stearothermophilus.* The large subunit was found to be a bottom-strand specific NEase when cloned separately in *E. coli* (Xu, unpublished observations). A similar observation has been made in BtsI where the large subunit makes a strand-specific nick at the target sequence (Zhu and Xu, unpublished results). The top-strand cleavage activity of BfiI [ACTGGG(5/4)] has also been reported to be inhibited at low pH, resulting in a bottom-strand specific nicking enzyme (8).

The uses of nicking endonucleases are still being explored. NEases can generate nicked or gapped duplex DNA for studies of DNA mismatch repair and for diagnostic applications. The long overhangs that nicking enzymes make can be used in DNA fragment assembly. Nt.BbvCI has been used to generate long and non-complementary overhangs when used with XbaI in the USER Friendly Cloning Kit* (NEB #E5500S). Nicking endonucleases are also useful for isothermal DNA amplifications, which rely on the production of site-specific nicks. Isothermal DNA amplification using Nt.BstNBI in concert with Vent (exo$^-$) DNA Polymerase (NEB #M0257) (EXPAR) has been reported for detection of a specific DNA sequence in a sample (9). Another isothermal DNA amplification technique has also been described using the 3-base cutter Nt.CviPII and *Bst* DNA Polymerase I [Nicking Endonuclease Mediated-DNA Amplification (NEMDA)] (7). Frequent-cutting NEases can generate short partial duplex DNA fragments from genomic DNA. These fragments can be used for cloning or used as probes for hybridization-based applications.

Nicking Endonucleases Available at NEB

Enzyme	NEB Catalog #	Cleavage site	Recommended reaction temperature (upper limit)
7-base cutters			
Nb.BbvCI RR	R0631	C C T C A G C G G A G T↑C G	37°C (47°C)
Nt.BbvCI RR	R0632	C C↓T C A G C G G A G T C G	37°C (47°C)
6-base cutters			
Nb.BsmI RR	R0706	G A A T G C N C T T A C↑G N	65°C
Nb.BsrDI RR	R0648	G C A A T G N N C G T T A C↑N N	65°C
5-base cutters			
Nt.BstNBI RR	R0607	G A G T C N N N N↓N C T C A G N N N N N	55°C
Nt.AlwI RR	R0627	G G A T C N N N N↓N C C T A G N N N N N	37°C (55°C)

For more information about NEases and REases, you can visit www.neb.com. Alternatively, REBASE (rebase.neb.com) offers a comprehensive database of enzyme properties and useful resources of restriction-modification systems. REBASE also includes citations of all relevant literature as well as links to resources such as structural data and genomic sequences when they are available.

† Down-arrows (↓) indicate cleavage at the top strand; up-arrows (↑) indicate cleavage at the bottom strand.

* The USER™ (Uracil-Specific Excision Reagent) Friendly Cloning Kit offers an extremely fast, simple and efficient method for the cloning of PCR products.

References:

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2. Heiter, D.F., Lunnen, K.D. and Wilson, G.G. (2005) *J. Mol. Biol.* 348, 631–640.
3. Xu, Y. et al. (2001) *Proc. Natl. Acad. Sci.* USA 98, 12990–12995.
4. Samuelson, J.C., Zhu, Z. and Xu, S.Y. (2004) *Nucl. Acids Res.* 32, 3661–3671.
5. Zhu, Z. et al. (2004) *J. Mol. Biol.* 337, 573–583.
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9. Van Ness, J. et al. (2003) *Proc. Natl. Acad. Sci.* USA 100, 4504–4509.

Assaying Nicking Endonucleases

Nicking endonucleases are simple to use. Since the nicks generated by 6- or 7- base NEases do not fragment DNA, their activities are monitored by conversion of supercoiled plasmids to open circles. Alternatively, substrates with nicking sites close enough on opposite strands to create a double-stranded cut can be used instead.

New Restriction Endonucleases from NEB

NEB maintains an aggressive research program in the discovery, cloning and overexpression of restriction endonucleases. This allows us to offer the largest selection of these essential reagents. Four of our newest enzymes are listed below. For a more up to date list of restriction endonucleases, please see our website, www.neb.com.

BtsCI (Recombinant)

BtsCI is a recombinant isoschizomer of BstF5I with a lower optimum incubation temperature and a 2-fold unit increase.

#R0647S 2,000 units
#R0647L 10,000 units

5´. . . G G A T G N N▼. . . 3´
3´. . . C C T A C▲N N . . . 5´

Nb.BsrDI (Recombinant)

Nb.BsrDI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate.

#R0648S 1,000 units
#R0648L 5,000 units

5´. . . G C A A T G N N . . . 3´
3´. . . C G T T A C▲N N . . . 5´

CviKI-1 (Recombinant)

CviKI-1 is a restriction endonuclease with four expected recognition sites as well as up to eleven relaxed non-cognate sites (star sites).

#R0710S 250 units
#R0710L 1,250 units

5´. . . R G▼C Y . . . 3´
3´. . . Y C▲G R . . . 5´

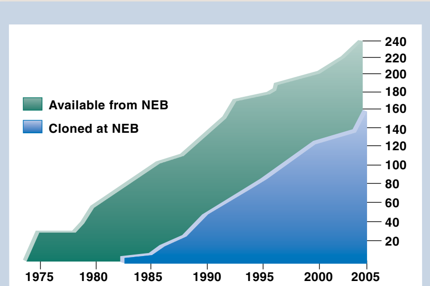
TspMI

TspMI is a thermophilic XmaI isoschizomer that cuts plasmids efficiently.

#R0709S 200 units
#R0709L 1,000 units

5´. . . C▼C C G G G . . . 3´
3´. . . G G G C C▲C . . . 5´

RR = Recombinant



Our aggressive restriction endonuclease screening and cloning programs enable us to maintain a position at the forefront of this field. NEB now supplies over 230 restriction enzymes, of which over 150 are recombinant.

Optimizing Restriction Endonuclease Reactions

There are several key factors to consider when setting up a restriction endonuclease digest. Using the proper amounts of DNA, enzyme and buffer components in the correct reaction volume will allow you to achieve optimal digestion without any star activity. By definition, 1 unit of restriction enzyme will completely digest 1 µg of substrate DNA in a 50 µl reaction in 60 minutes. This enzyme : DNA : reaction volume ratio can be used as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, where a 10-fold overdigestion is recommended to overcome variability in DNA source, quantity and purity. NEB offers the following tips to help you to achieve maximal success in your restriction endonuclease reactions.

Enzyme

- Keep on ice when not in the freezer.
- Should be the last component added to reaction.
- Mix components prior to addition of enzyme 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.

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents, or excessive salts.
- Methylation of DNA can inhibit digestion with certain enzymes. For more information about methylation, see pages 266-267 of our 2005-06 catalog or www.neb.com.

Buffer

- Use at a 1X concentration.
- If necessary, add BSA to a final concentration of 100 µg/ml.
- 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 1 µg of substrate.
- Smaller reaction volumes are more susceptible to pipetting errors.
- Keep glycerol concentration at less than 5% of total reaction volume to prevent star activity.
- The restriction enzyme (supplied in 50% glycerol) should not exceed 10% of the total reaction volume.

Incubation Time

- Can often be decreased by using an excess of enzyme, or by using one of our Time-Saver qualified enzymes (see page 3).
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours. For more information, see page 257 of our 2005-06 catalog or www.neb.com.

Stopping a reaction

If no manipulation of DNA is required:

- Terminate with a stop solution [50% glycerol, 50 mM EDTA (pH 8.0), and 0.05% bromophenol blue]. Use 10 µl per 50 µl reaction.

When manipulation of DNA is required:

- Heat inactivation can be used (refer to page 256 of our 2005-06 catalog or www.neb.com to determine if the enzyme can be heat inactivated).
- Remove enzyme by using a spin column or phenol/chloroform extraction.

A "Typical" Restriction Digest	
Restriction Enzyme	10 units is sufficient Generally 1 µl is used
DNA	1 µg
10X NEBuffer	5 µl (1X)
BSA	Add to a final concentration of 100 µg/ml (1X) if necessary
Total Reaction Volume	50 µl
Incubation Time	1 hour
Incubation Temperature	Enzyme dependent

Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Experimental DNA without restriction enzyme to check for contamination in the DNA preparation or reaction buffer.
- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or adenovirus-2 DNA) with restriction enzyme to test enzyme viability.
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.

Nomenclature of Restriction Endonucleases

The nomenclature of NEB restriction enzymes is being updated to eliminate the space between the organism name and the Roman numeral. We are currently changing our literature to reflect the proper nomenclature. Please note that this change will be taking place gradually and names will be in either format for a limited time. As the leader in enzyme technology, it is important for us to properly present the names of these essential reagents. For additional information on the nomenclature of restriction enzymes, please see the following reference:

Roberts R.J., et al. (2003). *Nucl. Acids Res.* 31, 1805–1812.

Gaussia Luciferase Transcriptional Reporter

New products that utilize this extremely sensitive and naturally secreted luciferase

Choose the enhanced performance and convenience of *Gaussia* Luciferase (GLuc) for your mammalian gene expression experiments. This ideal transcriptional reporter is extremely sensitive, naturally secreted and very stable. New England Biolabs now offers several products so that you can experience the considerable advantages of this new reporter gene.

pGLuc-Basic Vector

For cloning promoter sequences to assess their transcriptional regulatory functions

#N8082 20 µg

pCMV-GLuc Control Plasmid

For constitutive expression of GLuc

#N8081 20 µg

pNEBR-X1 GLuc Control Plasmid

GLuc is placed under the control of GAL4 and a minimal promoter. Compatible with Rheoswitch and other GAL4 activators.

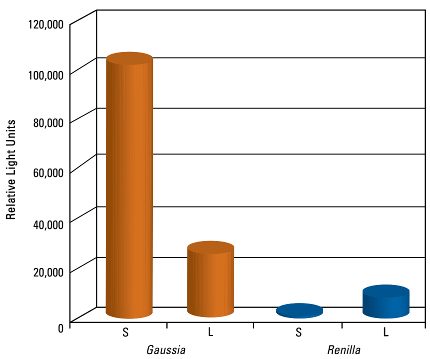
#N8080 20 µg

Gaussia Luciferase Assay Kit

Easy-to-use kit to measure luciferase activity

#E3300S 100 assays
#E3300L 1,000 assays

Extreme Sensitivity of the *Gaussia* Luciferase Reporter



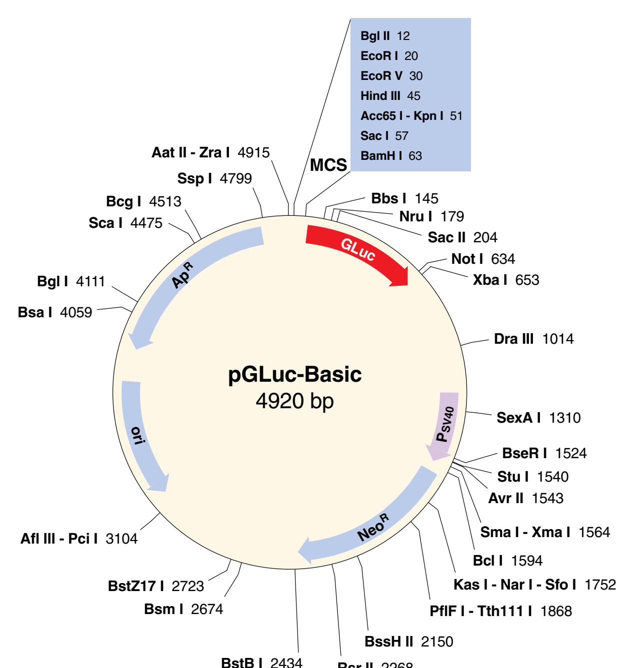
HeLa cells were transfected with 1 µg of pCMV-GLuc or pCMV-Renilla, and light emission was measured for the supernatant (S) or cell lysate (L). Note that Gaussia Luciferase is a naturally secreted reporter, while Renilla is not.

Top reasons to choose *Gaussia* Luciferase as a transcriptional reporter

Many unique features of the GLuc reporter gene coupled with the Gaussia Luciferase Assay Kit make it the ideal choice as a transcriptional reporter.

1. **Speed**
   Secreted GLuc can be detected in the culture medium only a few hours after transcription. Just collect a few µl of the medium and monitor transfection efficiency, cell viability, and gene expression.
2. **Naturally Secreted**
   Since the culture medium can be used to monitor GLuc expression without the need for cell lysis, the cells can be used for additional assays (e.g Western blots, immunocytochemistry, etc).
3. **Extreme Sensitivity**
   Gaussia Luciferase produces a significantly higher bioluminescent intensity that surpasses Firefly and *Renilla* luciferases. For most applications, such as transient transfection assays, higher sensitivity offers the advantage of using less material, which is more practical when working with hard to transfect cells, for example.
4. **Stability**
   Gaussia Luciferase can be stored for several days at 4°C without loss of activity. This allows multiple assays from the same experiment over a long period of time.
5. **Compatibility with other formats**
   Secreted *Gaussia* Luciferase is most conveniently measured in the culture medium. Since it is so sensitive, a significant amount of activity can also be measured from cell lysates, which are commonly used in other assays. It is also compatible with other secreted reporters (e.g. SEAP) as well as standard assays used for transfection normalization (e.g. beta-galactosidase or firefly luciferase).

Tannous et al. (2005) *Mol. Ther.* 11, 435–443.



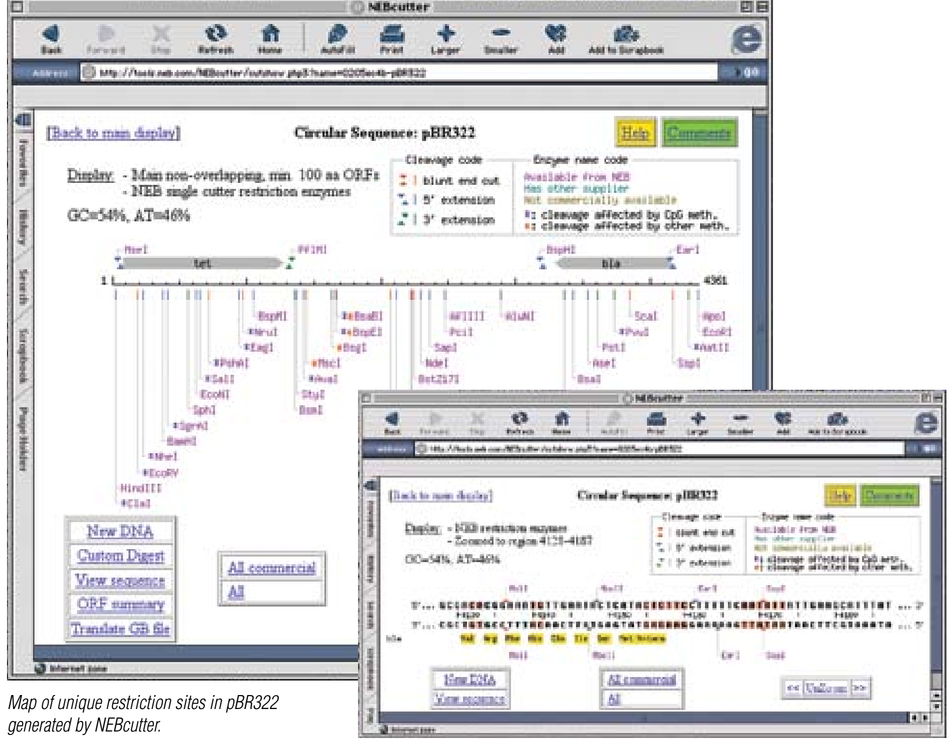

```
             BglII    EcoRI      EcoRV           HindIII
1   GACGGATCGGGAGATCTTGGAATTCTGCAGATATCCTCGAGCCCAAGCTT   50

    KpnI  SacI  BamHI
51  GGTACCGAGCTCGGATCCAGCCACCATGGGAGTCAAAGTTCTGTTTGCCC   100
                              M  G  V  K  V  L  F  A...
                                       GLuc
```

The pGLuc-Basic Vector allows transcriptional regulatory functions of a specific promoter sequence to be measured.

NEBcutter V2.0

innovative web tool for experimental design

The technical reference section of our website provides several web based programs to aid in experimental design. NEBcutter allows you to input sequence data and find large ORFs, identify restriction sites and generate custom digests. The virtual digests display fragment length, and allow you to choose appropriate markers, gel types, and methylation sensitivity.



Map of unique restriction sites in pBR322 generated by NEBcutter.

Acquire sequence information by expanding a region of interest on the plasmid map.

Features in Version 2.0

- Input up to 20,000 bp from local sequence file, GenBank or by cutting and pasting.
- GCG, DNAStar and EMBL formats accepted.
- Up to 40 custom oligonucleotides can be specified.
- Options include Type I and III enzymes, homing endonucleases and nicking enzymes, as well as all commercial or all known specificities.
- Up to 5 ambiguous nucleotides are allowed in any 20 nt window of input sequence.
- All NEB isoschizomers are displayed on the maps.
- Enzymes listed by number of sites produced, alphabetically or by cut frequency.
- Sequences submitted are maintained locally for 2 days and then discarded for your privacy.
- Silent mutation sites can be introduced into ORF sequence by a single mouse click.
- Enzymes may be selected by their site length or cut frequency.

NEBcutter:
tools.neb.com/NEBcutter2/index

User Guide:
tools.neb.com/NEBcutter2/help/guide

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