Winter 2008, vol. 2.4

NEB expressions

a scientific update from New England Biolabs

Welcome to the winter edition of NEB Expressions focusing on restriction enzymes, a core component of the NEB product line. The feature section chronicles the crucial role of NEB in the research and development of these important reagents, and reports on the latest research at NEB that enables us to introduce a line of High Fidelity (HF) restriction enzymes. These HF enzymes offer the benefit of reduced star activity, a concern for some enzymes when being used under suboptimal conditions.

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

inside:

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

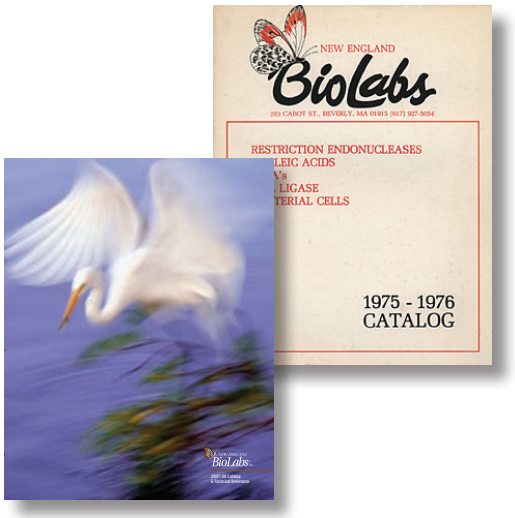
Restriction Enzymes at NEB: Over 30 Years of Innovation

the discovery, cloning and engineering of these essential reagents

Richard J. Roberts, Chief Scientific Officer, NEB and 1993 Nobel Laureate in Physiology or Medicine.

The early days

In 1975, New England Biolabs (NEB) became the first company to sell restriction enzymes, thereby providing researchers with a key set of tools that proved invaluable for the early development of the biotechnology industry. Many of those early restriction enzymes had been discovered in my laboratory at Cold Spring Harbor Laboratory (CSHL). Summer visitors would stop by with a tube of their favorite DNA in their pocket, just to see if we had an enzyme that would convert it into some useful fragments. At that time, there was no commercial source for reagents, and I was unsuccessful in persuading CSHL to consider being that source and using the proceeds to fund basic research. A short time later, I met Dr. Donald Comb, who had recently started a company, New England Biolabs, based on this very same principle. We struck up a lasting partnership, and I was able to serve as a consultant to NEB, providing strains for many enzymes, while performing quality control experiments in my lab at CSHL prior to their commercial availability at NEB. Soon NEB was selling most of the known specificities and had begun a vigorous program to clone the genes for these enzymes in order to improve their quality and efficiency of production.



The first NEB catalog, released in 1975, contained 8 restriction endonucleases. Today NEB offers more than 230 enzymes, of which over 180 are recombinant.

(continued on page 2)

High Fidelity (HF) Restriction Enzymes

engineered for reduced star activity

NEB introduces a line of restriction enzymes that has been engineered for reduced star activity. Star activity (relaxed specificity) can be problematic for some enzymes when setting up reactions with extended incubation times, smaller reaction volumes or increased number of units. These new High Fidelity (HF) enzymes offer maximum performance, convenience and flexibility.

Advantages

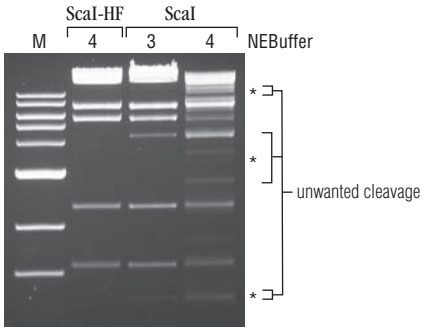
- The same specificity as the wild type enzyme
- Available for the same price as the wild type
- Reduced star activity offers added flexibility
- Supplied with NEBuffer 4, which may simplify double digest reactions
- Time-Saver™ qualified

For more information and the introductory list of HF enzymes, see page 4.

Introductory Offer

Receive a 50% discount on our new HF restriction enzymes†

Through March 31, 2008



ScaI is one of the most frequently reported enzymes to exhibit star activity (unwanted cleavage). While star activity is observed with ScaI in NEBuffer 3 (supplied buffer) as well as NEBuffer 4, star activity is significantly reduced with ScaI-HF. 20 µl reactions were set up containing 2 µl of enzyme and incubated for 1 hour. Marker M is the 1 kb DNA Ladder (NEB #N3232).

† No other discounts apply.

Restriction Enzymes at NEB

(continued from page 1)

In addition, NEB embarked on an ambitious research program to find out as much as possible about restriction enzymes and their properties. That program continues today and we have arrived at the point where we have cloned, sequenced and characterized the genes for all but a handful of the restriction enzymes we sell. Along the way we have established wide-ranging academic collaborations to obtain structures for many of these enzymes. We also regularly provide purified enzymes and clones to our academic colleagues interested in researching these fascinating enzymes.

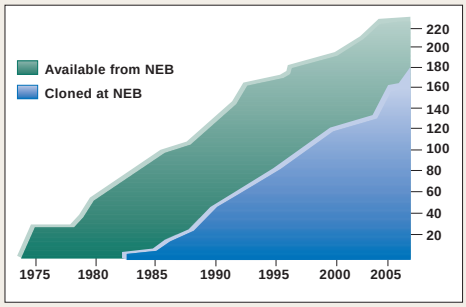
The discovery and screening process

As NEB grew and became known as a premier supplier of restriction enzymes, it was natural to begin screening microorganisms for new ones. This began in earnest in 1978, and over the years more than 500 restriction enzymes have been discovered by NEB scientists. This includes not only in-house scientists, but also many collaborating investigators from overseas. We have supported a series of screening labs in China, Cameroon, Vietnam, Nicaragua, Uganda and Portugal, which has helped these labs become established within their own countries.

For the most part screening was accomplished by the original methods. This involved growing locally isolated novel organisms, breaking them open and testing either crude extracts or column fractions for activities able to give specific fragmentation patterns on bacteriophage lambda DNA. By testing candidate enzymes on several different DNAs, it was often possible to show a new activity even before detailed characterization had taken place. Today, almost half of the enzymes sold by NEB were discovered here.

Cloning and engineering of restriction enzymes

As NEB expanded its offering of restriction enzymes it soon became apparent that the demand for some enzymes was greater than the supply. Also, some enzymes were notoriously difficult to purify from native sources due to the presence of contaminating activities, such as non-specific nucleases, that routinely co-purified with the restriction enzyme. Since the fledgling biotechnology industry was focused on producing useful proteins by recombinant DNA methodology, it became clear that NEB should do the same for restriction enzymes. If overexpression could be achieved, purification protocols would be simplified. For many enzymes, overexpression has enabled NEB to reduce prices 5- to 20-fold. In cases where the native organism contained more than one restriction enzyme, the cloned version would guarantee greater purity with no cross-contamination. Furthermore, having access to cloned and sequenced genes would offer opportunities to increase our basic knowledge about these enzymes and lead to the possibility of improving their properties.



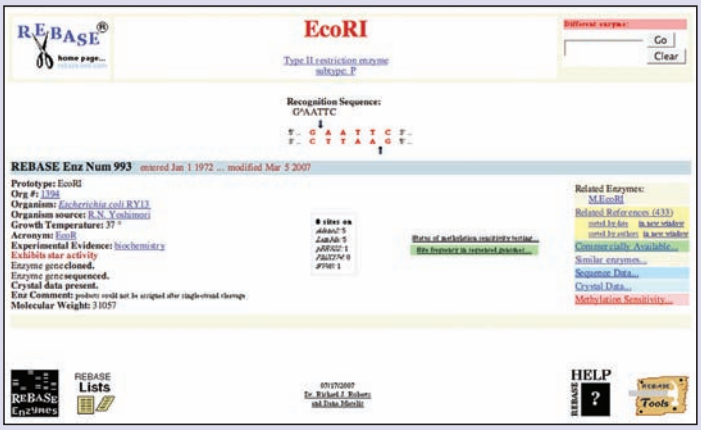
NEB now supplies over 230 restriction enzymes, of which over 180 are recombinant. The aggressive screening and cloning programs at NEB has enabled us to maintain a position at the forefront of this field.

The first restriction-modification (R-M) system cloned at NEB was PstI. Soon to follow were EcoRI, MspI, HindIII and TaqI. As the number of researchers working in this area grew, BamHI, FokI and BglII were cloned, as well as the important and useful 8-base cutters SfiI, NotI, and PacI. Some systems were cloned using bacteriophage to select for recombinants that expressed the restriction phenotype. Others were cloned using variations of an enzymatic method to select for recombinants that expressed the modification phenotype (1,2). In many cases, the R (restriction) and M (methylase) genes could be transferred simultaneously into *E. coli*. In other cases, it was necessary to clone them sequentially, first M and then R (3). Occasionally, additional non-cognate M genes were needed to provide complete protection from self-restriction. Cloning M genes alone was often problematic until it was discovered that *E. coli* synthesizes enzymes that specifically destroy modified DNA (4). Numerous obstacles were encountered in the course of cloning and over-expressing restriction enzymes – stemming mainly from the fact that each is unique – and even today they can still present exasperating problems. However, as a result of our extensive research activities in this area we now have unparalleled knowledge of the organization and properties of these systems – knowledge that has been directly applied to our ability to manufacture the very highest quality enzymes available.

One of the research focuses at NEB has been to clone and sequence the genes encoding all of the restriction enzymes that we sell. So far, this has been accomplished for 222 of 231 restriction enzymes; to date, over 180 are

REBASE – the restriction enzyme database

In 1974, the first list of known restriction enzymes was produced for a meeting on Restriction and Modification Systems in Ghent, Belgium. It was an instant hit and after the meeting many investigators wanted a personal copy of the list. Initially the contents of the list were maintained on an IBM memory typewriter at CSHL, but soon they were transferred to an early PDP computer and held in a hierarchical database that became known as REBASE – **R**estriction **E**nzyme data**BASE**. As time went on and the list grew, not to mention the accompanying publications documenting their properties, REBASE was transferred to a relational database model where it is maintained today. REBASE is available directly over the web as well as from the NEB website (www.neb.com). In addition to containing information about all of the restriction enzymes for which biochemical and/or genetic experimental data is available, REBASE also contains information about potential RM systems that can be identified by bioinformatic analysis of GenBank. This includes annotation information for the more than 600 completely sequenced genomes held in RefSeq. Partial support for these activities is provided by the National Library of Medicine and the contents of REBASE are freely available to everyone. REBASE also contains links to many useful online tools available from New England Biolabs, including NEBcutter, which can provide both lists and graphic representation of the action of restriction enzymes on a user's DNA (18).



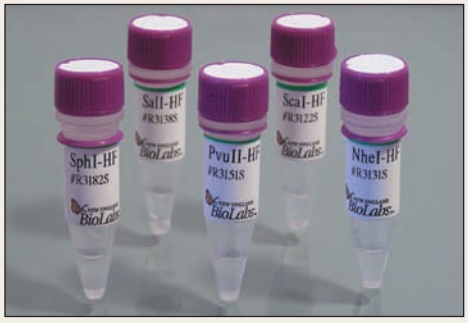
REBASE contains data such as recognition sequences, cleavage sites, methylation sensitivity, isoschizomers and commercial availability of enzymes.

already commercially available. This work has provided valuable information that we use when screening newly sequenced genomes for the presence of Type II restriction enzyme genes. It is often possible to identify new examples of well known specificities on the basis of sequence similarity, but more importantly it has enabled us to identify potential genes that might encode new specificities. This is now a key part of our screening efforts to find new restriction enzymes.

Once a restriction enzyme gene had been cloned, it became natural to try to change its properties by engineering. Until recently, changing specificity proved troublesome, and only a few non-useful changes had been described in the literature (5,6,7). One set of useful mutants that has been produced is able to selectively nick one strand but not the other (8). Many of these nicking endonucleases are now commercially available, including Nb.BbvCI and Nt.BstNBI. These novel enzymes are generated both by incapacitating or omitting individual subunits of heterodimeric restriction enzymes (9,10) or by preventing dimerization of others (11). Most recently, scientists at NEB have had great success in engineering Type IIG enzymes, a specific subtype of restriction enzymes. It is now possible to generate a wide variety of new sequence specificities in a directed fashion by discrete amino acid substitution (12). Additionally, fusions between different types of restriction enzymes have introduced new properties into them such as cleaving on both sides of their recognition sequence (13), while mutagenesis has had some dramatic effects on the star activity of many restriction enzymes (14).

Infidelity among the restriction enzymes

While most restriction enzymes cleave their recognition sequences with great fidelity, some are notorious for their propensity to cleave at secondary sites that are closely related to their cognate sites. One of the earliest observations of this promiscuous behavior was seen for EcoRI (cognate recognition site: GAATTC), which was found to cleave at sites differing from this "proper" site by one base (15). It was found that under certain buffer conditions, such as low ionic strength, low Mg^{2+} or the presence of organic solvents, sites such as NAATTC, GNATTC or GANTTC could be cleaved, albeit at reduced efficiency. This unwanted cleavage became known as star activity, and has been the bane of researchers looking for faithful cleavage ever since.



The newly released High Fidelity (HF) restriction enzymes have the same specificity as the wild type enzyme, and have all been engineered to offer the benefit of reduced star activity. See page 4 for more details.

In addition to EcoRI, a number of other enzymes have been shown to exhibit star activity, including BamHI, PvuII and EcoRV (16). The conditions leading to this additional cleavage are now well documented. A quick glance at most catalogs containing restriction enzymes show that a rather large number of enzymes exhibit this unwanted behavior. Fortunately, under ideal buffer conditions, which can change substantially from one enzyme to another, star activity can be eliminated or at least greatly reduced in many cases. Since it is often desirable to perform a restriction digestion immediately following another reaction (i.e. ligation or amplification) or to use two restriction enzymes simultaneously, buffer conditions for one of these reactions is often far from ideal. In these circumstances, the need to change buffers can be time-consuming, may lead to sample loss or the desired products are formed at low yield. Recent innovations at NEB are poised to help.

Several major advances in restriction enzyme technology have been made that can alleviate problematic star activity. Researchers at NEB and the Indian Institute of Science in Bangalore described a mutant of KpnI that shows reduced star activity (17). Now, mutants of a number of other restriction enzymes have been prepared at NEB that can effectively eliminate or greatly reduce star activity for many commonly used enzymes. These newly released, high fidelity (HF) enzymes are sold as separate products at the same low price as their wild type counterparts. For more information on HF enzymes see page 4.

The current offering of HF enzymes with reduced star activity is only the beginning of improved products in the restriction enzyme field that we will be offering. Twenty-plus years of research has been rewarding in terms of knowledge acquired about these systems. From that knowledge has sprung an understanding of how these reagents might be improved for maximum utility. One of our goals is to only be selling restriction enzymes that are prepared from clones, thus ensuring unparalleled purity. Also, we will be offering additional engineered variants with altered and much improved performance and convenience. Once again our dedication to research, which is an integral part of our business philosophy, has paid off in better products and a deeper understanding of the products we sell.

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Acknowledgements

New England Biolabs expresses its thanks to the many individuals who, over the years, have played a role in cloning restriction enzymes, including Brian Anton, Janet Barsomian, Jack Benner, Tanya Bhatia, Joan Brooks, Siu-Hong Chan, Rebecca Croft, Dan Heiter, Alexey Fomenkov, Lucia Greenough, Ellen Guthrie, Lauren Higgins, Kimberly Howard, William Jack, Tineka Jager-Quinton, Huimin Kong, Mary Looney, Keith Lunnen, Yvette Luyten, Laurie Mazzola, Elizabeth McLeod, Julie Menin, Fana Mersha, Richard Morgan, Iain Murray, Peter Nathan, Celine Nkenfou, Donald Nwankwo, Carol Polisson, Elisabeth Raleigh, James Samuelson, Ira Schildkraut, Karen Silber, Timothy Simcox, Barton Slatko, Laura Sznyter, Ruth Trimarchi, Romas Vaisvila, Elizabeth Van Cott, Phyllis Waite-Rees, Paul Walsh, Geoffrey Wilson, Shuang-Yong Xu, Yan Xu and Zhenyu Zhu.

New The Latest Innovation in Restriction Enzyme Technology

High Fidelity (HF) restriction enzymes

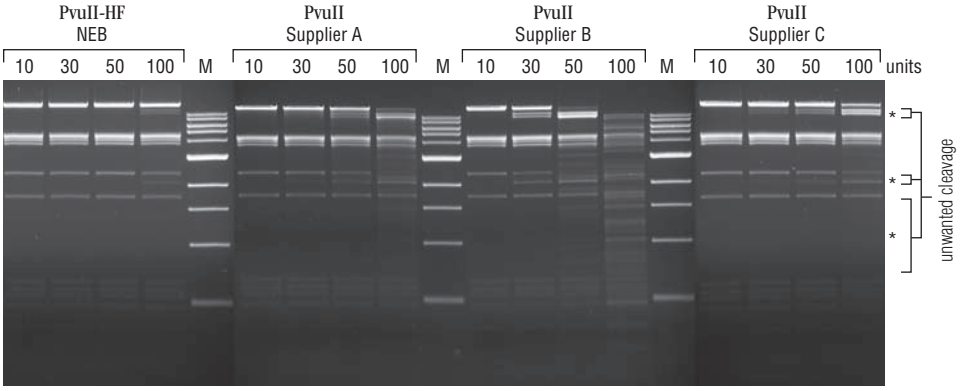
New England Biolabs provides customers with high quality tools for a wide range of molecular biology applications. As part of our ongoing commitment to the study and improvement of restriction enzymes, we are pleased to introduce a line of High Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as their established counterparts. However, certain properties have been altered, including buffer requirements and enzyme fidelity. These modifications provide customers with more flexibility in setting up their restriction enzyme digests. The overall goal of engineering restriction enzymes is to provide improved enzymes that will allow more flexibility with respect to reaction volume, incubation time and buffer compatibility. Each of these enzymes has been purified to the same high standards as our other restriction enzymes, and are available at the same low price.

The introductory selection of engineered restriction enzymes offers the benefit of reduced star activity. Star activity, or relaxed specificity, 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 carefully under the recommended conditions to avoid unwanted cleavage (see page 6 for tips on avoiding star activity). Different 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. These new products with reduced star activity will offer increased flexibility to reaction setup, maximizing results under a wider range of conditions.

In addition to reduced star activity, all of these engineered enzymes work optimally in NEBuffer 4, which has the highest level of enzyme compatibility, and may simplify double digest reactions. They are also Time-Saver qualified, and will digest substrate DNA in five minutes.

In order to distinguish these engineered enzymes, the letters -HF™ have been added to the restriction enzyme name. An icon designating that the enzyme has been engineered will appear with the product entry, on the datacard and on the website. In addition, icons for the enhanced properties that these new enzymes possess will also be included. These enzymes will be packaged with a purple cap to distinguish them from our existing restriction enzymes.

Visit our website, www.neb.com to learn more about the latest innovation in restriction enzyme technology from New England Biolabs!



PvuII from various suppliers produces the correct banding pattern when 10 units is used, however, star activity is observed with larger amounts of enzyme. Star activity is not observed with PvuII-HF, even at higher enzyme amounts. Reactions were set up according to recommended reaction conditions of each manufacturer. Reactions contained 1 µg Lambda DNA in a 50 µl reaction volume and were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).

Cloned at NEBiolabs	37° Incubation Temperature
RR Recombinant Enzyme	dam Methylation Sensitivity
Time-Saver Qualified	Yes Heat Inactivation
NEB4 Optimum Buffer	Blue/White Certified
BSA Requires BSA	

New icons:

e Indicates that the enzyme has been engineered	★- Indicates that the enzyme has reduced star activity

References:

1. New England Biolabs, unpublished observations.
2. Nasri, M. and Thomas, D. (1987) *Nucleic Acids Res.,* 15, 7677.
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For limited time take advantage of our 50% off discount*

ScaI-HF™

5′...AGT▼ACT...3′
3′...TCA▲TGA...5′

ScaI is one of the enzymes most frequently reported to exhibit star activity (1). ScaI-HF™ has significantly reduced star activity. ScaI-HF has the same specificity as ScaI, and the recommended buffer has been shifted from NEBuffer 3 to 4. Note that engineered enzymes have different activity in the four NEBuffers. For more information, see datacard or website.

		Reg Price	Intro Price*
#R3122S	1,000 units	$58	$29

PvuII-HF™

5′...CAG▼CTG...3′
3′...GTC▲GAC...5′

PvuII has been reported to exhibit star activity when used under suboptimal conditions, including non-recommended buffer, smaller reaction volumes and extended incubation times (2). PvuII-HF™ has significantly reduced star activity. PvuII-HF has the same specificity as PvuII, and the recommended buffer has been shifted from NEBuffer 2 to 4. Note that engineered enzymes have different activity in the four NEBuffers. For more information, see datacard or website.

		Reg Price	Intro Price*
#R3151S	5,000 units	$53	$26.50

SalI-HF™

5′...G▼TCGAC...3′
3′...CAGCT▲G...5′

Star activity has been noted for wild type SalI in the recommended buffer (3) and can be observed when digesting pBR322 with increased enzyme concentration and incubation time. This activity cannot be detected with SalI-HF™. SalI-HF has the same specificity as SalI, and the recommended buffer has been shifted from NEBuffer 3 to 4. In addition, BSA is no longer required. Note that engineered enzymes have different activity in the four NEBuffers. For more information, see datacard or website.

		Reg Price	Intro Price*
#R3138S	2,000 units	$56	$29

* No other discounts apply.

SphI-HF™

5′...GCATG▼C...3′
3′...C▲GTACG...5′

Star activity has been reported in the literature for SphI (4) and can be observed in digests carried out in small volumes. Star activity is not observed with SphI-HF™ under these conditions. SphI-HF has the same specificity as SphI, and the recommended buffer has been shifted from NEBuffer 2 to 4. Note that engineered enzymes have different activity in the four NEBuffers. For more information, see datacard or website.

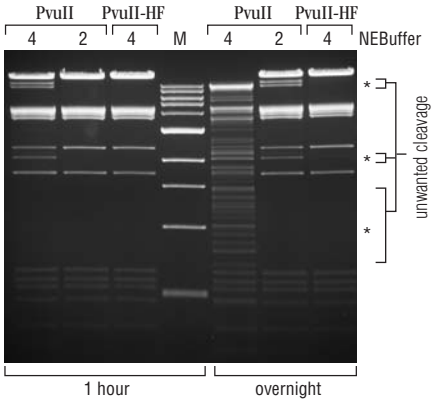
		Reg Price	Intro Price*
#R3182S	500 units	$61	$30.50

NheI-HF™

5′...G▼CTAGC...3′
3′...CGATC▲G...5′

Star activity is not reported in the literature for NheI, but can be observed in digests carried out in small volumes. This is not seen with NheI-HF™. While NheI-HF has the same specificity as NheI, the recommended buffer has been shifted from NEBuffer 2 to 4, and BSA is still recommended. Note that engineered enzymes have different activity in the four NEBuffers. For more information, see datacard or website.

		Reg Price	Intro Price*
#R3131S	1,000 units	$61	$30.50



PvuII can exhibit star activity when used under suboptimal reaction conditions. This activity is significantly reduced with PvuII-HF, even under extended incubation times. 20 µl reactions were set up using 2 µl units of enzyme and incubated for the indicated time at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).

Five reasons to choose New England Biolabs as your restriction enzyme supplier

1 Selection

NEB is the only company that continues an aggressive program to clone and overexpress restriction enzymes. As a result, we are proud to offer the largest number of commercially available specificities. NEB scientists have discovered more than 300 different sequence specificities, and NEB supplies over 220 specificities, over half of which have been discovered here. In addition to Type II enzymes that cleave DNA at defined positions close to or within their recognition sequence, we also offer unique enzymes such as 8-base cutters, Type IIS and Type IV enzymes, nicking and homing endonucleases.

2 Quality

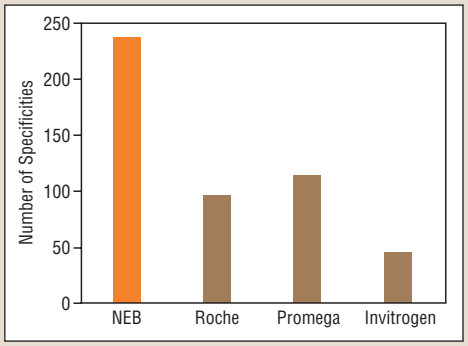
As a result of our active restriction enzyme screening and cloning program, NEB is able to provide the largest selection of recombinant enzymes available. Cloning improves enzyme purity by using a defined host thereby removing the uncertainties of less defined native contaminants. It also improves enzyme yields and simplifies purification, resulting in a higher quality product with no lot-to-lot variation.

3 Value

The availability of recombinant enzymes allows us to offer our products at a lower cost/unit, in turn enabling our customers to experience substantial savings while benefitting from improved purity and consistency of product.

4 Support

One of the focuses of the NEB research program is to understand the regulation of restriction/modification systems and how they interact with DNA. Our dedication to research enables NEB to remain the best resource for questions regarding restriction enzymes and for support in experimental design. The same scientists who purify and quality control our products also answer technical questions. Also, our research staff is always available as a technical resource, assuring customers that they will receive the best possible answers. In addition, the NEB Catalog & Technical Reference is an invaluable tool, and many common restriction enzyme questions can be answered using the comprehensive charts, protocols and technical tips found here. This information can also be accessed through our website, www.neb.com. The NEB website also offers several web-based tools designed to aid in your cloning experiments, including NEBcutter, Enzyme Finder, the Double Digest Finder and REBASE.



NEB offers over 220 cleavage specificities, the largest selection commercially available (as of 10/07).

5 Accessibility

In an effort to deliver your materials as quickly as possible, NEB provides same day delivery to most of the Boston and Cambridge area on orders placed before 10:00 AM (EST), and overnight delivery coast to coast if your order is received Monday through Friday by 8:00 PM (EST). Many of our customers also benefit from the convenience of on-site access to our products through Freezer Programs. To learn more about the Freezer Program network, visit the Freezer Program Locator at www.neb.com.

Avoiding Star Activity

tips for preventing unwanted cleavage in restriction enzyme digests

It is well known that 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. It has been suggested that star activity is a general property of restriction endonucleases (1) and that any restriction endonuclease will cleave noncanonical sites under certain extreme conditions, some of which are listed below. Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog or 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, so a good rule of thumb is the amount of enzyme added should not exceed 10% of the total reaction volume. This reduces the final glycerol concentration in the reaction. Use the standard 50 µl reaction volume; this will reduce evaporation during incubation. Evaporation results in an increased glycerol concentration which contributes to star activity.
High units of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	Use as few units as possible to get 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 strength and pH may contribute to star activity.
Prolonged reaction time	Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (2), ethylene glycol, dimethylacetamide, dimethylformamide, sulfolane (3)]	Make sure the reaction is free of any organic solvents such as alcohols which 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 caution must be taken to follow the steps listed above to avoid star activity. **Alternatively, using our new line of HF restriction enzymes will allow some flexibility in reaction setup.** Please visit our website frequently to learn about new enzymes available in this growing product line.

For more information on star activity, visit our website, or page 305 of the 2007–08 NEB Catalog & Technical Reference.

References:
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3. Tikchonenko, T.I., et al. (1978) *Gene,* 4, 195.

New recombinant enzymes available at NEB:

Enzyme	Catalog #	Recognition Site
FatI	R0650S/L	/CATG
FauI	R0651S/L	CCCGC(4/6)
PspOMI	R0653S/L	G/GGCCC
Bpu10I	R0649S/L	CC/TNAGC
BmtI	R0658S/L	GCTAG/C
ZraI	R0659S/L	GAC/GTC
PspXI	R0656S/L	VC/TCGAGB
AfeI	R0652S/L	AGC/GCT
PciI	R0655S/L	A/CATGT

For information on pricing and sizes available, please visit www.neb.com.

V = A or C or G (not T)
B = C or G or T (not A)

IsoAmp® II Universal tHDA Kit

amplification without the need for thermocycling

The IsoAmp® II Universal tHDA Kit can be used to amplify and detect short DNA sequences (70–120 bp) at a constant temperature. This kit utilizes thermophilic Helicase Dependent Amplification (tHDA), a novel method for isothermal amplification which utilizes helicase to separate the DNA. Ideal for diagnostic applications, this second generation kit replaces the original IsoAmp tHDA Kit.

Advantages

- Amplify a single copy of DNA under optimized conditions
- Addition of ET SSB to reaction offers enhanced speed and sensitivity
- Suitable for a variety of templates, including microbial genomic DNA, viral DNA, plasmid DNA, human genomic DNA and cDNA
- Compatible with a number of detection methods, including real-time and probe-based detection
- Applications include tHDA, RT-HDA, qHDA and qRT-HDA

Kit Includes:

IsoAmp Enzyme Mix and dNTP Solution

10X Annealing Buffer II

$MgSO_4$ and NaCl

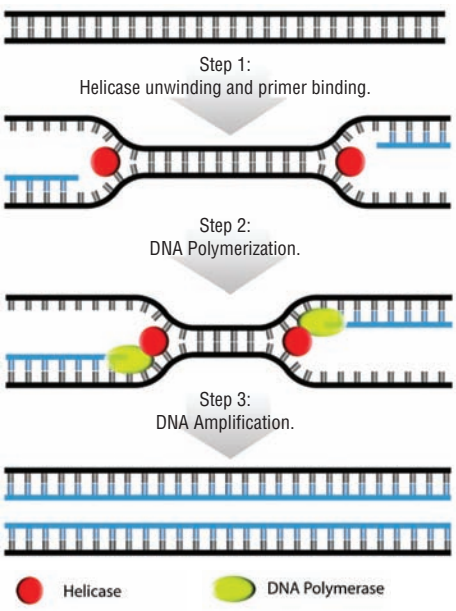
Control Template (1 ng/µl plasmid)

Control Forward and Reverse Primers

#H0100S 50 reactions $250

Developed and produced by BioHelix Corp, a NEB-affiliated company.

Helicase Dependent Amplification



HDA technology

23rd Annual Molecular Biology Workshop

coming this summer

This intensive summer course held at Smith College in Northampton, MA, emphasizes hands-on molecular biology laboratory work and covers a wide variety of topics and techniques, including:

- Gene cloning
- Gene expression analysis
- PCR and qRT-PCR
- Genomics and bioinformatics
- DNA sequencing and fingerprinting
- RNAi and microarrays

This course is now offered in one- and two-week sessions. No previous experience in molecular biology is required or expected. For additional information, course dates and to fill out an application, visit the Summer Workshop website: http://www.science.smith.edu/neb

NEB FAQ Spotlight

restriction digests with EcoRI and BamHI

Q I would like to digest DNA with EcoRI and XbaI at the same time. The Double Digest Finder recommends a sequential digest, using each enzyme in its supplied NEBuffer. However, both of these enzymes show 100% activity in NEBuffer 2. Why is a double digest not recommended with these enzymes?

A Although EcoRI shows 100% activity in NEBuffer 2, it also exhibits significant star activity in this buffer, resulting in non-specific, unintended cleavage. This is also observed when using NEBuffer 4. For this reason, a sequential digest is recommended. However, research has shown that EcoRI exhibits less star activity in NEBuffers 1 and 3. Since XbaI has 75% activity in NEBuffer 3 a double digest could be performed in this buffer, provided that the reactions are set up according to the recommended reaction conditions for avoiding star activity (see page 6).

Q Why is BamHI now supplied with NEBuffer 3 rather than a unique buffer? Why is BamHI not recommended for use in NEBuffer 2 or 4, even though it is listed as being 100% active in these buffers?

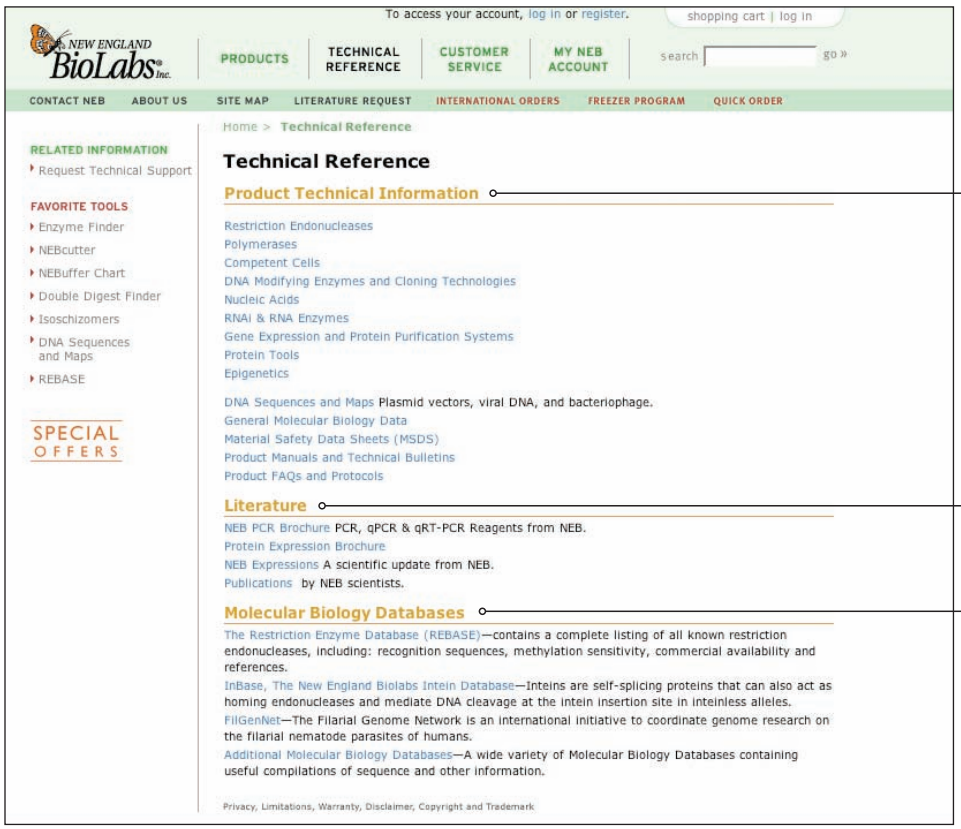
A In an effort to simplify our buffer system, BamHI is now supplied with NEBuffer 3. BamHI has been carefully purified and characterized so there is no loss of activity in this buffer. BamHI works in most of our buffers, but like EcoRI has significant star activity, particularly in those buffers with low salt concentrations. Therefore, BamHI is not recommended in NEBuffers 1, 2 and 4.

Q What is the most convenient method of using BamHI with another restriction enzyme that requires a low salt buffer?

A Perform the first digest in the low salt buffer. Then supplement with 100–150mM NaCl using a concentrated NaCl stock solution (i.e. 5 M NaCl). Alternatively, perform the low salt digest in a 20 µl reaction volume and then increase the reaction volume to 50 µl, by adding 5 µl 10X NEBuffer 3, BamHI, BSA and water.

Technical Reference

The Technical Reference section of our website contains all of the technical information for NEB products. Use this link to access comprehensive selection charts, technical tips, protocols, maps and FAQs. Much of the literature we offer, including brochures, newsletters and product manuals can also be found here. Direct links to several databases that are related to NEB products can also be accessed through this page, www.neb.com/nebcomm/tech_reference.



Technical information for all NEB products, including selection charts, technical tips, protocols and maps can be found here.

The literature section contains information found in NEB brochures and newsletters as well as recent publications from NEB scientists.

Several useful molecular biology databases can also be accessed through the NEB website.

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