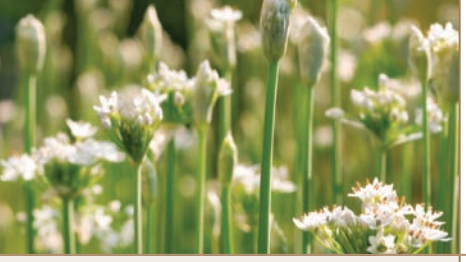


Spring 2009, vol. 4.1

NEB expressions

a scientific update from New England Biolabs

Welcome to the spring edition of NEB expressions. This issue introduces the 2009–10 NEB Catalog & Technical Reference, and highlights several components of our catalog, including new products, reference appendix updates and environmental minireviews. The feature article focuses on the importance of high quality sample preparation for emerging technologies, such as next-generation sequencing, microarrays and library construction. In addition to our standalone reagents, NEB now offers the NEBNext™ Sample Prep Reagent Set 1 to facilitate sample preparation.

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

inside:

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

The Next Generation of Reagents for Sample Preparation

Fiona Stewart, Ph.D. and Andrew Gardner,
New England Biolabs, Inc.

NEBNext™ DNA Sample Prep Reagent Set 1 facilitates sample preparation for downstream analysis. See page 2 for details.

Sample preparation is a critical step in a wide variety of analytical techniques in which a sample is biochemically or enzymatically treated for downstream analysis. In the realm of nucleic acid manipulation, sample preparation relates to the steps that occur following initial purification of DNA. Recently, the number of applications that require upstream preparation of nucleic acid samples has grown dramatically. Techniques such as next-generation sequencing, microarrays and library construction all require efficient, uniform and unbiased processing of nuclear material for accurate analysis. As a result, the demand for high quality sample preparation reagents has grown, as well as the need for novel reagents, novel formats of existing reagents and more stringent quality controls.

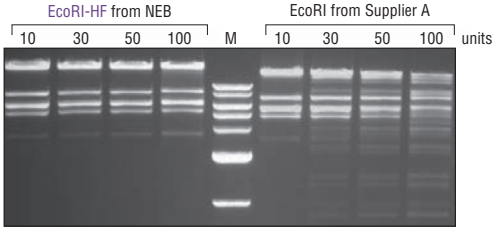
Next Generation Sequencing

In 2003, the completion of the human genome sequence marked the end of a large international project, but also the beginning of novel sequencing methods. During the following year, the National Human Genome Research Institute (NHGRI) began funding sequencing projects with the goal of lowering the cost of sequencing a human genome from $3 billion (1) down to $100,000 – and ultimately to $1,000. Since then, several strategies for massively parallel sequencing have been commercialized or are in development. These include pyrosequencing (Roche 454), sequencing by synthesis (Illumina, Intelligent Bio-Systems, Helicos BioSciences, LaserGen), sequencing by ligation (Applied Biosystems/Life Technologies, Complete Genomics, Polonator), real-time sequencing (Pacific Biosciences) and nanopore sequencing (Oxford Nanopore Technologies, Sequenom).

(continued on page 2)

High Fidelity (HF) Restriction Enzyme Sampler

High Fidelity (HF) Restriction Enzymes have been engineered to maximize performance under a wider range of conditions, including reaction volume, incubation time and buffer compatibility. For a limited time, purchase the HF Restriction Enzyme Sampler, containing sample sizes (20 µl each) of a selection of our most popular restriction enzymes in HF format. The exceptional value of the Sampler makes it the perfect way to try out an HF enzyme.



HF enzymes reduce star activity (non-specific cleavage), even at higher enzyme amounts. 50 µl reactions were set up according to manufacturer's recommendations using 1 µl λ DNA and incubated at 37°C overnight.

Introductory Offer

For a limited time, try the
HF Restriction Enzyme Sampler for $15

Advantages of HF enzymes

- Same specificity and price as wild type*
- Reduced star activity offers added flexibility
- 100% activity in NEBuffer 4, simplifying double digests
- Time-Saver qualified – digests DNA in 5 minutes

The HF Sampler includes

- EcoRI-HF™
- BamHI-HF™
- SalI-HF™
- NotI-HF™
- BSA
- NEBuffer 4

High Fidelity (HF) Restriction Enzyme Sampler
#R3000S.....$15

See page 6 for additional HF offers.

*BamHI-HF is offered at a different $/unit, with a lower cost/vial.

The Next Generation of Reagents for Sample Preparation

(continued from page 1)

In recent years, the advent of these technologies has revolutionized the world of sequencing and biology. There is an insatiable appetite for DNA sequencing across diverse scientific disciplines including microbiology, evolution, ecology, forensics, epidemiology, comparative genomics, and diagnostic applications.

Parallel sequencing methods have enabled whole genome resequencing of human genomes (2,3), analysis of chromatin binding sites along the entire genome by ChIP analysis (4,5), methylation analysis (6), transcriptome analysis (7), metagenomic surveys, and sequencing of ancient DNA (8,9).

Reagents for Sample Preparation

DNA and RNA sample preparation is one of the most important aspects of optimization of the quality and quantity of nucleic acid material for next generation sequencing. The exquisite sensitivity of these technologies places new demands on all the reagents involved, including those required for the upstream sample preparation process, which is outlined in Figure 1. During sample preparation, genomic DNA is sheared into 150 – 800 bp fragments. This can be done by nebulization, acoustic shearing or by treatment with enzymes. DNA sheared by nebulization or acoustic shearing may contain ends that are incompatible (3´ or 5´ overhangs and 3´-phosphates or 5´-OH) with subsequent ligation steps and therefore must be repaired. In some cases, it may be necessary to convert a mixture of heterogeneous ends to a homogenous population of blunt ended DNA fragments with 5´-phosphates and 3´-OH. Furthermore, if dA tailing is required for subsequent ligation, a 3´ dAMP must be added to a blunt ended fragment. Adaptors are then ligated either to blunt ended or dA tailed fragments and the entire library is amplified.

Because sample preparation is a series of coordinated reactions, each step must be as efficient as possible to maximize yield while at the same time maintaining convenience and cost effectiveness. The efficiency of end repair, dA tailing, ligation and amplification are all dependent on the purity of the enzymes that perform these steps.

For 35 years, New England Biolabs has been committed to providing enzymes that drive advances in molecular biology. The breadth of NEB products is built on a foundation of core enzymes that has expanded to include enzymes with unique properties, as well as new applications and combinations of existing enzymes. In fact, scientists have been successfully using NEB reagents for sample preparation of nucleic acid material for many years, and to meet the demands of our customers, NEB offers a broad selection of reagents that facilitate sample preparation of DNA (Figure 2). Additionally, NEB has taken a dual approach to developing products for sample preparation:

1.) Develop novel solutions for multiple steps in sample preparation processes. For example, the PreCR Repair Mix (NEB #M0309) is an optimized enzyme master mix that repairs damaged DNA and also offers a simplified workflow.

2.) Offer additional formulations of reagents for increased ease-of-use in specific applications. An example of this approach is the NEBNext™ DNA Sample Prep Reagent Set 1, a set of enzymes, nucleotides and buffers ideally suited for sample preparation for next-generation sequencing and expression library construction (see side bar for more information).

Quality Assurance

Our priority is to provide product formulations that appropriately address existing and future demanding applications, thereby providing maximum convenience and quality to customers. As new and more sensitive applications develop, quality controls and functional assays must evolve to ensure that the more stringent demands placed on reagents are met. For example, each component of the NEBNext DNA Sample Prep Reagent Set 1 undergoes rigorous quality controls and validation assays. In addition to the standard quality controls for contaminating endonuclease and exonuclease activities, each component also meets further

NEBNext™ DNA Sample Prep Reagent Set 1

The NEBNext™ DNA Sample Prep Reagent Set 1 is a set of reagents that facilitates sample preparation for downstream applications including next-generation sequencing, library construction and microarrays. Each of these components must pass rigorous quality control standards and are functionally validated by preparation of a genomic library sequenced on an Illumina Genome Analyzer II, and by construction of an expression library.

Enzymes included in set:

- T4 DNA Polymerase RR
- DNA Polymerase I, Large (Klenow) Fragment RR
- T4 Polynucleotide Kinase RR
- Klenow Fragment (3´→ 5´ exo-) RR
- Quick T4 DNA Ligase RR
- 2X Phusion™ High-Fidelity PCR Master Mix*

Buffers and additional components included in set:

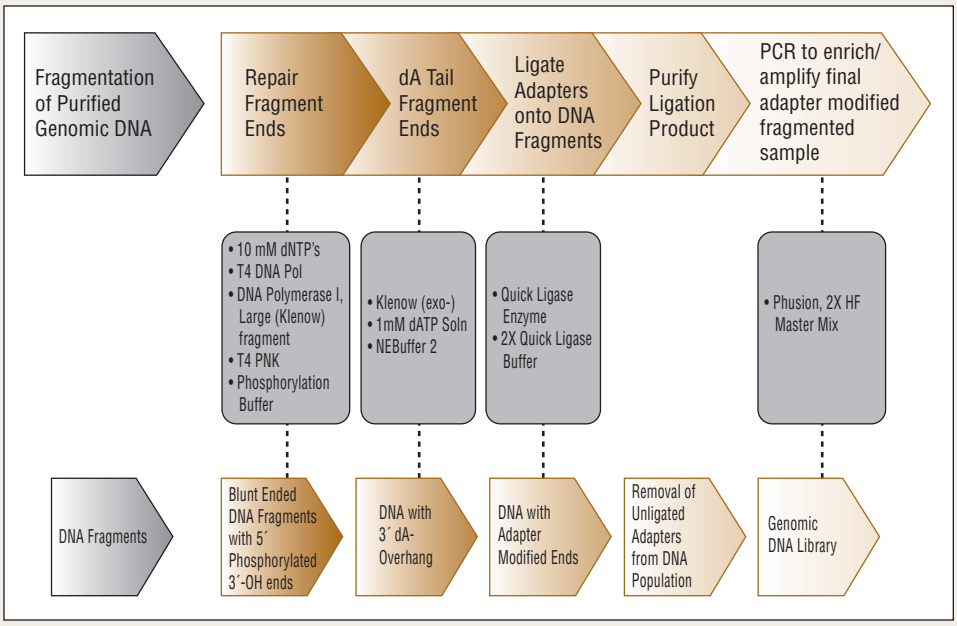
- 10X Phosphorylation Buffer
- Deoxynucleotide Solution Mix (10 mM)
- dATP Solution (1 mM)
- 10X NEBuffer 2
- Quick Ligation Reaction Buffer

NEBNext™ DNA Sample Prep Reagent Set 1

#E6000S	10 reactions	$500
#E6000L	50 reactions	$2,000

*Produced by Finnzymes, distributed by New England Biolabs
Phusion is a trademark of Finnzymes, Oy.

Figure 1: Genomic library construction using NEB reagents†



† *For construction of expression libraries, dA tailing is optional, and blunt/A-tailed fragments are ligated to linearized vector. PCR Amplification is also optional for this application.*

stringent quality controls relevant for these applications, including screening for RNase activity, phosphatase activity, functional activity assays and physical purity. Finally, these reagents are functionally validated together by preparation of a genomic DNA library that is sequenced on an Illumina Genome Analyzer II, and by preparation of an expression library.

Examples of the individual quality control assays performed for Quick T4 DNA Ligase, a component of the NEBNext DNA Sample Prep Reagent Set 1, are seen in the sidebar. Specific details of each quality control are supplied on the data card accompanying the product.

For more information regarding the NEBNext product line, please contact NEBNext@neb.com.

Customized Solutions

Additionally, to meet the demands of high-throughput customers, NEB reagents can be supplied in bulk quantities, with customized formulations and supply formats available. NEB is committed to working closely with larger consumption customers at the research, development and production levels to optimize reagent integration specific to technology platforms.

For more information regarding customized solutions and OEM opportunities, please contact oem@neb.com.

New England Biolabs is committed to meeting the current and emerging needs of scientists in this rapidly evolving area of research.

References

(1) Collins, F. S., et al. (2003). "The Human Genome Project: Lessons from a large-scale biology." *Science*, 300, 286–290.

(2) Bentley, D. R., et al. (2008). "Accurate whole human genome sequencing using reversible terminator chemistry." *Nature*, 456, 53–9.

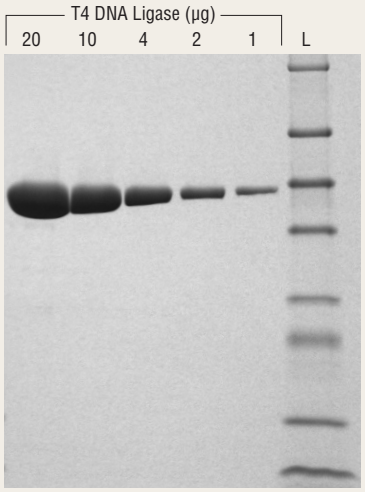


Figure 3. *A dilution series of Quick T4 DNA Ligase analyzed by SDS-PAGE illustrates physical purity. L is the Protein Ladder (NEB #P7703).*

(3) Wheeler, D. A., et al. (2008). "The complete genome of an individual by massively parallel DNA sequencing." *Nature*, 452, 872–6.

(4) Johnson, D. S., et al. (2007). "Genome-wide mapping of *in vivo* protein-DNA interactions." *Science*, 316, 1497–502.

(5) Mikkelsen, T. S., et al. (2007). "Genome-wide maps of chromatin state in pluripotent and lineage-committed cells." *Nature*, 448, 553–60.

(6) Meissner, A., et al. (2008). "Genome-scale DNA methylation maps of pluripotent and differentiated cells." *Nature*, 454, 766–70.

(7) Tang, F., et al. (2009). "mRNA-Seq whole-transcriptome analysis of a single cell." *Nat. Methods*, 6, 377–82.

(8) Ermini, L., et al. (2008). "Complete mitochondrial genome sequence of the Tyrolean Iceman." *Curr. Biol.*, 18, 1687–93.

(9) Green, R. E., et al. (2008). "A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing." *Cell*, 134, 416–26.

Scientific Contributors

Cynthia Henrickson, Ph.D. and Derek Robinson – development of quality controls

Quality Control Assays for Quick T4 DNA Ligase, a component of NEBNext DNA Sample Prep Reagent Set 1††

- *Physical Purity:* SDS-PAGE analysis is used to determine the physical purity of the enzyme. (Figure 3).
- *16-Hour Incubation:* An incubation with linear DNA substrate for 16 hours is performed to detect any non-specific nuclease degradation.
- *Endonuclease Activity:* Incubation with supercoiled plasmid DNA for 4 hours is used to detect any non-specific endonuclease activity.
- *Phosphatase Activity:* The enzyme is tested for contaminating phosphatase activity by incubation with 2.5 mM *p*-nitrophenyl phosphate and detection of *p*-nitrophenylene anion by spectrophotometric analysis.
- *RNase Activity:* RNase contamination is detected by a fluorescence-based assay using an RNA transcript.
- *Exonuclease Activity:* Contaminating exonucleases are assayed for by monitoring release of a radioactively labeled DNA substrate.
- *Functional Activity (Blunt End Ligation):* Blunt ended DNA fragments are ligated together using Quick T4 DNA Ligase and monitored on an agarose gel.
- *Functional Activity (Cohesive End Ligation):* DNA fragments with 5' overhang cohesive ends are ligated using Quick T4 DNA Ligase. To confirm that the ligation was specific and that the 5´ ends were intact, the ligation junctions are cleaved with a restriction enzyme.
- *Functional Activity (Adapter Ligation):* Adapters are ligated by Quick T4 DNA Ligase and monitored on an agarose gel.
- *Functional Activity (Transformation):* Dephosphorylated vector and compatible insert fragments are ligated using Quick T4 DNA ligase and transformed into competent *E. coli* cells.

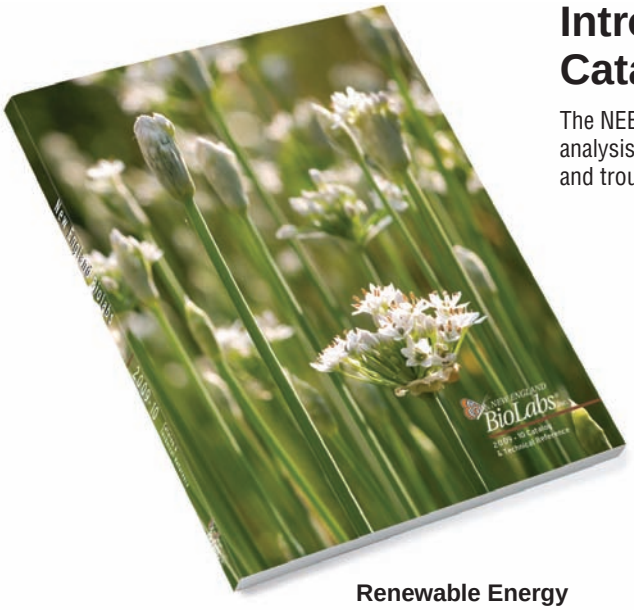
†† Specific details of each quality control are supplied on the datacard.

Figure 2: Reagents for DNA Sample Preparation

DNA SAMPLE PREPARATION	REPAIR OF ISOLATED DNA	END BLUNTING	3´ dA TAILING	ADAPTOR LIGATION	AMPLIFICATION	PAIRED ENDS
NEBNext™ DNA Sample Prep Reagent Set 1	✔	✔	✔	✔	✔	✔
PreCR™ Repair Mix R	✔					
Quick Blunting Kit R		✔				
T4 DNA Polymerase R		✔				
Klenow DNA Polymerase R		✔				
T4 Polynucleotide Kinase R		✔				
Klenow (exo-) DNA Polymerase R			✔			
Quick Ligase R				✔		
T4 DNA Ligase R				✔		
Phusion™ DNA Polymerase** R					✔	
Bst DNA Polymerase R					✔	
MmeI R						✔
EcoP15I R						✔

**Produced by Finnzymes, distributed by New England Biolabs. Phusion is a trademark of Finnzymes, Oy.

R = recombinant



Introducing the 2009-10 NEB Catalog and Technical Reference

The NEB Catalog & Technical Reference contains many new reagents for PCR, gene expression, cellular analysis, markers and ladders, competent cells and RNA analysis. Up-to-date technical charts, protocols and troubleshooting tips have also been added to aid experimental design. Catalog highlights include:

- High Fidelity (HF) enzymes – the next generation in restriction enzyme technology
- A comprehensive offering of PCR reagents
- The latest innovation in competent cells for protein expression
- An extensive selection of markers and ladders for DNA, RNA and protein analysis
- A new Gene Expression & Cellular Analysis section showcasing a powerful protein labeling technology unique to NEB
- A range of expression systems, including a novel kit for cell-free expression
- Tools for glycobiology
- Tools to enable RNA research
- Over 60 products with price decreases/unit size increases resulting in a lower cost/unit

Renewable Energy

Each edition of the NEB catalog contains a collection of minireviews that address various environmental topics. The theme of this catalog is "Renewable Energy". Generated from natural resources, renewable energy can come from wind power, wave power, tidal power, solar power, geothermal energy, biomass and biofuels. According to the official energy statistics from the US government, renewable energy contributed 7% to the Nation's total energy demand in 2007, and is expected to grow in the future.

Featured products in the new catalog

New Cellular Imaging & Analysis

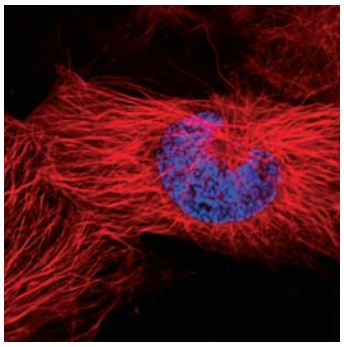
New England Biolabs introduces new tools for the specific labeling of fusion proteins with synthetic probes for examining various aspects of protein function in live cells and in cell lysates. SNAP-, CLIP-, MCP- and ACP-tag technologies bring simplicity and versatility to the imaging of proteins in live and fixed cells, as well as *in vitro* protein studies. A single gene construct yields a tagged fusion protein capable of forming a covalent linkage to a variety of functional groups, including fluorophores, biotin, or beads. Once cloned and expressed, a single construct can be used for numerous downstream applications such as:

- Simultaneous dual protein labeling inside live cells
- Protein localization and translocation
- Pulse-chase experiments
- Receptor internalization studies
- Selective cell surface labeling
- Protein pull down assays
- Protein detection in SDS-PAGE
- Flow cytometry
- High throughput binding assays in microtiter plates
- Biosensor interaction experiments
- FRET-based binding assays

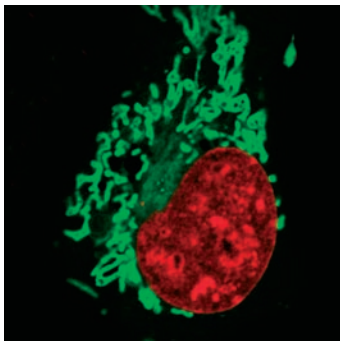
To help you get started, NEB offers several starter kits that include a plasmid encoding a tag, a control plasmid, two fluorophores and blocking agent (in most cases). Choose a starter kit to become familiar with the technology and build experience with the techniques.

To view the entire product line, including starter kits, fluorescent substrates, blocking agents, cloning vectors and plasmids and building blocks, please visit www.neb.com.

PRODUCT	NEB #	PRICE
SNAP-Cell™ Starter Kit	E9100S	$225
SNAP-Surface™ Starter Kit	E9120S	$225
CLIP-Cell™ Starter Kit	E9200S	$225
CLIP-Surface™ Starter Kit	E9230S	$225
ACP-Surface Starter Kit	E9300S	$225



SNAP-Cell™: *Live COS-7 cells transfected with pSNAP-tubulin β. Cells were labeled with SNAP-Cell™ TMR-Star (red) for 30 minutes and counterstained with Hoescht 33342 (blue) for nuclei.*



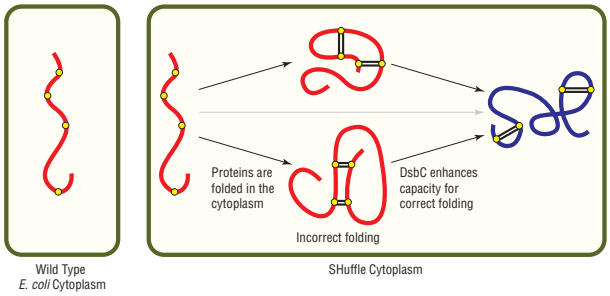
CLIP-Cell™: *Live COS-7 cells transfected with pCLIP-H2B (Histone H2B) and pSNAP-Cox8A (mitochondrial cytochrome oxidase 8A). Cells were labeled with CLIP-Cell™ TMR-Star (red) and SNAP-Cell™ Oregon Green (green) for 30 minutes.*

New SHuffle™ Competent *E. coli*

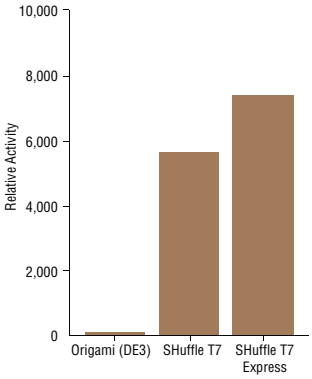
Exclusively from NEB, SHuffle™ Competent *E. coli* strains are engineered for cytoplasmic expression of proteins with increased disulfide bond complexity. The introduction of the isomerase DsbC to the SHuffle chromosome further enhances the correct formation of disulfide bonds in proteins that require them for biological activity. SHuffle is ideal for expression of antibodies, hormones, proteases, toxins and cellular signaling proteins.

Advantages:

- High level expression of proteins requiring disulfide bond formation
- Increased yield of biologically active protein
- Cytoplasmic expression
- A wide range of antibiotic resistance
- High Transformation efficiency
- Value pricing



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.



Express higher levels of biologically active protein with SHuffle. *Plasmodium falciparum chitinase (PfCHT1) with three cysteines was expressed from a plasmid under the regulation of T7 promoter. After induction, cells were harvested and crude cell lysates were prepared. PfCHT1 was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.*

PRODUCT	NEB #	SIZE	PRICE
E. coli K12 STRAINS			
SHuffle™ Competent *E. coli*	C3025H	6 x 0.05 ml	$60
SHuffle™ T7 Competent *E. coli*	C3026H	6 x 0.05 ml	$60
SHuffle™ T7 *lysY* Competent *E. coli*	C3027H	6 x 0.05 ml	$60
E. coli B STRAINS			
SHuffle™ Express Competent *E. coli*	C3028H	6 x 0.05 ml	$60
SHuffle™ T7 Express Competent *E. coli*	C3029H	6 x 0.05 ml	$60
SHuffle™ T7 Express *lysY* Competent *E. coli*	C3030H	6 x 0.05 ml	$60

For added convenience:

SHuffle Sampler Pack

Find the optimal SHuffle strain by purchasing the SHuffle Sampler. The Sampler is an exceptional value when compared to purcahsing each strain separately.

#C3032I.....$180

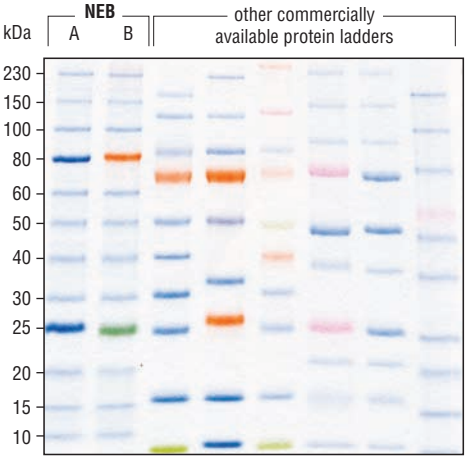
New Protein Ladder

The Protein Ladder is a mixture of recombinant, highly purified proteins that resolve into 12 sharp, evenly spaced bands when analyzed by SDS-PAGE. The Protein Ladder is now available in three convenient formats: unstained for accurate molecular weight determination, prestained, and prestained with two additional colored bands for easy identification.

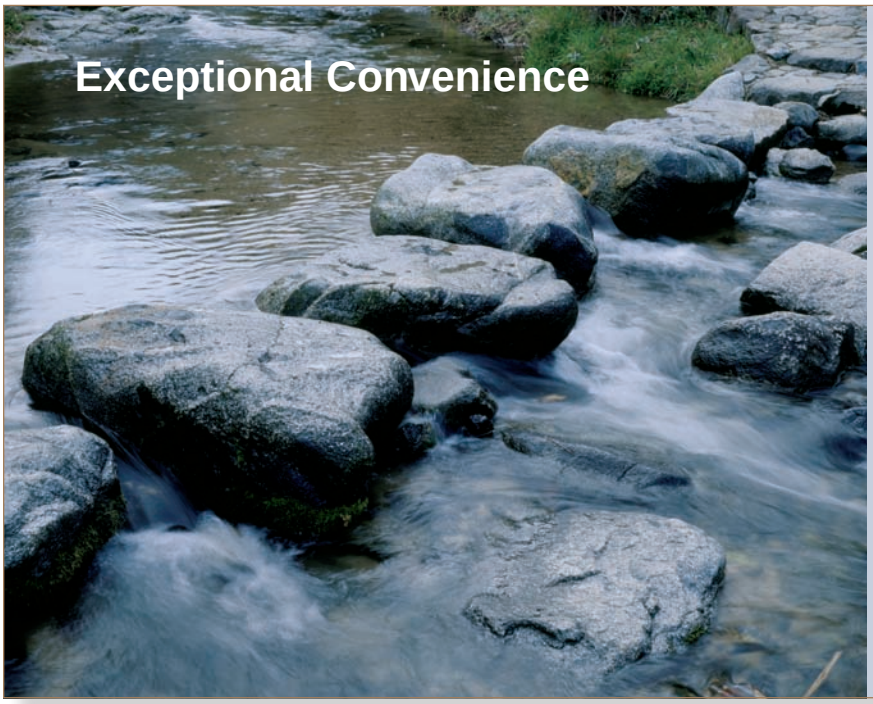
Advantages:

- Suitable for analysis of a wide range of proteins
- Uniform band intensities
- Convenient band spacing
- Easy-to-identify reference bands
- Value pricing

PRODUCT	NEB #	SIZE	PRICE
Protein Ladder (10-250 kDa)	P7703S	100 mini-gel lanes	$70
Prestained Protein Ladder, Broad Range (10-230 kDa)	P7710S	100 mini-gel lanes	$85
ColorPlus Prestained Protein Ladder, Broad Range (10-230 kDa)	P7711S	100 mini-gel lanes	$95



Choose the NEB ladder for sharp, evenly spaced bands, convenient band spacing and choice of formats. (A) ColorPlus Prestained Protein Ladder (NEB #P7711), (B) Prestained Protein Ladder (NEB #P7710).



NEB Restriction Enzymes

New England Biolabs offers unmatched convenience when selecting a restriction enzyme. With over 160 enzymes recommended for use in a single buffer, reactions can be set up quickly and easily. For additional flexibility, try a High Fidelity (HF) enzyme with reduced star activity. Reaction times can be shortened to as little as 5 minutes using one of our Time-Saver qualified enzymes. With over 220 specificities to choose from, NEB enzymes deliver convenience you can count on.

- **Selection** – More specificities than any other supplier
- **Convenience** – Optimal activity for over 160 enzymes in a single buffer (NEBuffer 4)
- **Quality** – State-of-the-art production and stringent QCs
- **Innovation** – HF enzymes engineered for reduced star activity
- **Performance** – Guaranteed...

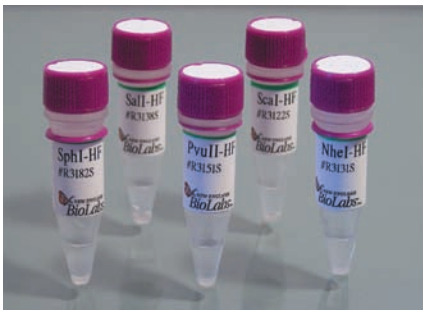
Reduce Star Activity with High Fidelity Restriction Enzymes only from NEB

PRODUCT NAME	NEB #	BUFFER†	MAXIMUM UNITS WITH NO STAR ACTIVITY*	HF FACTOR**
BamHI-HF™	R3136	4	4,000	125
BamHI	R0136	3 + BSA	32	
EagI-HF™	R3505	4	500	2
EagI	R0505	3	250	
EcoRI-HF™	R3101	4	16,000	64
EcoRI	R0101	U	250	
EcoRV-HF™	R3195	4	64,000	64
EcoRV	R0195	3 + BSA	1,000	
MfeI-HF™	R3589	4	500	15
MfeI	R0589	4	32	
NcoI-HF™	R3193	4	16,000	133
NcoI	R0193	3	120	
NheI-HF™	R3131	4 + BSA	32,000	266
NheI	R0131	2 + BSA	120	
NotI-HF™	R3189	4 + BSA	64,000	16
NotI	R0189	3 + BSA	4,000	
PvuII-HF™	R3151	4	500	31
PvuII	R0151	2	16	
SacI-HF™	R3156	4 + BSA	4,000	33
SacI	R0156	1 + BSA	120	
SalI-HF™	R3138	4	2,000	500
SalI	R0138	3 + BSA	4	
SbfI-HF™	R3642	4	250	31
SbfI	R0642	4	8	
ScaI-HF™	R3122	4	250	62
ScaI	R0122	3	4	
SphI-HF™	R3182	4	2,000	62
SphI	R0182	2	32	
SspI-HF™	R3132	4	500	ND
SspI	R0132	U	ND	

Special Offer

Through 7/31, receive a **50% discount** on any **High Fidelity (HF) restriction enzyme**

Use coupon code QHFEXP when placing an order. Offer valid on online orders in the US only. No additional discounts apply.



Visit our website to view the growing list of HF enzymes from NEB.

U = Unique
ND = Not determined

† *Wild type enzymes were tested in supplied buffer for comparisons.*
* *Wei, H. et al (2008) Nucleic Acids Reseach 36, e50.*
** *The HF factor refers to the x-fold fidelity increase achieved by choosing an HF enzyme.*

Restriction Enzyme Troubleshooting Guide

Problem	Possible Cause	Solution
Incomplete or no digestion	DNA is contaminated with an inhibitor	1. Assay a mixture of substrate and control DNA. Control DNA will not cleave if an inhibitor is present. 2. Purify DNA with a spin column, resin or drop dialysis, or increase volume to dilute contaminant. Note: Miniprep DNA is particularly susceptible to contamination.
	Reaction conditions are not optimal	1. Use recommended buffer supplied with restriction enzyme 2. Follow recommendations for double digestion, or try a sequential digest 3. Repeat with fresh buffer. Additives present in buffer (e.g., DTT, SAM) may degrade over time.
	Enzyme concentration is too low	Some supercoiled plasmids or genomic DNAs may require up to 10–20 units/µg
	Additive is missing	Repeat reaction setup, being sure that enzyme and/or additive (e.g., BSA) is added
	DNA concentration is not optimal	NEB recommends 1 µg of DNA in a 50 µl reaction. Excess DNA may result in incomplete cleavage.
	Incubation time was too short	Some enzymes can exhibit slower cleavage towards specific sites. In most cases, 1–2 hours are sufficient.
	Enzyme has not been stored properly or mishandled	1. Test enzyme on control DNA with known multiple sites 2. Enzyme should be stored at –20°C. Enzymes stored at –70°C will freeze, and repeated freeze/thaw cycles may reduce enzyme activity.
	Recognition site is not present	Confirm DNA sequence
	Cleavage is blocked by methylation	1. Some recognition sites are blocked by Dam or Dcm methylation. If site is blocked, DNA should be passed through a *dam-/dcm-* strain (NEB #C2925). 2. Eukaryotic genomic DNA may be blocked by CpG methylation. This can be overcome by cloning into a bacterial host. Note: PCR products are not methylated
	DNA may be supercoiled	Restriction enzymes cleave supercoiled DNA with varying efficiency. Additional enzyme may be required.
	Recognition site may be too close to the end of the DNA fragment	As a general rule, add 6 bases pairs on either side of the recognition site for efficient cleavage
	Site preference	Some enzymes require two recognition sites for efficient cleavage (e.g., SfiI)
Unexpected Cleavage Pattern	Incomplete cleavage	Extra bands may be due to uncut sites. Add additional enzyme or increase incubation time. Also, see star activity below.
	DNA sample is contaminated	Prepare a new DNA sample
	Additional recognition sites are present in DNA	Confirm DNA sequence
	Star Activity	See tips for avoiding star activity (see 2009-10 NEB Catalog & Technical Reference page 326) and/or use a High Fidelity Restriction Enzyme (see 2009-10 NEB Catalog & Technical Reference page 327).
Smearing of DNA on gel	Enzyme has a high binding affinity to DNA and will not dissociate well	Add SDS to the gel loading dye/stop solution to a final concentration of 0.1–0.5% to help dissociate the enzyme from the DNA
	Nuclease contamination	Care should be taken to avoid cross-contamination when setting up reactions
	Agarose gel running conditions	Use fresh running buffer and appropriate voltage to avoid over heating

NEB FAQ Spotlight

High Fidelity (HF) Restriction Enzymes

Q Why choose an HF enzyme?

A 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 will simplify double digest reactions. They are all Time-Saver qualified and will digest substrate DNA in five minutes. As with all of our restriction enzymes, HF enzymes undergo stringent quality controls and offer the highest quality that you have come to expect from NEB.

Q Why does the HF version of the enzyme have a different recommended buffer than the wild type?

A In many cases, the mutation introduced into the HF enzyme results in significant changes in buffer preference. For example, wild type SalI has a strict requirement for NEBuffer 3, a high ionic strength buffer. However, SalI-HF works well in NEBuffer 4, which is a moderate ionic strength buffer.

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New England Biolabs, Inc.
240 County Road
Ipswich, MA 01938-2723
1-800-NEB-LABS
www.neb.com

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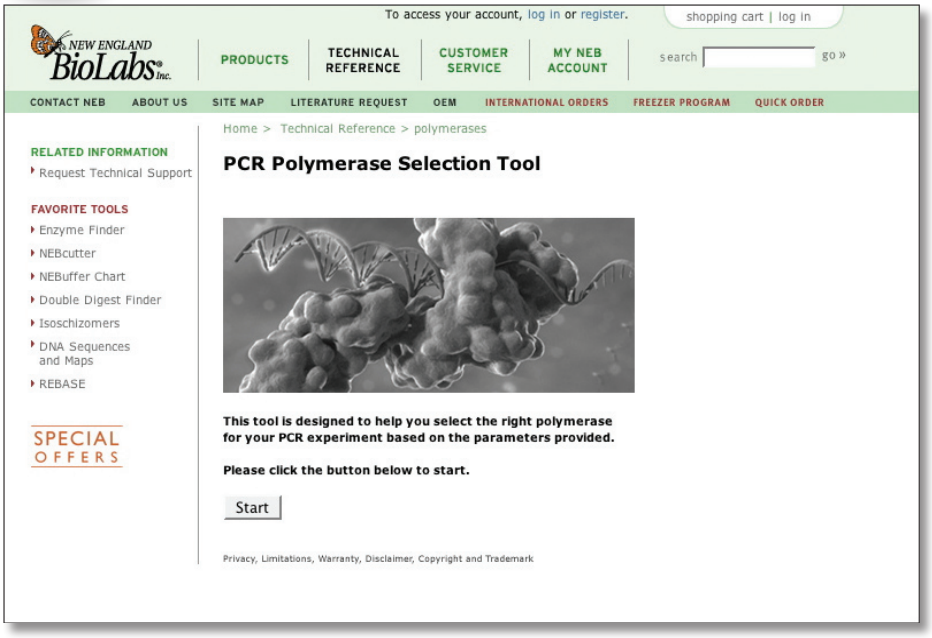
Web Tool Focus

New PCR Selection Tool



Our new PCR Polymerase Selection Tool will recommend the optimal DNA polymerase for your experiment based on the parameters provided. NEB offers a wide range of PCR polymerases in several formats for specific research applications, various template lengths and particular buffer requirements.

www.neb.com/nebecomm/PcrPolymeraseSelectionTool.asp

Upcoming Conferences

NEB will be exhibiting at the following conferences:

- 23rd Annual Symposium of the Protein Society
  July 25-29, 2009
  Boston, Massachusetts
  Boston Marriott Copley Place

- Cambridge Healthtech Institute's The Bioprocessing Summit
  August 24-27, 2009
  Royal Sonesta Hotel
  Cambridge, MA

- Cambridge Healthtech Institute Exploring Next Generation Sequencing
  September 21-23, 2009
  Rhode Island Convention Center
  Providence, RI

- Donald Danforth Plant Science Symposium
  September 23-25, 2009
  Donald Danforth Center
  St. Louis, MO

Stop by the booth to learn about new products and special offers.