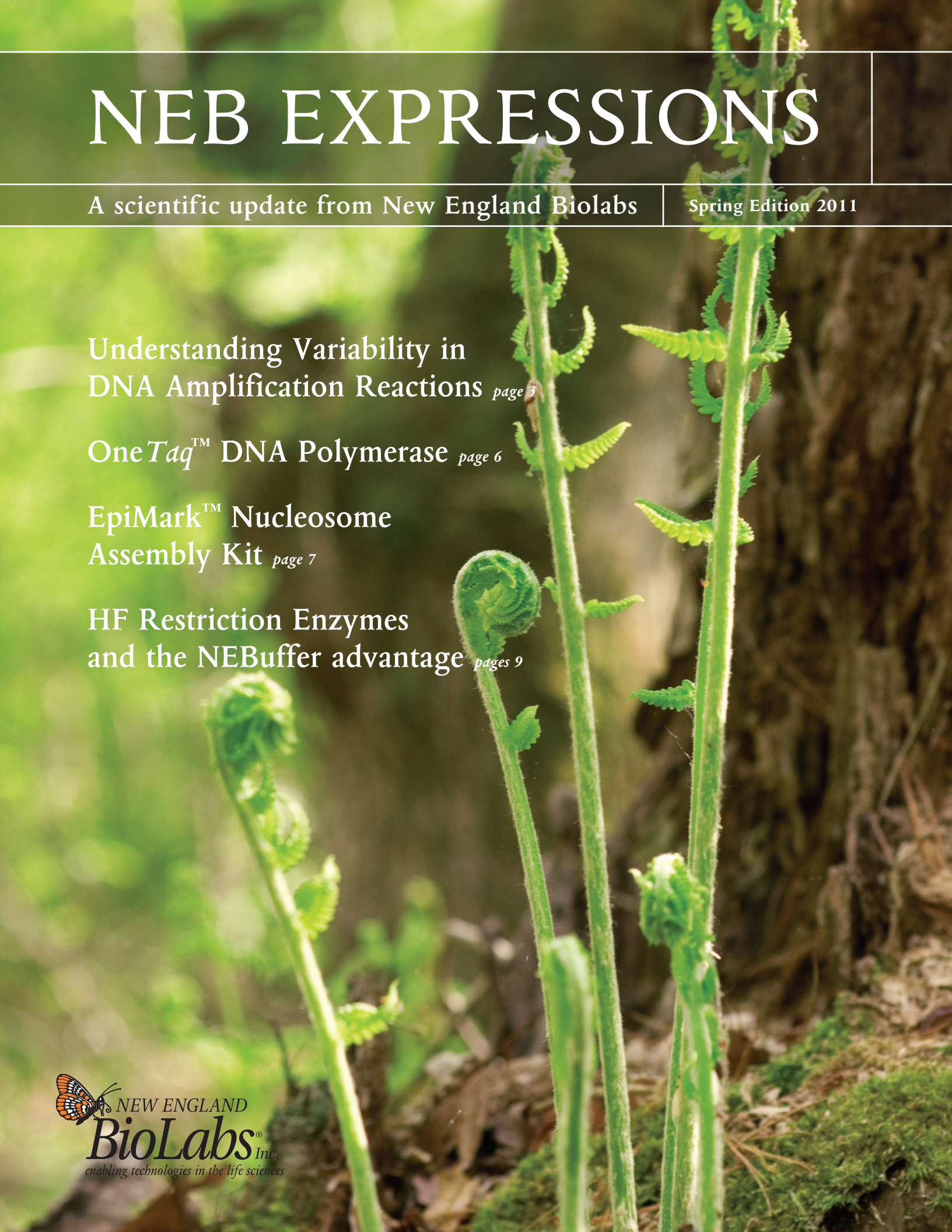
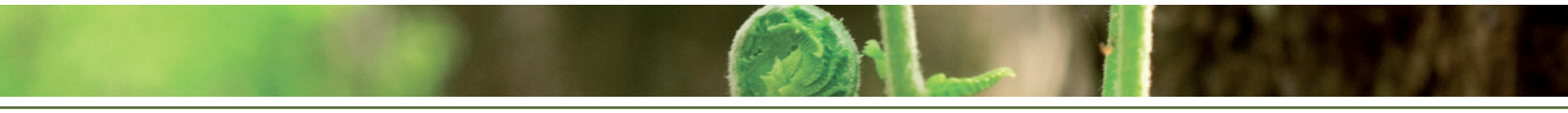
NEB EXPRESSIONS

A scientific update from New England Biolabs | Spring Edition 2011



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



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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **ESHG, European Human Genetics Conference 2011**
  May 28 - 31, 2011
  Amsterdam RAI, The Netherlands,
- **EMBO Conference Series: Chromatin and Epigenetics**
  June 1 - 5, 2011
  EMBL Heidelberg, Germany
- **36th FEBS Congress (Federation of European Biochemical Societies)**
  June 25 - 30, 2011
  Torino (Turin), Italy

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A Letter from NEB

Dear Researcher,

For over 30 years, New England Biolabs, Inc. has been at the forefront of the isolation, characterization and cloning of polymerases. Our experience and understanding of these integral reagents allows us to develop new polymerases that meet the needs of our customers, as well as optimize their use in PCR. The importance of understanding the interplay between variables in a PCR experiment is explored in the feature article. We also introduce our PCR Test Panel, a systematic approach designed to better understand the contribution of the numerous reagents and conditions in an amplification reaction.

The PCR Test Panel has helped us develop a new polymerase that is ideal for routine or problematic amplicons. One*Taq*™ DNA Polymerase offers a robust solution to the vast majority of endpoint PCR needs and shows exceptional performance against other commercially available polymerases, especially when amplifying difficult or GC-rich templates.

Wishing you continued success in your research,

New England Biolabs

Spring at New England Biolabs' campus.

Try One*Taq*™ for a Chance to Win an iPad®

Try our new One*Taq*™ DNA Polymerase! Send us your feedback, including data, and receive a free NEB lab timer and a chance to win one of six iPads®*.

For contest rules, visit
www.neb.com/OneTaqContest

This offer is valid globally, while supplies last. One submission per person.

Submit early to increase your chances of winning; iPad winners will be drawn weekly on March 14, March 21, March 28 and April 4, and then monthly on May 2 and June 6, 2011.

iPad® is a registered trademark of Apple Computer, Inc.

Understanding Variability in DNA Amplification Reactions

The polymerase chain reaction (PCR, 1) is arguably the most common technique of molecular biology. As such, it has significantly impacted research and development in fields such as biochemistry, medicine, bioengineering and beyond. Success or failure in PCR is influenced by a myriad of factors including primer design, cycling conditions, and the quality and concentration of reaction substrates and solutions. By understanding the interplay of these variables, PCR-based tools and techniques are bolstered, and difficult amplifications become routine.

Nicole M. Nichols, Ph.D., New England Biolabs

The range of variables that impact amplification reactions is masked by the high success rate of typical PCR experiments. It is only after unsuccessful amplification attempts that these variables become evident. Ideally, various components can be altered to achieve success and even to favor a desired outcome (e.g., specificity over yield, sensitivity over specificity, etc.). Practical issues, such as very high (or low) template GC content, the presence of inhibitors, limitations of primers, source materials or time can limit the ability either to follow optimized PCR guidelines or to systematically evaluate a sufficient number of variables to ensure success.

The PCR Test Panel:

With the long-standing goal of enabling the research of our customers and our own scientists, NEB continues to devote resources both to basic DNA polymerase research and to the development of new amplification products. As part of these efforts, we have created a quantitative, microfluidic-based, PCR Test Panel. The goals of the PCR Test Panel are to:

1. Systematically manipulate the numerous variables present in an amplification reaction.
2. Understand the contributions of each variable to the desired signal-to-noise outcome.
3. Facilitate the development of enhanced tools (e.g., polymerases, buffers, etc.) that will broaden the definition of "routine PCR" to encompass situations that are currently challenging.

The PCR Test Panel is enabled by a microfluidic, agarose gel-based mimic (LabChip® GX platform, Caliper Life Sciences, Hopkinton, MA) that allows rapid end-point quantitation of amplification reactions in a 96-well plate format. A supporting, internal database has been designed to link the results from the microfluidic analysis (e.g., yield and purity of each expected product) to the detailed contents of each well (e.g., identity and concentration of polymerase, template, buffer, additives). Cycling conditions, thermocycler ID and other relevant details are also tracked.

By varying large numbers of conditions and quantitating both specificity and yield of a PCR product, the contribution of each reaction component can be systematically evaluated. Some of the variations in template and primer components that are assessed by the PCR Test Panel are described in Table 1. Importantly, this system is easily adapted and can be scaled to assess new components and conditions as they arise.

In practice, the PCR Test Panel involves a typical workflow from experimental planning to data analysis, with crucial steps of database integration. The workflow is comprised of the following steps:

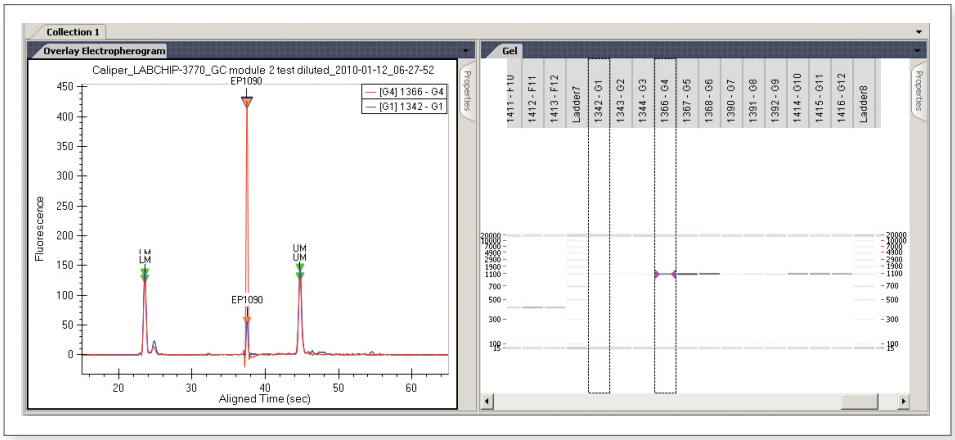
1. Design PCR experiment and capture relevant details in tracking database.
2. Run PCR experiments in triplicate (various module/experiment combinations to make 96-well plates).
3. Run 96-well plates through quantitative microfluidic analysis.
4. Export results from microfluidic platform into database to link quantitation with experimental details.

Experiments are performed in triplicate and relevant information about the contents of each well is captured in the database. The data tracking system communicates the expected product sizes for each well/reaction to the microfluidic system. The output of microfluidic analysis is an electropherogram, which for familiarity is also represented as a typical gel-based image (Figure 1). The instrument software identifies the expected peaks and quantitates the yield and percent purity of each (described in more detail below). Finally, results from the analysis are imported into the database and connected with well contents and additional experimental information, forming the foundation of an expanding data warehouse of PCR results. Simple data analysis can be accomplished within the database, and IGOR Pro (Wavemetrics, Inc., Portland, OR) has been used for more complex analysis and graphical display.

Advances in Research and Development as a result of the PCR Test Panel:

The PCR Test Panel has furthered our understanding of many aspects of amplification reactions, one example being the best use for Lambda PCR tests. The functional tests of many commercially available polymerases employ Lambda as a substrate. However, the Lambda amplification

Figure 1. Electropherogram and interpreted gel image of primary PCR Test Panel data.



The electropherogram (left) shows overlaid results from two samples (G1, G4) selected from the interpreted gel (right). Expected peaks (EP) are identified by the software from information provided by the database. For product quantitation and size assessment, a DNA ladder is run after every 12 samples and upper and lower markers (UM, LM) are mixed 1:1 with each sample immediately prior to analysis.

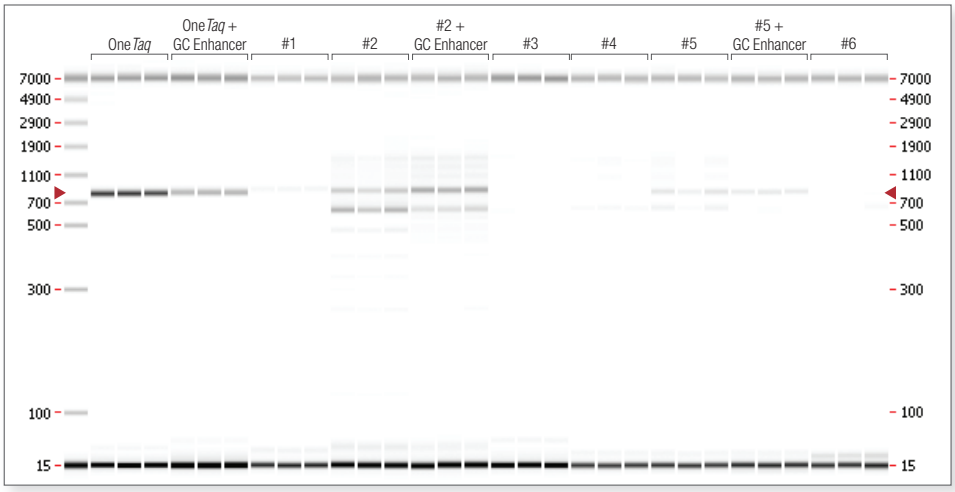
Table 1: Examples of Test Panel Variables

VARIABLE	EXAMPLES
Template Source	Genomic DNA from simple (e.g., Lambda) and complex (e.g., plant, mouse, human) organisms
Length	From <100 bases to over 10 kb
GC/AT Content	From ~80% GC to ~ 80% AT
Repeats	Homopolymers, CpG islands, triplet repeats, etc.
Primer design	Typical and suboptimal primer pairs

reactions that were part of the PCR Test Panel were so consistently robust that they did not serve as good indicators for how a polymerase would perform with real world, complex templates, even when matched for GC content. Interestingly, at typical template concentrations for Lambda, an increase in enzyme concentration resulted in a linear increase in the yield of amplified product (akin to enzyme titer experiments). A similar, linear response was not observed at working concentrations for more complex genomic targets ≤ 1kb. Instead, high variation in yield was detected for individual amplicons in response to increased enzyme concentration in the reaction. These results demonstrate that Lambda can be used as an appropriate template for quality control assays, whereas templates relevant to daily laboratory experiments are better suited for optimizing product use recommendations.

Recently, the PCR Test Panel was used in the development of the One*Taq* line of amplification products. Extensive testing was used not only to guide formulations and eventual usage recommendations, but also to assess performance relative to a broad field of competitors. These comparisons demonstrated that One*Taq* polymerases and buffers performed well in "routine" amplifications, but unlike many of their peers, continued to offer robust performance with more difficult amplicons, such as those with high GC or AT content. Figure 2 shows the gel-based image of triplicate reactions with One*Taq* Hot Start DNA Polymerase (with and without the High GC enhancer) and six other hot start polymerases (plus competitor's GC enhancers where provided) on a single high GC amplicon (68%). In addition, the quantitative primary output of microfluidic analysis (yield as ng/µl and % product purity as a function of the total signal in each reaction lane) allows a more condensed view of the data, which is more compatible with the scale of the PCR Test Panel. In Figure 3 (next page), the triplicate reactions shown in Figure 2 were condensed into a single column (boxed area in Figure 3) and are shown as part of a series of reactions with high GC, human genomic amplicons (ranging from 66%-80% GC content). One routine amplicon (55% GC) was also included as a control. Amplifications were all performed according to each manufacturer's specific recommendations. Percent product purity is represented as circles in Panel A. Product yield is expressed as a bar chart in Panel B. Reactions were set up in the absence (solid bars) and presence (striped bars) of GC enhancers, where provided by the manufacturer. Percent purity scores account for contributions from primer-dimer formation and any other non-specific products formed by the reaction. Low purity scores can arise from a prominent secondary product, from a smear of non-specific products, or from significant primer dimer interference (compare boxed area in Figure 3 to Figure 2 which shows the gel-based representation of the same data).

Figure 2. Gel view of triplicate reactions on a single 68% GC amplicon for One*Taq* Hot Start DNA Polymerase and hot start competitors (lanes #1 to #6).



A familiar gel output provides visual information about product yield and purity. Data from these triplicate reactions were averaged to create a single panel (*) of the graphs shown in Figure 3. Amplifications were all performed according to each manufacturer's specific recommendations.

Although it would be convenient to suggest a single solution to all PCR difficulties, the reality is more complex and nuanced. For example, considering only GC content, we observed that the percent GC content of a template was not always an adequate predictor of reaction difficulty, nor could it alone define the need for a GC-specific buffer or enhancer. One striking example can be seen in Figure 3, where a 73% GC amplicon could only be robustly amplified using One*Taq* in GC Buffer with the High GC Enhancer, whereas an 80% GC amplicon did not require the enhancer. Trends that emerge from studies of this scale help inform product guidelines for a broad range of product applications. For One*Taq* polymerase, these include guidelines for increasing enzyme concentration when amplifying products over 3 kb and the use of a 68°C extension temperature. More information on One*Taq* can be found on page 6 and at www.neb.com/OneTaq.

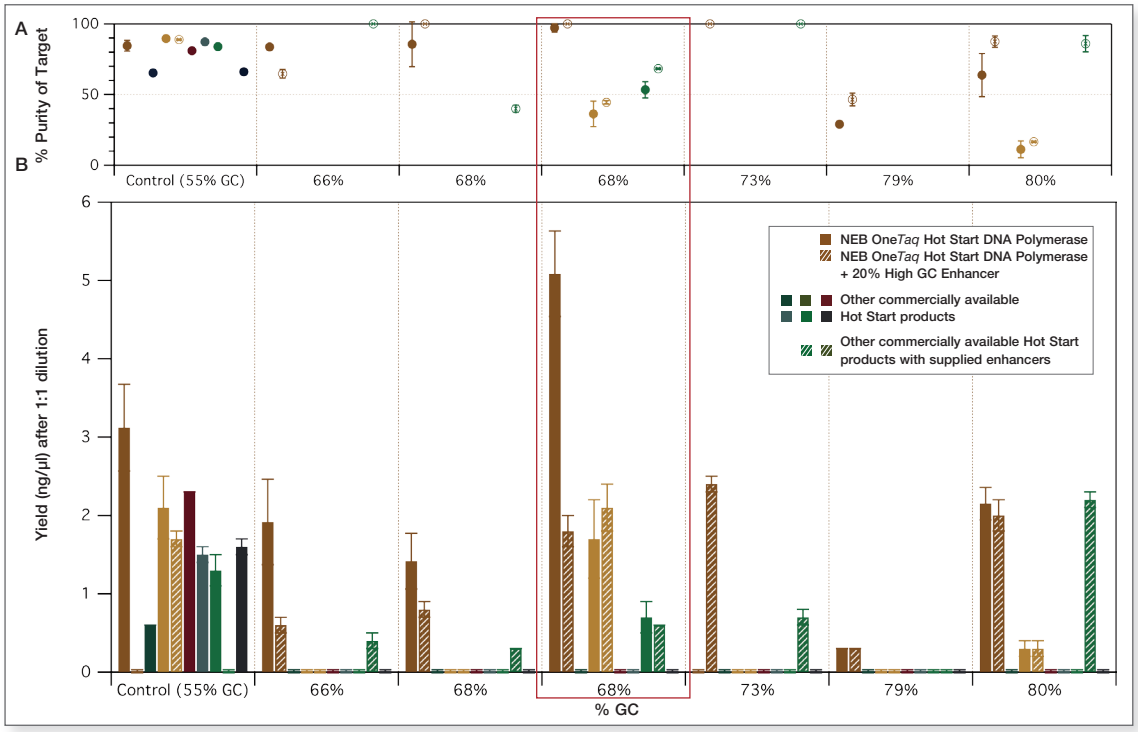
Conclusion:

Testing new products under a wide variety of conditions on a multitude of primer/template sets provides a comprehensive view of our products (and others in the market). Although it is interesting to study all the variables that affect PCR, we also realize that successful amplification of a desired target is what is important to our customers. The scope and scale of the PCR Test Panel allows us to see past the natural variability produced by the rugged PCR landscape where a single amplicon or reaction condition can be found to prove nearly any point desired. Data at this scale serves as a vantage point to build upon the strengths of various polymerases, buffers and protocols to expand the useful range of PCR conditions and the definition of what is routine. It allows our researchers at NEB to develop solutions to challenging amplifications and support your future polymerase-based applications.

References:

1. Saiki, et al., (1988) *Science* 239, 487-491

Figure 3: Examples of data from the PCR test panel: Comparison of One*Taq* Hot Start DNA Polymerase to other commercially available hot start polymerases on high GC amplicons.

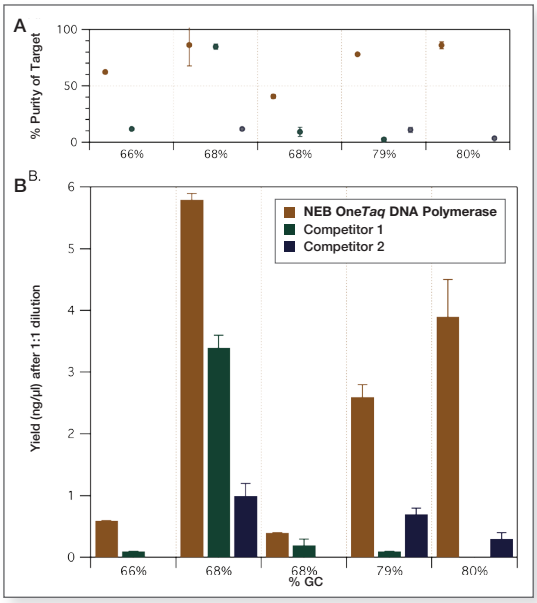


Reactions containing high GC human genomic DNA templates were set up at room temperature. PCR experiments included 30 cycles. Purity (A) and Yield (B) were calculated via microfluidic analysis from triplicate reactions. OneTaq polymerase was used in the absense (brown, solid) or presense (brown, striped) of High GC Enhancer. Competitor polymerases were cycled according to manufacturer's recommendations and included GC enhancers when supplied (striped bars).

One*Taq*: Robust amplification across a wide range of templates

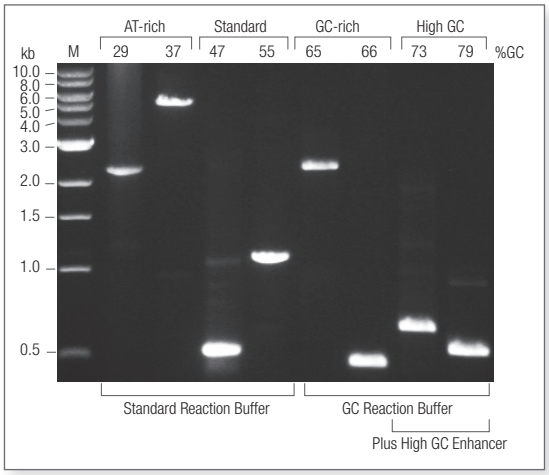
One*Taq* DNA Polymerase is supplied with two 5X buffers (Standard and GC), as well as a High GC Enhancer solution. For most routine and/or AT-rich amplicons or complex amplicons with up to ~65% GC content, One*Taq* Standard Reaction Buffer provides robust amplification. For GC-rich amplicons, the One*Taq* GC Reaction Buffer can improve both performance and yield. For particularly high GC amplicons (>65%) or difficult amplicons, the One*Taq* High GC Enhancer can be added to reactions containing One*Taq* GC Buffer. These formulations ensure maximum performance for routine, AT- or GC-rich amplicons.

Comparison of One*Taq* DNA Polymerase to other commercially available polymerases (non-hot start).



Amplification of a selection of high GC human genomic DNA templates demonstrates OneTaq performance. PCR experiments included 30 amplification cycles. Purity (A) and Yield (B) were calculated via microfluidic analysis from triplicate reactions. Competitor polymerases were cycled according to manufacturer's recommendations.

Achieve robust amplification for routine, AT- and GC-rich templates with One*Taq*



Amplification of a selection of sequences with varying AT and GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

Ordering Information

PRODUCT	NEB #	SIZE
One*Taq*™ DNA Polymerase	M0480S/L/X	200/1,000/5,000 units
One*Taq*™ 2X Master Mix with Standard Buffer	M0482S/L	100/500 reactions (50 µl vol)
One*Taq*™ 2X Master Mix with GC Buffer	M0483S/L	100/500 reactions (50 µl vol)
One*Taq*™ Quick-Load® 2X Master Mix with Standard Buffer	M0486S/L	100/500 reactions (50 µl vol)
One*Taq*™ Quick-Load® 2X Master Mix with GC Buffer	M0487S/L	100/500 reactions (50 µl vol)
One*Taq*™ Hot Start DNA Polymerase	M0481S/L/X	200/1,000/5,000 units
One*Taq*™ Hot Start 2X Master Mix with Standard Buffer	M0484S/L	100/500 reactions (50 µl vol)
One*Taq*™ Hot Start 2X Master Mix with GC Buffer	M0485S/L	100/500 reactions (50 µl vol)
One*Taq*™ Hot Start Quick-Load® 2X Master Mix with Standard Buffer	M0488S/L	100/500 reactions (50 µl vol)
One*Taq*™ Hot Start Quick-Load® 2X Master Mix with GC Buffer	M0489S/L	100/500 reactions (50 µl vol)

EpiMark™ Nucleosome Assembly Kit

NEB's line of EpiMark™ validated reagents for epigenetics studies now includes the EpiMark Nucleosome Assembly Kit. This kit contains the components necessary to form an unmodified recombinant human nucleosome using your own target DNA or the supplied control DNA. The protocol requires the mixing of already formed and purified recombinant human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer in the presence of DNA at high salt, followed by dialysis down to low salt to make nucleosomes. One tetramer associates with two dimers to form the histone octamer on the DNA, generating a nucleosome. A method for assaying nucleosome formation by gel shift assay is also provided. These nucleosomes may serve as a better substrate for enzymes that are inactive on the DNA or one of the core histones alone. Each described reaction creates nucleosomes from ~50 pmol of a 208 bp DNA and may be scaled depending on the experiment.

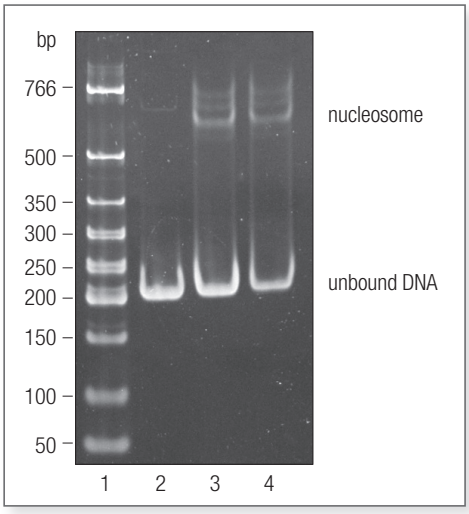
Advantages

- Highly pure, recombinant system
- Pre-formed histone dimer and tetramer complexes simplify octamer formation
- Components stable for one year
- Dilution protocol only requires a few hours for assembly

Applications

- ChIP assay
- HAT assay
- Enzyme modification assays (i.e., methylation studies)

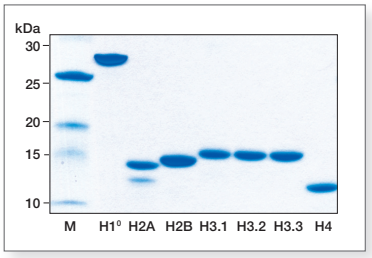
Gel shift assay to visualize nucleosome assembly



Samples from nucleosome assembly reactions were run on 6% polyacrylamide gel in 0.5X TBE.
Lane 1: Low Molecular Weight DNA Ladder (NEB #N3233)
Lane 2: Nucleosome Control DNA
Lane 3: 0.5:1 ratio of Octamer to DNA*
Lane 4: 1:1 ratio of Octamer to DNA*

**Octamer = 2:1 mix of Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer.*

Purity of NEB Histones



The EpiMark Nucleosome Assembly Kit Includes:

- Histone H2A/H2B Dimer
- Histone H3.1/H4 Tetramer
- Control DNA

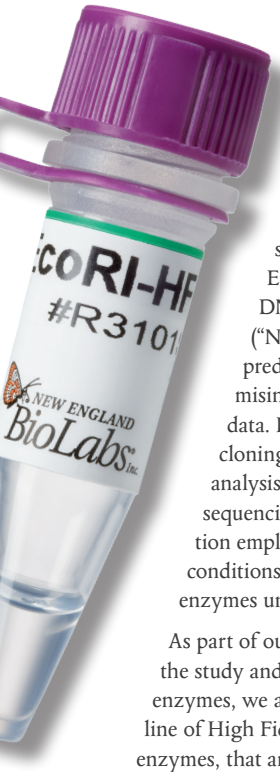
Ordering Information

PRODUCT	NEB #	SIZE
EpiMark™ Nucleosome Assembly Kit	E5350S	20 reactions
COMPANION PRODUCTS		
Histone H3.1/H4 Tetramer Human, Recombinant	M2509S	1 nmol
Histone H2A/H2B Dimer Human, Recombinant	M2508S	2 nmol
Nucleosome Control DNA	N1202S	0.2 nmol

Visit **www.epimark.com** to learn about additional products for epigenetics research available from NEB, including our broad line of recombinant human histones.

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 offer a line of restriction enzymes that have been engineered for maximum performance, convenience and flexibility.



EcoRI, BamHI, NotI, KpnI, HindIII, ...these restriction enzymes are some of the most commonly used workhorses of today's molecular biologists. However, under certain conditions these enzymes are reported to exhibit star activity. Star activity, or relaxed specificity, is an intrinsic property of restriction enzymes that leads to unwanted cleavage. In addition to its canonical consensus sequence "GAATTC" EcoRI for example may cut DNA at noncanonical sites ("NAATTC"), leading to non-predictable banding pattern and misinterpretation of experimental data. Different techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and often require the use of enzymes under suboptimal conditions.

As part of our ongoing commitment to the study and improvement of restriction enzymes, we are pleased to offer a superior line of High Fidelity (HF™) restriction enzymes, that are compatible with our existing "wildtype" restriction enzymes:

EcoRI-HF™ is one of 20+ currently available optimized High Fidelity restriction enzymes. It has been engineered by NEB's scientists by exchanging functional amino acid residues e. However, EcoRI-HF™ has the same specificity as the original established enzyme but with 64-fold reduced star activity ★-!

All engineered High Fidelity (HF™) enzymes offer the highest level of buffer compatibility, i.e. they work optimally in NEBuffer 4 NEB4. This will simplify double digest reactions, since more than 160 of NEB's restriction enzymes are supplied with NEBuffer 4 - the largest selection of restriction enzymes supplied in a single optimal buffer!

HF™ restiction enzymes have been engineered for robustness and flexibility to accelerate your reseach: all HF™ enzymes are also Time-Saver™ Qualified ◐ and allow for an optional 5 min digest as well as overnight incubations.

To distinguish these engineered enzymes, the letters -HF™ have been added to the restriction enzyme name and they are packaged in unique purple-capped tubes.

NEB offers the new High Fidelity (HF™) restriction enzymes at the same price as the original enzymes ! - We recommend using HF™ restriction enzymes whenever possible in your DNA digests!

High Fidelity (HF™) Restriction Enzymes - Advantages:

- e **Engineered for performance**
- ★- **Dramatically reduced Star Activity -** Up to 64,000-fold reduced star activity compared to wildtyp enzyme
- NEB4 **Highest Buffer Compatibility -** NEBuffer 4 is the optimal reaction buffer for over 160 restriction enzymes, including all HF™ enzmyes
- ◐ **Speed -** HF™ enzymes are Time-Saver™ qualified for 5 min digests
- ! **Value -** HF™ enzymes are offered at the same fair price as the original enzymes

Use HF™ Restriction Enzymes in all applications for optimal results and convenience!

Enzyme	optimal NEBuffer†	HF-Factor: reduced Star Activity compared to Wildtype*
AgeI-HF™	4 + BSA	≥ 8x
BamHI-HF™	4	≥ 125x
BsaI-HF™	4 + BSA	≥ 250x
DraIII-HF™	4 + BSA	≥ 1,000x
EagI-HF™	4	2x
EcoRI-HF™	4	64x
EcoRV-HF™	4	≥ 64x
HindIII-HF™	4	≥ 2.000x

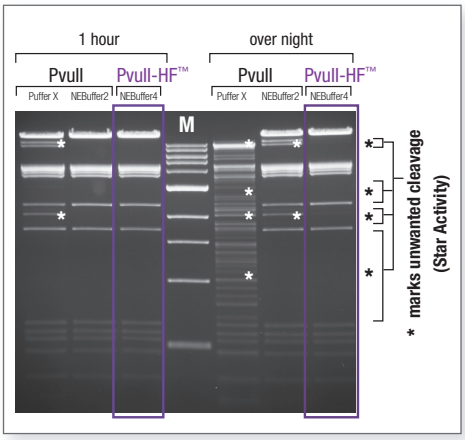
Enzyme	optimal NEBuffer†	HF-Factor: reduced Star Activity compared to Wildtype*
KpnI-HF™	4	≥ 62.500x
MfeI-HF™	4	≥ 16x
NcoI-HF™	4	≥ 530x
NheI-HF™	4 + BSA	≥ 266x
NotI-HF™	4 + BSA	≥ 16x
PstI-HF™	4	33x
PvuI-HF™	4 + BSA	≥ 32x
PvuII-HF™	4	32x

Enzyme	optimal NEBuffer†	HF-Factor: reduced Star Activity compared to Wildtype*
SacI-HF™	4 + BSA	≥ 266x
SalI-HF™	4	≥ 8,000x
SbfI-HF™	4	32x
ScaI-HF™	4	62x
SphI-HF™	4	250x
SspI-HF™	4	16x
StyI-HF™	4	125x

† *Wild type enzymes were tested in supplied buffer for comparisons.*

* *Ref.: Wei, H. et al (2008) Nucleic Acids Reseach 36, e50.*

Dramatically reduced star activity with HF restriction enzymes



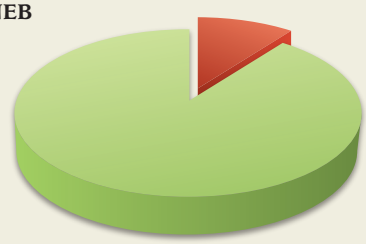
PvuII shows 100% activity in all four NEBuffers, but exhibits intrinsic star activity in buffers other than the recommended NEBuffer 2 or under extended incubation times (i.e. overnight). Star activity is not observed with PvuII-HF™, even in overnight digests! (20 µl reactions, 2 µl of enzyme and incubated at 37°C. Marker M = 1 kb DNA Ladder #N3232.

Unrivaled buffer compatibility of NEB Restriction Enzymes: Save your budget with NEB!

New England Biolabs provides the largest selection of restriction enzymes that exhibit 100% activity in a single buffer. In fact, 169 Restriction Enzymes are supplied with NEBuffer 4; over 190 Restriction Enzymes are 100% active in this buffer!

Of the Top20 most commonly used restriction enzymes NEB provides 18 enzymes with NEBuffer 4. This unrivaled buffer compatibility results in unmatched convenience in reaction set-up and great savings to your budget!

NEB



NEB provides 18 enzymes with a single buffer NEBuffer4. Two different buffer are supplied with the Top20.

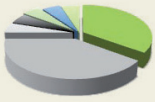
Invitrogen/ Life Technologies:

Six different buffers supplied with the Top20.

Roche:

Six different buffers supplied with the Top20.

Promega:



Seven different buffers supplied with the Top20.

Thermo/Fermentas:

Eight different buffers supplied with the Top20.*

**FastDigest Enzymes excluded*

Top20 Enzymes: BamHI-HF, BglII, DpnI, EcoRI-HF, EcoRV-HF, Hinc II, Hind III-HF, KpnI-HF, NcoI-HF, NheI-HF, NotI-HF, PstI-HF, PvuI-HF, SacI-HF, SalI-HF, SmaI, SpeI, SphI-HF, Xba I, XhoI

Save money through NEB's buffer compatibility

Example: Double Digest EcoRI/XbaI:

NEB:
EcoRI-HF/XbaI both exhibit 100% activity in NEBuffer 4 NEB4, i.e. each enzyme works at the regular 1-fold concentration to obtain 100% cleavage.

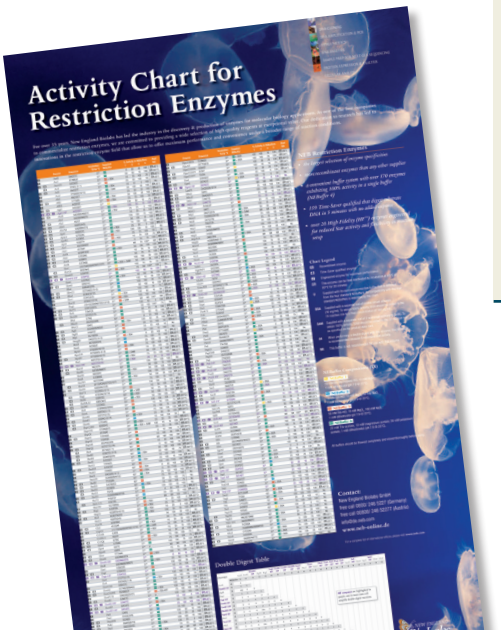
vs.

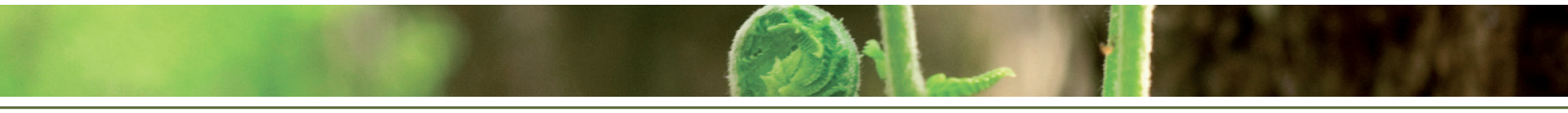
Thermo/Fermentas:
There is no buffer available in which both enzymes exhibit 100% activity! Therefore an expensive 2-fold or 4-fold excess of XbaI is needed to get the job done!

Screenshot Fermentas Webpage Feb. 2011

Updated free NEB Activity Chart Poster available!

Request your free updated "NEB Activity Chart for Restriction Enzymes" Poster (75 x 45 cm) at your local distributor

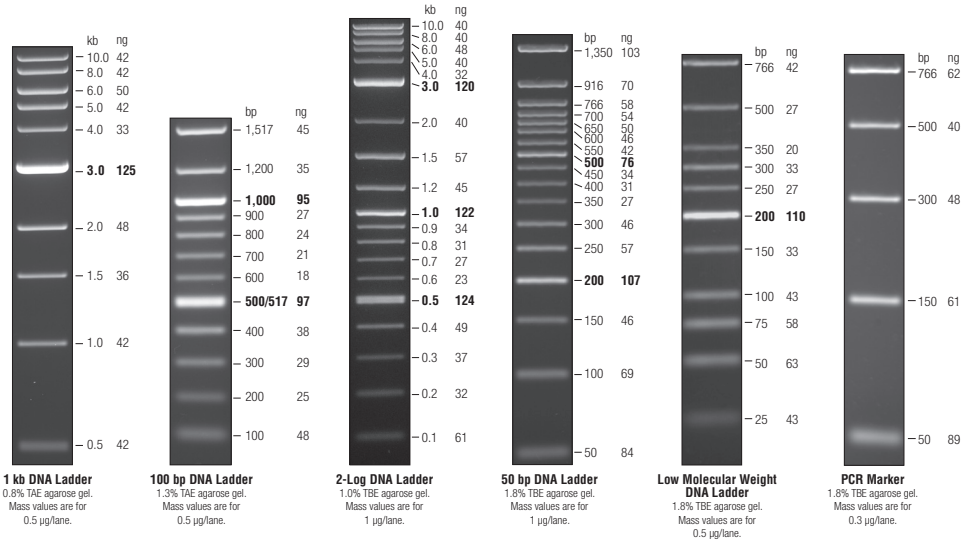




Featured Product

DNA Ladders

NEB offers a variety of DNA Ladders with sizes ranging from 25 bp to 10 kb for use in agarose gel electrophoresis.



For PCR applications involving small DNA fragments try the special designed PCR Marker.
More DNA, RNA and Protein ladders as well as conventional markers can be found at www.neb.com.

Ordering Information

PRODUCT	NEB #	SIZE
CONVENTIONAL LADDERS AND PCR MARKER		
1 kb DNA Ladder	N3232S/L	200/1,000 gel lanes
100 bp DNA Ladder	N3231S/L	100/500 gel lanes
2-Log DNA Ladder	N3200S/L	100/500 gel lanes
50 bp DNA Ladder	N3236S/L	100/500 gel lanes
Low Molecular Weight DNA Ladder	N3233S/L	100/500 gel lanes
PCR Marker	N3234S/L	30/150 µg
Supercoiled DNA Ladder	N0472S	100 gel lanes
QUICK-LOAD® DNA LADDERS		
Quick-Load 2-Log DNA Ladder	N0469S/L	125/375 gel lanes
Quick-Load 1 kb DNA Ladder	N0468S/L	125/375 gel lanes
Quick-Load 100 bp DNA Ladder	N0467S/L	125/375 gel lanes
Quick-Load 50 bp DNA Ladder	N0473S	125 gel lanes
Quick-Load Low Molecular Weight DNA Ladder	N0474S	125 gel lanes
Quick-Load PCR Marker	N0475S	125 gel lanes
TRIDYE™ DNA LADDERS		
TriDye 2-Log DNA Ladder	N3270S	125 gel lanes
TriDye 1 kb DNA Ladder	N3272S	125 gel lanes
TriDye 100 bp DNA Ladder	N3271S	125 gel lanes

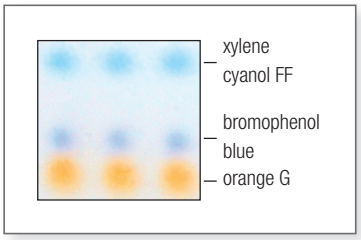
Advantages

- Stable at room temperature
- Sharp uniform bands
- Easy-to-identify reference bands
- Can be used for sample quantitation
- No extra backbone DNA present

Convenient Formats

The **Quick-Load DNA Ladders** are pre-mixed, ready-to-load molecular weight markers containing bromophenol blue as a tracking Dye.

The **TriDye DNA Ladders** are pre-mixed, ready-to-load molecular weight markers containing 3 dyes which serve as visual aids to monitor the progress of migration during agarose gel electrophoresis.



TriDye™ Ladders: 1 kb, 50 bp, 100 bp and 2-Log DNA Ladders are available in ready-to-load format containing xylene cyanol FF, bromophenol blue and orange G.

Novel Protocol for Small RNA Sample Prep for Next Gen Sequencing

New England Biolabs has expanded its line of NEBNext reagents to include NEBNext Small RNA Sample Prep Set 1 for sequencing on the Illumina® platforms, and NEBNext Small RNA Sample Prep Set 3 for sequencing on Life's SOLiD platform. These new products offer a new and unique protocol, which results in higher yields and lower adaptor-dimer formation, as compared to other protocols. With oligos, enzymes and buffers included, both sets provide substantial cost savings, and the master mix format streamlines the workflow by reducing the number of vials and pipetting steps required.

	NEBNext® Small RNA Sample Prep Set 1 Reagents Supplied (Illumina Compatible)	NEBNext® Small RNA Sample Prep Set 3 Reagents Supplied (SOLiD Compatible)
NEB #	E6120S/L	E6160S/L
Size	10/50 reactions	10/50 reactions
3´ Ligation	• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor 1	• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor 3
Primer Hybridization	• SR RT Primer 1	• SR RT Primer 3
5´ Ligation	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor 1 • Nuclease-Free Water	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor 3 • Nuclease-Free Water
First Strand Synthesis	• RNase Inhibitor, Murine • dNTPs	• RNase Inhibitor, Murine • dNTPs
PCR Amplification	• LongAmp® *Taq* 2X Master Mix • SR Primer F1 • SR Primer R1	• LongAmp® *Taq* 2X Master Mix • SR Primer F3 • SR Primer R3
Size Selection	• Gel Loading Dye, Orange (6X) • Quick-Load® Low Molecular Weight DNA Ladder • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE	• Gel Loading Dye, Orange (6X) • Quick-Load® Low Molecular Weight DNA Marker • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE

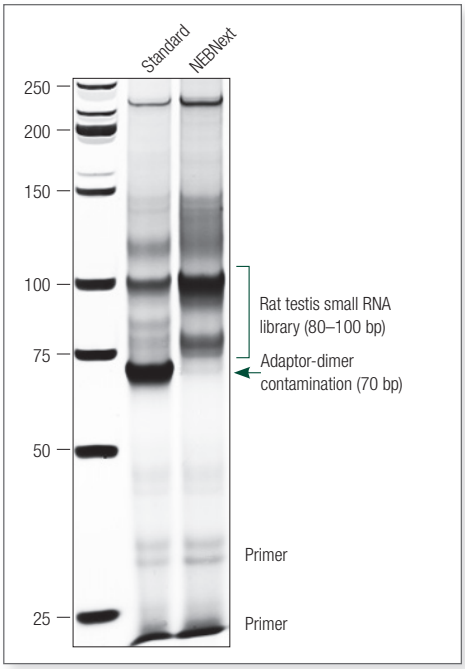
KEY
RNA = Red
Adaptor = Green
RT Primer = Aqua
cDNA = Blue
DNA Product = Black

For the complete listing of NEBNext reagents available, please visit www.nebnext.com

Advantages of Small RNA Sets

- **Convenient formats** – Enzymes, buffers and nucleotides are included at the appropriate concentrations and in appropriate volumes, and are available in set or master mix format
- **Novel Protocol for small RNA** – Higher yield and substantially reduced adaptor dimer formation suitable for methylated and unmethylated small RNAs
- **Functional Validation** – Each set is functionally validated by preparation of a library from a standard reference RNA, followed by Illumina or SOLiD sequencing
- **Stringent Quality Controls** – Additional QCs ensure maximum quality and purity
- **Value Pricing**

NEBNext Small RNA sample prep increases library yield and substantially reduces adaptor-dimer contamination



Standard and NEBNext protocols for small RNA Library construction were carried out using 5 µg of rat testis total RNA. Note: miRNAs as well as other small RNA species (including piRNAs that are very abundant in rat testis), are efficiently captured by the NEBNext protocol.

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- A new epigenetics section showcasing novel methods for the identification and quantitation of 5-mC and 5-hmC within a DNA locus
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