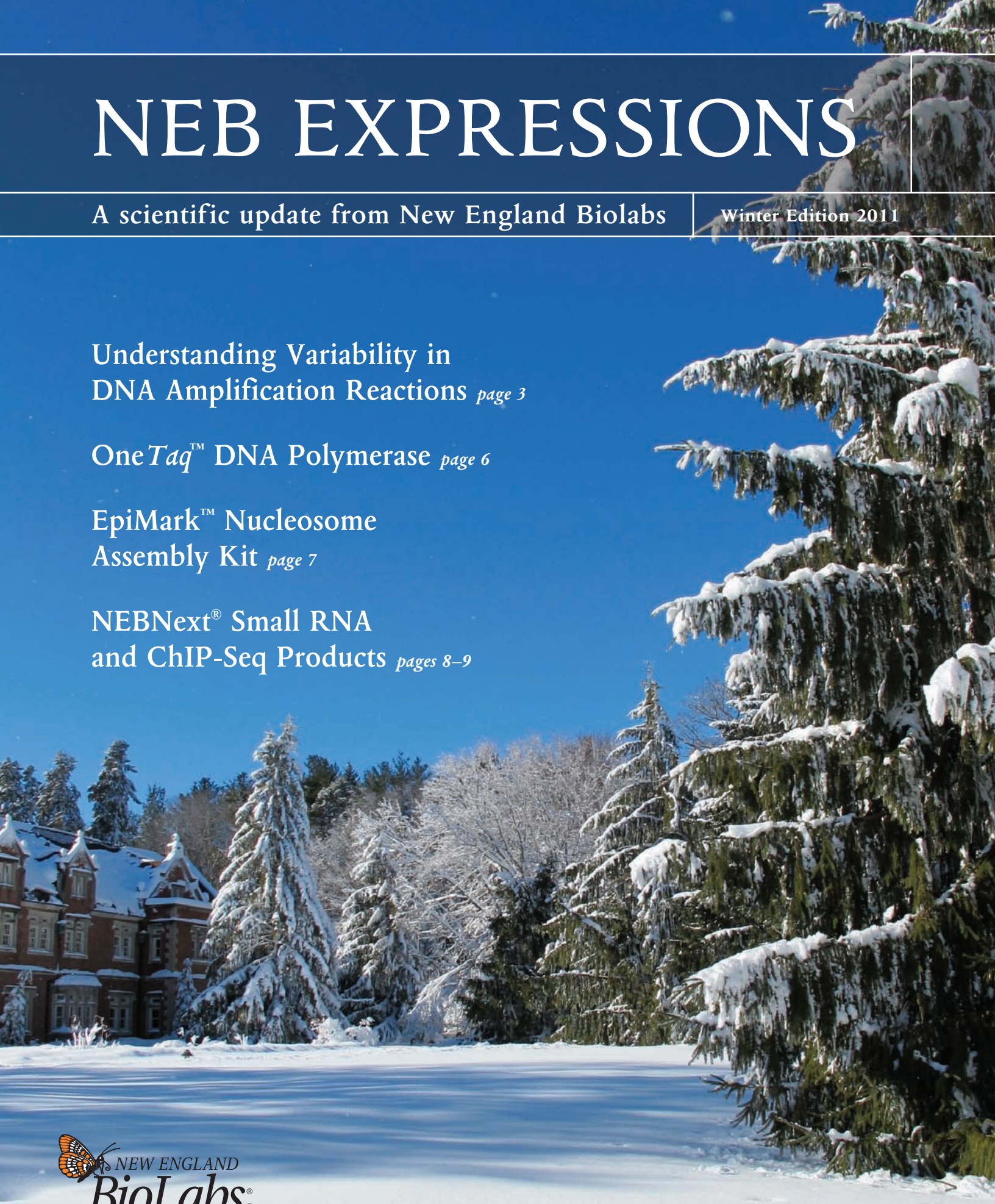
# NEB EXPRESSIONS

A scientific update from New England Biolabs | Winter 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:

- **XGen Congress: Applying Next Generation Genomic Technologies for Now Generation Discoveries**
  March 15–19, 2011
  San Diego, CA, USA
  www.healthtech.com/xgn/2010

- **Experimental Biology (FASEB)**
  April 9–13, 2011
  Washington, DC, USA
  http://experimentalbiology.org/content/default.aspx

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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.

In addition to developing high quality, innovative research tools, NEB has always had a unique, personalized approach to technical support. NEB is proud of its tech support model, which puts the customer in direct contact with scientists who are developing and manufacturing products. This approach is discussed in more detail on page 10.

Wishing you continued success in your research,

New England Biolabs

*Two otters visit the 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

**PCR (1) is arguably the most common technique in 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 DNA amplification reactions is masked by the relatively high success rate of 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.). However, 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 products for DNA amplification. 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. Provide information to enable researchers to modify a limited set of variables depending on the desired outcome (or inherent limitation) of their particular set of amplifications.
4. 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.

Use of the PCR Test Panel is simplified 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, etc.). Cycling conditions, thermocycler ID and other relevant details are also tracked.

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 |

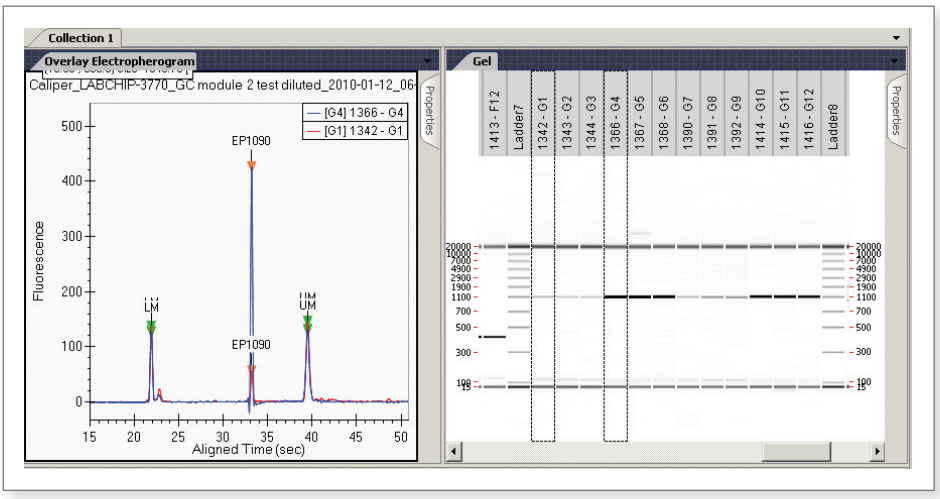
By varying large numbers of conditions and quantitating both specificity and yield of a PCR product, the contribution of each reaction component can be systematically evaluated. Some of the variations in template and primer components that are assessed by the PCR Test Panel are described in Table 1. 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 PCR 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 are utilized 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-like" image (Figure 1). The instrument software identifies the expected peaks and quantitates the yield and percent purity

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.*

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 set of data of PCR results. Simple data analysis can be accomplished within the database, and IGOR Pro (Wavemetrics, Inc., Portland, OR) is 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 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 (≤1 kb). 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 product quality control assays, whereas "real-world" templates 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* DNA 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-like 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%). This type of output provides familiar visual information of both product yield and purity. However, 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 panel (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-like representation of the same data).

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 have observed that the percent GC content of a template is 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* DNA 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:

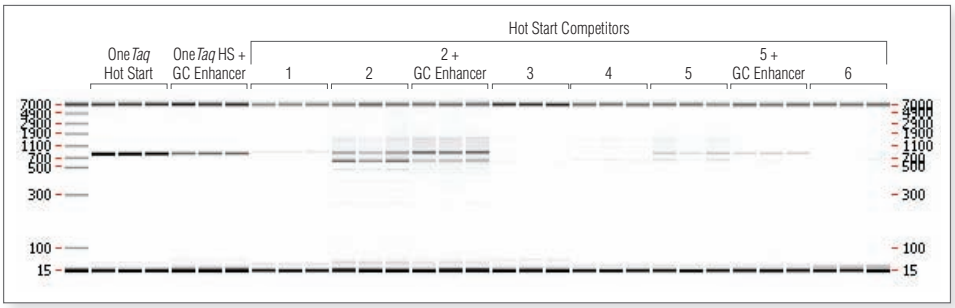
Using a wide variety of conditions and a multitude of primer/template sets has allowed NEB to develop a comprehensive view of its PCR products (and others in the market) that is not possible with a smaller number of amplification reactions. Although it is interesting to study all the variables that affect 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 has allowed 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 researchers at NEB to develop solutions for challenging amplifications and support future polymerase-based applications.

**References:**

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

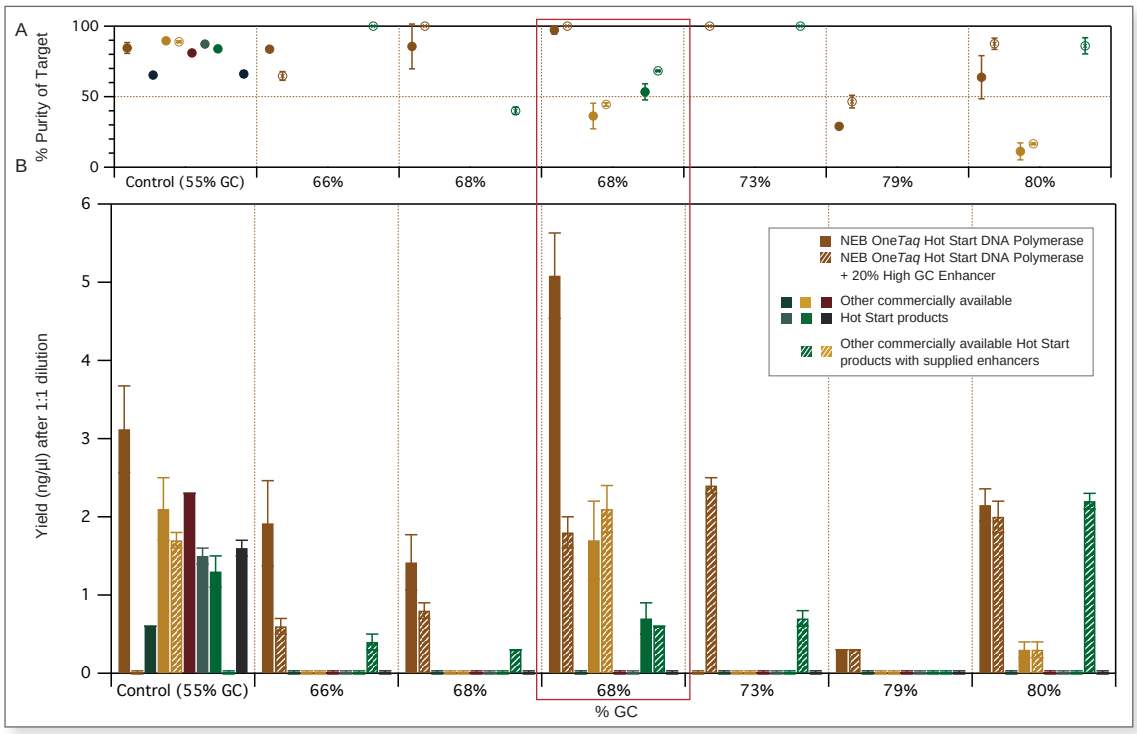


Figure 2. Gel view of triplicate reactions on a single 68% GC amplicon for One*Taq* Hot Start DNA Polymerase and hot start competitors.




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

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 DNA polymerase was used in the absence (brown solid bar) or presence (brown striped bar) of High GC Enhancer. Competitor polymerases were cycled according to manufacturer's recommendations and included GC enhancers when supplied (striped bars).*

*Introducing*

# One*Taq*™ DNA Polymerase

An optimized blend of *Taq* and Deep Vent$_R$® DNA polymerases, One*Taq*™ and One*Taq* Hot Start DNA Polymerases offer robust amplification across a wide range of templates. The 3´–5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robustness of *Taq*, and the hot start formulation combines convenience with decreased interference from primer-dimers and secondary products. Available in convenient product formats, including master mixes, One*Taq* shows exceptional performance against other commercially available polymerases, especially when amplifying difficult or GC-rich templates.

## Advantages

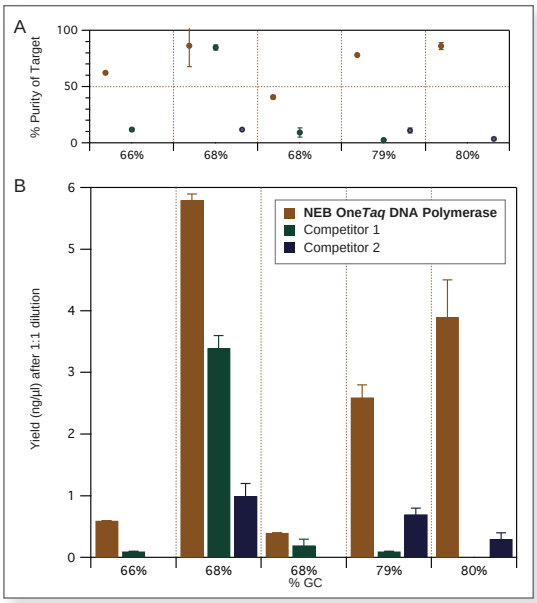
- Exceptional performance in endpoint PCR across a wide range of templates
- Robust yields with minimal optimization
- Convenient product formats (stand-alone enzyme, master mixes, and Quick-Load® formats)
- Hot start version allows room temperature reaction setup and does not require a separate activation step
- Compatible with standard *Taq* protocols

*See page 6 for more information*

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

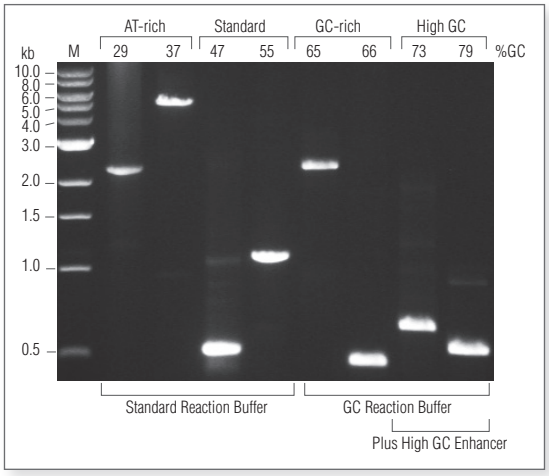
One*Taq* and One*Taq* Hot Start DNA Polymerases are supplied with two 5X buffers (Standard and GC), as well as a High GC Enhancer solution. For most routine and/or AT-rich amplicons or complex amplicons with up to ~65% GC content, One*Taq* Standard Reaction Buffer provides robust amplification. For GC-rich 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.




*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

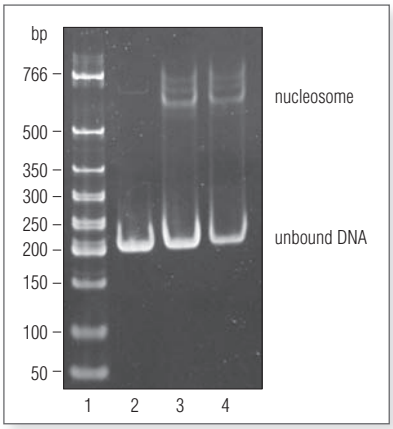
| PRODUCT | NEB # | SIZE |
|---|---|---|
| One*Taq*™ DNA Polymerase | M0480S/L/X | 200/1,000/5,000 units |
| One*Taq*™ 2X Master Mix with Standard Buffer | M0482S/L | 100/500 reactions (50 µl vol) |
| One*Taq*™ 2X Master Mix with GC Buffer | M0483S/L | 100/500 reactions (50 µl vol) |
| One*Taq*™ Quick-Load® 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 reagents and easy-to-follow protocols to simplify nucleosome formation using your own target DNA or the supplied control DNA. These recombinant nucleosomes may serve as a better substrate for enzymes (e.g., acetylases, methylases, sumomylases) or other chromatin interacting proteins that are unable to bind and/or modify individual histones or DNA alone.

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.*

## 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)

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 |

*Coming this Summer...*

## 26th Annual Molecular Biology Workshop

This intensive, two-week 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, siRNA and microarrays

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.

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

# 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, that 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<br>• 3´ Ligation Reaction Buffer (2X)<br>• 3´ SR Adaptor 1 | • 3´ Ligation Enzyme Mix<br>• 3´ Ligation Reaction Buffer (2X)<br>• 3´ SR Adaptor 3 |
| **Primer Hybridization** | • SR RT Primer 1 | • SR RT Primer 3 |
| **5´ Ligation** | • 5´ Ligation Enzyme Mix<br>• 5´ Ligation Reaction Buffer (10X)<br>• 5´ SR Adaptor 1<br>• Nuclease-Free Water | • 5´ Ligation Enzyme Mix<br>• 5´ Ligation Reaction Buffer (10X)<br>• 5´ SR Adaptor 3<br>• Nuclease-Free Water |
| **First Strand Synthesis** | • RNase Inhibitor, Murine<br>• dNTPs | • RNase Inhibitor, Murine<br>• dNTPs |
| **PCR Amplification** | • LongAmp® *Taq* 2X Master Mix<br>• SR Primer F1<br>• SR Primer R1 | • LongAmp® *Taq* 2X Master Mix<br>• SR Primer F3<br>• SR Primer R3 |
| **Size Selection** | • Gel Loading Dye, Orange (6X)<br>• Quick-Load® Low Molecular Weight DNA Ladder<br>• DNA Gel Elution Buffer (1X)<br>• Linear Acrylamide<br>• TE | • Gel Loading Dye, Orange (6X)<br>• Quick-Load® Low Molecular Weight DNA Ladder<br>• DNA Gel Elution Buffer (1X)<br>• Linear Acrylamide<br>• TE |

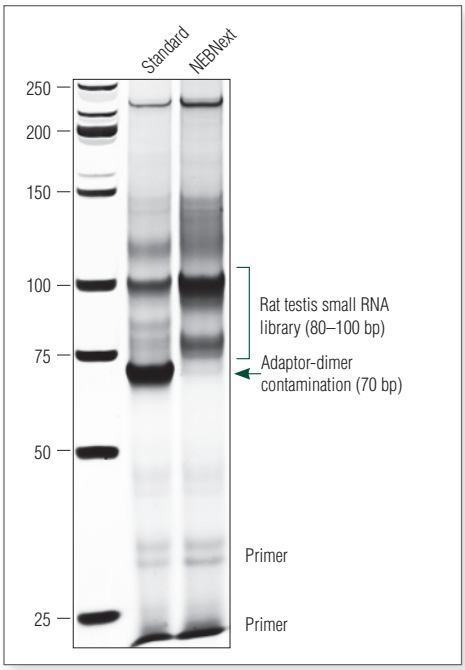
KEY
RNA = Red
Adaptor = Green
RT Primer = Aqua
cDNA = Blue
DNA Product = Black

*Illumina® is a registered trademark of Illumina, Inc.*
*SOLiD™ is a trademark of Life Technologies.*

## Advantages of Small RNA Sets

- **Convenient formats** – Enzymes, adaptors, primers, buffers and nucleotides are included at the appropriate concentrations and in appropriate volumes
- **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

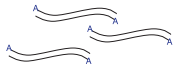



*Standard and NEBNext protocols for small RNA Library construction were carried out using 5 µg of rat testis total RNA. Note: miRNAs as well as other small RNA species (including piRNAs that are very abundant in rat testis), are efficiently captured by the NEBNext protocol.*



# NEBNext® Reagents for ChIP-Seq Sample Preparation

For sample preparation of a ChIP-Seq DNA library, NEB offers the NEBNext ChIP-Seq Sample Prep Reagent Set 1 and Master Mix Set 1 for Illumina sequencing, and the NEBNext ChIP-Seq Sample Prep Master Mix Set 3 for SOLiD sequencing. With similar workflows to the NEBNext DNA Sample Prep Kits, the ChIP-Seq kits require lower amounts of input DNA and are compatible with commercially available ChIP kits.

| | NEBNext ChIP-Seq Sample Prep Reagent Set 1 (Illumina Compatible) | NEBNext ChIP-Seq Sample Prep Master Mix Set 1 (Illumina Compatible) | NEBNext ChIP-Seq Sample Prep Master Mix Set 3 (SOLiD Compatible) |
|---|---|---|---|
| **NEB #** | E6200S/L | E6240S/L | E6260S/L |
| **Size** | 10/50 reactions | 10/50 reactions | 10/50 reactions |
| **End Repair**  | • T4 DNA Polymerase<br>• T4 Polynucleotide Kinase<br>• T4 DNA Pol I, Large (Klenow) Fragment | • NEBNext End Repair Enzyme Mix | • NEBNext End Repair Enzyme Mix |
| | • Phosphorylation Reaction Buffer<br>• Deoxynucleotide Solution Mix | • NEBNext End Repair Reaction Buffer | • NEBNext End Repair Reaction Buffer |
| **dA Tailing**  | • NEBuffer 2 for Klenow Fragment (3´→5´ exo⁻)<br>• Deoxyadenosine 5´-Triphosphate (dATP) | • NEBNext dA-Tailing Reaction Buffer | |
| | • Klenow Fragment (3´->5´ exo-) | • Klenow Fragment (3´->5´ exo-) | |
| **Adaptor Ligation**  | • Quick T4 DNA Ligase | • Quick T4 DNA Ligase | • Quick T4 DNA Ligase |
| | • NEBNext Quick Ligation Reaction Buffer (2X) | • NEBNext Quick Ligation Reaction Buffer (2X) | • NEBNext Quick Ligation Reaction Buffer (5X) |
| **PCR Enrichment of Adaptor-Ligated cDNA Library**  | • Phusion® High-Fidelity DNA Polymerase* | • Phusion® High-Fidelity DNA Polymerase* | • LongAmp® *Taq* 2X Master Mix |
| | • Phusion® HF Buffer (5X)* | • Phusion® HF Buffer (5X)* | |
| | • Deoxynucleotide Solution Mix | • Deoxynucleotide Solution Mix | |

KEY
DNA = Black
Adaptors = Orange/Yellow

* Developed and manufactured by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion is a registered trademark and property of Thermo Fisher Scientific.

## Advantages of NEBNext ChIP-Seq Sets

- **Convenient formats** – All of the required enzymes, buffers and nucleotides are included, and are available in set or master mix format.
- **Functional Validation** – Each reagent set, master mix set and module is functionally validated by preparation of a ChIP-Seq DNA library followed by Illumina or SOLiD sequencing
- **Stringent Quality Controls** – Additional QCs ensure maximum quality and purity
- **Value Pricing**

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

# TS Technical Support at New England Biolabs: For Scientists, By Scientists

Ana L. Egana, Ph.D. and Maurice W. Southworth, Ph.D.

New England Biolabs was established in the mid-1970s as a cooperative laboratory of experienced scientists. It was conceived to be a part of the scientific community who contributed to the advancement of science by providing top quality tools and experimental expertise. This basic philosophy continues today and has led to long-standing relationships with many of our fellow scientists. NEB's commitment to its customers begins before the purchase of a product, and is supported by our catalog, website and technical support staff.

NEB's technical support model is unique as it utilizes many of the scientists at NEB. Any question regarding a product is dealt with by the product manager, who is the scientist responsible for manufacturing the product, by a product development scientist, who may have helped develop the product, or by a researcher, who uses the product in their daily research. Instead of having a small tech support group at NEB, all of our scientists participate in technical support.

Today, when a customer contacts us by phone with a technical support question, they have the option of speaking directly with the product manager by entering the product catalog number in our phone system. If the product manager is not available, the call will be routed to our technical support group. Every scientist at NEB, whether they are in Production, Development or Research is assigned to a technical support group once a month on a rotation basis. This system guarantees that a customer will always speak with a scientist that has current hands-on experience at the bench. If the question requires a higher level of expertise, the call will be transferred to a scientist within the company who can best answer the question. In some cases, the appropriate scientist may not be available and we will arrange to have the scientist contact the customer. It is our goal to provide our customers with the most scientifically accurate information in the shortest period of time possible.

In addition to calling NEB for technical support during business hours, customers have the option of submitting questions directly into our tech support system with our online Technical Support Form at www.neb.com/techsupport. Questions can also be submitted by e-mail to info@neb.com. In both instances, the question is entered into a database that allows easy monitoring of questions and helps ensure a timely response.

Our international customers can contact the NEB subsidiary or distributor from which they purchased their product directly. The contact information for our international subsidiaries and distributors can be found in our website at http://www.neb.com/nebecomm/international.asp.

With over 35 years of research history, NEB scientists have accumulated a large amount of technical information and have made much of it accessible on our website. The Technical Reference section on neb.com contains a wide range of information related to our products, including how to set-up a restriction enzyme digest, guidelines for PCR optimization with different DNA Polymerases, and competent cell strain selection, to name a few. In addition, FAQ's containing technical information are available for many products, as well as some commonly used and requested protocols. NEB has also developed online tools, such as NEBcutter which generates restriction enzyme maps and display restriction digests of a DNA sequence, Enzyme Finder to find a restriction enzyme that will cut a specific DNA sequence, or the Double Digest Finder, which gives recommendations for restriction enzyme double digests. The NEB website also provides customers with access to several comprehensive databases such as REBASE, InBASE, PolBase and FilGenNet. Technical information for our products is constantly being generated and added to our website.

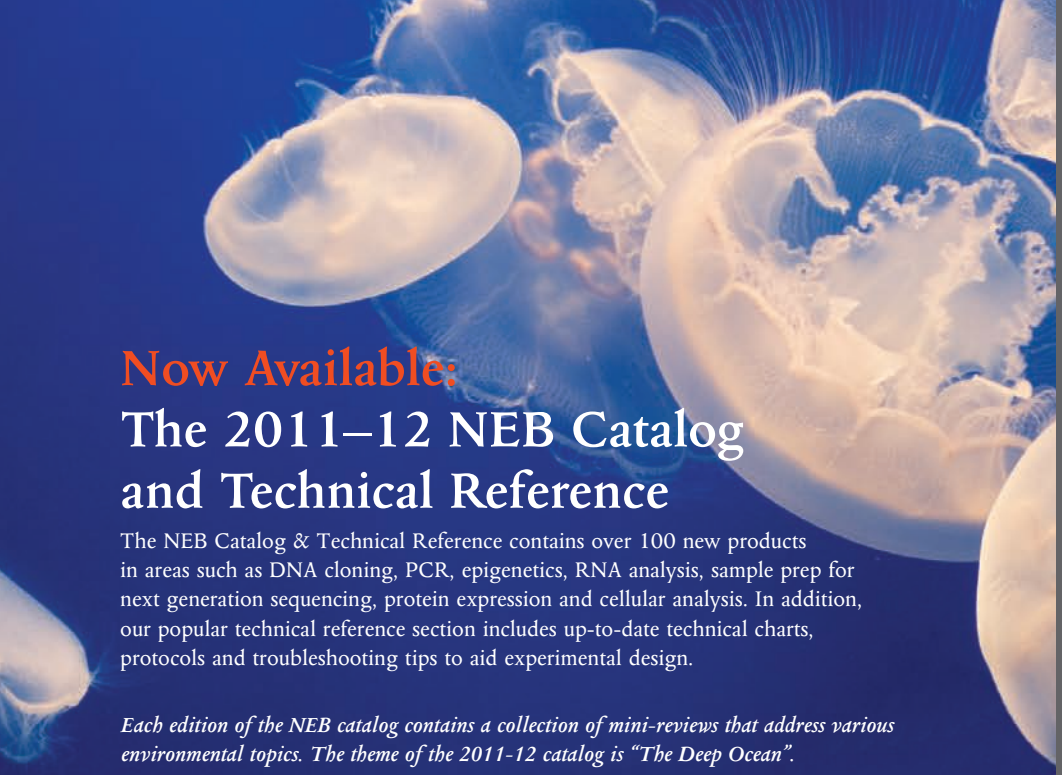
The success of our unique approach to technical support is reflected in the positive feedback that we receive from customers, a few of which are highlighted below:

*"Honestly, I did not expect such thorough help. I really appreciate it. Most importantly, I got help from … who actually had hands-on experience on the matter. It was splendid. I am communicating with him as follow up"*
*– Professor, University of Nebraska Medical Center*

*"… was provided with some specific concentration details that a whole afternoon of Internet and Electronic Journal searching did not provide."*
*– Postdoctoral Fellow, SUNY at Buffalo*

*"Without a doubt the best technical support I have encountered. Well informed, nice, and willing to put in time and effort to answer my questions, even if that means doing bench work. Keep up the great work!"*
*– Undergraduate Student, Dartmouth College*

At NEB, we always strive to improve our technical support. To identify the areas for improvement, we need your input. If you have any feedback, comments or suggestions, please don't hesitate to contact us by using our "Make a Suggestion" form: **www.neb.com/suggestions**



# Now Available:
# The 2011–12 NEB Catalog and Technical Reference

The NEB Catalog & Technical Reference contains over 100 new products in areas such as DNA cloning, PCR, epigenetics, RNA analysis, sample prep for next generation sequencing, protein expression and cellular analysis. In addition, our popular technical reference section includes up-to-date technical charts, protocols and troubleshooting tips to aid experimental design.

*Each edition of the NEB catalog contains a collection of mini-reviews that address various environmental topics. The theme of the 2011-12 catalog is "The Deep Ocean".*

## The Deep Ocean

Earth is called the "Blue Planet" for a reason: Oceans cover 71 percent of its surface and store almost 97 percent of its water. Needless to say, that's a lot of $H_2O$ – about 320 million cubic miles of it, weighing well over a million, trillion tons. It dominates our planet to the extent that the land masses we call home are really just islands in a vast, interconnected body of saltwater. In essence, our world has just one giant ocean, although for convenience geographers, and cartographers have divided this sprawling body into five oceans (Arctic, Atlantic, Indian, Pacific, and Southern), plus a number of smaller seas, bays, and gulfs.

Our planet looks blue (as seen from space) because water absorbs red light while scattering bluer wavelengths. The deeper one goes into the ocean, the more light that is absorbed or scattered. Venturing about 200 meters down to the lower edge of the so-called photic zone, less than one percent of the sunlight hitting the surface remains. Enough sunlight penetrates into the photic zone to sustain photosynthesis, but below this surface layer, it is simply too dark and primary energy production shuts down.

This is roughly where the "deep ocean" – containing about 95 percent of the total water by volume – begins. The word "deep" is appropriate, since average ocean depths are about two-and-a-half miles. The deepest spots yet measured extend downward almost seven miles. This realm constitutes the Earth's largest continuous habitat, as well as one of the planet's last great frontiers. "Less than five percent of the ocean has been seen even once, let alone explored or understood," writes the oceanographer, Sylvia Earle.

We have mapped out the surface of the moon, Mars, and Venus in far greater detail than we have surveyed the ocean floor. We can point our telescopes at other planets and take pictures, waiting, if necessary, for a cloudless night to do so. But the deep ocean is opaque, and no matter how long we wait, we'll never get clear, unobstructed views from the surface.

Fortunately, new tools have become available in recent decades to observe phenomena previously beyond reach. These include manned submersibles, robotic vehicles, subsurface floats, undersea laboratories and seafloor drills. We now know that, far from being mostly devoid of life, the oceans are filled with an astonishing variety of creatures of all sizes and at all depths, even extending into the subsurface.

Despite its enormous size, the ocean is not immune to human insults – pollution, dumping, mining, and overfishing – all of which can have profound effects on marine ecosystems in ways we don't fully appreciate. But the lesson is clear nevertheless: We can no longer take the deep ocean, and its resources, for granted by continuing to act as if out of sight is out of mind.

## Catalog highlights:

- An expanded line of High Fidelity (HF) Restriction Enzymes engineered for reduced star activity and convenience
- A comprehensive offering of PCR reagents
- A new section highlighting NEBNext reagents for sample preparation of DNA and RNA for next generation sequencing
- The latest innovation in competent cells for protein expression
- A new epigenetics section showcasing novel methods for the identification and quantitation of 5-mC and 5-hmC within a DNA locus
- Tools to enable RNA research
- An extensive selection of markers and ladders for DNA, RNA and protein analysis
- A powerful protein labeling technology unique to NEB
- A range of expression systems, including a novel kit for cell-free expression
- Tools for glycobiology

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