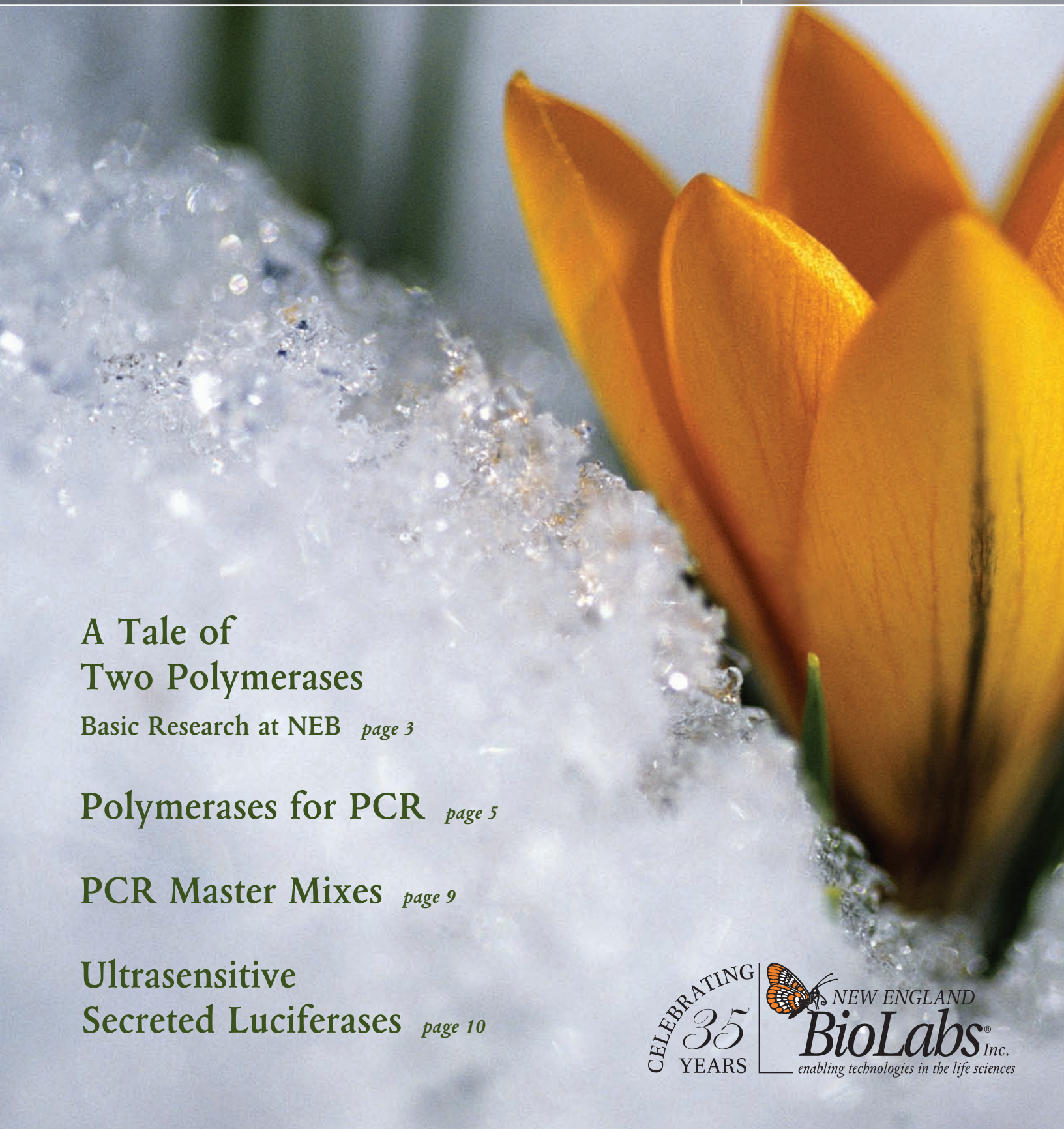
NEB EXPRESSIONS

A scientific update from New England Biolabs | Early Spring Edition 2010



CELEBRATING 35 YEARS

NEW ENGLAND BioLabs® Inc.

enabling technologies in the life sciences

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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **American Association for Cancer Research** (AACR) – April 17-21, 2010 – Washington, DC
  www.aacr.org
- **Experimental Biology** (EB) – April 24-28, 2010 – Anaheim, CA
  www.experimentalbiology.org

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A Letter from NEB

Dear Researcher,

For over 30 years, New England Biolabs has been at the forefront of the isolation, characterization and cloning of polymerases. Part of our basic research program includes a focus on understanding the structure and function of these critical enzymes, and making this information available to our customers. When choosing a polymerase from NEB, you can be sure that you are receiving a reagent that has been fully characterized, giving you greater confidence that it will work for your application.

This issue highlights advances in polymerase research made by NEB scientists. Additionally, we are pleased to introduce several new products to our growing line of PCR reagents, including two hot start polymerases and a line of products that enable direct amplification without the need for DNA extraction. As a supplier of one of the largest selection of PCR polymerases commercially available, NEB is committed to providing high quality products and outstanding technical support.

Wishing you continued success
in your research,

New England Biolabs

35th Anniversary Offers

NEB would like to thank its customers for 35 years of support. Join us in celebrating our 35th anniversary by visiting **www.neb.com** to find 12 months of exciting offers, including significant product discounts and giveaways.

Past offers have included significant discounts on HF restriction enzymes, T4 DNA Ligase and DNA ladders, as well as prize drawings for NEB Klean Kanteens and ice buckets filled with valuable NEB products.

Look for the 35th Anniversary Offers icon on our website to learn about the next monthly special offer.

As we celebrate our 35th year, share your thoughts or comments about your experience with NEB through our **online guestbook** by visiting http://nebiolabs.wordpress.com/

A Tale of Two Polymerases – Basic Research at NEB

Scientists at New England Biolabs have always had an interest in understanding the structure and function of polymerases, from both a commercial and scientific perspective. NEB commercialized its first DNA polymerase over 30 years ago, and was the first company to sell a hyperthermophilic DNA polymerase, *Taq* DNA Polymerase, in 1987. Moreover, it has always been our priority to extensively characterize our polymerases in order to provide the highest quality products. In addition to product development, DNA polymerases are the focus of a sustained research effort that spans over 20 years. This article highlights two important polymerase research projects at NEB, and discusses how these discoveries have helped to drive advances in PCR polymerase discovery and performance, facilitate DNA sequencing technologies and provide insights into nucleotide selectivity during primer extension.

Thomas C. Evans Jr., Ph.D. and Andrew F. Gardner, Ph.D., New England Biolabs, Inc.

Genomic replication and maintenance rely on several types of DNA polymerases. Free-living organisms encode multiple DNA polymerases, each optimized for a particular cellular role. Replicative polymerases, in complex with multiple protein cofactors, copy standard DNA quickly and faithfully (1). By comparison, translesion DNA polymerases are slow and inaccurate when replicating a standard DNA template, but are more effective than the replicative enzymes in polymerizing past lesions in a DNA template (2). This befits their role in responding to DNA damage. Currently, there are six recognized families of DNA-dependent DNA polymerases (Table 1). Although each has biological relevance, Family A and B DNA polymerases have had the greatest impact on molecular biology and biotechnology.

Table 1: DNA Polymerase Families

FAMILY	EXAMPLE
A	*E. coli* DNA Polymerase I
B	*E. coli* DNA Polymerase II
C	*E. coli* DNA Polymerase III α-subunit
D	*P. furiosus* DNA Polymerase II
X	Human Polymerase β
Y	*E. coli* DNA Polymerase V

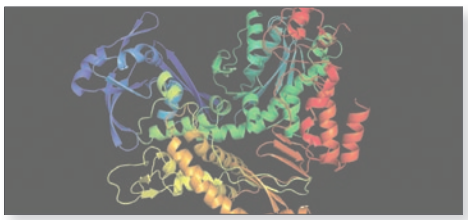
Vent$_R$® DNA Polymerase: The discovery of a hyperthermophilic polymerase

Taq DNA Polymerase is the most widely used polymerase for PCR. While it is a robust enzyme, it is not ideal for some reactions, as it does not possess proof-reading activity and has a half-life of <10 minutes at 100°C. In order to investigate the utility of other hyperthermophilic DNA polymerases, scientists at NEB entered into a collaboration with Dr. Holger Jannasch of the Woods Hole Oceanographic Institute. Dr. Jannasch had isolated an organism, *Thermococcus litoralis*, from a thermal vent near Naples, Italy (3). This hyperthermophilic archaea can be cultured and grows at temperatures of up to 98°C.

Initially, Vent DNA Polymerase was purified from the native source, which required culturing *T. litoralis*. Unlike *Escherichia coli*, which grows at 37°C, *T. litoralis* prefers a scorching 90°C growth temperature. This presented a challenge when isolating larger amounts of enzyme, particularly at production scale. Additionally, it was discovered that the presence of sulfur in the growth media significantly increased the growth rate, even though it was not required. Challenging growth conditions coupled with the increased quality offered by recombinant enzymes made cloning Vent DNA Polymerase a high priority for NEB in the late 1980's.

During this time, little was known about Archaea, and nothing was known about archaeal DNA polymerases. To find the gene encoding Vent DNA Polymerase it was first necessary to purify it from the native host. Cultured *T. litoralis* was lysed and the cellular proteins separated by column chromatography. Polymerase activity was narrowed to a single fraction containing four prominent proteins, as determined by SDS-PAGE. Scientists at NEB determined that the 90 kDa protein in this fraction contained all the DNA polymerase activity. Given today's technology, the Vent DNA polymerase gene could be quickly identified using mass spectral analysis of tryptic digests, coupled with sequencing the whole genome of *T. litoralis*. With these techniques not available, other tools, such as Western blotting and immunoblotting became necessary to clone the Vent DNA Polymerase gene.

Ultimately, the cloning of Vent DNA Polymerase was accomplished by creating a genomic expression library that was probed using an antibody raised against the partially purified Vent DNA Polymerase. A genomic DNA library was constructed using λ-Zap, as well as a λ-gt11 expression library. Considering that high quality



9°N$_m$ DNA Polymerase Structure (Coordinates from Rodriguez, A.C. et al. (2000) J. Mol. Biol. 299, 447–462.)

T. litoralis genomic DNA was used for several types of libraries, it was surprising that no colonies expressed the full-length DNA polymerase.

Using affinity-purified antibody, clones expressing gene fragments recognized by the antibody were identified and sent for sequencing. In the age where sequencing instruments routinely produce 20–40 gigabases worth of information per run, it is hard to imagine that 20 years ago it could take months to reliably sequence 2–3 kilobases of an unknown sequence. When the sequence data for the gene fragments became available, a few of the known conserved DNA polymerase regions were identified. With known sequence confirming that this was a polymerase gene, restriction enzyme fragments could be used as probes in Southern blotting to find the remainder of the gene. These probes, coupled with a new genomic library, allowed the subcloning of a 10 kb fragment that hybridized with the probes into pBR322. Unfortunately, there was still no detectable polymerase activity.

As more sequencing data became available, it was apparent that only the first part of the conserved DNA polymerase motif B was recognizable. Nothing was known about archaeal DNA polymerases at that time and, therefore, it was not clear whether a divergence in motif B was typical of archaeal DNA polymerases or whether some artifactual gene rearrangements were occurring during cloning. Human polymerase alpha (hPolα), a Family B DNA polymerase, was being used for comparison and the Vent DNA Polymerase gene diverged significantly in

primary amino acid sequence at the asparagine-serine (NS) amino acid residues in motif B of hPolα. More interestingly, the sequencing data revealed that another NS appeared about 500 amino acid residues after the first NS, and this was followed by other conserved amino acid residues indicative of motif B. It appeared the polymerase gene was split. It was postulated that perhaps archaeal genes contain introns, but this intervening sequence (IVS) was an open reading frame that was also in-frame with the hypothetical polymerase gene and lacked known intron indicators.

While this was very intriguing, it was also frustrating because no polymerase activity could be detected from expression of the subcloned gene. Moreover, subcloning of the entire gene in an expression vector led to deletions in the polymerase gene. NEB scientists began designing experiments to remove this IVS from the gene. It wasn't until the deletion construct was made that DNA polymerase activity was detected (4). This was a seminal event in the study of archaeal DNA polymerases, as well as for the new field of protein splicing elements (inteins). Ultimately, two IVS's were identified in the Vent DNA Polymerase gene, and both turned out to be inteins. These thermophilic inteins were subsequently used by NEB scientists to elucidate the mechanism of protein splicing (5,6).

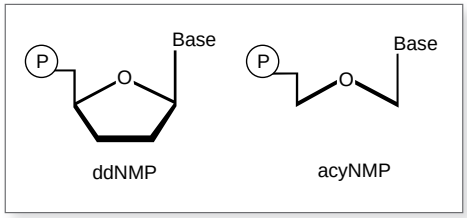
The cloning of an archaeal DNA polymerase and the polymerase gene DNA fragments created during this work facilitated the discovery and cloning of additional archaeal DNA polymerases (7). Genomic libraries constructed from additional isolates from hydrothermal vent sites led to the rapid discovery of five more hyperthermophilic archaeal DNA polymerases, including genes encoding Deep Vent$_R$® and 9°N$_m$ DNA Polymerases. These, along with Vent DNA Polymerase, were the first sequenced and cloned archaeal DNA polymerase genes reported, and set the stage for improving PCR performance. It is interesting to note that all commercially available high-fidelity PCR polymerases on the market today are archaeal DNA polymerases. Additionally, the oligonucleotide probes developed to screen for Vent DNA Polymerase were also used to type archaeal species using RFLP and Southern analysis (8).

Therminator™ DNA Polymerase: Incorporation of modified nucleotides

In addition to improvements in quality and performance, the availability of cloned genes permitted mutagenic studies to determine structure/function relationships in the archaeal DNA polymerases. All living cells contain high concentrations of both ribonucleotides and deoxyribonucleotides, and a DNA polymerase must be able to discriminate between the two pools. As the process for doing this was not well understood at the time, scientists at NEB decided to investigate the structural basis of this discrimination in the little studied archaeal Family B DNA polymerases. Building upon work by Catherine Joyce and co-workers, NEB scientists demonstrated that amino acid residues in motif B were important for discriminating deoxyribo- from ribonucleotides and dideoxyribonucleotides (9).

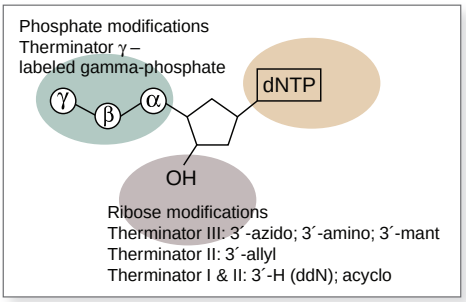
It was determined that the Family B archeaeal DNA polymerases have a bias toward acyclonucleotides as compared to dideoxynucleotide chain terminators (Figure 1). Family A DNA polymerases, such as those routinely used in Sanger sequencing reactions, display an opposite bias. Combining this natural bias with directed mutations to motif B resulted in mutant DNA polymerases with significantly enhanced incorporation of acyclonucleotide chain terminators (10,11). This resulted in the commercialization of four DNA polymerases created to incorporate various modified nucleotides (Figure 2).

Figure 1: ddNMP and acyNMP comparison



For example, Therminator™ DNA Polymerase, a mutant of 9°N$_m$ DNA Polymerase, was genetically engineered to more efficiently incorporate a variety of modified nucleotides and nucleotide terminators, including acycloNTPs, ddNTPs and rNTPs. Therminator DNA Polymerase has been subsequently shown by other research groups to efficiently incorporate a variety of nucleotide analogs with further modifications on the ribose and base moeities (12,13,14,15). Further mutagenesis resulted in development of Therminator™ II DNA Polymerase which allows incorporation of rNTPs and 3´-modified-dNTPs (16,17). Therminator™ III DNA Polymerase was selected for incorporation of nucleotides with large 3´-dNTP modifications, including 3´-azido-dNTPs and 3´-amino-dNTPs, and may be useful for sequencing-by-synthesis. In collaboration with LI-COR®

Figure 2: Different dNTP modifications recognized by DNA polymerase mutants



Biosciences, Therminator™ γ DNA Polymerase was developed to incorporate gamma-phosphate labeled dNTPs. As a result, 9°N$_m$ DNA Polymerase mutants, such as Therminator, have contributed to our understanding of DNA polymerase structure/function relationships and have emerged as important tools for DNA sequencing and genotyping.

New England Biolabs continues to have an active research program involved in both basic and applied research projects. The goal of our research is to contribute to general scientific knowledge, produce novel products, and continually improve our current products. The work of NEB scientists to clone and characterize hyperthermophilic archaeal DNA polymerases exemplified this idea and set the stage for the creation of the latest generation of high fidelity PCR enzymes, including Phusion® and Phire® DNA Polymerases.

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(1) Pomerantz, R.T. and O'Donnell, M. (2007) *Trends Microbiol.* 15, 156–164.
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(11) Gardner, A.F., et al. (2004) *J. Biol. Chem.* 279, 11834–11842.
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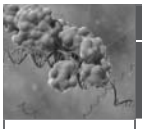
(16) Gardner A.F. and Jack W.E. (1999) *Nucleic Acids Res.* 27, 2545–2553.
(17) Ju J., et al. (2006) *Proc. Natl. Acad. Sci.* USA, 103, 19635–19640.

NEB has the PCR polymerase for your application

New England Biolabs offers a wide range of DNA polymerases and through our commitment to research, ensures the development of innovative, high quality tools for PCR. We are pleased to provide an unsurpassed offering of state-of-the-art products for PCR combined with the service and quality for which NEB is known.
Visit **confidentpcr.com** to learn more, and to find PCR-related special offers.



	APPLICATIONS											
	Routine PCR	High Fidelity	High Yield	Hot Start	Fast	Long Amplicon	Difficult (GC-rich) Templates	High Throughput	Multiplex PCR	Extraction-free PCR	DNA-labeling	Site-directed Mutagenesis
Phusion®*	•	•	•		•	•	•	•				
Phusion® Hot Start II*	•	•	•	•	•	•	•	•				•†
Phusion® Flash*	•	•	•	•	•	•	•	•				
Taq	•		•					•	•††		•	
LongAmp™ *Taq*	•	•	•			•	•					
Crimson *Taq*	•		•					•				
Vent®R	•	•										
Deep Vent®R	•	•					•					
DyNAzyme™ II Hot Start*	•		•	•				•				
DyNAzyme™ Ext*			•			•	•					
Phire® Hot Start II*	•		•	•	•			•				
Hemo KlenTaq									•	•		
Phusion® Blood Direct*		•	•	•	•					•		
Phire® Animal Tissue Direct PCR Kit*			•	•	•					•		
Phire® Plant Direct PCR Kit*			•	•	•					•		

* *Manufactured by Finnzymes Oy. Distributed by New England Biolabs, Inc.*

† *Use Phusion® Site Directed Mutagenesis Kit.*

†† *Use Multiplex PCR 5X Master Mix.*

Phusion and Phire are registered trademarks of Finnzymes, Oy.

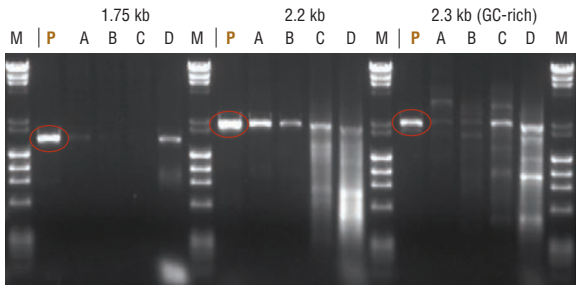
Phusion® Hot Start II High-Fidelity DNA Polymerase

Phusion® Hot Start II High-Fidelity DNA Polymerase is an extremely robust, rapid and accurate polymerase. This new and improved version allows the use of an even wider range of primers, including those with lower melting temperatures. Phusion Hot Start II is ideal for routine PCR, highly demanding applications such as cloning, or for high throughput reactions.

Advantages

- Robust amplification
- Fast reaction times
- High yields
- Extreme fidelity

Phusion Hot Start II DNA Polymerase provides extreme specificity and abundant yields



P: Phusion® Hot Start II High-Fidelity DNA Polymerase
A-D: Proofreading DNA polymerases from other leading suppliers

Five proofreading DNA polymerases from major suppliers were used to amplify 1.7-2.3 kb amplicons from human genomic DNA. All amplifications were performed in accordance with manufacturers' instructions.

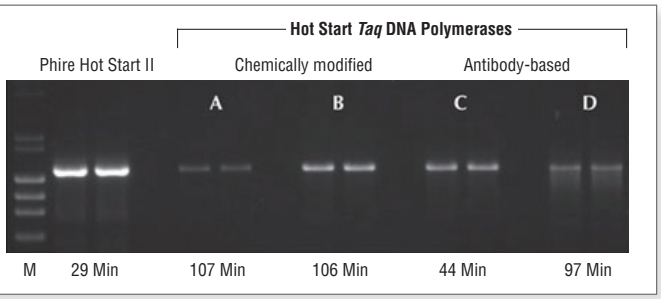
Phire® Hot Start II DNA Polymerase

Phire® Hot Start II DNA Polymerase is an extremely fast and robust polymerase, and is more effective than *Taq*-based hot start polymerases at amplifying long DNA fragments with high yields. Phire Hot Start II is ideal for routine, fast and high throughput PCR applications.

Advantages

- Robust amplification
- Fast reaction times
- High yields

Abundant yields in shorter time with Phire Hot Start II DNA Polymerase



A 1.5 kb fragment from the human Cathepsin K gene was amplified with five different hot start DNA polymerases according to suppliers' recommendations.

Ordering Information

PRODUCT	NEB #	SIZE
Phusion® Hot Start II High-Fidelity DNA Polymerase	F-549S/L	100/500 rxns (50 µl vol)
Phire® Hot Start II DNA Polymerase	F-122S/L	200/1,000 rxns (50 µl vol)

Manufactured by Finnzymes, Oy and distributed by New England Biolabs, Inc.
Phusion and Phire are registered trademarks of Finnzymes, Oy.

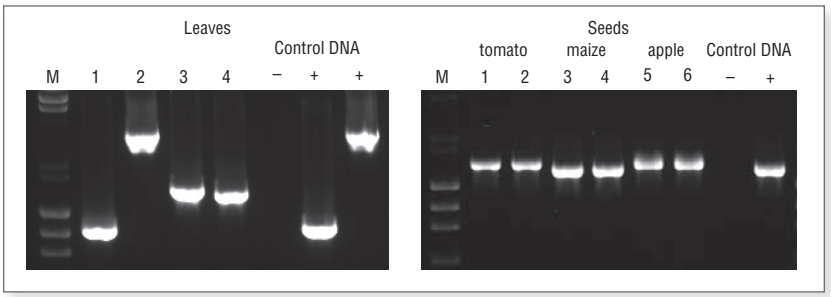
Extraction-free PCR

Amplification of DNA directly from starting materials such as blood or tissue has been problematic due to the presence of inhibitors in the sample (1). Purification of genomic DNA, as well as the use of specialized buffers or pre-treatments can overcome this difficulty, but often adds considerable time and expense to the process (2,3). As an alternative, NEB offers several kits and polymerases that can be used for direct amplification of DNA. These products offer robust activity and will save valuable laboratory time and expense.

Advantages

- No need for DNA extraction
- Robust amplification
- Kits available for extraction directly from blood, plant or animal-derived tissue samples
- Minimal sample material required

Amplification from various plant leaves and seeds with Phire Plant Direct PCR Kit



Leaves: *Lane 1: Arabidopsis, Mt DNA (1 kb); Lane 2: Arabidopsis, gDNA (3.5 kb); Lane 3: Capsicum, Mt DNA (1.4 kb); Lane 4: Maize, Mt DNA (1.4 kb).* ***Seeds:*** *Lanes 1-6: Mt DNA (~1.4 kb, depending on species)*

References
1. Wang, Y. (2004) *Nucleic Acids Res.* 32, 1197–1207
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3. McCusker, J. (1992) *Nucleic Acids Res.* 20, 6747.

Ordering Information

PRODUCT	NEB #	SIZE
Hemo KlenTaq™	M0332S/L	200/1000 rxns (25 µl vol)
Phusion® Blood Direct PCR Kit*	F-547S/L	100/500 rxns (20 µl vol)
Phire® Plant Direct PCR Kit*	F-130S	200 rxns (50 µl vol)
Phire® Animal Tissue Direct PCR Kit*	F-140S	200 rxns (50 µl vol)
DNARelease Additive*	F-355S	1 set

** Produced by Finnzymes, Oy. and distributed by New England Biolabs, Inc.*

Introducing NEB Tools – A new iPhone™ App from NEB

NEB's popular web tools, Double Digest Finder and Enzyme Finder, are now available for the iPhone™ and iPod® Touch. These tools to help design restriction enzyme experiments are often used on the NEB website, www.neb.com. Making them available as free downloads brings their accessibility closer to the bench.

Double Digest Finder recommends optimum conditions for a double digest, including buffer, incubation temperature and supplement requirements. Simple scrolling lists allow you to choose two enzymes and get all the information you need quickly.

Enzyme Finder allows you to sort restriction enzymes by category, sequence or name, and provides detailed information for each enzyme. The tool contains links to product pages, as well as allowing you to send the results directly to your e-mail account.

Watch for exciting updates to the NEB Tools App!

iPod is a registered trademark of Apple Inc., registered in the US and other countries.
iPhone is a trademark of Apple Inc.

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Additional tips for optimizing reactions can be found in the technical reference section of our website, www.neb.com.

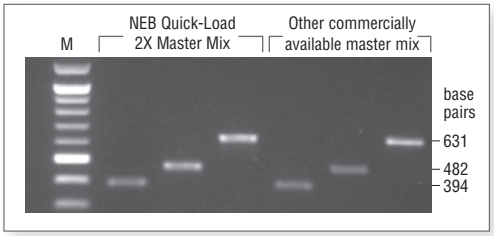
PROBLEM	POSSIBLE CAUSE	SOLUTION
SEQUENCE ERRORS	Low fidelity polymerase	• Choose a higher fidelity polymerase (see selection chart on page 5)
	Reaction conditions are not optimal	• Reduce number of cycles • Decrease extension time • Decrease Mg^{++} concentration present in reaction • Increase the amount of template present
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
	Template DNA has been damaged	• Start with a fresh template
	Faulty primer preparation	• Repeat reaction with new primers
	UV damage	• UV wavelength exposure time should be limited to 254–312 nm when using a light box to analyze or excise PCR products
	Unbalanced nucleotide concentrations	• Prepare fresh nucleotide mix
INCORRECT PRODUCT SIZE	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{++} concentration	• Adjust Mg^{++} concentration in 0.5 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
NO PRODUCT	Primer annealing temperature too high	• If amplifying with Phusion or Phire DNA Polymerases, verify annealing temperature using the Finnzymes T_m calculator (www.finnzymes.fi/tm_determination.html) • Lower annealing temperature in 2°C increments • Perform "Touchdown" PCR (1)
	Poor primer design	• Check polymerase datacard/manual for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Increase primer concentration to 0.1–0.5 µM
	Missing reaction component	• Repeat reaction setup
	Target sequence not present in template	• Try other sources of template DNA
	Poor reaction conditions	• Optimize Mg^{++} concentration, annealing temperature and extension time • Thoroughly mix Mg^{++} solution • Check primer concentrations • Perform "Touchdown" PCR
	Questionable template quality	• Analyze DNA via gel electrophoresis after incubation with Mg^{++}
	Presence of inhibitor in reaction	• Decrease sample volume • Purify template DNA by alcohol precipitation or drop dialysis • Try an extraction-free PCR product (see page 7), designed for amplification directly in the presence of inhibitors such as blood, plant or animal tissue
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents and new tubes
	Complex template	• For GC-rich templates, we recommend Phusion® DNA Polymerase with GC buffer • For longer templates, we recommend LongAmp™ *Taq* DNA Polymerase
MULTIPLE OR NON-SPECIFIC PRODUCTS	Premature replication (non-hot start polymerases)	• Set up reactions on ice using chilled components. Add samples to thermocycler preheated to the denaturation temperature • Try using a hot start polymerase, such as Phire Hot Start II and Phusion Hot Start II DNA Polymerases
	Primer annealing temperature too low	• Raise annealing temperature in 2°C increments • If using Phusion or Phire, verify annealing temperature using Finnzymes T_m calculator • Perform "Touchdown" PCR (1)
	Insufficient mixing of reaction buffer	• Thoroughly mix reaction buffer
	Incorrect Mg^{++} concentration	• Adjust Mg^{++} concentration in 0.5 mM increments
	Poor primer design	• Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Reduce primer concentration to 0.1–0.5 µM
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• Use 1 pg–1 ng/50 µl rxn of phage or plasmid DNA • Use 1 ng–1 µg/50 µl rxn of genomic DNA

(1) Don, R.H., et al. (1991) *Nucleic Acids Res.* 19, 4008.

Looking to speed up reaction setup? Try one of our PCR Master Mixes

For additional convenience, many polymerases from NEB are available in a master mix format. NEB master mixes have been carefully tested so that you can be confident they will offer the same high performance as the individual polymerases. With the polymerase, buffer and dNTPs included, master mixes reduce the number of pipetting steps and increase reaction throughput.

NEB Quick-Load® 2X Master Mix offers robust amplification for a variety of templates



Amplification of three templates according to manufacturers' recommended conditions. Amplicon sizes are indicated next to gel. Marker M is the 2-Log DNA Ladder (NEB #N3200).

Advantages

- Fast reaction setup
- Minimal pipetting errors
- Large selection for a variety of applications
- Ideal for high throughput
- Value pricing

Visit **confidentPCR.com** to request a sample

Master Mix Selection Chart

PRODUCT	NEB #	FEATURES
Taq 5X Master Mix	M0285S/L	Routine high yield
Taq 2X Master Mix	M0270S/L	Routine high yield
LongAmp™ *Taq* 2X Master Mix	M0287S/L	Long amplicons
Quick-Load® *Taq* 2X Master Mix	M0271S/L	Ready-to-load
Multiplex PCR 5X Master Mix	M0284S	Multiple templates
Phusion® Flash High-Fidelity PCR Master Mix*	F-548S/L	High fidelity, robust, fast
Phusion® High-Fidelity PCR Master Mix with GC Buffer*	F-532S/L	High fidelity, robust, fast
Phusion® High-Fidelity PCR Master Mix with HF Buffer*	F-531S/L	High fidelity, robust, fast

* *Produced by Finnzymes, Oy and distributed by New England Biolabs, Inc. Phusion is a registered trademarks of Finnzymes, Oy.*

FAQ Spotlight

Q: Why should I choose a PCR master mix?

A: *PCR master mixes offer greater convenience during reaction setup. Master mixes reduce pipetting steps and subsequently minimize the chances for pipetting errors. This leads to greater amplification accuracy and reaction consistency. The Quick-Load Taq 2X PCR Master Mix reduces pipetting steps even further by including a gel loading dye in the mix so that PCR reactions can be loaded directly onto an agarose gel for analysis.*

Q: What applications can benefit from the use of a PCR master mix?

A: *Any applications where Taq or Phusion would normally be used can benefit from the use of a PCR master mix. High throughput experiments are particularly suitable for master mix use.*

Q: Why is the *Taq* PCR Master Mix available in both 2X and 5X concentrations?

A: *For users with more dilute DNA samples or those requiring higher concentrations of starting template, the Taq 5X PCR Master Mix allows for larger volumes of template or primers to be added to the reaction. When sample volumes are not an issue, the standard 2X format is available.*

Ultrasensitive Secreted Luciferases

NEB offers a unique line of compatible reporter products that utilize secreted *Gaussia* Luciferase (GLuc) and *Cypridina* Luciferase (CLuc). The sensitivity and secretory properties of these luciferases make them ideal choices for a wide range of reporter applications routinely used in the study of mammalian cell biology.

Why choose *Gaussia* and *Cypridina* Luciferase as reporter systems?

There are many unique features of *Gaussia* (GLuc) and *Cypridina* (CLuc) Luciferases that make them ideal choices as reporter systems. When expressed in mammalian cells, both GLuc and CLuc are naturally secreted into the growth media. This feature allows the user to sample activity from the same source multiple times over the course of an experiment. Not only does non-destructive sample collection expedite the assay protocol, but it also keeps the cells intact for downstream assays such as RT-PCR, Western blots, RNA expression analysis, live cell imaging and cell viability assays.

These luciferases are the brightest luciferase reporters commercially available, allowing for the detection of activity in small samples or from weak expression. While the majority of the expressed GLuc or CLuc is secreted, the sensitivity of these systems allows for the detection of activity in cell lysates. This remarkable range of sensitivity makes these luciferases ideal for high throughput applications.

Additionally, GLuc and CLuc utilize different substrates, permitting the detection of one luciferase without any cross reactivity from the other. This makes GLuc and CLuc ideal reporter partners to be used in co-expression experiments in mammalian cells.

NEB offers a selection of luciferase expression vectors, as well as several kits for assaying activity. Use the selection charts below to help choose which products are ideal for your experiments.

Advantages

- **Naturally secreted** – Amenable to live cell assays
- **Sensitivity** – Brightest luciferases available; enables single cell applications
- **Stability** – Samples can be stored for several days with no loss of activity
- **Easy-to-use** – Cell lysis not necessary
- **Non-destructive** – Living cells can be used in downstream assays
- **Compatibility** – *Gaussia* and *Cypridina* Luciferases can be used alone or together as a dual system

Luciferase plasmids are available with a variety of promoters

PLASMID/ VECTOR	NEB #	PROMOTER	PROPERTIES	SELECTION
pGLuc-Basic	N8082S	–	Promoterless vectors for promoter evaluation and screening	neo
pGLuc Mini-TK	N8086S	Minimal	Minimal promoter for promoter or enhancer screening	neo
pCMV-GLuc	N8081S	CMV	Constitutive promoter, high expression vector	neo
pTK-GLuc	N8084S	Thymidine Kinase (TK)	Constitutive promoter, medium expression vector	neo
pCLuc-Basic 2	N0317S	–	Promoterless vectors for promoter evaluation and screening	neo
pSV40-CLuc	N0318S	SV40	Constitutive promoter, high expression vector	–

Gaussia and *Cypridina* Luciferase Assay Kits can be used alone or together as a dual system

ASSAY KIT	NEB #	FEATURES
BioLux™ *Gaussia* Luciferase Assay Kit	E3300S/L	Contains reagents for assaying GLuc activity
BioLux™ *Gaussia* Luciferase Flex Assay Kit	E3308S/L	Reagents for GLuc activity include a more concentrated substrate and stabilizer; ideal for high throughput screening
BioLux™ *Cypridina* Luciferase Assay Kit	E3309S/L	Contains reagents for assaying CLuc activity
BioLux™ *Cypridina* Luciferase Starter Kit	E3314S	Contains the reagents for assaying CLuc activity, as well as two CLuc encoding plasmids

For licensing information, visit www.neb.com or contact us at busdev@neb.com.

Coming this Summer...

25th 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:

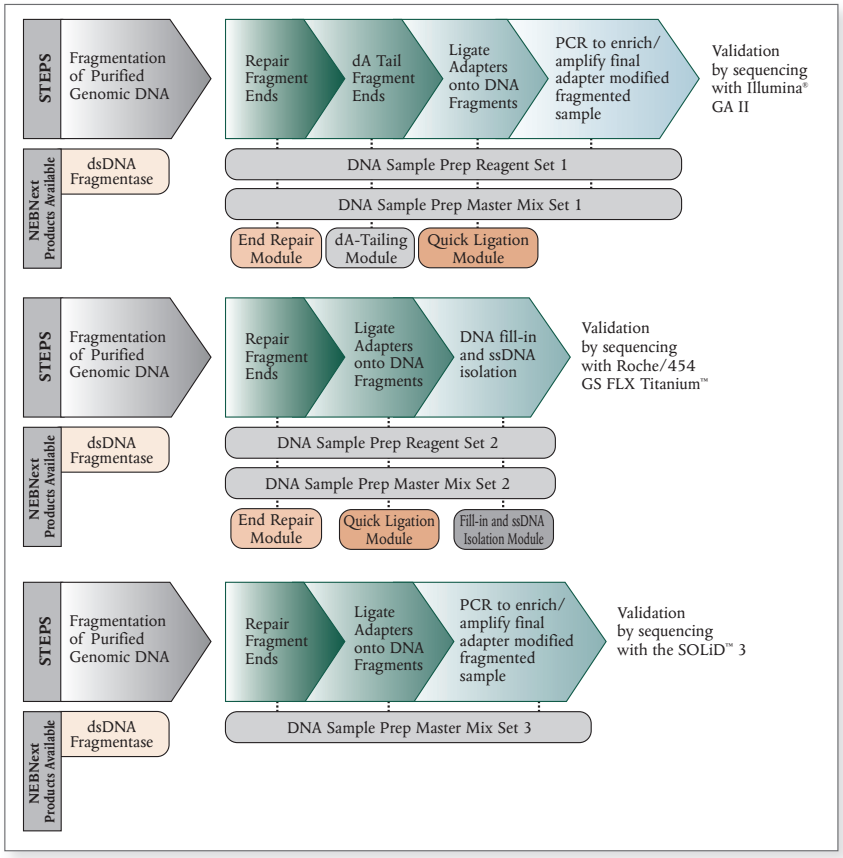
- gene cloning
- gene expression analysis
- PCR and qRT-PCR
- genomics and bioinformatics
- DNA sequencing and fingerprinting
- RNAi, siRNA and microarrays

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

NEBNext™ Reagents for Sample Prep – now available for the SOLiD™ System

The NEBNext™ series of reagents has expanded to include DNA Sample Prep Master Mix Set 3, which has been validated for the SOLiD™ System. NEBNext reagents are a series of highly pure reagents that facilitate sample preparation of DNA for downstream applications, including next generation sequencing. All reagents undergo stringent quality controls and functional validation, ensuring maximum yield, convenience and value.

NEBNext offers a solution for each step of Genomic Library Construction Workflow



Advantages

- **Convenient formats** – All of the required enzymes, buffers and nucleotides are included in master mix format. Modules offer the ability to customize sample preparation.
- **Functional Validation** – Each reagent set or module is functionally validated on the SOLiD platform.
- **Stringent Quality Controls** – Additional QCs ensure maximum quality and purity.
- **Value Pricing**

Applications

- **Next generation sequencing sample preparation**
- **Expression library construction**

Ordering Information

PRODUCT	NEB #	SIZE	COMPATIBLE SEQUENCING PLATFORM
NEBNext™ DNA Sample Prep Reagent Set 1	E6000S/L	10/50 rxns	Illumina
NEBNext™ DNA Sample Prep Reagent Set 2	E6020S/L	10/50 rxns	454
NEBNext™ DNA Sample Prep Master Mix Set 1	E6040S/L	10/50 rxns	Illumina
NEBNext™ DNA Sample Prep Master Mix Set 2	E6070S/L	10/50 rxns	454
NEBNext™ DNA Sample Prep Master Mix Set 3	E6060S/L	10/50 rxns	SOLiD
NEBNext™ End Repair Module	E6050S/L	20/100 rxns	Illumina, 454, SOLiD
NEBNext™ dA-Tailing Module	E6053S/L	20/100 rxns	Illumina
NEBNext™ Quick Ligation Module	E6056S/L	20/100 rxns	Illumina, 454, SOLiD
NEBNext™ Fill-in and ssDNA Isolation Module	E6071S/L	20/100 rxns	454
NEBNext™ dsDNA Fragmentase™	M0348S/L	50/250 rxns	Illumina, 454, SOLiD

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