Summer 2008, vol. 3.2

NEB expressions

a scientific update from New England Biolabs

Welcome to the summer edition of NEB expressions. This issue highlights our comprehensive PCR product line, that includes a wide selection of polymerases suited for a variety of PCR conditions, ultrapure deoxynucleotides and DNA ladders that will bring accuracy to your DNA sample analysis. A comprehensive polymerase selection chart is provided to help determine which enzyme is best suited for your application.

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

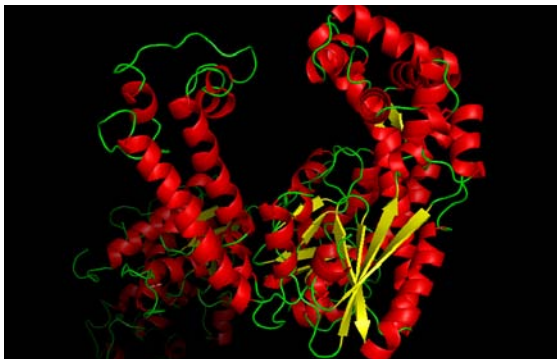
inside:

NEW ENGLAND BioLabs® Inc.
leader in enzyme technology

Anatomy of a Polymerase

how structural properties can affect function

Thomas C. Evans, Ph.D,
New England Biolabs, Inc.



Taq DNA Polymerase, one of the first thermophilic polymerases discovered, helped to facilitate the amplification process.

Accurate genome replication is critical for the viability of an organism. The general concept for copying DNA was evident upon the elucidation of DNA's double helical structure and the identification of base pair complementarity (1). Within a decade of these discoveries, an agent was purified from *E. coli* that catalyzed DNA strand duplication (2). This agent was termed a "polymerase". *E. coli* DNA Polymerase I, the first DNA polymerase discovered was not the primary replicative polymerase, but instead one involved in Okazaki fragment resolution and DNA repair. This foreshadowed future discoveries of many DNA polymerase families, each serving specific cellular requirements.

Polymerases play a key role in the life sciences for the same reason that they are critical in nature: they copy DNA. Additional polymerase applications include DNA labeling, sequencing and amplification. One specific amplication protocol, the polymerase chain reaction (PCR) is a widely used technique that employs thermophilic polymerases to exponentially amplify specific DNA segments (3).

Accuracy

PCR puts the same basic demands on a polymerase as a cell puts on its replicative polymerase. Essentially, it should be reliable, accurate and fast. Polymerase "accuracy" or "fidelity" refers to the propensity to incorporate the correct nucleotide as specified by the template strand. The standard PCR enzymes are not surprisingly quite accurate. Even *Thermus aquaticus* (*Taq*) DNA polymerase, a low fidelity PCR polymerase, only makes a mistake once in approximately 100,000 nucleotide insertions, depending on reaction conditions (4).

(continued on page 2)

New Crimson *Taq*™ DNA Polymerase

for loading PCR samples directly on a gel

Experience the robust and reliable performance of *Taq* DNA Polymerase in a more convenient format. Ideal for high throughput applications, Crimson *Taq* Reaction Buffer is optimized for robust amplification, and contains tracking dye that allows samples to be loaded directly on a gel.

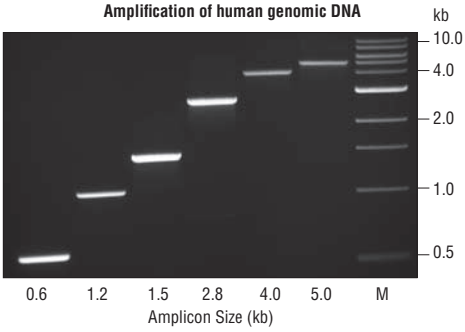
Advantages

- Robust and reliable reactions
- Samples can be loaded directly on a gel
- Ideal for high throughput applications
- Value pricing

See pages 4 and 5 for additional related offers.

Introductory Offer

Try the **Crimson *Taq* PCR Sampler**, containing everything you need for setting up a PCR reaction at an exceptional value. See page 5 for more details.



Choose Crimson Taq DNA Polymerase for robust reactions in a convenient format. *Amplicon sizes are indicated below gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

How Structure Effects Function

(continued from page 1)

The basis of polymerase accuracy is an exciting area of investigation. Although most work on the mechanism of DNA polymerization has been performed with mesophilic polymerases, it is believed that the general trends can be extrapolated to related thermophilic enzymes.

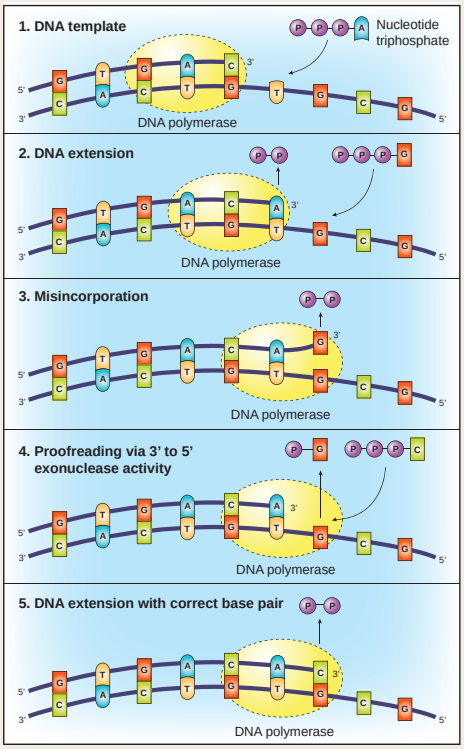


Figure 2: *Polymerases that have a 3´→5´ exonuclease activity are able to excise mismatched base pairs when an error is encountered, thereby increasing enzyme fidelity.*

Geometric Selection

At first thought, a reasonable method to discriminate between correct and incorrect nucleotides would be based on forming the correct hydrogen bond to the template base. However, hydrogen bonding between complimentary bases is not enough to explain the high fidelity displayed by DNA polymerases. Studies using a deoxynucleotide triphosphate with a non-standard difluorotoluene base (dF) demonstrated the importance of base pair size and shape, as opposed to hydrogen bonding during incorporation (5). The pyrimidine ring of difluorotoluene is isosteric with thymine but the oxygens are replaced by fluorine atoms and therefore dF cannot form effective hydrogen bonds in water. In synthetic oligonucleotides, dF was found to base pair equally poorly with all four of the standard bases. If hydrogen bonding was the most critical parameter dictating incorporation accuracy, then it would be expected that dF would be incorporated into a template poorly and with low fidelity. However, it was incorporated only 40-fold less efficiently than dT. This finding could be explained if the polymerase active site accommodates only proper base pairs of the correct size and shape. The "geometric selection" mechanism (6) is an elegant method of ensuring accurate incorporation is extremely effective, but there are also more active ways to ensure high fidelity replication.

Proofreading

A perhaps better-known method of increasing fidelity is for the polymerase to have 3´→5´ exonuclease activity, termed "proofreading". Considering the complexity of DNA polymerization, *Taq* DNA polymerase is incredibly accurate, but proofreading enzymes can have even higher fidelity. This is accomplished by the polymerase "checking" whether the correct nucleotide has been inserted into the template. If a mismatch is detected the DNA is transferred from the polymerization domain to an N-terminal 3´→5´ exonuclease domain of the polymerase. The incorrectly incorporated nucleotide is excised and the DNA is moved back into the polymerization domain, permitting copying to resume (Figure 2).

Bacteriophage T4 proved to be a useful experimental system for evaluating the importance of 3´→5´ exonuclease activity for accurate DNA replication (6). Mutations in T4 gene 43 were identified that either decreased or increased fidelity. By defining an exonuclease/polymerase (N/P) activity ratio for a particular mutant enzyme it was found that polymerases with low N/P ratios were more error prone than those with high N/P ratios. An explanation for this observation is that upon incorporation of a mismatched base it is more likely that the exonuclease will remove the nucleotide before the polymerase activity extends it in enzymes with higher N/P ratios. Interestingly, the proofreading effectiveness of a polymerase can show sequence dependence. For example, AT-rich sequences are more effectively proofread than GC regions. This is thought to be due to the lower stability of AT stretches that facilitates melting and therefore, proofreading activity.

The absence of 3´→5´ exonuclease activity may have ramifications other than fidelity in PCR. The lack of proofreading activity in *Taq* DNA Polymerase has been proposed to limit the amplicon size possible with this enzyme (7). As a generality, *Taq* performs best when amplifying DNA fragments < 2 kb, but works on fragments up to 3–4 kb. When kept to this amplicon size, *Taq* is a robust, easily optimized enzyme. However, above ~3 kb it quickly drops in effectiveness. During PCR, *Taq* DNA Polymerase will misincorporate nucleotides at a particular rate leading to mismatch formation. This is termed "error rate". *Taq*, and polymerases in general, will stall at these mismatched bases and are more likely to dissociate before extending as compared to correctly base paired 3´ ends. Therefore, at a certain amplicon size and polymerase error rate enough mismatched 3´ ends may accumulate to effectively inhibit the PCR process. These mismatched 3´ ends are particularly problematic for *Taq* because it lacks the 3´→5´ exonuclease activity to remove them. By adding in a small amount of proofreading enzyme such as Deep Vent$_R$™ DNA Polymerase, amplification of fragments ≥ 20 kb can be achieved (Figure 3). Since the vast majority of the enzyme in the blend is *Taq* DNA Polymerase it is probably doing the bulk of the primer extension. The proofreading polymerase is most likely removing the inhibitory 3´ mismatches generated by *Taq*.

Processivity

The importance of proofreading activity to PCR has been widely known for nearly two decades, but another property, processivity, has recently gained attention. "Processivity" is a term that refers to the number of nucleotides incorporated by a polymerase before it dissociates. *Taq* DNA polymerase adds approximately 50 nucleotides per binding event (8). Why does this matter? A distributive polymerase extends a population

Amplification of Human Genomic DNA with LongAmp™ *Taq* DNA Polymerase

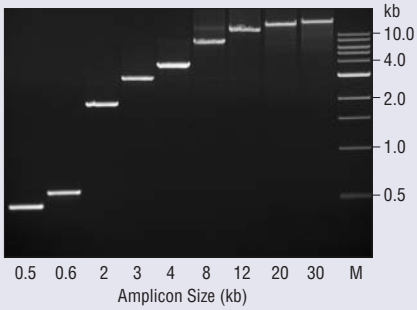


Figure 3: *A unique blend of Taq and Deep Vent$_R$® DNA Polymerases, LongAmp Taq DNA Polymerase enables amplification of larger PCR products with a higher fidelity than Taq DNA Polymerase alone. Amplicon sizes are indicated below the gel. Marker M is the NEB 1 kb DNA Ladder (NEB #N3232).*

Advantages of LongAmp™ *Taq*

- Robust amplifications
- Amplification up to 40 kb from low complexity templates and up to 30 kb from difficult templates (i.e., human genomic DNA)
- Increased fidelity compared to *Taq* DNA Polymerase alone

For ordering information, see page 5.

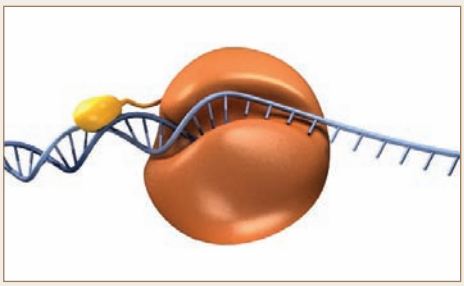


Figure 4: *Phire Hot Start DNA Polymerase is constructed by fusing a DNA polymerase (orange) and a small dsDNA-binding protein (yellow). This technology increases the processivity of the polymerase and improves its overall performance. For more information on Phire Hot Start DNA Polymerase, see page 7.*

of 1 kb templates in a noticeably different manner than a processive polymerase. The distributive polymerase binds to a template, adds a couple of nucleotides, and dissociates, leaving a population of templates that can be extended equally with time. The processive polymerase binds a template and extends to its end. Therefore, it is an all-or-nothing extension, where a fraction of the templates are fully copied and the remaining fraction are unextended. It would follow that given enough time the outcome of either a processive or distributive polymerase would be a population of copied templates. However, in certain circumstances it is possible that the processive polymerase has superior performance. The *E. coli* polymerase III α subunit, part of the main replicative polymerase, has a processivity of < 10 base pairs and a speed of < 20 nucleotides/second (nt/s). However, when the subunit associates with the other replisome subunits, particularly the sliding clamp, the effective processivity and replication speed increase to > 50 kb and 1,000 nt/s, respectively (9). The term "effective processivity" is used because there is data indicating the polymerase subunit can exchange in the replisome, but the replisome as a whole maintains fast, processive DNA replication (10). In order to take advantage of processivity in PCR, researchers have fused a DNA binding domain to an archaeal polymerase (11). This chimeric enzyme has a number of improved properties, but notably it is able to amplify DNA with shorter extension times, thus shortening overall thermocycling times. This fusion is the base of Phusion™ High-Fidelity DNA Polymerase and Phire™ Hot Start High-Fidelity DNA Polymerase, two polymerases available from NEB (Figure 4).

Future directions

Many properties affect the efficacy and utility of a PCR polymerase. Polymerase active site architecture and proofreading activity affect the accuracy of the final product. Polymerase blends and fusion to a DNA binding protein confer superior PCR performance for amplicon length and, in the case of the chimera, reaction speed. Other important advances in PCR, such as hot-start polymerases to increase reaction specificity, multiplex PCR (Figure 5) and qPCR have also revolutionized many aspects of the life sciences. As demonstrated by engineered blends and chimeras, properties of the polymerase itself can be modulated to improve PCR performance. In the future, it is likely that polymerase properties will increasingly be tailored to specific PCR applications, and as such, this is an important area of research at NEB.

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Amplification of Multiple PCR Products from Human Genomic DNA with the Multiplex PCR 5X Master Mix

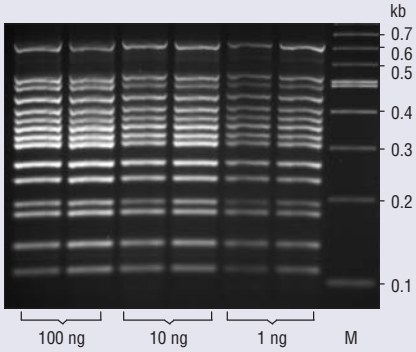


Figure 5: *The Multiplex PCR 5X Master Mix includes Taq DNA Polymerase and buffer that has been optimized for amplification of multiple targets. Its performance is illustrated in a 15-plex reaction with various amounts of human genomic DNA as template (shown below gel).*

Advantages

- Optimized for high yield and robust performance
- 5X formulation facilitates reaction setup

For ordering information, see page 5.

Make NEB Your Choice for PCR

Performance, Convenience and Results

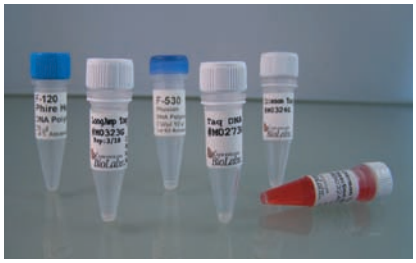
Looking for the right solution for your PCR application? New England Biolabs offers a wide selection of polymerases ideal for a variety of PCR conditions. Whether your focus is fidelity, speed, or amplification of a challenging template, NEB has the polymerase to suit your needs. For added convenience, many of these polymerases are available as kits or master mixes. In addition, choose from our selection of high quality DNA ladders or ultrapure deoxynucleotides and make NEB your one-stop solution for PCR reagents.

Polymerase Selection Chart

Characteristic	Polymerase	Feature
High Fidelity	Phusion™ High-Fidelity DNA Polymerase	Enhanced yield, speed and robustness
	Phusion™ Hot Start High-Fidelity DNA Polymerase	Enhanced specificity, yield, speed and robustness
	Phusion™ Flash High-Fidelity PCR Master Mix	Enhanced speed, yield and robustness
	Deep Vent$_R$™ DNA Polymerase	Extremely thermostable enzyme for difficult templates
	Vent$_R$® DNA Polymerase	Thermostable enzyme for routine PCR
	LongAmp™ *Taq* DNA Polymerase	Long templates (< 40 kb)
	Phire™ Hot Start DNA Polymerase	Enhanced specificity and speed for routine PCR
	DyNAzyme™ EXT DNA Polymerase	Long or difficult templates (< 40 kb)
High Yield	*Taq* DNA Polymerase	Industry standard for routine PCR
	Crimson *Taq*™ DNA Polymerase	Load directly onto a gel
	LongAmp™ *Taq* DNA Polymerase	Long templates (< 40 kb)
	Phusion™ High-Fidelity DNA Polymerase	Extreme fidelity, speed and robustness
	Phusion™ Hot Start High-Fidelity DNA Polymerase	Enhanced fidelity, specificity, speed and robustness
	Phusion™ Flash High-Fidelity PCR Master mix	Enhanced speed and robustness
	Phire™ Hot Start DNA Polymerase	Enhanced specificity and speed for routine PCR
	DyNAzyme™ EXT DNA Polymerase	Long or difficult templates (< 40 kb)
	DyNAzyme™ II Hot Start DNA Polymerase	Hot start PCR
Hot Start	Phusion™ Hot Start High-Fidelity DNA Polymerase	Extreme fidelity, yield, speed and robustness
	Phire™ Hot Start DNA Polymerase	High speed and yield
	DyNAzyme™ II Hot Start DNA Polymerase	Routine PCR
Fast PCR	Phusion™ High-Fidelity DNA Polymerase	High fidelity with extension times 15-30s/kb
	Phusion™ Hot Start High-Fidelity DNA Polymerase	High fidelity, specificity with extension times 15-30s/kb
	Phusion™ Flash High-Fidelity PCR Master Mix	Extreme speed with extension times < 15s/kb
	Phire™ Hot Start DNA Polymerase	Extension times 20s/kb
Long PCR	LongAmp™ *Taq* DNA Polymerase	Robust reactions for templates (< 40 kb)
	Phusion™ High-Fidelity DNA Polymerase	Extreme fidelity, yield and robustness (< 20 kb)
	Phusion™ Hot Start High-Fidelity DNA Polymerase	Enhanced specificity (< 20 kb)
	Phusion™ Flash High-Fidelity PCR Master mix	Enhanced speed (< 20 kb)
	DyNAzyme™ EXT DNA Polymerase	Difficult templates (< 40 kb)
Difficult templates	Phusion™ High-Fidelity DNA Polymerase	Available with buffer for GC-rich templates
	LongAmp™ *Taq* DNA Polymerase	Robust and reliable reactions
Convenience	Phusion™ High-Fidelity DNA Polymerase	High fidelity, master mix or kit format
	Phusion™ Flash High-Fidelity PCR Master Mix	Master mix format for fast PCR
	Taq DNA Polymerase	Robust reactions, master mix or kit format
	LongAmp™ *Taq* DNA Polymerase	Long PCR, master mix or kit format
qPCR	DyNAmo™ qPCR Kits	Available for SYBR® Green and Probe chemistries
	DyNAmo™ Flash qPCR Kits	Fast protocols for SYBR® Green and Probe chemistries
PCR of multiple templates	Multiplex PCR 5X Master Mix	High yield and robust performance

Special Offer

Through September 30th, receive a FREE DNA polymerase sample with any purchase. Select from the polymerases listed below, and include the appropriate product number(s) with your order.



Select from five different PCR polymerase samples.

Choose from:

Taq DNA Polymerase with Standard Taq Buffer ..... #M0273G
for standard PCR reactions

Crimson *Taq*™ DNA Polymerase ..... #M0324G
for added convenience

LongAmp™ *Taq* DNA Polymerase ..... #M0323G
for larger PCR products

Phusion™ High Fidelity DNA Polymerase ..... #F-530G
for extreme fidelity, speed and robustness

Phire™ Hot Start DNA Polymerase ..... #F-120G
for speed and enhanced specificity

Offer valid in the U.S. only while supplies last. Limit one each per customer.

Deoxynucleotides for PCR

NEB offers ultrapure deoxynucleotide triphosphates (dNTPs) for use in your PCR reactions. These high quality reagents are available separately, or as a convenient mix to accommodate a variety of applications.

Deoxynucleotide Solution Mix
#N0447S 8 µmol $63
#N0447L 40 µmol $252

Deoxynucleotide Solution Set
#N0446S 25 µmol of each $184

Taq DNA Polymerase

robust and reliable reactions in convenient formats

NEB now offers an expanded selection of recombinant *Taq* DNA Polymerase-based products in a variety of formats. To further optimize your reactions, choose from a wider selection of buffers, including Crimson *Taq* Reaction Buffer, which has been optimized for performance and contains a crimson tracking dye that allows samples to be loaded directly onto a gel.

Taq Buffer Selection Chart

Choice of Buffer	Mg-Control	NEB Product #
Standard *Taq* Reaction Buffer: Detergent-free and designed to be compatible with detergent-sensitive assay systems	*Taq* DNA Polymerase with Standard *Taq* Buffer	#M0273
	Taq DNA Polymerase with Standard *Taq* (Mg-free) Buffer	#M0320
ThermoPol Buffer: Designed to optimize yields and specificity	*Taq* DNA Polymerase with ThermoPol Buffer	#M0267
	Taq DNA Polymerase with ThermoPol II (Mg-free) Buffer	#M0321
Crimson *Taq* Reaction Buffer: Contains crimson tracking dye, allowing samples to be loaded directly onto a gel	Crimson *Taq* DNA Polymerase	#M0324
	Crimson *Taq* DNA Polymerase with (Mg-free) Buffer	#M0325

Ordering Information

New Crimson *Taq*™ DNA Polymerase
#M0324S 200 units.....$44
#M0324L 1,000 units.....$176

New Crimson *Taq*™ DNA Polymerase with (Mg-free) Buffer
#M0325S 200 units.....$44
#M0325L 1,000 units.....$176

LongAmp™ *Taq* 2X Master Mix
#M0287S 100 rxns (50 µl vol).....$135
#M0287L 500 rxns (50 µl vol).....$540

LongAmp™ *Taq* DNA Polymerase
#M0323S 500 units.....$75
#M0323L 2,500 units.....$300

LongAmp™ *Taq* PCR Kit
#E5200S 100 rxns (50 µl vol).....$105

New Multiplex PCR 5X Master Mix
#M0284S 100 rxns (50 µl vol) $210

Quick-Load® *Taq* 2X Master Mix
#M0271S 100 rxns (50 µl vol).....$63
#M0271L 500 rxns (50 µl vol).....$252

Taq with Standard *Taq* Buffer
#M0273S 400 units.....$58
#M0273L 2,000 units.....$232
#M0273X 4,000 units.....$418

Taq with Standard *Taq* (Mg-free) Buffer
#M0320S 400 units.....$58
#M0320L 2,000 units.....$232

Taq 2X Master Mix
#M0270S 100 rxns (50 µl vol).....$63
#M0270L 500 rxns (50 µl vol).....$252

Taq 5X Master Mix
#M0285S 100 reactions.....$63
#M0285L 500 reactions.....$252

Taq PCR Kit
#E5000S 200 rxns.....$95
#E5100S 200 rxns (w/ controls).....$105

Taq with ThermoPol Buffer
#M0267S 400 units.....$58
#M0267L 2,000 units.....$232
#M0267X 4,000 units.....$418

Taq with ThermoPol II (Mg-free) Buffer
#M0321S 400 units.....$58
#M0321L 2,000 units.....$232

Through September 30th, order Crimson *Taq*™ DNA Polymerase and receive a free NEB Crimson Sharpie®.*

* Offer valid on purchases of M0324, M0325 and E0547. Valid in the US only.

> Introductory Offer

Crimson *Taq*™ PCR Sampler

The Crimson *Taq* PCR Sampler contains sample sizes of everything you need for setting up a PCR reaction, at an exceptional value.

The sampler contains:

- 50 units of Crimson *Taq* DNA Polymerase
- 50 µl Deoxynucleotide Solution Mix (10 mM)
- Quick-Load® 1 kb DNA Ladder (12 gel lanes)

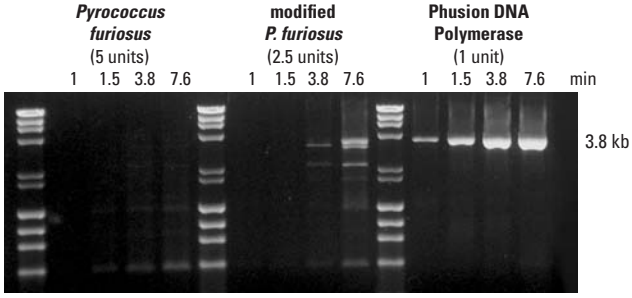
Crimson *Taq*™ PCR Sampler
#E0547S 40 rxns.....$35

> Produced by Finnzymes, Oy
Distributed by New England Biolabs, Inc.

Phusion™ High-Fidelity DNA Polymerase

extreme precision with unparalleled speed and robustness

With Phusion™ High-Fidelity DNA Polymerase, there is no need to compromise any aspect of PCR performance. A superior choice for cloning, this recombinant polymerase has an error rate 50-fold lower than *Taq* DNA Polymerase, making it the most accurate thermostable polymerase available. Phusion DNA Polymerase is supplied in a variety of formats, or with a hot start modification for increased specificity.



Experience extreme speed and yield with Phusion DNA Polymerase.
A 3.8 kb fragment from human beta globin gene was amplified according to suppliers' recommendations using varying extension times. One unit of Phusion DNA Polymerase was able to amplify the fragment with a combined annealing and extension step of only 1 minute, with higher yields than 2.5 or 5 units of Pyrococcus furiosus DNA Polymerases.

Ordering Information

Phusion™ High-Fidelity DNA Polymerase
#F-530S 100 units $100
#F-530L 500 units $400

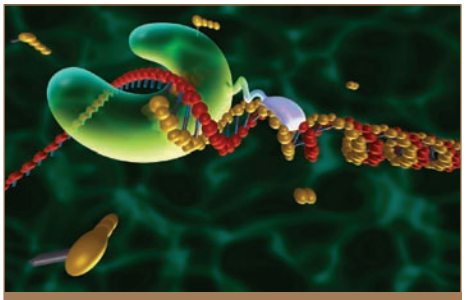
Phusion™ Hot Start High-Fidelity DNA Polymerase
#F-540S 100 units $120
#F-540L 500 units $480

Phusion™ High-Fidelity PCR Master Mix with HF Buffer
#F-531S 100 rxns (50 µl vol) $162
#F-531L 500 rxns (50 µl vol) $650

Phusion™ High-Fidelity PCR Master Mix with GC Buffer
#F-532S 100 rxns (50 µl vol) $162
#F-532L 500 rxns (50 µl vol) $560

Phusion™ High-Fidelity PCR Kit
#F-553S 50 rxns (50 µl vol) $62
#F-553L 200 rxns (50 µl vol) $210

Phusion™ Flash High-Fidelity PCR Master Mix
#F-548S 100 rxns (20 µl vol) $75
#F-548L 500 rxns (20 µl vol) $300



Tips for successful amplification with Phusion

- Use Phusion High-Fidelity DNA Polymerase at 0.5 – 1.0 units per 50 µl reaction volume. Do not exceed 2 units/50 µl reaction.
- Use 15 – 30 sec/kb for extension. Do not exceed 1 min/kb.
- Use 200 µM dNTPs. Do not use dUTP.
- Use 98°C for denaturation. (Due to high salt concentration in buffer)
- Anneal at T_m+3°C (For oligos > 20 nt) or use 2-step protocol.
- Note: Phusion DNA Polymerase produces blunt end products.

NEB FAQ Spotlight

PCR reactions with Taq and Phusion DNA Polymerases

Q The conditions I used with *Taq* DNA Polymerase do not work with Phusion DNA Polymerase. What changes should I make?

A The annealing temperatures that are optimal when using *Taq* DNA Polymerase are not always optimal when using Phusion DNA Polymerase. The T_m of the primers should be calculated using the Finnzymes T_m calculator, available at www.finnzymes.com

Q You sell *Taq* DNA Polymerase with many different buffers. How do I know which product to order?

A Crimson *Taq* DNA Polymerase (NEB #M0324) is most useful for applications where convenience is advantageous. The buffer is detergent-free and reactions can be loaded directly onto the gel for analysis, without the addition of stop dye. *Taq* with ThermoPol Buffer (NEB #M0267) is useful for standard PCR, colony PCR, and primer extension. ThermoPol buffer has been optimized for use with NEB *Taq* DNA Polymerase. ThermoPol DF (Detergent-free) Reaction Buffer (NEB #B9013) is also available for customers that prefer the ThermoPol formulation, but cannot have detergent in the reaction. *Taq* with Standard *Taq* Reaction Buffer (NEB #M0273) utilizes a detergent-free buffer and is designed to be compatible with detergent-sensitive assay systems. All buffer formulations are available in Mg-free versions (NEB #M0320, #M0321, #M0325) for applications that require maximum control over Mg^{++} concentration.

> Application note

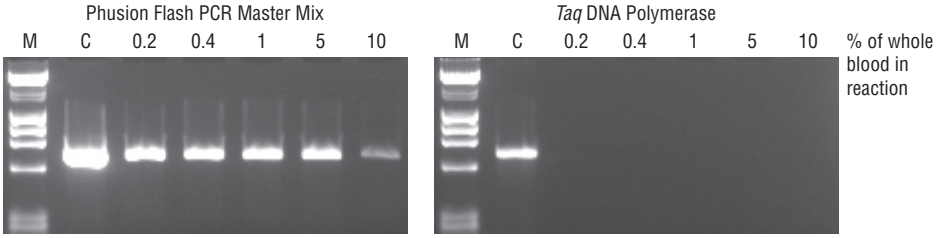
Efficient PCR Under Inhibitory Conditions

direct amplification from whole blood or mouse tissue

Scientists at Finnzymes have demonstrated that Phusion™ Flash High-Fidelity PCR Master Mix and Phire Hot Start DNA Polymerase can be used in PCR reactions in the presence of inhibitors, bypassing the need for template purification. Two application notes were recently released that describe direct amplification from whole blood or mouse tissue using Phusion Flash and Phire. Application notes can be downloaded by visiting www.finnzymes.com/application_notes.html.

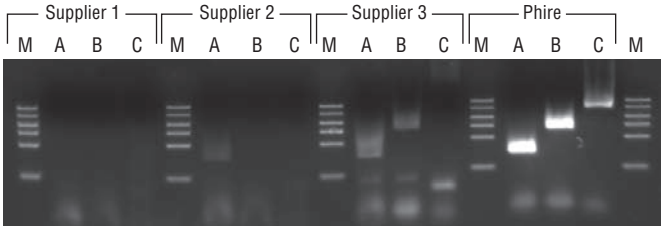
Whole blood is often used as a DNA source for PCR tests. Unfortunately, *Taq* DNA polymerase, the most commonly used enzyme for PCR, is known to be completely inhibited by even small quantities of blood in the reaction (1). Several strategies have been used to overcome this difficulty, including specialized purifications, buffers and pretreatments (2,3). All of these methods have drawbacks, adding time and cost, and often producing inconsistent results. As a consequence, purification of genomic DNA from whole blood remains a common practice, even though it adds considerable time and expense.

The **Phusion Flash PCR Master Mix** works well in the presence of strong PCR activity inhibitors. Researchers at Finnzymes compared *Taq* DNA polymerase to Phusion Flash to PCR inhibition in the presence of whole blood. The results showed successful amplification of exogenous amplicon (λ DNA, not shown) and human genomic DNA in the presence of whole blood using Phusion Flash, while *Taq* only worked in the absence of whole blood.



Direct amplification from whole blood with Phusion Flash PCR Master Mix. *Taq DNA polymerase was unable to amplify a 0.7 kb fragment from human genomic DNA in the presence of 0.2% whole blood, while the Phusion Flash Master Mix amplified the product in the presence of as much as 5% whole blood. Taq reactions were carried out using a conventional thermal cycler. Phusion Flash reactions were performed using Finnzymes high speed Piko™ Thermal Cycler, which enables results in dramatically reduced reaction times. Note that Phusion Flash will produce similar results using a conventional thermal cycler with longer reaction times.*

Phire Hot Start DNA Polymerase was successfully used to amplify DNA fragments from mouse tissue without any prior purification. Phire is a specially engineered enzyme that has been fused to a ds-DNA binding domain (1), a combination that performs well even in the presence of strong PCR inhibitors. Compared to Phire, hot-start *Taq*-based polymerases performed poorly, producing weak and/or non-specific bands. Phire successfully amplified all PCR products tested, up to 1.5 kb.



Amplicon Sizes
M = Size Marker
A = 194 bp
B = 466 bp
C = 1500 bp

Direct amplification from mouse tissue with Phire Hot Start DNA Polymerase: *Phire Hot Start DNA Polymerase was compared to three commercially available hot start Taq DNA Polymerases. Reactions were set up according to manufacturer's recommendations. Both Taq and Phire reactions were performed using the Piko Thermal Cycler. PCR products were run on FlashGel® System with DNA Release Additive in the FlashGel Loading Dye. Note that Phire will produce similar results using a conventional thermal cycler with longer reaction times.*

Excerpts from the following application notes were reprinted with permission from Finnzymes, Oy.:

- *Yang, P. and Andre, C. (2007) Efficient PCR from Whole Blood Using Finnzymes High Performance PCR Solution.*
- *Yang, P. and Andre, C. (2008) Direct PCR from Mouse Tissue using Phire Hot Start DNA Polymerase and Piko Thermal Cycler.*

Information on Finnzyme's High Performance PCR solution, including the high speed Piko™ Thermal Cycler can be found at www.hppcr.com.

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Phusion™ Flash High-Fidelity PCR Master Mix

save valuable laboratory time

A novel reagent developed for fast PCR, Phusion Flash High-Fidelity PCR Master Mix can amplify both low and high complexity DNA with an extension time of only 15 seconds or less per kb.

Advantages

- Extreme speed
- High fidelity (25X *Taq)*
- Robust yields in reduced time
- Minimal optimization required

Phusion™ Flash High-Fidelity PCR Master Mix
#F-548S 100 rxns (20 µl vol) $75
#F-548L 500 rxns (20 µl vol) $300

New Phire™ Hot Start DNA Polymerase

speed and specificity for high performance PCR

This novel PCR enzyme outperforms *Taq*-based hot start polymerases. The polymerase's DNA binding domain shortens extension times, improves yields and increases fidelity 2-fold compared to *Taq*. In addition, the Affibody®-based hot start technology does not require any cycling time adjustments. Phire is ideal for routine and high throughput applications.

Advantages

- Activation step is eliminated
- Amplification up to three times faster than hot start *Taq* polymerases
- High yield
- Amplification of longer products compared to other hot start *Taq* polymerases

Phire™ Hot Start DNA Polymerase
#F-120S 200 rxns (50 µl vol) $110
#F-120L 1,000 rxns (50 µl vol) $440

References

1. Wang, Y. (2004) *Nucleic Acids Res.* 32, 1197–1207
2. Panaccio, M. and Lew, A. (2007) *Nucleic Acids Res.* 19, 1151.
3. McCusker, J. (1992) *Nucleic Acids Res.* 20, 6747.

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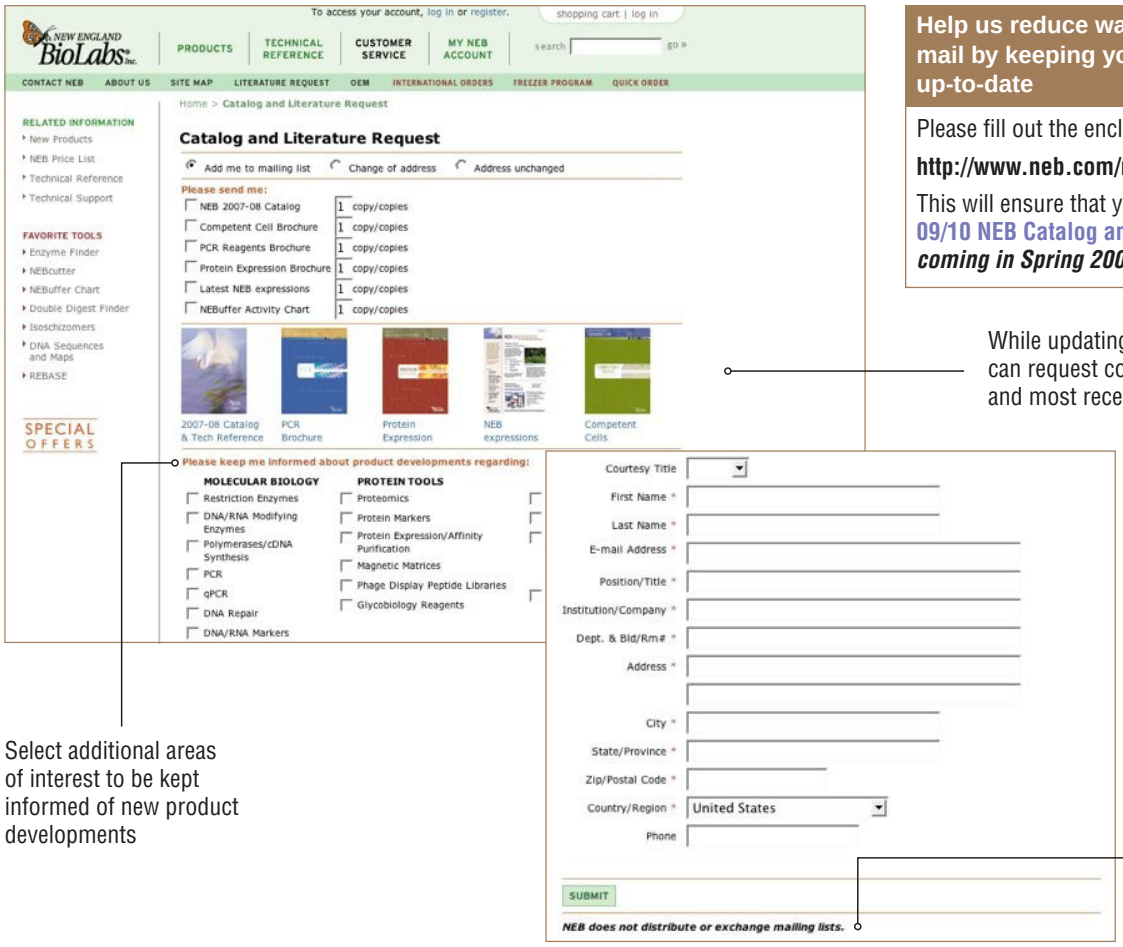

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