



## Polymerases from NEB

NEB supplies DNA polymerases differing in properties such as temperature preference, proofreading exonuclease activity, processivity and strand displacement. In order to facilitate matching the ideal DNA polymerase to a particular research application, the chart below and accompanying text describes properties associated with specific enzymes.

CLONING & MAPPING

**DNA AMPLIFICATION & PCR**

RNA ANALYSIS

PROTEIN EXPRESSION & ANALYSIS

GENE EXPRESSION & CELLULAR ANALYSIS

DNA POLYMERASES	3' → 5' PROOF-READING	STRAND DISPLACEMENT	PRIMARY APPLICATIONS
<b>Mesophilic DNA Polymerases</b>			
phi29 DNA Polymerase	++++	+++++	Strand Displacement Applications
T4 DNA Polymerase	+++++	-	Polishing Ends, 2nd Strand Synthesis
DNA Polymerase I	++	-*	Nick Translation
DNA Polymerase I, Large (Klenow) Fragment	++	++	Polishing Ends
Klenow Fragment (3' → 5' exo-)	-	+++	Labeling
T7 DNA Polymerase (unmodified)	++++	-	Site Directed Mutagenesis
Terminal Transferase	-	n/a	3' Terminal Tailing
<b>Thermophilic DNA Polymerases</b>			
• Crimson <i>Taq</i> <sup>™</sup> DNA Polymerase	-	-*	PCR (routine, ready-to-load)
• LongAmp <sup>™</sup> <i>Taq</i> DNA Polymerase	+	-*	PCR (high-fidelity)
• Crimson LongAmp <sup>™</sup> <i>Taq</i> DNA Polymerase	+	-*	PCR (high-fidelity)
<i>Taq</i> DNA Polymerase	-	-*	PCR (routine), Primer Extension
Vent <sub>R</sub> <sup>®</sup> DNA Polymerase	+++	++	PCR (high-fidelity), Primer Extension
Vent <sub>R</sub> <sup>®</sup> (exo-) DNA Polymerase	-	+++	PCR, Sequencing
Deep Vent <sub>R</sub> <sup>™</sup> DNA Polymerase	+++	++	PCR (high-fidelity), Primer Extension
Deep Vent <sub>R</sub> <sup>™</sup> (exo-) DNA Polymerase	-	+++	PCR (long), Primer Extension
9 <sup>o</sup> N <sub>m</sub> DNA Polymerase	+	+++	Primer Extension
Therminator <sup>™</sup> DNA Polymerase	-	+	Chain Terminator Applications
Therminator <sup>™</sup> II DNA Polymerase	-	+	Incorporation of Modified Substrates
<i>Bst</i> DNA Polymerase, Large Fragment	-	+++	Strand Displacement Applications
<b>Other Polymerases</b>			
M-MuLV Reverse Transcriptase	-	+++	cDNA Synthesis
AMV Reverse Transcriptase	-	+++	cDNA Synthesis
<i>E. coli</i> Poly(A) Polymerase	-	n/a	3' Labeling of RNA
phi6 RNA Polymerase	-	n/a	dsRNA Synthesis
T7 RNA Polymerase	-	n/a	High Yield RNA Production
SP6 Polymerase	-	n/a	RNA Production

## Proofreading

A 3'→5' proofreading exonuclease moiety is intrinsic to most DNA polymerases. It allows the enzyme to check each nucleotide during DNA synthesis, and excise mismatched nucleotides in the 3' to 5' direction. The proofreading domain also enables a polymerase to remove unpaired 3' overhanging nucleotides to create blunt ends. Protocols such as high-fidelity PCR, 3' overhang polishing and second strand synthesis require the presence of a 3'→5' exonuclease.

In contrast, some applications are enhanced by use of polymerases without proofreading activity. For example, the efficiency of DNA labeling is enhanced by the absence of proofreading because it prevents excise of incorporated bases, allowing for the use of less of the modified base.

Modified base incorporation assays such as multicolor analysis of gene expression, gene mapping, and in situ hybridization, which utilize DNA that has been labeled with a fluorescent nucleotide to facilitate detection, are well matched to NEB's exonuclease-deficient DNA polymerases. Non-proofreading polymerases are also indispensable when partially filling in 5' overhangs with only selected dNTPs. Addition of an untemplated residue at the 3' terminus of blunt ends, a requirement for TA cloning, is also promoted by non-proofreading enzymes. In addition to several wild type polymerases in each of these categories, NEB offers genetically altered versions of several proofreading polymerases where the proofreading exonuclease has been attenuated or abolished.

- New
- \* Degrades displaced strand.



## Thermostability

DNA is a dynamic molecule whose structure is stabilized by a large number of weak interactions. The stability of the DNA double helix depends on a variety of factors, including DNA sequence, pH, ionic strength, solvents and temperature. In particular, as the temperature is increased, the weak interactions are sequentially disrupted, first resulting in localized denaturation of the terminal and selected internal sequences, and finally in complete separation of DNA strands. The degree to which this destabilization is desired or tolerated depends on the application.

For example, cloning procedures such as end-polishing are maximized when the termini are stabilized, suggesting use of a polymerase derived from a mesophilic organism. Second strand cDNA synthesis and nick-translation are other applications that have traditionally used mesophilic enzymes. Such enzymes are maximally

active at temperatures of 25–40°C and retain significant activity at even lower temperatures. They commonly can be heat-inactivated and work in the same buffers used by restriction endonucleases and ligases, obviating the need for subsequent DNA purification.

A variety of other molecular biology applications such as PCR require high temperature to denature the DNA prior to primer annealing or during polymerization to reduce secondary structure, thus reducing polymerase pausing. Archaeal DNA polymerases such as Vent<sub>R</sub><sup>®</sup> and 9°N<sup>™</sup> are derived from hyperthermophiles and are extremely resistant to heat inactivation, even at 100°C, and display maximal polymerase activity at 75–85°C. Bacterial thermophiles have yielded enzymes such as *Taq* and *Bst* DNA polymerases, which have similar polymerization temperature optima, but somewhat reduced stability at 95°C when compared with archaeal counterparts.

## Strand Displacement

The term strand displacement describes the ability to displace downstream DNA encountered during synthesis. Protocols such as the isothermal amplification method Strand Displacement Amplification (SDA) exploit this activity. NEB produces DNA polymerases with varying degrees of strand displacement activity as well as a few whose ability to strand displace is temperature dependent. Polymerases lacking strand displacement activity are used in gap-filling reactions such as those employed in site-directed mutagenesis protocols.

In contrast to strand displacement, some polymerases degrade an encountered downstream strand via a 5'→3' exonuclease activity. This activity is employed for nick-translation protocols.

## Focus: *Taq* DNA Polymerase

*Taq* DNA Polymerase is the industry standard for routine PCR. NEB provides high quality recombinant *Taq* at an exceptional value. To accommodate a variety of PCR applications, *Taq* is available with different reaction buffers. Standard *Taq* Buffer is designed to support existing PCR platforms and is an ideal choice for DHPLC and high-throughput applications. ThermoPol Buffer is formulated to promote high product yields, even under demanding conditions.

### *Taq* Buffer Selection Chart

CHOICE OF BUFFER	MG-CONTROL	CATALOG #
<b>Standard <i>Taq</i> Reaction Buffer:</b> Detergent-free and designed to be compatible with existing assay systems	<i>Taq</i> with Standard <i>Taq</i> Buffer	#M0273
	<i>Taq</i> with Standard <i>Taq</i> (Mg-free) Buffer	#M0320
<b>ThermoPol Buffer:</b> Designed to optimize yields and specificity	<i>Taq</i> with ThermoPol Buffer	#M0267
	<i>Taq</i> with ThermoPol II (Mg-free) Buffer	#M0321
<b>Crimson <i>Taq</i> Reaction Buffer:</b> Contains crimson tracking dye, allowing samples to be loaded directly onto a gel	Crimson <i>Taq</i>	#M0324
	Crimson <i>Taq</i> with (Mg-free) Buffer	#M0325

### Advantages:

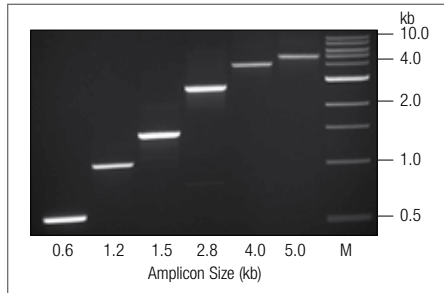
- **Value** - Industry standard for routine PCR at a low price
- **Versatility** - Will amplify a wide range of templates with minimal optimization
- **Flexibility** - Able to incorporate dUTP, dITP and fluorescently-labeled nucleotides
- **Choice** - Reaction buffers accommodate a variety of PCR applications
- **Convenience** - Master mixes, buffer choice, tracking dye and kits available



## Crimson *Taq* delivers additional convenience

Experience the robust and reliable performance of *Taq* DNA Polymerase in a more convenient format. Ideal for both routine and high throughput applications, Crimson *Taq* Reaction Buffer is optimized for robust amplification and contains tracking dye that allows samples to be loaded directly onto a gel. The Sampler contains sample sizes of Crimson *Taq* DNA Polymerase, dNTPs and the Quick-Load® 1 kb DNA Ladder at a value price.

### Amplification with Crimson *Taq*



Amplification of specific sequences from human genomic DNA using Crimson *Taq* DNA Polymerase. Amplicon sizes are indicated below gel. Marker M is NEB 1 kb DNA Ladder (NEB #N3232).

### Crimson *Taq* DNA Polymerase



Crimson *Taq* DNA Polymerase offers the convenience of loading samples directly onto a gel.

PRODUCT	NEB #	SIZE
Crimson <i>Taq</i> ™ DNA Polymerase	M0324S/L	200/1,000 units
Crimson <i>Taq</i> ™ DNA Polymerase with (Mg-free) Buffer	M0325S/L	200/1,000 units
Crimson LongAmp <i>Taq</i> ™ DNA Polymerase	M0326S/L	250/1,250 units
Crimson <i>Taq</i> ™ PCR Sampler	E0547S	40 rxns
Hemo KlenTaq	M0332S/L	200/1,000 rxns (25 µl vol)
LongAmp™ <i>Taq</i> 2X Master Mix	M0287S/L	100/500 rxns (50 µl vol)
LongAmp™ <i>Taq</i> DNA Polymerase	M0323S/L	500/2,500 units
LongAmp™ <i>Taq</i> PCR Kit	E5200S	100 rxns (50 µl vol)
QuickLoad® <i>Taq</i> 2X Master Mix	M0271S/L	100/500 rxns (50 µl vol)
<i>Taq</i> DNA Polymerase with Standard <i>Taq</i> Buffer	M0273S/L/X	400/2,000/4,000 units
<i>Taq</i> DNA Polymerase with Standard (Mg-free) Buffer	M0320S/L	400/2,000 units
<i>Taq</i> 2X Master Mix	M0270S/L	100/500 rxns (50 µl vol)
<i>Taq</i> 5X Master Mix	M0285S/L	100/500 reactions
<i>Taq</i> PCR Kit	E5000S	200 rxns
<i>Taq</i> PCR Kit with Controls	E5100S	200 rxns
<i>Bst</i> DNA Polymerase, Large Fragment	M0275S/L	1,600/8,000 units
<i>Bst</i> DNA Polymerase, Full Length	M0328S/L	500/2,500 units
<i>Bsu</i> DNA Polymerase, Large Fragment	M0330S/L	200/1,000 units
<i>Taq</i> DNA Polymerase with ThermoPol Buffer	M0267S/L/X	400/2,000/4,000 units
<i>Taq</i> DNA Polymerase with ThermoPol II (Mg-free) Buffer	M0320S/L	400/2,000 units
Vent <sub>r</sub> ® DNA Polymerase	M0254S/L	200/1,000 units
Vent <sub>r</sub> ® (exo-) DNA Polymerase	M0257S/L	200/1,000 units
Deep Vent <sub>r</sub> ™ DNA Polymerase	M0258S/L	200/1,000 units
Deep Vent <sub>r</sub> ™ (exo-) DNA Polymerase	M0259S/L	200/1,000 units
9°N <sub>m</sub> DNA Polymerase	M0260S/L	200/1,000 units



PRODUCT	NEB #	SIZE
<i>Sulfolobus</i> DNA Polymerase IV	M0327S/L	100/500 units
Therminator™ DNA Polymerase	M0261S/L	200/1,000 units
Therminator™ II DNA Polymerase	M0266S/L	200/1,000 units
Therminator™ III DNA Polymerase	M0333S/L	200/1,000 units
Therminator™ $\gamma$ DNA Polymerase	M0334S/L	200/1,000 units
<b>Mesophilic Polymerases</b>		
phi29 DNA Polymerase	M0269S/L	250/1,250 units
T7 DNA Polymerase (unmodified)	M0274S/L	300/1,500 units
DNA Polymerase I ( <i>E. coli</i> )	M0209S/L	500/2,500 units
DNA Polymerase I, (Klenow) Lg Frag	M0210S/L/M	200/1,000/ 1,000 (5x conc) units
Klenow Fragment (3' $\rightarrow$ 5' exo-)	M0212S/L/M	200/1,000/ 1,000 (5x conc) units
T4 DNA Polymerase	M0203S/L	150/750 units
Terminal Transferase	M0315S/L	500/2,500 units
<b>Other Polymerases</b>		
M-MuLV Reverse Transcriptase	M0253S/L	10,000/50,000 units
T7 RNA Polymerase	M0251S/L	5,000/25,000 units
SP6 RNA Polymerase	M0207S/L	2,000/10,000 units
AMV Reverse Transcriptase	M0277S/L	200/1,000 units
<i>E. coli</i> Poly(A) Polymerase	M0276S/L	100/500 units
Poly(U) DNA Polymerase	M0337S	60 units
phi6 RNA Polymerase (RdRP)	M0255S/L	60/300 units