

# Polymerase Overview

New England Biolabs, Inc. offers a wide range of DNA polymerases and through our commitment to research, ensures the development of innovative, high quality tools for PCR. Our product quality, enzyme expertise and outstanding technical support bring unparalleled confidence to your PCR experiments.

When choosing a polymerase for PCR, we recommend starting with One Taq® or Q5® DNA Polymerases (shown below in gold). Both offer robust amplification and can be used on a wide range of templates (routine, AT- and GC-rich). Q5 provides the benefit of maximum fidelity, and is also available in a formulation specifically optimized for next generation sequencing.

### PCR Polymerase Selection Chart

### ★ indicates recommended choice for application

3 1/1	STAND	IDARD PCR HIGH-FIDELITY PCR		R	SPECIALTY PCR			
100				HEST ELITY	MODERATE Fidelity	LONG Amplicons	BISULFITE SEQUENCING	BLOOD DIRECT PCR
Vi and	ONE <i>Taq®/</i> ONE <i>Taq</i> HOT START	<i>Taq/</i> HOT START <i>Taq</i>	Q5°/Q5 Hot Start	PHUSION <sup>©(1)</sup> / PHUSION <sup>(1)</sup> FLEX	VENT®/ DEEP VENT™	LONGAMP®/ Longamp Hot Start <i>Taq</i>	EPIMARK <sup>®</sup> Hot Start <i>Taq</i>	HEMO KLENTaq®
PROPERTIES								
Fidelity vs. <i>Taq</i>	2X	1X	> 100X	> 50X	5-6X	2X	1X	ND
Amplicon Size	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	≤ 1 kb	≤ 2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1,2 kb/min	1 kb/min	0,5 kb/min
Resulting Ends	3´ A/Blunt	3´ A	Blunt	Blunt	Blunt	3´ A/Blunt	3´ A	3´ A
3'→5' exo	Yes	No	Yes	Yes	Yes	Yes	No	No
5'→3' exo	Yes	Yes	No	No	No	Yes	Yes	No
Units/50 µl Reaction	1,25	1,25	1,0	1,0	0,5-1,0	5,0	1,25	N/A
Annealing Temperature	Tm-5	Tm-5	Tm+3	Tm+3	Tm-5	Tm-5	Tm-5	Tm-5
APPLICATIONS								
Routine PCR	*	•	•	•	•	•		
Colony PCR	*	•						
Enhanced Fidelity	•		*	•	•	•		
High Fidelity			*	•				
High Yield	*	•	*	•				
Fast			*	•				
Long Amplicon			*	•		*		
GC-rich Targets	*		*		•	•		
AT-rich Targets	*	•	*	•		•	*	
High Throughput	•	•	•	•			•	
Multiplex PCR	•	★(2)	•	•				
Extraction-free PCR								*
DNA Labeling		*						
Site-directed Mutagenesis			*	•				
Bisulfite Sequencing							*	
NGS APPLICATIONS								
NGS Library Amplification	•		★(3)	•				
FORMATS								
Hot Start Available	•	•	•	•		•	•	
Kit		•	•	•		•		
Master Mix Available	•	•	•	•		•		
Direct Gel Loading		•						

Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

<sup>(3)</sup> Use NEBNext Hot Start HiFi PCR Master Mix.



# Why is Polymerase Fidelity Important?

# What is fidelity?

The fidelity of a DNA polymerase refers to its ability to accurately replicate a template. A critical aspect of this is the ability of the DNA polymerase to read a template strand, select the appropriate nucleoside triphosphate and insert the correct nucleotide at the 3′ primer terminus, such that canonical Watson-Crick base pairing is maintained. The rate of misincorporation is known as the polymerase's error rate. In addition to effective discrimination for correct over incorrect nucleotide incorporation, some DNA polymerases possess a 3′→5′ exonuclease activity. This activity, also termed proofreading, is used to excise incorrectly incorporated mononucleotides that are then replaced with the correct nucleotide. High-fidelity PCR utilizes DNA polymerases that couple low misincorporation rates with proofreading activity to give faithful replication of the DNA target of interest.

### For what applications is fidelity important?

Fidelity is important for applications in which the DNA sequence must be correct after amplification, including:

- Cloning/subcloning from in vitro amplified material (PCR, WGA, etc) for protein expression or gene studies
- SNP analysis by cloning and sequencing
- RNA analysis by RT-PCR
- · Applications that involve sequencing of in vitro amplified material

Fidelity is less important if the PCR amplified product is directly sequenced by Sanger sequencing (without an intervening cloning step), where the signal is an average of the input amplicons. Fidelity is also less important for diagnostic applications in which sequencing is not required after amplification, and the read-out is the presence or absence of a product. It is more important for next generation and single molecule sequencing techniques.

# How does a high-fidelity polymerase ensure that the correct base is inserted?

High-fidelity DNA polymerases have several checkpoints to protect against making and propagating mistakes while copying DNA.

- High-fidelity polymerases have a significant binding preference for the correct versus the incorrect nucleotide triphosphate during polymerization.
- If an incorrect nucleotide does bind in the polymerase active site, incorporation is slowed due to the sub-optimal architecture of the active site complex. This time increases the opportunity for the incorrect nucleotide to dissociate before incorporation, thereby allowing the process to start again (and for a correct nucleotide triphosphate to bind) (1,2).
- If an incorrect nucleotide is inserted, proofreading DNA polymerases have an extra line of defense. They can "sense" the perturbation caused by the mispaired bases and move the 3′ end of the growing DNA chain into a proofreading 3′→5′ exonuclease domain. There, the incorrect nucleotide is removed by the 3′→5′ exonuclease activity before the chain is moved back into the polymerase domain, where polymerization can continue with the correct nucleotide.

### **TOOLS & RESOURCES**

Visit www.neb.com/tools-and-resources/ tutorials to find the latest PCR videos from NEB Scientists, including:

- · Choosing the right polymerase for your PCR
- How to amplify GC-rich DNA
- · Why choose Q5 High-Fidelity DNA Polymerase
- Important tips for Q5 High-Fidelity DNA Polymerase
- · Tips for amplifying large amplicons
- · Amplification of GC-rich regions
- · Tips for setting up PCR
- Types of PCR
- · Why is Tm important?



### References:

- Johnson, K. A. (2010) Biochim. et. Biophys. Acta, 1804, 1041–1048
- Joyce, C. M. and Bencovic, S. (2004) *Biochemistry*, 43, 14317–14324.



# High-Fidelity DNA Polymerase

# Hot Start High-Fidelity DNA Polymerase

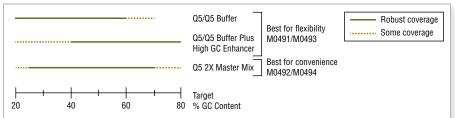
Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (> 100X higher than Taq and 2X higher than Thermo Scientific® Phusion®), Q5 DNA Polymerase results in ultra-low error rates. Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability of performance.

The Q5 buffer system is designed to provide superior performance with minimal optimization across a broad range of amplicons, regardless of GC content. For routine or complex amplicons up to ~65% GC content, Q5 Reaction Buffer provides reliable and robust amplification. For amplicons with high GC content (> 65% GC), addition of the Q5 High GC Enhancer ensures continued maximum performance. Q5 DNA Polymerase is available as a standalone enzyme, in hot start, master mix or kit format. Master mix formulations include dNTPs, Mg++ and all necessary buffer components. The kit contains Q5 High-Fidelity 2X Master Mix, nuclease-free water, gel loading dye and the Quick-Load® 2-log DNA Ladder.

### Also available: Q5 High-Fidelity DNA Polymerase optimized for NGS applications. Visit NEBNext.com for details.

Q5 High-Fidelity DNA Polymerase	. M0491S/L
Q5 High-Fidelity 2X Master Mix	. M0492S/L
Q5 Hot Start High-Fidelity DNA Polymerase	. M0493S/L
Q5 Hot Start High-Fidelity 2X Master Mix	. M0494S/L
Q5 High-Fidelity PCR Kit	E0555S/L
NEBNext® Q5 Hot Start HiFi PCR Master Mix	M0543S/L
NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L
NEBNext Ultra™ II Q5 Master Mix	M0544S/L

### Q5 DNA Polymerases offer exceptional coverage over the entire range of GC composition



The stand-alone enzyme comes with a reaction buffer that supports robust amplification of high AT to routine targets. Addition of the High GC Enhancer allows amplification of GC rich and difficult targets. For added convenience, the master mix formats allow robust amplification of a broad range of targets with a single formulation.

### **POLYMERASE DETAILS**

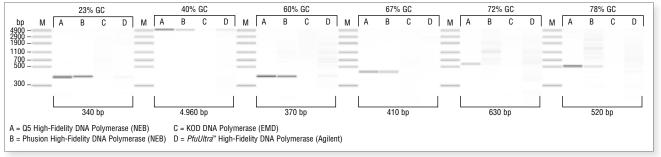
EXTENSION Mate
Amplicon Size ≤ 20 kb
Fidelity
Units/50 µl rxn
Resulting Ends
3´→5´ Exonuclease Activity Yes
5´→3´ Exonuclease Activity No
Supplied Buffer Q5 Rxn Buffer
Supplied Enhancer Q5 High
GC Enhancer
Compatible w/Other Buffers with Reduced
Activity Profile

Product Formats	
Hot Start Available Yes	S
- Activation Required	0
Master Mix Available Yes	S
PCR Kit Available Yes	S
NGS Version Available Yes	S
Applications	
High-Fidelity PCR Yes	S
Difficult PCR Yes	S
High GC PCR Yes	S



Learn how Q5 can be used in multiplex PCR in our application note at Q5PCR.com

Q5 DNA Polymerase offers superior amplification for a wide range of templates, even with high GC content



Amplification of a variety of human genomic amplicons from low to high GC content demonstrates the broad performance of Q5 High-Fidelity DNA Polymerase. All reactions were conducted using 20 ng of input template and included 30 cycles of amplification. Results were visualized by microfluidic LabChip® analysis. Competitor polymerases were cycled according to manufacturer's recommendations. For the final three amplicons, GC Buffers or enhancers were used when supplied with the polymerase.

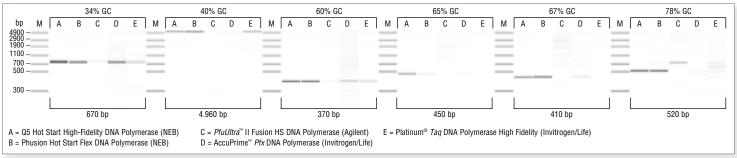
### Q5 Hot Start High-Fidelity DNA Polymerase

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.



High-fidelity polymerases benefit from a Tm<sup>+</sup>3 annealing temperature. Use the NEB Tm Calculator to ensure successful PCR at TmCalculator.neb.com.

Q5 Hot Start DNA Polymerase offers superior amplification for a wide range of templates, even with high GC content



Amplification of a variety of human genomic amplicons from low to high GC content demonstrates the broad performance of Q5 Hot Start High-Fidelity DNA Polymerase. All reactions were set up at room temperature using 20 ng of input template and included 30 amplification cycles. Results were visualized by microfluidic LabChip analysis. Competitor polymerases were cycled according to manufacturer's recommendations. For the final three amplicons, GC Buffers or enhancers were used when provided with the polymerase.

### Comparison of high-fidelity polymerases

PRODUCT NAME (Supplier)	POLYMERASE FIDELITY (Reported by supplier)	MAXIMUM AMPLICON LENGTH <sup>6</sup>	EXTENSION TIME <sup>6</sup> (For simple templates <sup>5</sup> )	EXTENSION TIME <sup>6</sup> (For complex templates <sup>5</sup> )
Q5 High-Fidelity DNA Polymerase (NEB)	> 100X Taq <sup>1,2</sup>	20 kb simple; 10 kb complex	10 s/kb	10 s/kb (< 1 kb) 20–30 s/kb (> 1 kb)
Phusion High-Fidelity DNA Polymerase* (NEB)	> 50X Taq <sup>1,2</sup>	20 kb simple; 10 kb complex	15 s/kb	30 s/kb
AccuPrime™ <i>Pfx</i> (Life)	26X <i>Taq</i> <sup>1</sup>	12 kb <sup>4</sup>	60 s/kb⁴	
PfuUltra™ II Fusion HS (Agilent)	20X <i>Taq</i> <sup>1</sup>	19 kb <sup>4</sup>	15 s/kb (< 10 kb <sup>4</sup> ) 30 s/kb (> 10 kb <sup>4</sup> )	
<i>PfuUltra</i> High-Fidelity DNA Polymerase (Agilent)	19X <i>Taq</i> <sup>1</sup>	17 kb simple; 6 kb complex	60 s/kb (< 10 kb) 120 s/kb (> 10 kb)	60 s/kb (< 6 kb) 120 s/kb (> 6 kb)
Platinum <i>Taq</i> HiFi (Life)	6X Taq 1	20 kb <sup>4</sup>	60 s/kb <sup>4</sup>	
KOD DNA Polymerase (EMD)	4X Taq³	6 kb simple; 2 kb complex	10-20 s/kb	30–60 s/kb

PCR-based mutation screening in lacZ (NEB), lacl (Agilent) or rpsL (Life)

4 Template not specified.

<sup>&</sup>lt;sup>2</sup>Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is difficult to measure in a statistically significant manner. Although measurements from assays done side-by-side with *Taq* yield Q5 fidelity values from 100–200X *Taq*, we report ">-100X *Taq* as a conservative value.

 $<sup>^{\</sup>star}$  Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

<sup>&</sup>lt;sup>3</sup> Takagi et. al. (1997) Appl. Env. Microbiol. 63, 4504-4510.

<sup>5</sup> Simple templates include plasmid, viral and E. coli genomic DNA. Complex templates include plant, human and other mammalian genomic DNA.

<sup>6</sup> Values provided by individual manufacturers.



# One Taq® DNA Polymerase One Taq Hot Start DNA Polymerase

An optimized blend of *Taq* and Deep Vent<sub>R</sub> 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*. Additionally, One *Taq* Reaction Buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

One Taq DNA Polymerase is supplied with two 5X buffers (Standard and GC), as well as a High GC Enhancer solution. For most routine, AT- rich or complex amplicons with up to  $\sim$ 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 (> 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.

For direct and fast agarose gel-loading after routine-PCRs such as genotyping or colony-PCR etc., One *Taq* DNA Polymerase is also available in a Quick-Load format. It is supplied with a densitiy and tracking dye containing 5x One *Taq* Quick-Load Reaction Buffer for direct gel loading in addition to the regular "color-less" 5x One *Taq* Reaction Buffer.

### Master Mix Formulations

In addition to standalone enzymes, both One *Taq* and One *Taq* Hot Start DNA Polymerases are available in master mix and Quick-Load master mix formats. Master mix formulations include dNTPs, MgCl<sub>2</sub> and other buffers and stabilizers. The Quick-Load master mix formulations also include two tracking dyes for use with downstream visualization (i.e., agarose gels). With these convenient formats, the addition of primers and template are all that is required for robust amplification.

One Taq DNA Polymerase	M0480S/L/X
One Taq Quick-Load DNA Polymerase	M0509S/L/X
One Taq 2X Master Mix with Standard Buffer	M0482S/L
One Taq 2X Master Mix with GC Buffer	M0483S/L
One Taq Quick-Load 2X Master Mix with Standard Buffer	M0486S/L
One Taq Quick-Load 2X Master Mix with GC Buffer	M0487S/L
One Taq Quick-Load 2X Master Mix with GC Buffer	M0487S/L

### One Taq Buffer Recommendations

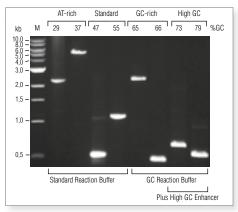
AMPLICON % GC BUFFER	RECOMMENDED DEFAULT BUFFER	OPTIMIZATION NOTES
< 50% GC	One <i>Taq</i> Standard Reaction Buffer	Adjust annealing temperature, primer/ template concentration, etc. if needed.
50–65% GC	One <i>Taq</i> Standard Reaction Buffer	One <i>Taq</i> GC Reaction Buffer can be used to enhance performance of difficult amplicons.
> 65% GC	One Taq GC Reaction Buffer	One <i>Taq</i> GC Reaction Buffer with 10–20% One <i>Taq</i> High GC Enhancer can be used to enhance performance of difficult amplicons.

### **POLYMERASE DETAILS** Amplicon Size $\ldots \le 6 \text{ kb}$ 3´→5´ Exonuclease Activity . . . . . Yes 5´→3´ Exonuclease Activity . . . . . Yes Supplied Buffer . . . . . . One Taq Std Rxn Buffer, One Tag GC Rxn Buffer\* Supplied Enhancer . . . . One Taq High GC Enhancer\* Compatible w/Other Buffers . . . . . . with Reduced Activity Profile **Product Formats** Hot Start Available . . . . . . . . Yes - Activation Required . . . . . . . . . . . . No Master Mix Available . . . . . . . . . Yes Direct Gel-loading Available. . . . . . . . Yes PCR Kit Available . . . . . . . . . . . . . . . . . No **Applications** Routine PCR . . . . . Yes SNP Detection . . . . . . . . . Yes T/A, U/A Cloning . . . . . Yes Colony PCR. . . . . . Yes \*not supplied with OneTaq Quick-Load DNA Polymerase



Visit www.neb-online.de/onetaq for more information.

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).



### One Taq Hot Start DNA Polymerase allows room temperature reaction setup with no separate activation step

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's One Taq Hot Start utilizes aptamer technology. This aptamer/inhibitor binds to the polymerase through non-covalent interactions, blocking polymerase activity at temperatures below 45°C. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. One Taq Hot Start DNA Polymerase does not require a separate high temperature incubation step to activate the enzyme and can be used in typical Taq-based cycling protocols. This ultimately shortens reaction times and increases ease of use.

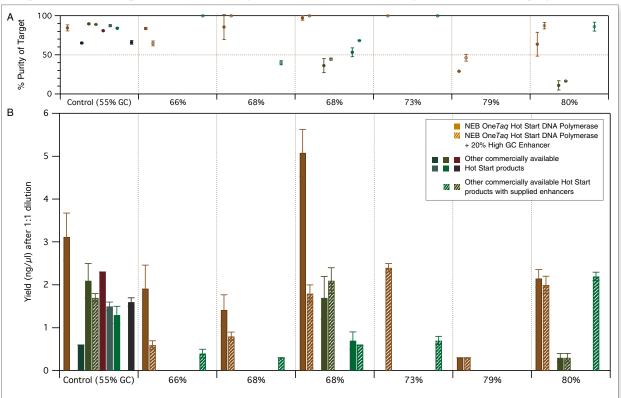
One Taq Hot Start DNA Polymerase	M0481S/L/X
One Taq Hot Start 2X Master Mix with Standard Buffer	M0484S/L
One Taq Hot Start 2X Master Mix with GC Buffer	M0485S/L
One Taq Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S/L
One Tag Hot Start Quick-Load 2X Master Mix with GC Buffer	M0489S/L



To learn how One Tag can be used in colony PCR, download the application note at

www.neb-online.de/onetaq

Comparison of One Taq Hot Start DNA Polymerase to other commercially available hot start polymerases.



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. OneTag polymerases were used with GC Buffer. Some OneTag reactions also contained High GC Enhancer (striped bars). Competitor polymerases were cycled according to manufacturer's recommendations and included GC enhancers when supplied (striped bars).

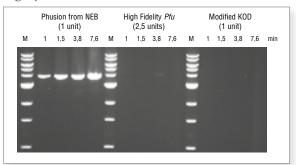


# Phusion® High-Fidelity DNA Polymerase

DNA polymerases with high fidelity are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific® Phusion High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel *Pyrococcus*-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Product selection includes a standalone enzyme, master mix and kit format, as well as a choice of reaction buffers for amplification of difficult templates. Phusion Hot Start Flex DNA Polymerase is available as standalone enzyme or in a master mix format and enables high specificity amplification of a broad range of templates with the flexibility of room temperature setup.

Phusion High-Fidelity DNA Polymerase	M0530S/L
Phusion High-Fidelity PCR Kit	E0553S/L
Phusion High-Fidelity PCR Master Mix with HF Buffer	M0531S/L
Phusion High-Fidelity PCR Master Mix with GC Buffer	M0532S/L
Phusion Hot Start Flex	M0535S/L
Phusion Hot Start Flex 2X Master Mix	M0536S/L

# Phusion DNA Polymerase generates amplicons with high yield and much shorter extension times



A 3,8 kb fragment was amplified from 50 ng of Jurkat gDNA using different polymerases. Reactions were carried out according to the manufacturer's recommended conditions. Extension times are indicated (in minutes). Ladder M is a 1 kb DNA Ladder (NEB #N3232).

### Phusion Buffer Selection Chart

CHOICE OF BUFFER	APPLICATION	NEB #
Phusion HF Buffer Pack	Default buffer for high-fidelity amplification	B0518S
Phusion GC Buffer Pack	For long, difficult or GC-rich templates (when HF buffer fails)	B0519S

Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

POLYMERASE DETAILS	
Extension Rate  Amplicon Size  Fidelity  Units/50 µl rxn  Resulting Ends  3'→5' Exonuclease Activity  5'→3' Exonuclease Activity  Supplied Buffer  Supplied Enhancer  Compatible w/Other Buffers	≤ 20 kb> 50X <i>Taq</i> 1 unitsBluntYesNo 5X Phusion HF Buffer, 5X Phusion GC Buffer100% DMSO
Product Formats	
Hot Start Available	No Yes
Applications	
High-Fidelity PCR T/A, U/A Cloning Colony PCR. Blunt Cloning	No No



High-fidelity polymerases benefit from a Tm<sup>+</sup>3 annealing temperature. Use the NEB Tm Calculator to ensure successful PCR at TmCalculator.neb.com.

# Vent<sub>R</sub>® DNA Polymerase

Vent<sub>R</sub> DNA Polymerase is a recombinant, high-fidelity thermophilic DNA polymerase with the lowest cost per reaction of any moderate-fidelity PCR polymerase. It has an error rate 5-fold lower than Taq DNA Polymerase, a characteristic derived in part from an intrinsic  $3' \rightarrow 5'$  proofreading exonuclease. In addition, greater than 90% of the polymerase activity remains following a 1 hour incubation at 95°C, ensuring maximal activity over the course of the PCR reaction. For enhanced-fidelity amplification of routine targets,  $Vent_{R}$  DNA Polymerase offers exceptional value.

 $Vent_R$  (exo<sup>-</sup>) DNA Polymerase has been genetically engineered to eliminate the 3' $\rightarrow$ 5' proofreading exonuclease activity resulting in higher yield PCR.

Vent <sub>R</sub> DNA Polymerase	10254S/L
Vent, (exo <sup>-</sup> ) DNA Polymerase	10257S/L

# Extension Rate 1 kb/min Amplicon Size ≤ 6 kb Fidelity 5X Taq Resulting Ends Blunt 3´→5´ Exonuclease Activity Yes (M0254) 5´→3´ Exonuclease Activity No Supplied Buffer ThermoPol® Rxn Buffer Compatible w/Other Buffers No Applications Blunt Cloning Yes Enhanced Thermostability Yes

# 

Deep  $\operatorname{Vent}_R$  DNA Polymerase is a recombinant, moderate-fidelity DNA polymerase with unsurpassed thermostability. This feature makes Deep  $\operatorname{Vent}_R$  an ideal choice for PCR amplification of DNA targets with a high degree of secondary structure, even in the absence of additives. It has an error rate 6-fold lower than  $\operatorname{Taq}$  DNA Polymerase, a characteristic derived in part from an integral  $3' \rightarrow 5'$  proofreading exonuclease. Deep  $\operatorname{Vent}_R$ 's combination of extreme thermostability and moderate-fidelity make it an excellent choice for accurate PCR amplification of GC-rich sequences or templates with secondary structures.

Deep Vent<sub>R</sub> (exo<sup>-</sup>) DNA Polymerase has been genetically engineered to eliminate the  $3' \rightarrow 5'$  proofreading exonuclease activity resulting in higher yield PCR.

Deep Vent <sub>R</sub> DNA Polymerase	M0258S/L
Deep Vent <sub>R</sub> (exo <sup>-</sup> ) DNA Polymerase	M0259S/L

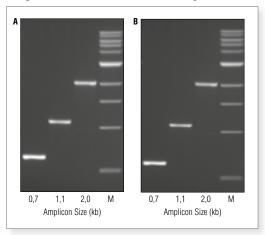
### **POLYMERASE DETAILS**

Extension Rate
Amplicon Size $\leq$ 6 kb
Fidelity
Resulting Ends
3´→5´ Exonuclease Activity Yes (M0258)
5´→3´ Exonuclease Activity No
Supplied Buffer ThermoPol Rxn Buffer
Compatible w/Other Buffers No

### **Applications**

Blunt Cloning								Yes
Enhanced Thermostability								Yes

### Amplification with Vent and Deep Vent DNA Polymerases



Amplification of Jurkat Genomic DNA with Vent<sub>R</sub> (A) and Deep Vent<sub>R</sub> (B) DNA Polymerases. Amplicon sizes are indicated below gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).



# *Taq* DNA Polymerase

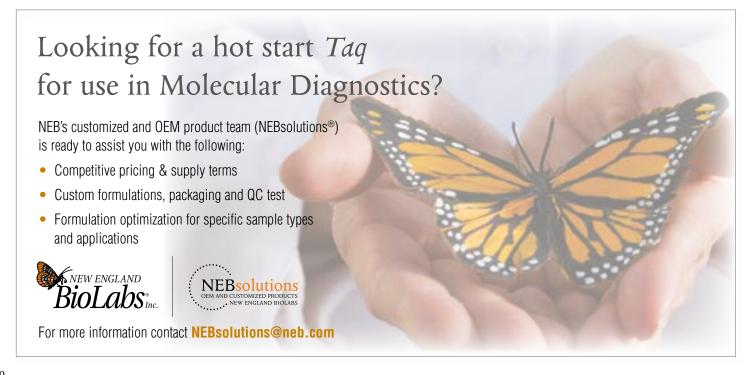
For routine amplification, where cost per reaction and yield are the priorities, *Taq* DNA Polymerase is the industry standard. NEB provides high quality recombinant *Taq* at an exceptional value. To accommodate a variety of PCR applications, *Taq* is available with different reaction buffers. Standard *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 DNA Polymerase with Standard Taq Buffer	M0273S/L/X
Taq DNA Polymerase with Standard Taq (Mg-free) Buffer	M0320S/L
Taq DNA Polymerase with ThermoPol Buffer	M0267S/L/X/E
Taq PCR Kit	E5000S
Taq PCR Kit with Controls	E5100S
Taq 5X Master Mix	M0285L
Taq 2X Master Mix	M0270L
Quick-Load Taq 2X Master Mix	M0271L
Hot Start Taq DNA Polymerase	M0495S/L
Hot Start Taq 2X Master Mix	M0496S/L

### Taq Buffer Selection Chart

CHOICE OF BUFFER	MG-CONTROL	NEB #
Standard <i>Taq</i> Reaction Buffer: Detergent-free and designed to be	<i>Taq</i> with Standard <i>Taq</i> Buffer	M0273S/L/X
compatible with existing assay systems	Taq with Standard Taq (Mg-free) Buffer	M0320S/L
ThermoPol Buffer: Designed to optimize yields and specificity	Taq with ThermoPol Buffer	M0267S/L/X/E

### **POLYMERASE DETAILS** Amplicon Size . . . . . . . . . $\leq$ 5 kb 3´→5´ Exonuclease Activity . . . . . . . . No 5´→3´ Exonuclease Activity . . . . . Yes Supplied Buffer . . . . . Standard Taq Rxn Buffer, or ThermoPol Rxn Buffer Compatible w/Other *Taq* Buffers . . . . . . . . . Yes **Product Formats** Hot Start Available . . . . . . . Yes - Activation Required . . . . . . . . . . . . No Direct Gel-loading Available. . . . . . Yes PCR Kit Available . . . . . . . . . Yes **Applications** Routine PCR . . . . . . . . . Yes T/A, U/A Cloning . . . . . . . . Yes Colony PCR. . . . . . Yes



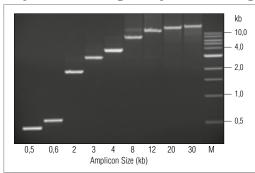


### LongAmp® Taq enables extension of longer amplicons

An optimized blend of Taq and Deep  $Vent_R$  DNA Polymerases, LongAmp Taq DNA Polymerase enables amplification of up to 30 kb PCR products with a fidelity higher than Taq DNA Polymerase alone.

LongAmp Taq DNA Polymerase	M0323S/L
LongAmp Taq PCR Kit	E5200S
LongAmp Taq 2X Master Mix	M0287S/L
LongAmp Hot Start Taq DNA Polymerase	M0534S/L
LongAmp Hot Start Taq 2X Master Mix	M0533S/L

### Amplification of longer templates with LongAmp Taq



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

### **POLYMERASE DETAILS**

Extension Rate 1,2 kb/min
Amplicon Size $\leq$ 30 kb
Fidelity
Units/50 $\mu$ l rxn 5 units
Resulting Ends 3´ A/Blunt
3´→5´ Exonuclease Activity Yes
5´→3´ Exonuclease Activity Yes
Supplied Buffer LongAmp or <i>Taq</i> Rxn Buffer
Compatible w/Other <i>Taq</i> Buffers with Reduced
Activity Profile

### **Product Formats**

Hot Start Available	Yes
- Activation Required	. No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes

### **Applications**

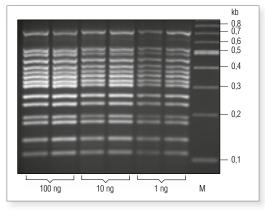
Long Amplicons.											Yes
Routine PCR											Yes
T/A, U/A Cloning											Yes
Colony PCR											Yes

### Multiplex PCR 5X Master Mix for multiple templates

Multiplex PCR can simultaneously detect two or more products in a single reaction. Multiplex PCR can also be used for semi-quantitative gene expression analysis using cDNA templates. The NEB Multiplex PCR 5X Master Mix is an easy-to-use solution featuring high quality recombinant *Taq* DNA Polymerase. The mix is optimized for high yield and performance. Its performance is illustrated below in a 15-plex PCR reaction using human genomic DNA. The 5X formulation allows maximal flexibility for input of custom primers and template DNAs.

Multiplex PCR 5X Master Mix M0284S

### 15-plex PCR reaction



15-plex PCR using varying amounts of human genomic DNA. 1X Multiplex PCR 5X Master Mix was used with 0,15 µM of each primer. The cycling conditions were 95°C for 1 minute, 35 cycles of 95°C for 20 seconds, 60°C for 1 minute and 68°C for 2 minutes. Marker M is the 2-Log DNA Ladder (NEB #N3200).

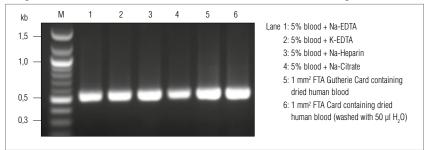


### Hemo KlenTaq® for PCR from blood

Hemo KlenTaq is a truncated version of Taq DNA Polymerase that contains mutations, making it resistant to inhibitors present in whole blood. Hemo KlenTaq offers the versatility of Taq and can successfully amplify samples containing up to 20% whole blood from human and mouse sources in a 50  $\mu$ l reaction volume.

Hemo KlenTaq M0332S/L

Amplification from human whole blood with Hemo Klen Taq



Percent blood present in sample and anticoagulant used are indicated in the legend. Ladder M is the 2-Log DNA Ladder (NEB #N3200).

# EpiMark® Hot Start *Taq* DNA Polymerase for bisulfite sequencing

EpiMark Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. This inhibitor binds reversibly to the enzyme, inhibiting polymerase activity below 45°C, but releases the enzyme during normal PCR cycling conditions. With a reaction buffer that has been optimized for AT-rich templates, EpiMark Hot Start *Taq* is an excellent choice for bisulfite-treated DNA.

EpiMark Hot Start Taq DNA Polymerase M0490S/L

### **POLYMERASE DETAILS**

Extension Rate 0,5 kb/min
Amplicon Size $\leq$ 2 kb
Units/50 $\mu$ l rxn 4 units
Resulting Ends
$3' \rightarrow 5'$ Exonuclease Activity No
5´→3´ Exonuclease Activity No
Supplied Buffer Hemo Klen <i>Taq</i> Rxn Buffer
Compatible w/Other Buffers Yes

### **Applications**

**	
Extraction-free PCR	Ye
T/A, U/A Cloning	Ye
Cloning PCR	Ye

### **POLYMERASE DETAILS**

Extension Rate
Amplicon Size $\leq 1$ kb
Units/50 µl rxn
Resulting Ends 3´A
3´→5´ Exonuclease Activity No
5´→3´ Exonuclease Activity Yes
Supplied Buffer Epimark Hot Start  Taq Rxn Buffer
Compatible w/Other <i>Taq</i> Buffers with Reduced Activity Profile

### **Product Formats**

Hot Start Available								Yes
- Activation Required .								. No
Applications								

# A/T Rich Targets. Yes Bisulfite-converted DNA Yes Routine PCR Yes T/A, U/A Cloning Yes

# PreCR® Repair Mix

The PreCR Repair Mix is a cocktail of enzymes formulated to repair damaged DNA *in vitro* prior to PCR. The repair pre-treatment can be applied to techniques such as whole genome amplification, DNA sequencing and microarray analysis.

The PreCR Repair Mix can repair a wide range of damaged DNA, resulting from exposure to heat, low pH, oxygen, and/or UV light. The lesions repaired by the PreCR Repair Mix do not include all possible types of damage. For example, it cannot repair DNA crosslinks, such as those that occur during exposure to formalin, nor can the mix effectively repair highly fragmented DNA.

PreCR Repair Mix M0309S/L

### **ADVANTAGES**

- Specific Treats damaged DNA without harming template
- Versatile Can be used in conjunction with any thermophilic polymerase
- Convenient PCR can be done directly on repair reaction
- Flexible Suitable for PCR, microarrays and other DNA technologies



# **Nucleotide Solutions**

### Deoxynucleotide (dNTP) Solution Set

The Deoxynucleotide Solution Set contains four separate 100 mM solutions of ultrapure nucleotides (dATP, dCTP, dCTP, and dTTP).

Deoxynucleotide Solution Set N0446S

### Deoxynucleotide (dNTP) Solution Mix

The Deoxynucleotide Solution Mix is an equimolar mixture of ultrapure dATP, dCTP, dGTP, and dTTP. Each nucleotide is present at a concentration of 10 mM in the mixture for a total dNTP concentration of 40 mM.

Deoxynucleotide Solution Mix N0447S/L

### 7-deaza-dGTP\*

A useful additive for PCR of GC-rich templates; contains a 5 mM solution of 7-deaza-GTP as a dilithium salt.

\* licensed from Roche Diagnostics GmbH

7-deaza-dGTP\* N0445S/L

### Acyclonucleotide Set

Acyclonucleotide Set contains four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP).

Acyclonucleotide Set N0460S

### dATP Solution

Contains 0,25 ml of 100 mM ultrapure dATP.

dATP Solution N0440S

### Ribonucleotide Solution Set

Ribonucleotide Solution Set consists of four separate 100 mM solutions of ATP, GTP, CTP and UTP.

Ribonucleotide Solution Set N0450S/L

### Ribonucleotide Solution Mix

The Ribonucleotide Solution Mix is an equimolar mixture of ribonucleotide triphosphates (rATP, rCTP, rGTP and rUTP). Each is supplied at a concentration of 80 mM for a total concentration of 320 mM.

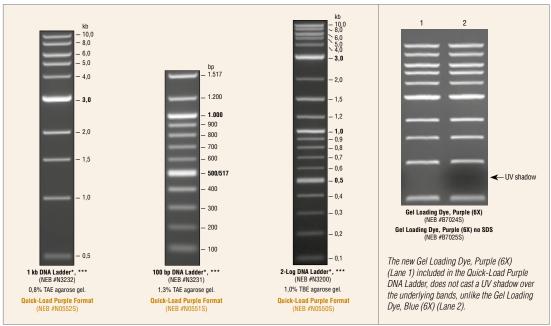
Ribonucleotide Solution Mix N0466S/L



# **DNA** Analysis

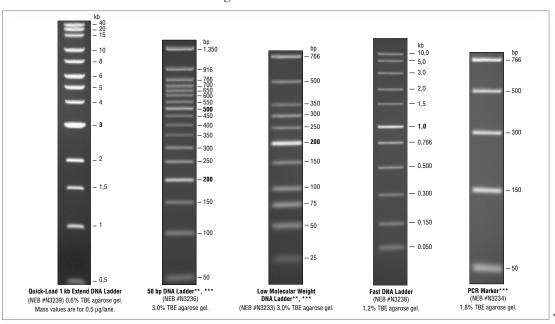
Agarose- or polyacrylamide-gel electrophoresis is the standard method used for separation, identification and purification of DNA fragments. DNA is visualized on a gel after soaking or pre-impregnating the gel with ethidium bromide, a DNA intercalating agent that fluoresces under UV illumination. Using the marker or ladder as a reference, it is possible to determine the size and relative quantity of the DNA of interest. The original DNA markers were made of genomic DNAs digested with a restriction enzyme to exhibit a banding pattern of known fragment sizes. Later, markers were made of fragments with evenly-spaced sizes and the resulting banding pattern resembles a ladder. The bands are visible under UV illumination; since the bands of the marker/ladder are not visible under normal lighting conditions. To track the progress of the gel as it runs, the marker contains a dye or combination of dyes that identify the leading edge of well contents, also called the dye front.

### The following DNA Ladders are Now Available in Quick-Load Purple Format



- \* Available in Quick-Load and TriDye™ formats
- \*\*\* Free Loading Dye included

### Additional DNA Ladders from New England Biolabs



- \*\* Available in Quick-Load format
- \*\*\* Free Loading Dye included

# 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, **international.neb.com**.

Continuity polymentals	PROBLEM	POSSIBLE CAUSE	SOLUTION						
Decision of Marcalization Intolliances   Decision and American Intolliances   Pergane Intolliances Intolliances   Pergane Intolliances		Low fidelity polymerase							
Personal Procession of the Control Process		Suboptimal reaction conditions	·						
Prompiate DNA has been damaged   "Tyrequerine DNA seroption with the PPDOP Region MA (DER 4M0309)   "Tyrequerine DNA seroption with control processing PCH product from the gell		Unbalanced nucleotide concentrations	Prepare fresh deoxynucleotide mixes						
Nonemak patients may be taken to hate   less allow-capy number bording vector	errors	Template DNA has been damaged	• Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309)						
No product   Negotimes   Negotimes   Negotimes   Negotimes have no additional complementary regions within the template DNA   Improve Mg* concentration   A. Aljust Mg* concentration in 0.2 - I mill incoments   Nuclease continuation   Nuclease continuation   A. Beginst reactions using them bottoms of the standing attrophent of the primer of the prim		Desired sequence may be toxic to host							
Improper Mg* concentration   Adjust Mg* concentration in 0,2—1 mM increments		Incorrect annealing temperature	Recalculate primer Tm values using the NEB Tm calculator (TmCalculator.neb.com)						
No locises contamination   Repeat reactions using thesis solutions		Mispriming	Verify that primers have no additional complementary regions within the template DNA						
Recalculate primer in values using grey MEST in calculator (verw reb. com/TmCalculator)	product size	Improper Mg <sup>2+</sup> concentration	Adjust Mg <sup>2+</sup> concentration in 0,2–1 mM increments						
lest an amealing temperature   lest an amealing temperature gradient, starting at 5°C below the lower Tm of the primer pair		Nuclease contamination	Repeat reactions using fresh solutions						
Poor primer design   Verify that primers are non-complementary, both internally and to each other		Incorrect annealing temperature	· · · · · · · · · · · · · · · · · · ·						
Insufficient primer concentration   Primer concentration can range from 0.05—1 µM in the reaction. Please see specific product literature for ideal conditions		Poor primer design	Verify that primers are non-complementary, both internally and to each other						
Missing reaction component  Roproduct  No product  Poor template quality  Poor template quality  Poor template quality  Poor template quality  Presence of inhibitor in reaction  Insufficient number of cycles  Incorrect thermocycler programming  Incorrect thermocycler programming  Contamination of reaction tubes  or solutions  Complex template  Presence of inhibitor in reaction  Poor template quality  Presence of inhibitor in reaction  Insufficient number of cycles  Incorrect thermocycler programming  Incorrect thermocycler block temperature  Contamination of reaction tubes  or solutions  Complex template  Complex template  Presence of inhibitor in reaction  Presence of inhibitor in reaction with more cycles  Incorrect Memory in reaction  Presence of inhibitors  Presence of inhibitors or use new reagents  Presence of inhibitors  Presence of inhibitors  Presence of inhibitors  Presence of inhibitors  Presence of		Poor primer specificity	Verify that oligos are complementary to proper target sequence						
No product   Poor template quality   Continue Mg* concentration by testing 0,2-1 mM increments		Insufficient primer concentration	• Primer concentration can range from 0,05–1 μM in the reaction. Please see specific product literature for ideal conditions						
No product   Poor template quality   Presence of inhibitor in reaction   Poor template quality   Presence of inhibitor in reaction   Poor template purify starting temperature by testing an annealing temperature, starting at 5°C below the lower Tm of the primer pair of Check 260/280 ratio of DNA template   Presence of inhibitor in reaction   Presence and inhibitor in reaction   Presence of inhibitor in reaction   Presence assempte volume   Presence assempte vo		Missing reaction component	Repeat reaction setup						
Presence of inhibitor in reaction Presence of inhibitor in reaction Insufficient number of cycles Incorrect thermocycler programming Inconsistent thermocycler block temperature Contamination of reaction tubes or solutions  Premature replication Premature replication Premature replication Incorrect Mg²- concentration can range from 0,05-1 µM in the reaction. Please see specific product literature for ideal conditions. Incorrect template concentration Incorrect template concentration Incorrect template concentration Incorrect template concentration Incorrect temp		Suboptimal reaction conditions	Thoroughly mix Mg <sup>2+</sup> solution and buffer prior to adding to the reaction						
Presence of influibitor in reaction    Decrease sample volume	No product	Poor template quality							
Incorrect thermocycler programming Inconsistent thermocycler plock temperature  Contamination of reaction tubes or solutions  Complex template  Complex template  Premature replication  Premature replication  Primer annealing temperature too low Incorrect Mg²²- concentration  Incorrect Mg²²- concentration  Multiple or non-specific products  Excess primer  Contamination with excess primer  Contamination with excess primer  - For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 µl reaction		Presence of inhibitor in reaction							
Inconsistent thermocycler block temperature Contamination of reaction tubes or solutions  Complex template  Complex temp		Insufficient number of cycles	Rerun the reaction with more cycles						
Contamination of reaction tubes or solutions  - Autoclave empty reaction tubes prior to use to eliminate biological inhibitors - Prepare fresh solutions or use new reagents  - Use One Tary DNA Polymerases - For GC-rich template suse One Tary DNA Polymerase (NEB #M0480) with One Tary GC Reaction Buffer (plus One Tary High GC Enhancer, if necessary) or 05 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer - For longer templates, we recommend LongAmp Tary DNA Polymerase  Premature replication  - Use a hot start polymerase, such as OS Hot Start High-Fidelity (NEB #M0493) or One Tary Hot Start (NEB #M0481) DNA Polymerases - Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature  Primer annealing temperature too low - Recalculate primer Tm values using the NEB Tm Calculator (TmCalculator.neb.com) - Increase annealing temperature - Recalculate primer Tm values using the NEB Tm Calculator (TmCalculator.neb.com) - Increase annealing temperature - Adjust Mg² in 0,2-1 mM increments - Check specific product literature for recommended primer design - Verify that primers are non-complementary, both internally and to each other - Increase length of primer - Avoid GC-rich 3' ends - Primer concentration can range from 0,05-1 µM in the reaction. Please see specific product literature for ideal conditions.  - Use positive displacement pipettes or non-aerosol tips - Set-up dedicated work area and pipetter for reaction setup - Wear gloves during reaction setup - Wear gloves during reaction setup - For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 µl reaction		Incorrect thermocycler programming	Check program, verify times and temperatures						
or solutions  Prepare fresh solutions or use new reagents  Use One Pag DNA Polymerases For GC-rich template suse One Pag DNA Polymerase (NEB #M0480) with One Pag GC Reaction Buffer (plus One Pag High GC Enhancer, if necessary) or Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer For longer templates, we recommend LongAmp Tag DNA Polymerase Premature replication  Premature replication  Primer annealing temperature too low Incorrect Mg² concentration  Primer annealing temperature too low Incorrect Mg² concentration  Poor primer design  Poor primer design  Poor primer design  Excess primer  Contamination with exogenous DNA  Primer concentration  Primer concentration  Primer annealing temperature Primer concentration  Primer annealing temperature Poor primer design  Poor primer design  Poor primer design  Poor primer design  Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  Primer concentration can range from 0,05–1 µM in the reaction setup  Primer concentration can setup  Primer concentration setup		Inconsistent thermocycler block temperature	• Test calibration of heating block						
For GC-rich templates, use One Tag DNA Polymerase (NEB #M0480) with One Tag GC Reaction Buffer (plus One Tag High GC Enhancer, if necessary) or GS High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer   For longer templates, we recommend LongAmp Tag DNA Polymerase   For longer templates, we recommend LongAmp Tag DNA Polymerases   Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature enterperature									
Premature replication  One Taq Hot Start (NEB #M0481) DNA Polymerases  Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature  Primer annealing temperature too low Incorrect Mg²- concentration  Adjust Mg²- in 0,2-1 mM increments  Check specific product literature for recommended primer design  Verify that primers are non-complementary, both internally and to each other  Incorrect Mg²- concentration  Excess primer  Excess primer  Contamination with exogenous DNA  Incorrect template concentration  One Taq Hot Start (NEB #M0481) DNA Polymerases  Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation (TmCalculator.neb.com)  Recalculate primer Tm values using the NEB Tm Calculator (TmCalculator.neb.com)  Incorrect Mg²- concentration  Adjust Mg²- in 0,2-1 mM increments  Check specific product literature for recommended primer design  Verify that primers are non-complementary, both internally and to each other  Incorrect template concentration  Use positive displacement pipettes or non-aerosol tips  Set-up dedicated work area and pipettor for reaction setup  Wear gloves during reaction setup  For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg—10 ng of DNA per 50 µl reaction		Complex template	For GC-rich templates, use One Taq DNA Polymerase (NEB #M0480) with One Taq GC Reaction Buffer (plus One Taq High GC Enhancer, if necessary) or Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer						
Multiple or non- specific products  Multiple or non- specific products  Multiple or non- specific products  Poor primer design  Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  Use positive displacement pipettes or non-aerosol tips Set-up dedicated work area and pipettor for reaction setup  Wear gloves during reaction setup  Poor primer design  Poo		Premature replication	One Taq Hot Start (NEB #M0481) DNA Polymerases - Set up reactions on ice using chilled components and add samples to thermocycler preheated to the						
Multiple or non- specific products  Poor primer design  - Check specific product literature for recommended primer design - Verify that primers are non-complementary, both internally and to each other - Increase length of primer - Avoid GC-rich 3' ends  - Primer concentration can range from 0,05–1 µM in the reaction. Please see specific product literature for ideal conditions.  - Use positive displacement pipettes or non-aerosol tips - Set-up dedicated work area and pipettor for reaction setup - Wear gloves during reaction setup - For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction		Primer annealing temperature too low							
Multiple or non-specific products       Poor primer design       • Verify that primers are non-complementary, both internally and to each other         Excess primer       • Primer concentration can range from 0,05–1 μM in the reaction. Please see specific product literature for ideal conditions.         Contamination with exogenous DNA       • Use positive displacement pipettes or non-aerosol tips         Set-up dedicated work area and pipettor for reaction setup       • Set-up dedicated work area and pipettor for reaction setup         Incorrect template concentration       • For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 μl reaction		Incorrect Mg <sup>2+</sup> concentration	• Adjust Mg <sup>2+</sup> in 0,2–1 mM increments						
ideal conditions.  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  For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg—10 ng of DNA per 50 µl reaction		Poor primer design	Verify that primers are non-complementary, both internally and to each other     Increase length of primer						
- Set-up dedicated work area and pipettor for reaction setup - Wear gloves during reaction setup - Wear gloves during reaction setup - For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 µl reaction		Excess primer							
Incorrect template concentration			Set-up dedicated work area and pipettor for reaction setup						
		Incorrect template concentration							

<sup>\*</sup>Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.



# General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

### Setup Guidelines

### **DNA** Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 μl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng $-1~\mu g$  of DNA per 50  $\mu l$  reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly when a high number of cycles are run

### **Primers**

- Primers should typically be 20–40 nucleotides in length
- Ideal primer content is 40-60% GC
- Primer Tm calculation should be determined with NEB's Tm Calculator (www.neb.com/TmCalculator)
- Annealing temperatures should be determined according to specific enzyme recommendations. Please note that Q5 and Phusion annealing temperature recommendations are unique.
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers

- Final concentration of each primer should be 0,05–1 μM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5' to the site
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

### Magnesium Concentration

- Optimal Mg<sup>++</sup> concentration is usually 1,5–2,0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg<sup>++</sup> at 1X concentrations. Please refer to the specific product information for Mg<sup>++</sup> content.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg<sup>++</sup> can be added for applications that require complete control over Mg<sup>++</sup> concentration
- Further optimization of  $Mg^{++}$  concentration can be done in 0,2–1  $\mu M$  increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM  $Mg^{++}$  in the reaction
- Insufficient Mg<sup>++</sup> concentrations may cause reaction failure

### Deoxynucleotides

- Ideal dNTP concentration is typically 200  $\mu$ M of each, however, some enzymes may require as much as 400  $\mu$ M each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg<sup>++</sup> and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal-based PCR polymerases. Use One *Taq* or *Taq* DNA Polymerases for these applications.

### **Enzyme Concentration**

- Optimal enzyme concentration in the reaction is specific to each polymerase.
   Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

### Starting Reactions

- Unless using a hot start enzyme (e.g., One Taq Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme(e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).



### Cycling Guidelines

### Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

### Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (TmCalculator.neb.com)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion
  High-Fidelity DNA Polymerase\*, annealing
  temperatures are usually set at 2°-5°C below
  the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA
   Polymerase or Phusion High-Fidelity DNA
   Polymerase\*, annealing temperatures should be set at 0°–3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair

Ideally, primer Tm values should be less than
the extension temperature. However, if Tm
values are calculated to be greater than the extension temperature, a two-step PCR program
(combining annealing and extension into one
step) can be employed.

### Extension

- Extension temperature recommendations range from 65°-72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields



For more information on polymerase properties and usage, visit http://international.neb.com.

<sup>\*</sup> Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.



# PCR Polymerases

PRODUCT	NEB #	SIZE
Deep Vent <sub>r</sub> DNA Polymerase	M0258S/L	200/1.000 units
Deep Vent <sub>R</sub> (exo <sup>-</sup> ) DNA Polymerase	M0259S/L	200/1.000 units
EpiMark Hot Start <i>Taq</i> DNA Polymerase	M0490S/L	100/500 reactions
Hemo KlenTaq DNA Polymerase	M0332S/L	200/1.000 reactions (25 µl reaction vol)
Hot Start <i>Taq</i> 2X Master Mix	M0496S/L	100/500 reactions (50 µl reaction vol)
Hot Start <i>Taq</i> DNA Polymerase	M0495S/L	200/1.000 units
LongAmp <i>Taq</i> 2X Master Mix	M0287S/L	100/500 reactions (50 µl reaction vol)
LongAmp <i>Taq</i> DNA Polymerase	M0323S/L	500/2.500 units
LongAmp <i>Taq</i> PCR Kit	E5200S	100 reactions (50 μl reaction vol)
LongAmp Hot Start <i>Taq</i> DNA Polymerase	M0534S/L	500/2.500 units
LongAmp Hot Start <i>Taq</i> 2X Master Mix	M0533S/L	100/500 reactions (50 µl reaction vol)
Multiplex PCR 5X Master Mix	M0284S	100 reactions (50 µl reaction vol)
NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 reactions
NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 reactions
NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 reactions
One <i>Taq</i> DNA Polymerase	M0480S/L/X	200/1.000/5.000 units
One <i>Taq</i> Quick-Load DNA Polymerase	M0509S/L/X	100/500/2.500 units
One <i>Taq</i> 2X Master Mix with Standard Buffer	M0482S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> 2X Master Mix with GC Buffer	M0483S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> Quick-Load 2X Master Mix with Standard Buffer	M0486S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> Quick-Load 2X Master Mix with GC Buffer	M0487S/L	100/500 reactions (50 μl reaction vol)
One <i>Taq</i> Hot Start DNA Polymerase	M0481S/L/X	200/1.000/5.000 units
One <i>Taq</i> Hot Start 2X Master Mix with Standard Buffer	M0484S/L	100/500 reactions (50 µl reaction vol)
One Taq Hot Start 2X Master Mix with GC Buffer	M0485S/L	100/500 reactions (50 µl reaction vol)
One Taq Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S/L	100/500 reactions (50 µl reaction vol)
One Taq Hot Start Quick-Load 2X Master Mix with GC Buffer	M0489S/L	100/500 reactions (50 μl reaction vol)
Phusion High-Fidelity DNA Polymerase	M0530S/L	100/500 units
Phusion High-Fidelity PCR Kit	E0553S/L	50/200 reactions (50 µl reaction vol)
Phusion High-Fidelity PCR Master Mix with HF Buffer	M0531S/L	100/500 reactions (50 µl reaction vol)
Phusion High-Fidelity PCR Master Mix with GC Buffer	M0532S/L	100/500 reactions (50 µl reaction vol)
Phusion Hot Start Flex DNA Polymerase	M0535S/L	100/500 units
Phusion Hot Start Flex 2X Master Mix	M0536S/L	100/500 reactions (50 µl reaction vol)
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 units
Q5 Hot Start High-Fidelity DNA Polymerase	M0493S/L	100/500 units
Q5 High-Fidelity 2X Master Mix	M0492S/L	100/500 reactions (50 µl reaction vol)
Q5 Hot Start High-Fidelity 2X Master Mix	M0494S/L	100/500 reactions (50 µl reaction vol)
Quick-Load <i>Taq</i> 2X Master Mix	M0271L	500 reactions (50 μl reaction vol)
Taq 2X Master Mix	M0270L	500 reactions (50 μl reaction vol)
Taq 5X Master Mix	M0285L	500 reactions (50 μl reaction vol)
Taq DNA Polymerase with Standard Taq Buffer	M0273S/L/X	400/2.000/4.000 units
Taq DNA Polymerase with Standard Taq (Mg-free) Buffer	M0320S/L	400/2.000 units
Taq DNA Polymerase with ThermoPol Buffer	M0267S/L/X/E	400/2.000/4.000/20.000 units
Tag PCR Kit	E5000S	200 reactions (50 μl reaction vol)
Vent <sub>R</sub> DNA Polymerase	M0254S/L	200/1.000 units
Vent <sub>R</sub> (exo <sup>-</sup> ) DNA Polymerase	M0257S/L	200/1.000 units

# Repair

PRODUCT	NEB#	SIZE
PreCR Repair Mix	M0309S/L	30/150 reactions

# **Companion Products**

1 kb DNA Ladder	N3232S/L	200/1.000 gel lanes
Quick-Load 1 kb Extend DNA Ladder	N3239S	125 gel lanes
100 bp DNA Ladder	N3231S/L	100/500 gel lanes
2-Log DNA Ladder (0,1–10,0 kb)	N3200S/L	100-200/500-1.000 gel lanes
50 bp DNA Ladder	N3236S/L	100-200/500-1.000 gel lanes
Low Molecular Weight DNA Ladder	N3233S/L	100/500 gel lanes
Fast DNA Ladder	N3238S	50 gel lanes
PCR Marker	N3234S	100/500 gel lanes
Quick-Load Purple 1 kb DNA Ladder	N0552S	125 gel lanes
Quick-Load Purple 100 bp DNA Ladder	N0551S	125 gel lanes
Quick-Load Purple 2-Log DNA Ladder (0,1-10,0 kb)	N0550S	125-250 gel lanes
Deoxynucleotide Solution Set	N0446S	25 µmol of each
Deoxynucleotide Solution Mix	N0447S/L	8 µmol of each/40 µmol of each
dATP Solution	N0440S	25 μmol
Acyclonucleotide Set	N0460S	0,5 µmol of each
7-deaza-dGTP	N0445S/L	0,15 µmol of each/0,3 µmol of each
Ribonucleotide Solution Set	N0450S/L	10 µmol of each/50 µmol of each
Ribonucleotide Solution Mix	N0466S/L	8 µmol of each/40 µmol of each

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### USA

New England Biolabs, Inc.
Telephone (978) 927-5054
Toll Free (USA Orders) 1-800-632-7

Fax (978) 921-1350 e-mail: info@neb.com

### Canada

New England Biolabs, Ltd. Toll Free: 1-800-387-1095 info.ca@neb.com

### China, People's Republic

New England Biolabs (Beijing), Ltd. Telephone: 010-82378265/82378266 info@neb-china.com

### France

New England Biolabs France Telephone: 0800 100 632 info.fr@neb.com

### Japar

New England Biolabs Japan, Inc. Telephone: +81 (0)3 5669 6191 info@neb-japan.com

### Singapore

New England Biolabs, PTE. Ltd Telephone +65 6776 0903 sales.sg@neb.com

### **United Kingdom**

New England Biolabs (UK), Ltd Call Free: 0800 318486 info.uk@neb.com

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