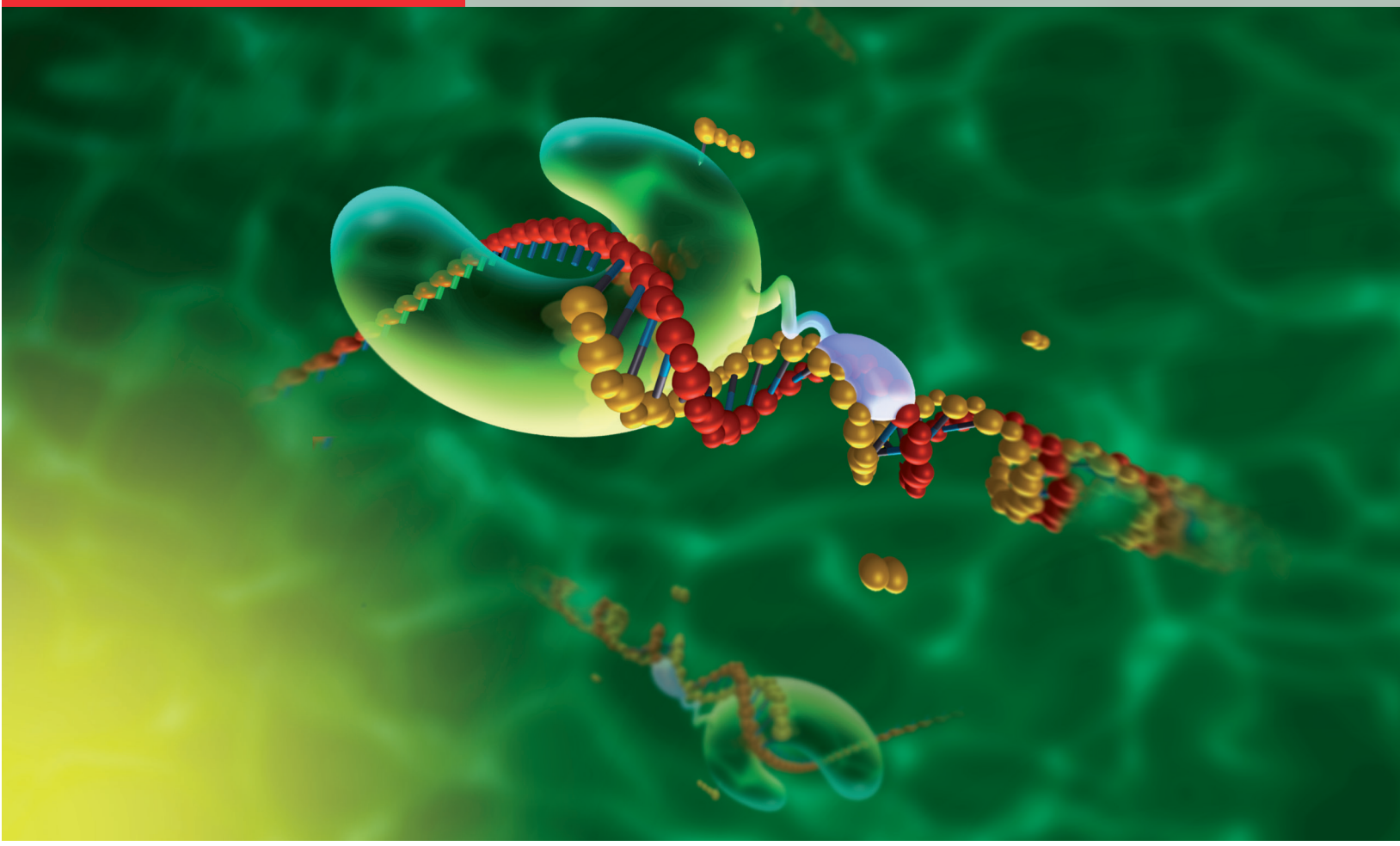
molecular biology

Thermo SCIENTIFIC



Thermo Scientific Phusion DNA Polymerases

Powerful, accurate, and fast polymerases for better PCR

VWR® We Enable Science

Upgrade to the gold standard for high-performance PCR

Since their introduction in 2003, Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerases have established the gold standard for high-performance PCR. Phusion products are referenced in thousands of publications and have become the first-choice DNA polymerases for a multitude of applications ranging from reconstruction,[1] design,[2] and massively-parallel, high-throughput sequencing of whole genomes.[3]

In Phusion High-Fidelity DNA Polymerase, a DNA-binding domain is fused to a *Pyrococcus*-like proofreading polymerase. Due to this unique fusion technique, Phusion DNA Polymerases generate PCR products with very high accuracy and speed. In addition, Phusion DNA Polymerases are tolerant of various inhibitors, allowing for robust amplification of PCR products with minimal optimization. For hot start PCR, Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase is an ideal choice allowing high specificity and improved robustness.

The processivity* of Phusion DNA Polymerases is approximately 10-fold greater than that of *Pfu* DNA polymerase and twice that of *Taq* DNA polymerase. This high processivity results in shorter extension times, more robust amplification and the ability to amplify long templates (up to 20 kb) in a fraction of the time. Phusion DNA Polymerases also produce higher yields while using less enzyme than traditional proofreading polymerase reactions.

Features

- **High fidelity**—52x more accurate than *Taq*, 6x more accurate than *Pfu*
- **Enhanced robustness**—fewer reaction failures and minimal optimization
- **High speed**—increased processivity allows shorter reaction times (extension 15–30 s/kb)
- **Improved yields**—high product yields with minimal enzyme amounts (0.5–1 U/50 µL reaction)
- **Enhanced specificity**—unique hot start technology with zero-time reactivation reduces nonspecific amplification and primer degradation
- **Simplified workflows**—Phusion Green DNA Polymerases allow direct loading of PCR products onto gels

Applications

- High-fidelity PCR
- Fast PCR
- Hot-start PCR
- Long range PCR (up to 20 kb)
- High-throughput PCR

The Thermo Scientific™ Phusion™ Green format is a combination of Phusion DNA Polymerases and 5X Green Reaction Buffer. The buffer includes a density reagent and two tracking dyes (blue and yellow) for direct loading of PCR products on gels. The green buffer does not interfere with the performance of Phusion DNA Polymerases and is compatible with downstream applications including DNA sequencing, ligation, and restriction digestion.

*Processivity measures the number of nucleotides the enzyme can incorporate into a growing DNA strand at one binding event during the extension step.

High fidelity

In many molecular biology applications including cloning, site-directed mutagenesis, and DNA translation, it is crucial to preserve the accurate DNA sequence during PCR amplification. An incorrectly incorporated nucleotide may result in the addition of the wrong amino acid, which, in turn, can affect folding and functional properties of the protein. Alternatively, deletion of a single nucleotide can destroy the correct reading frame.

Phusion DNA Polymerases have very high fidelity. The error rate of Phusion DNA Polymerase as determined by a modified *lac*I-based method[4] is approximately 50-fold lower than that of *Taq* DNA polymerase and six-fold lower than that of *Pfu* DNA polymerase (Figure 1).

The low error rate of Phusion DNA Polymerase was confirmed in studies using 454 sequencing[5] and Illumina sequencing methods[3] (Table 1).

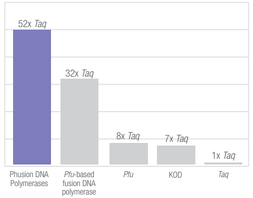


Figure 1. Relative fidelity values of different DNA polymerases. Fidelity = 1/error rate.

	KOD (%)	Phusion HF (%)	Pt *Taq* (%)	Expand HF (%)	FastStart HF (%)	SequalPrep Long (%)	*Pfu*Ultra HF (%)
Overall error rate[a]	0.21	0.11	0.34	0.25	0.23	0.29	0.23
Insertions	0.10	0.07	0.14	0.11	0.11	0.11	0.12
Deletions	0.06	0.02	0.08	0.07	0.05	0.06	0.05
Substitutions	0.01	0.01	0.07	0.04	0.03	0.07	0.01
Dots or Dot[b]	0.04	0.01	0.05	0.04	0.04	0.05	0.05

Table 1. Error rates for different DNA polymerases. Error rates for DNA polymerases were determined by 454 sequencing for following PCR amplification of four different exons from the human TP53 oncogene. The clonal TP53 plasmid was used as a starting template.

a Error rate, number of errors (miscalled bases, inserted or deleted bases) divided by total number of bases.
b Dots or Dot, three successive negative flows during 454 sequencing.
Table reprinted from Reference 5 with permission of the author and journal.

Successful amplification of targets up to 20 kb

Phusion DNA Polymerases are an ideal choice for amplification of long templates. The high enzyme processivity allows amplification of a wide variety of template sizes. Amplicons up to 20 kb are produced with high yields, short cycling times, and high fidelity.

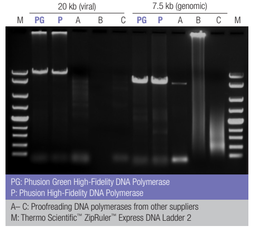


Figure 2. High yields of long PCR products. A 20 kb fragment from λ DNA and 7.5 kb fragment from human genomic DNA was amplified with Phusion DNA Polymerases and proofreading DNA polymerases from other suppliers.

Better yields with less enzyme

Due to their unique structure, Phusion DNA Polymerases are highly efficient and therefore require fewer units per reaction than conventional polymerases. Speed and efficiency result in high product yields in minimal time. In addition, Phusion polymerases are highly robust, minimizing the need for reaction optimization.

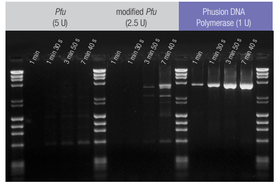


Figure 3. Higher efficiency with less enzyme. A 3.8 kb fragment from human beta globin gene was amplified with three different DNA polymerases. Phusion DNA Polymerase was able to amplify the 3.8 kb fragment with a combined annealing and extension step of only 1 minute. A single unit of Phusion DNA Polymerase produced higher yields than 2.5 or 5 units of the *Pfu* DNA polymerases.

High-specificity hot start PCR

Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase combines the Phusion DNA Polymerase and a reversibly bound, specific Affibody™ ligand. The ligand inhibits the polymerase activity at room temperature and thus prevents the amplification of nonspecific products. The reaction set-up can be done at room temperature enabling its use in high-throughput robotics. The Affibody ligand also inhibits the 3´→5´ exonuclease activity of the polymerase, preventing degradation of primers and template DNA during reaction set-up. At polymerization temperatures, the ligand is released, rendering the polymerase fully active. Phusion Hot Start II DNA Polymerase does not require a separate activation step in the PCR protocol as it is immediately reactivated at high temperatures.

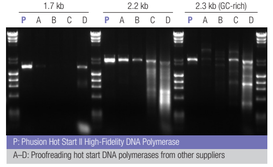


Figure 4. High specificity and yields. Five proofreading hot-start DNA polymerases were used to amplify 1.7–2.3 kb fragments from human genomic DNA. Phusion Hot Start II DNA Polymerase provided high yields of specific products, whereas other enzymes delivered zero or low yields, with some also amplifying nonspecific products.

Robust amplification of GC-rich templates

Optimized buffer system in synergy with the high enzyme processivity enables Phusion DNA Polymerase to amplify a broad range of DNA templates with different sequence content. Efficient amplification can be achieved even with difficult-to-amplify targets, including those with 85% GC content.

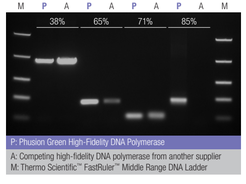


Figure 5. Amplification of DNA fragments with different sequence content. Four DNA fragments of different GC content were amplified with different DNA polymerases. Phusion Green DNA Polymerase produced all four amplicons with high yields. In contrast, a competing high-fidelity DNA polymerase was not able to efficiently produce the GC-rich product.

Fast PCR with high fidelity

Phusion DNA Polymerases incorporate a high number of nucleotides per binding event. This high processivity allows extremely short extension times and consequently reduced protocol times. Shortest protocol times can be achieved with Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix, a product developed specifically for fast PCR.

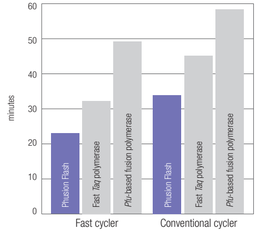


Figure 6. Shorter PCR run times with Phusion Flash High-Fidelity PCR Master Mix.

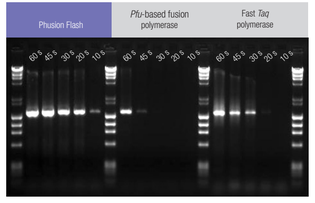


Figure 7. High speed and yields with Phusion Flash High-Fidelity PCR Master Mix. A 1.5 kb human cathepsin K gene was amplified with three different polymerases using varying extension times (10–60 seconds). Only Phusion Flash High-Fidelity PCR Master Mix was able to amplify the 1.5 kb gene with very short extension times of 10 and 20 seconds. It also produced superior yields of specific product compared to other enzymes tested.

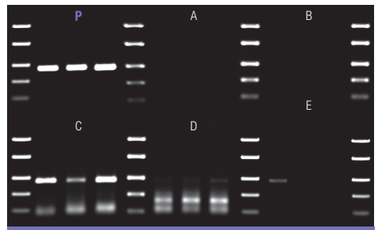
Engineered polymerase for uracil-tolerant PCR

Proofreading DNA polymerases are unable to amplify uracil-containing templates due to a so-called uracil-binding pocket, which detects uracil residues in the template strand and stalls further DNA synthesis. Thermo Scientific™ Phusion™ U DNA Polymerase carries a mutation in the uracil-binding pocket to overcome this limitation.

Thermo Scientific™ Phusion™ U Hot Start DNA Polymerase retains all features of Phusion family enzymes—great accuracy, speed, ability to amplify long amplicons up to 20 kb, and a high specificity with Affibody ligand–based hot start.

Applications

- Amplification of bisulfite-converted DNA
- Amplification of damaged or aged DNA
- Carryover contamination control
- Uracil excision–based (USER) cloning methods



P: Phusion U Hot Start DNA Polymerase
A: Proofreading fusion-type polymerase
B–D: Uracil-tolerant proofreading DNA polymerases from other vendors
E: Hot start *Taq* DNA polymerase

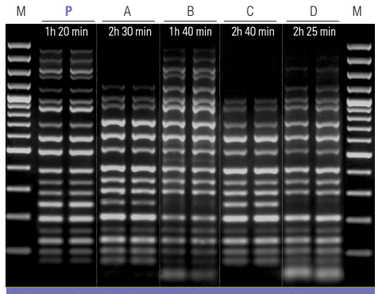
Figure 8. Highest yields and specificity with uracil-containing templates. Five proofreading DNA polymerases and hot start *Taq* polymerase were used to amplify a 798 bp fragment of bisulfite-treated human genomic DNA. Phusion U Hot Start DNA Polymerase provided high yields of specific products, whereas all other enzymes delivered zero or lower yields, with some also amplifying products nonspecifically.

Maximum performance in multiplex PCR

Thermo Scientific™ Phusion™ U Multiplex PCR Master Mix supports simultaneous amplification of multiple targets up to 2.5 kb over a wide range of template concentration and GC content. The master mix allows fast, sensitive, and inhibitor-tolerant multiplex PCR on any type of template DNA.

Applications

- Genotyping
- Pathogen detection
- Food testing
- Analysis of genetically modified organisms
- Amplification of microsatellites



P: Thermo Scientific™ Phusion™ U Green Multiplex PCR Master Mix
A–D: Multiplex PCR master mixes from other suppliers
M: Thermo Scientific™ GeneRuler™ 100 bp Plus DNA Ladder

Figure 9. Highly multiplexed PCR in the shortest time. 19 fragments (73–2,527 bp) from human genomic DNA were simultaneously amplified using different multiplex PCR master mixes according to manufacturers' recommendations. Phusion U Green Multiplex PCR Master Mix enabled amplification of all 19 fragments and resulted in the fastest PCR protocol.

References

1. Gibson DG et al. (2008) Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319:1215–1220.
2. Gibson DG et al. (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329:52–56.
3. Kinde I et al. (2011) Detection and quantification of rare mutations with massively parallel sequencing. *PNAS* 108:9530–9535.
4. Frey B, Suppmann B (1995) Demonstration of the Expand PCR System's greater fidelity and higher yields with a *lacI*-based PCR fidelity assay. *Biochemica* 2:8–9.
5. Vandenbroucke I et al. (2011) Minor variant detection in amplicons using 454 massive parallel pyrosequencing: experiences and considerations for successful applications. *Biotechniques* 51:167–177.

"After realizing that we could get the same number of cycles in roughly a quarter of the time (and at only slightly higher per unit cost), we changed exclusively to Phusion [Polymerase]."

PhD student
Department of Biological Sciences
Idaho State University, USA

Enzyme characteristics and formats

		Phusion High-Fidelity DNA Polymerase	Phusion Hot Start II High-Fidelity DNA Polymerase	Phusion Flash High-Fidelity DNA Polymerase	Phusion U Hot Start DNA Polymerase	Phusion U Multiplex PCR Master Mix
CHARACTERISTICS	Blunt or 3'A end	Blunt	Blunt	Blunt	Blunt	Blunt
	Target length, genomic/phage DNA	≤16/20 kb	≤16/20 kb	≤16/20 kb	≤20 kb	≤2.5 kb
	Hot start	No	Yes	Yes	Yes	Yes
	Recommended extension time	15–30 s/kb	15–30 s/kb	15 s/kb	15–30 s/kb	15–30 s/kb
	Fidelity vs. *Taq*	52x	52x	25x	25x	NA
	dUTP tolerance	No	No	No	Yes	Yes
FORMATS	Enzyme[1]	✓	✓	–	✓	–
	Green Buffer[2]	✓	✓	–	✓	✓
	Master mix[3]	✓	✓	✓	✓	✓
	Complete kit[4]	✓	–	–	–	–

1. DNA polymerase, buffer(s), DMSO, and $MgCl_2$
2. DNA polymerase supplied with Green Buffer, which includes density reagent and two tracking dyes for direct loading on gel
3. 2X master mix
4. All the necessary PCR reaction components including control template and primers

Ordering information

Product	Quantity	Cat. No.
Phusion High-Fidelity DNA Polymerases and Master Mixes		
Phusion High-Fidelity DNA Polymerase	100 U	F-530S
	500 U	F-530L
Phusion Green High-Fidelity DNA Polymerase	100 U	F-534S
	500 U	F-534L
Phusion High-Fidelity PCR Master Mix with HF Buffer	100 x 50 µL rxns	F-531S
	500 x 50 µL rxns	F-531L
Phusion High-Fidelity PCR Master Mix with GC Buffer	100 x 50 µL rxns	F-532S
	500 x 50 µL rxns	F-532L
Phusion High-Fidelity PCR Kit	50 x 50 µL rxns	F-553S
	200 x 50 µl rxns	F-553L
Phusion Hot Start II High-Fidelity DNA Polymerase	100 U	F-549S
	500 U	F-549L
Phusion Green Hot Start II High-Fidelity DNA Polymerase	100 U	F-537S
	500 U	F-537L
Phusion Hot Start II High-Fidelity PCR Master Mix	100 x 50 µL rxns	F-565S
	500 x 50 µL rxns	F-565L
Phusion Green Hot Start II High-Fidelity PCR Master Mix	100 x 50 µL rxns	F-566S
	500 x 50 µL rxns	F-566L
Phusion Flash High-Fidelity PCR Master Mix	100 x 20 µL rxns	F-548S
	500 x 20 µL rxns	F-548L

Product	Quantity	Cat. No.
Phusion U DNA Polymerases and Master Mixes		
Phusion U Hot Start DNA Polymerase	100 U	F-555S
	500 U	F-555L
Phusion U Green Hot Start DNA Polymerase	100 U	F-556S
	500 U	F-556L
Phusion U Hot Start PCR Master Mix	100 x 50 µL rxns	F-533S
	500 x 50 µL rxns	F-533L
Multiplex PCR Master Mixes		
Phusion U Multiplex PCR Master Mix	100 x 50 µL rxns	F-562S
	500 x 50 µL rxns	F-562L
Phusion U Green Multiplex PCR Master Mix	100 x 50 µL rxns	F-564S
	500 x 50 µL rxns	F-564L
Other Phusion Polymerase–based products		
Phusion Site-Directed Mutagenesis Kit	20 rxns	F-541

Country	Telephone Number	Fax Number	Email
Austria	01 97 002 0	01 97 002 600	info@at.vwr.com
Belgium	016 385 011	016 385 385	vwrbe@be.vwr.com
Czech Republic	+420 321 570 321	+420 321 570 320	info@cz.vwr.com
Denmark	43 86 87 88	43 86 87 90	info@dk.vwr.com
Finland	09 8045 5300	09 80 45 52 00	info@fi.vwr.com
France	0 825 02 30 30 (0,18 € TTC/min)	0 825 02 30 35 (0,18 € TTC/min)	info@fr.vwr.com
Germany *0,14 €/Min. aus d. dt. Festnetz	Freecall: 0800 702 00 07	0180 570 22 22*	info@de.vwr.com
Hungary	(52) 521-130	(52) 470-069	info@hu.vwr.com
Ireland /Northern Ireland	01 88 22 222	01 88 22 333	sales@ie.vwr.com

Country	Telephone Number	Fax Number	Email
Italy	02-3320311/02-487791	800 152999/02-40090010	info@it.vwr.com
The Netherlands	020 4808 400	020 4808 480	info@nl.vwr.com
Norway	02290	815 00 940	info@no.vwr.com
Poland	058 32 38 200	058 32 38 205	info@pl.vwr.com
Portugal	21 3600 770	21 3600 798/9	info@pt.vwr.com
Spain	902 222 897	902 430 657	info@es.vwr.com
Sweden	08 621 34 00	08 621 34 66	kundservice@se.vwr.com
Switzerland	044 745 13 13	044 745 13 10	info@ch.vwr.com
United Kingdom	0800 22 33 44	01455 55 85 86	uksales@uk.vwr.com

GO TO VWR.COM FOR THE LATEST NEWS, SPECIAL OFFERS AND DETAILS OF YOUR LOCAL VWR DISTRIBUTOR

VWR® | vwr.com
We Enable Science