

Phusion[®]

High-Fidelity DNA Polymerases

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Finnzymes' Phusion® DNA Polymerases offer PCR performance that no other enzyme can match. Phusion DNA Polymerases combine the fidelity you demand with unparalleled speed and robustness.

In Phusion® High-Fidelity DNA Polymerases, a unique dsDNA-binding domain is fused to a *Pyrococcus*-like proofreading polymerase. Due to the novel fusion technique, Phusion DNA Polymerases generate PCR products with accuracy and speed previously unattainable with a single enzyme, even on your most difficult templates. In addition, Phusion DNA Polymerases are capable of amplifying long amplicons (e.g. the 7.5 kb human genomic and 20 kb λ DNA used in Finnzymes' quality control assays). Phusion Hot Start DNA Polymerase does not require a separate activation step in the PCR protocol.

The Phusion® Technology

Incorporating an exciting new fusion protein technology, Phusion DNA Polymerases bring together a novel *Pyrococcus*-like proofreading polymerase with a processivity-enhancing domain. This domain increases the affinity of Phusion DNA Polymerases for double-stranded DNA, allowing incorporation of more nucleotides per binding event and decreasing the number of binding events required for elongation. The processivity of Phusion DNA Polymerases is approximately 10-fold greater than that of *Pyrococcus furiosus* DNA polymerase and twice that of *Thermus aquaticus* DNA polymerase. This dramatic increase in processivity results not only in shorter extension times, but more robust amplification and the ability to amplify long templates in a fraction of the time. Phusion DNA Polymerases also produce higher yields with lower enzyme amounts than traditional proofreading polymerases. Due to these unique characteristics, Phusion DNA Polymerases can be used for routine PCR as well as for demanding PCR applications.

Advantages

- **Accuracy** - The highest fidelity of any available thermostable polymerase
- **Speed** - Increased processivity allows shorter reaction times (extension 15-30 s/kb)
- **Robustness** - Fewer reaction failures and minimal optimization
- **High Yields** - Increase product yields with minimal enzyme amounts
- **Specificity** - Hot start modification reduces non-specific amplification and primer degradation

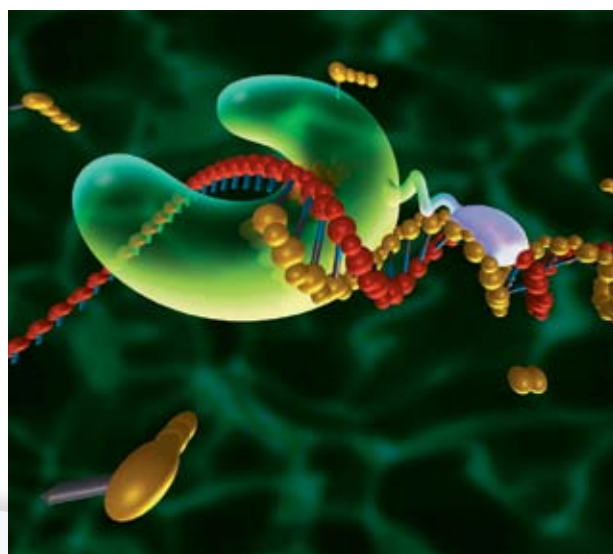


Figure 1. The schematic structure of Phusion® High-Fidelity DNA Polymerase. The double-strand DNA-binding domain (purple) is fused to a novel *Pyrococcus*-like proofreading enzyme (green) forming a unique high-performance polymerase - Phusion DNA Polymerase.

Extreme Fidelity

Phusion DNA Polymerases have extremely low error rates, thus setting a new standard for high-fidelity PCR. The error rate, determined by a modified *Ia*CI-based method¹, is 4.4×10^{-7} in HF Buffer. It is approximately 50-fold lower than that of *Thermus aquaticus* DNA polymerase and 6-fold lower than that of *Pyrococcus furiosus* DNA polymerase. Due to their low error rate, Phusion DNA Polymerases are ideally suited for cloning. Phusion DNA Polymerases produce blunt end PCR products.

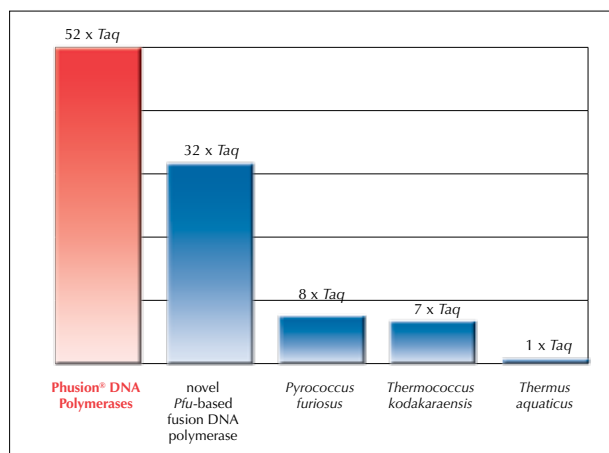


Figure 2. Relative fidelity values of different DNA polymerases. Fidelity = 1 / error rate.

Extreme Specificity with the Hot Start Modification

The Affibody[®]-based inactivation method of Phusion Hot Start DNA Polymerase increases the specificity of PCR amplification. Both the DNA polymerase and the proofreading activities of Phusion Hot Start DNA Polymerase are inactivated at room temperature. This prevents non-specific extension of the DNA template as well as degradation of the PCR primers during reaction setup. With Phusion Hot Start DNA Polymerase, the reaction setup can be done at room temperature, enabling its use in high-throughput robotics. In addition to improved specificity, Phusion Hot Start DNA Polymerase delivers improved robustness, allowing fewer reaction failures and minimizing optimization.

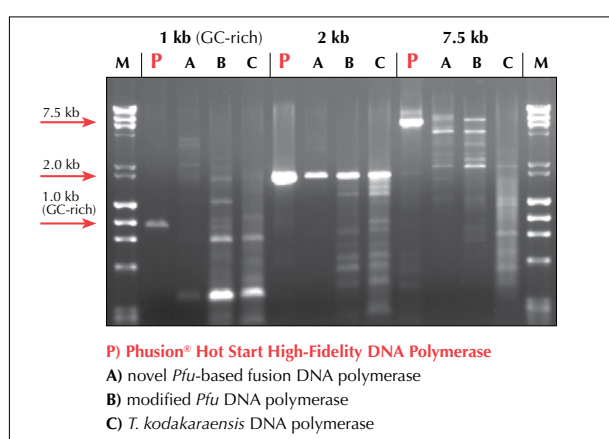


Figure 3. Four different hot start high-fidelity polymerases were used to amplify three different amplicons from human genomic DNA. The amplified fragments were: 1 kb from CEBPB gene (GC-rich), 2 kb and 7.5 kb from β -globin gene. The conditions were according to suppliers' recommendations.

Phusion Hot Start DNA Polymerase was the only polymerase capable of amplifying the GC-rich 1 kb fragment from human CEBPB gene. Furthermore, compared to the other polymerases, Phusion Hot Start DNA Polymerase amplified the 2 kb and 7.5 kb fragments with higher specificity and yield.

Extreme Speed and Yield

Reduced extension and overall cycling times

In a comparison of three DNA polymerases each amplifying a 3.8 kb human genomic DNA fragment, Phusion DNA Polymerase was significantly faster than the other two polymerases. Furthermore, significantly fewer units of the highly processive Phusion DNA Polymerase were required to complete the PCR. The extension times ranged from 1 min to 7 min 40 sec. Phusion DNA Polymerase gave strong specific bands even with the shortest extension time, completing the 3.8 kb fragment with a combined annealing and extension step of only 1 minute (total cycling time 1h). DNA polymerase from *Pyrococcus furiosus* failed to amplify any product even with the longest extension time (total cycling time more than 5 hours). The modified *Pyrococcus furiosus* DNA polymerase, in turn, amplified only a weak band with 3 min 50 s extension time (total cycling time 3 hours).

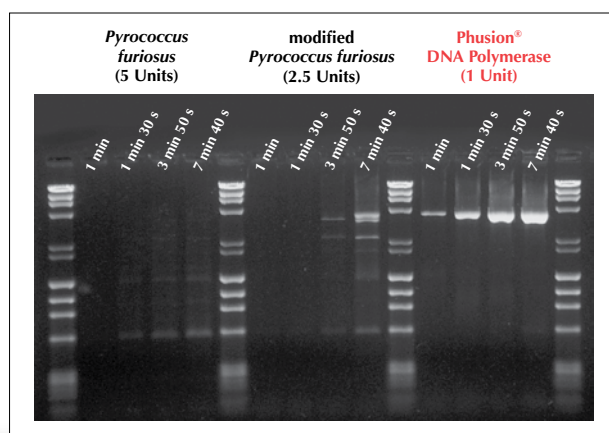


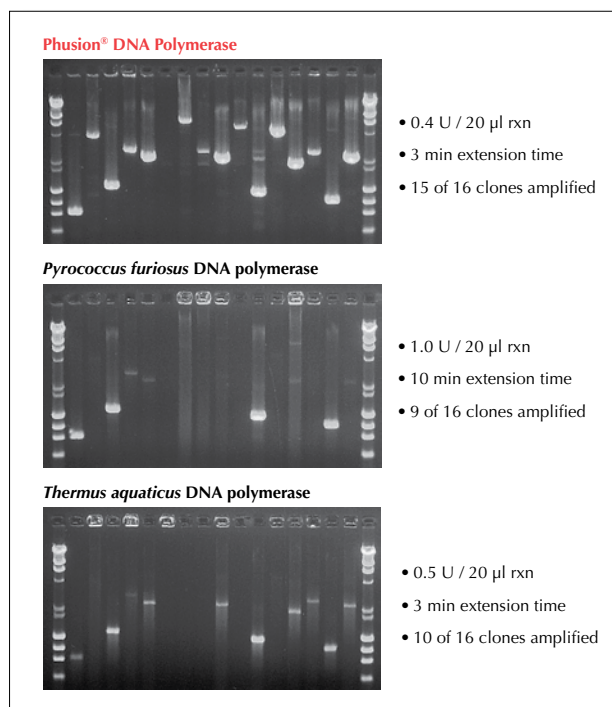
Figure 4. A 3.8 kb fragment from human beta globin gene was amplified with three different DNA polymerases according to suppliers' recommendations using varying extension times. Phusion DNA Polymerase was able to amplify the 3.8 kb genomic fragment with a combined annealing and extension step of only 1 minute, thus being significantly faster than the two other polymerases tested. A single unit of Phusion DNA Polymerase produced higher yields than 2.5 or 5 units of the *Pyrococcus furiosus* DNA polymerases.

Extreme Robustness

Minimize reaction failures

A set of 16 clones were randomly selected from a *Thermus sp.* genomic library and amplified with three different thermostable DNA polymerases. The results highlight the robustness and speed of Phusion DNA Polymerase, which was able to amplify 15 of the 16 randomly selected amplicons (94 %) with high yields. The success rate of *Pyrococcus furiosus* DNA polymerase was 56 % and *Thermus aquaticus* DNA polymerase 62 % with noticeably lower yields. Extension times and enzyme amounts used with Phusion DNA Polymerase (3 min, 0.4 U/20 µl) were significantly lower than those needed with *Pyrococcus furiosus* DNA polymerase (10 min, 1 U/ 20 µl) and comparable to those of *Thermus aquaticus* DNA polymerase (3 min, 0.5 U/20 µl), yet the yields were superior. These results further confirm the improved performance benefits of Phusion DNA Polymerase.

Figure 5 (on the right). A random set of 16 clones from a *Thermus sp.* genomic library was amplified from bacterial colonies. The amplicon size varied between 1-10 kb. Amplifications were done according to suppliers' instructions using same reaction conditions for all 16 amplicons.



Ordering Information

Phusion® Flash High-Fidelity PCR Master Mix	
F-548S/L	100 / 500 reactions in 20 µl volume
Phusion® Hot Start High-Fidelity DNA Polymerase	
F-540S/L	100 / 500 U (2 U/µl)
Phusion® High-Fidelity DNA Polymerase	
F-530S/L	100 / 500 U (2 U/µl)
Phusion® High-Fidelity PCR Master Mix with HF Buffer	
F-531S/L	100 / 500 reactions in 50 µl volume
Phusion® High-Fidelity PCR Master Mix with GC Buffer	
F-532S/L	100 / 500 reactions in 50 µl volume
Phusion® High-Fidelity PCR Kit	
F-553S/L	50 / 200 reactions in 50 µl volume
Detergent-free buffers available separately	
F-520S/L	Detergent-Free Phusion® HF Buffer Pack
F-521S/L	Detergent-Free Phusion® GC Buffer Pack

Other Phusion® DNA Polymerase Products

F-546	Phusion® RT-PCR Kit
F-541	Phusion® Site-Directed Mutagenesis Kit
F-547	Phusion® Blood Direct PCR Kit

References

1. Frey & Suppmann (1995) Biochemica 2: 34-35.

Improve PCR performance with Finnzymes' instruments and vessels



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