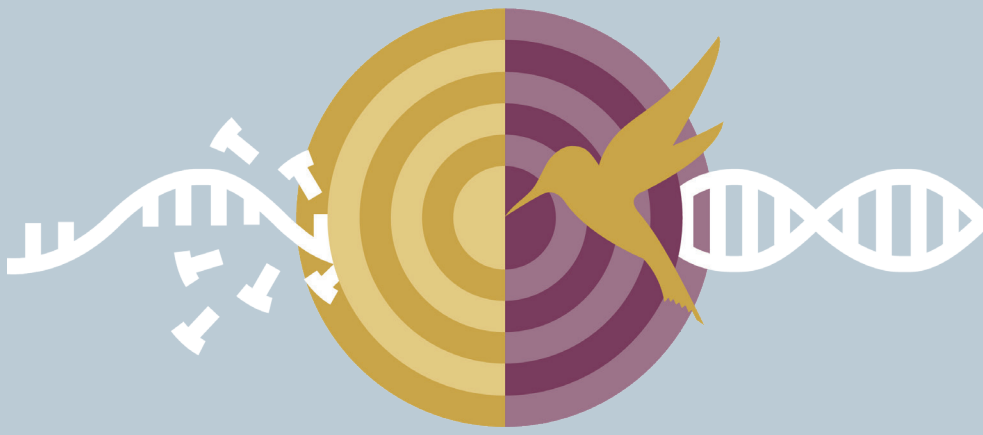
AMPLIQON
PCR ENZYMES & REAGENTS

AQ97 HIGH FIDELITY

DNA POLYMERASES & MASTER MIXES



AQ97 High Fidelity DNA Polymerase is a novel proofreading DNA polymerase developed and created by Ampliqon. AQ97 High Fidelity DNA Polymerase is composed of a novel chimeric DNA polymerase with Archaeal ancestry, fused to a processivity-enhancing DNA binding domain. Alongside very fast and robust amplification of complex and long targets, AQ97 High Fidelity DNA Polymerase displays a high fidelity ensuring accurate amplification.

AQ97 High Fidelity DNA Polymerase is well suited for PCR experiments that require amplification with very low error rates, such as cloning/sub-cloning, NGS applications, SNP analysis and mutagenesis.

A hot start version of AQ97 High Fidelity DNA Polymerase is available, enabling room temperature setup without the risk of primer degradation and decreased specificity.

Features:

- High fidelity: > 60x Taq fidelity
- High elongation rate: 10 sec/kb
- Long range amplification: 18 kb for human gDNA and 25 kb for λ DNA
- 3' to 5' proofreading exonuclease activity
- Hot start version: reaction setup at room temperature
- Hot start master mix versions available as clear and red

Applications:

- Cloning/sub-cloning
- Long range amplification
- NGS applications
- Mutagenesis
- Gene expression
- Construction of libraries
- SNP analysis

High fidelity

Fidelity values for AQ97 DNA Polymerase, two well-recognized high fidelity DNA polymerases P and Q and Taq DNA Polymerase were determined through a novel NGS-based analysis of nucleotide mis-incorporation during PCR.

Initially, PCR amplification was performed on a ~ 200 bp synthetic DNA target, generating PCR products for each of the tested polymerases (using recommended setup conditions).

Each product was purified and NGS-prepped, followed by sequencing using the MiSeq sequencing platform. In total, over 100 million reads were generated, with an average dataset size of 6 million reads. The substitution rate (error rate) was determined at each position within the DNA target (Figure 1) and subsequently summarized to determine an error rate of the entire target (Table 1).

The error rates found for AQ97 High Fidelity DNA polymerase and the high fidelity DNA polymerases P and Q were below the detection limit of this method, indicating that these polymerases generated very few substitution errors. The detection limit is estimated to be 8.4 x 10^{-6} errors per base per doubling, which corresponds to around 60x the fidelity of Taq DNA Polymerase. The error rates determined here may not be comparable with other error rates found in the literature due to technical and methodical differences.

	Error rate[a]	Fidelity (x Taq)
Taq	5 x 10^{-4} (± 4.3 x 10^{-6})	1x
AQ97	Below detection limit[b]	>60x
P	Below detection limit[b]	>60x
Q	Below detection limit[b]	>60x

Table 1. Error rates and corresponding fidelity values.
Errors per base per doubling. Standard deviations are given in brackets. Fidelity values for AQ97 High Fidelity DNA Polymerase and high fidelity DNA polymerases P and Q were compared to the fidelity values of Taq DNA Polymerase (1x).

[a] The presented error rates may not be comparable to those presented in other literature due to technical and methodical differences.
[b] Error rates were below the detection limit for the method. This limit is estimated to be 8.4 x 10^{-6}.

High fidelity (continued)

Diagram A in Figure 1 displays the distribution profile of the substitution rate across the amplification target for Taq DNA Polymerase, AQ97 High Fidelity DNA Polymerase and the two well-recognized high fidelity DNA polymerases P and Q. The diagram shows that the number of substitutions at each target position are much higher for Taq DNA Polymerase than for AQ97 High Fidelity DNA Polymerase and the two high fidelity DNA polymerases P and Q.

Furthermore, the number of substitutions at each target position for AQ97 High Fidelity DNA Polymerase and the two high fidelity DNA polymerases P and Q is close to the detection limit of the method.

Diagram B magnifies the area near the detection limit, displaying more information about the number of substitutions for AQ97 High Fidelity DNA Polymerase and the high fidelity DNA polymerases P and Q. Collectively, these diagrams show that AQ97 High Fidelity DNA Polymerase displays an extremely low number of substitutions. Furthermore, there is an indication that the substitution pattern of AQ97 High Fidelity DNA Polymerase is very similar to both high fidelity DNA polymerases P and Q.

Long range amplification

AQ97 High Fidelity DNA Polymerase provides the user with the ability to amplify a broad range of DNA targets from short and up to 18 kb for human genomic DNA.

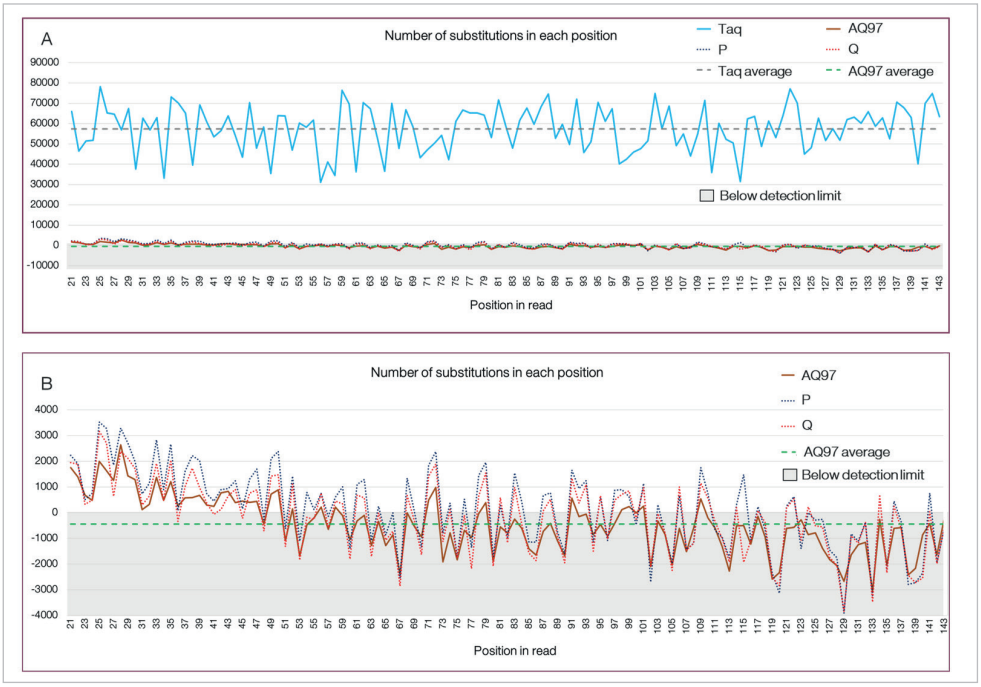


Figure 1. Distribution of substitutions. PCR was performed on a synthetic DNA target, using Taq DNA Polymerase, AQ97 High Fidelity DNA Polymerase and the two well-recognized high fidelity DNA polymerase P and Q. The amplified products were purified, NGS-prepped and sequenced.

The number of substitutions at each target position was calculated and plotted in diagrams A and B. Diagram B magnifies the area near the detection limit. Substitutions include misincorporated nucleotides and deletions. Non-polymerase errors are subtracted from the total number of errors to reveal true polymerase errors. Non-polymerase errors include mutations caused by thermocycling-induced DNA-damage, pre-NGS sample preparation and sequencing errors.

In these diagrams the average number of substitutions for Taq DNA Polymerase (Taq average) and for AQ97 High Fidelity DNA Polymerase (AQ97 average) is also plotted.

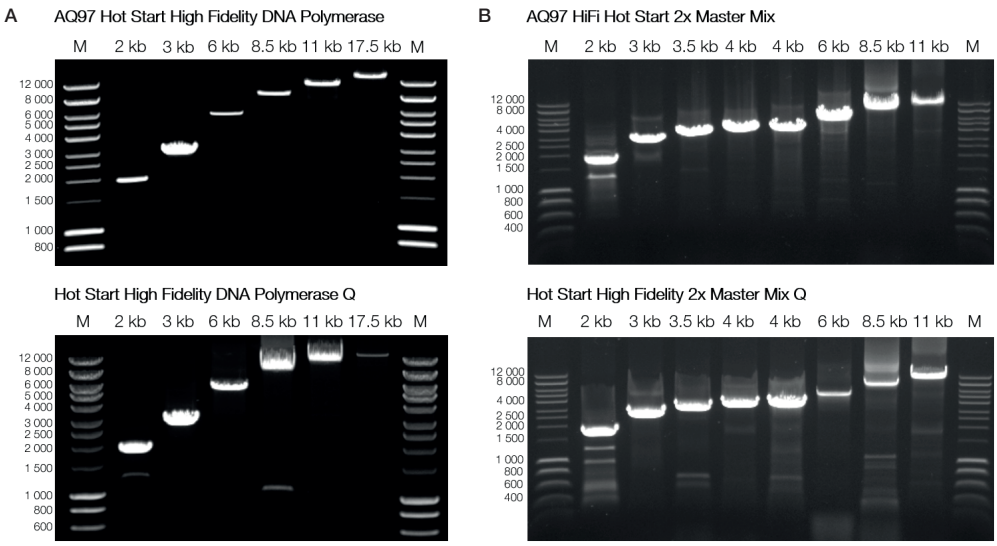


Figure 2. AQ97 enables long range amplification. A: Performance of AQ97 Hot Start High Fidelity DNA Polymerase (AQ97 HS HiFi) on large and complex amplicons was compared to a leading hot start high fidelity DNA Polymerase Q (Q). Six different targets of hgDNA ranging from 2-17.5 kb were amplified. Robust amplification was observed for all targets using AQ97 HS HiFi. Q generally resulted in higher yield, except for the longest target, where Q resulted in a weaker band and less yield. Amplification with AQ97 HS HiFi tends to minimize background noise and nonspecific bands compared to Q. Marker M: High Range DNA Ladder (Ampliqon, A610141).
B: Performance of AQ97 HiFi Hot Start 2x Master Mix was compared to a leading hot start high fidelity 2x master mix, Q. Eight different targets of hgDNA ranging from 2-11 kb were amplified. Generally, robust amplification was observed from both AQ97 HiFi Hot Start 2x Master Mix and master mix Q. For these targets AQ97 HiFi Hot Start 2x Master Mix tends to provide higher yield and fewer non specific bands than master mix Q. Marker M: High Range DNA Ladder (Ampliqon, A610141).

AQ97 HIGH FIDELITY

Robust amplification on AT-rich to GC-rich DNA targets

AQ97 High Fidelity DNA Polymerase and AQ97 Hot Start High Fidelity DNA Polymerase provide the user with robust and specific amplification of a variety of DNA targets with GC content ranging from ~ 30 – 80 % GC. The 5x AQ97 Buffer provided with the enzyme is recommended for highest fidelity and specificity. For DNA targets with a high GC content, more complex secondary structure or longer DNA targets, the addition of 1-2 M Betaine Enhancer Solution is recommended.

Hot start

AQ97 Hot Start High Fidelity DNA Polymerase shares the characteristics of its non-hot start counterpart, but distinguishes itself with the convenience of room temperature reaction setup.
Unlike non-hot start high fidelity polymerases, which can degrade primers and template DNA at room temperature, AQ97 Hot Start High Fidelity Polymerase remains inactive, thanks to the inactivation of both its polymerase and exonuclease domains through site-specific antibodies. The use of antibodies guarantees a stable yet quick release of inhibition.

An initial heating at 98 °C for 30 seconds is enough to fully active the enzyme.

The PCR performance of AQ97 Hot Start High Fidelity DNA Polymerase was compared to that of a hot start high fidelity DNA polymerase from a well-recognized competitor Q (Figure 3A). PCR was performed on eight different human genomic targets, 400 – 800 bp in length and with GC content ranging from 29 – 78 %.

Robust amplification was observed for all targets using AQ97 High Fidelity DNA Polymerase. High fidelity DNA polymerase Q provided results very similar to AQ97 High Fidelity DNA polymerase.

Hot start master mixes

The AQ97 Hot Start HiFi 2x Master Mix is a ready-to-use master mix containing AQ97 Hot Start HiFi DNA polymerase and an optimized buffer system designed to enhance enzyme performance and maximize PCR product yields.

The master mix is available in two variants: a classic clear formulation and a red variant, which includes a red dye for direct gel loading. Both master mixes remain liquid at -20 °C, eliminating the need for thawing prior to use.

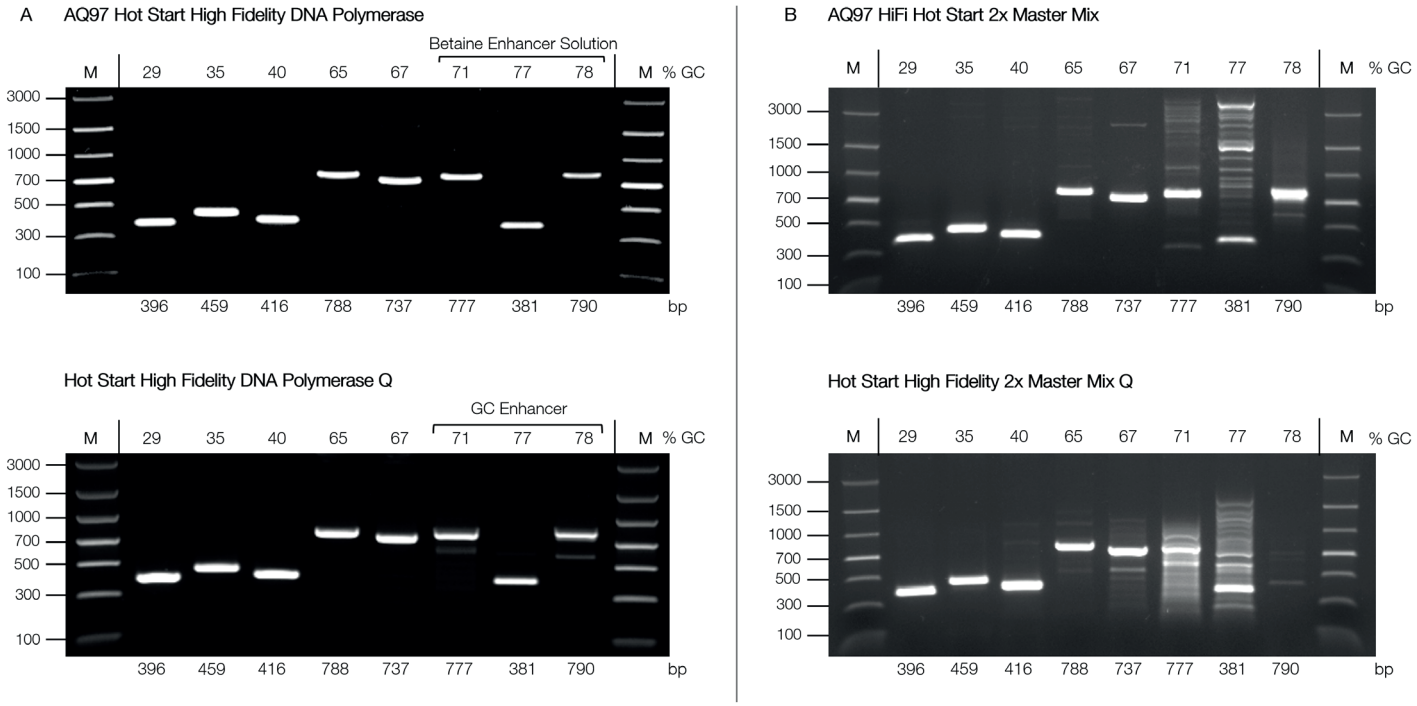


Figure 3. Robust amplification of AQ97 Hot Start High Fidelity DNA Polymerase and master mix.
A: Performance of AQ97 Hot Start High Fidelity DNA Polymerase (AQ97 HS HiFi) was compared to a leading hot start high fidelity DNA Polymerase, Q. Eight different human genomic DNA targets, 400 – 800 bp in length and with GC content ranging from 29 – 78 %, were amplified. Robust amplification was observed for all targets using AQ97 HS HiFi. DNA Polymerase Q provided very similar results to AQ97 HS HiFi. When amplifying GC-rich targets, 2 M Betaine Enhancer Solution (AQ97 HS HiFi) or a GC enhancer (DNA Polymerases Q) was included in the reaction mix. Marker M: Iqon PCR Ladder from Ampliqon (A610641).*

B: The above comparison was also performed between AQ97 HiFi Hot Start 2x Master Mix and a leading hot start high fidelity master mix, Q. Robust amplification was observed for all targets using AQ97 HS HiFi and master mix Q. For the GC-rich targets we observe some unspecific amplification using both master mixes. For the target with 78 % GC content, we observe a robust amplification with high yield using AQ97 HiFi Hot Start 2x Master Mix, whereas this target could not be amplified using master mix Q. Marker M: Iqon PCR Ladder from Ampliqon (A610641).*

**Amplification studies have been set up, as recommended by the manufacturers. The competitor's Tm calculator was used to calculate the annealing temperatures for all primers used in the studies. The calculated annealing temperatures were used for both AQ97 hot start products and the competitor products. The PCR programs used in all the studies for both AQ97 hot start products and the competitor products were the PCR programs recommended by the competitor.*

AQ97 HIGH FIDELITY

Technical chart	AQ97 High Fidelity DNA Polymerase	AQ97 HiFi 2x Master Mix	AQ97 Hot Start High Fidelity DNA Pol.	AQ97 HiFi Hot Start 2x Master Mix	AQ97 HiFi Hot Start 2x Master Mix RED
Hot start			✓	✓	✓
Setup at RT			✓	✓	✓
Master mix		✓		✓	✓
Direct gel loading					✓
Target length hgDNA, kb	≤18	≤11	≤18	≤11	≤11
Yield	+	+	++	+++	+++
Specificity	+	+	+++	++	++

+: high ++: very high +++: excellent

Ordering information

Product	Size	Cat #
AQ97 High Fidelity DNA Polymerase	100 Units	A767501
	500 Units	A767503
	1000 Units	A767504
	2500 Units	A767506
AQ97 Hot Start High Fidelity DNA Polymerase	100 Units	A787501
	500 Units	A787503
	1000 Units	A787504
	2500 Units	A787506
Betaine Enhancer Solution 5 M	5 x 1 ml	A351104
AQ97 HiFi 2x Master Mix	100 Reactions	A770201
	500 Reactions	A770203
	2500 Reactions	A770204
	5000 Reactions	A770206
AQ97 HiFi Hot Start 2x Master Mix	100 Reactions	A790901
	500 Reactions	A790903
	2500 Reactions	A790906
	5000 Reactions	A790907
AQ97 HiFi Hot Start 2x Master Mix RED	100 Reactions	A810801
	500 Reactions	A810803
	2500 Reactions	A810806
	5000 Reactions	A810807

For more information or ordering, please contact Ampliqon.

Email: enzyme@ampliqon.com
Phone: +45 70201169

Product link

AMPLIQON
PCR ENZYMES & REAGENTS

01.2025