AMPLIQON
PCR ENZYMES & REAGENTS

AQ97 HIGH FIDELITY DNA POLYMERASE

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.

Features:

- High Fidelity: > 60x Taq fidelity
- High elongation rate: 10 sec/kb
- Long range amplification: 18 kb for human gDNA
- 3' to 5' proofreading exonuclease activity

High fidelity

Fidelity values for AQ97 DNA Polymerase, AccuPol 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 misincorporation 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 2) and subsequently summarized to determine an error rate of the entire target (Figure 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.

A

	Error rate[a]
Taq	5 x 10^{-4} (± 4.3 x 10^{-6})
AccuPOL	1.1 x 10^{-4} (± 2.9 x 10^{-5})
AQ97	Below detection limit[b]
P	Below detection limit[b]
Q	Below detection limit[b]

B

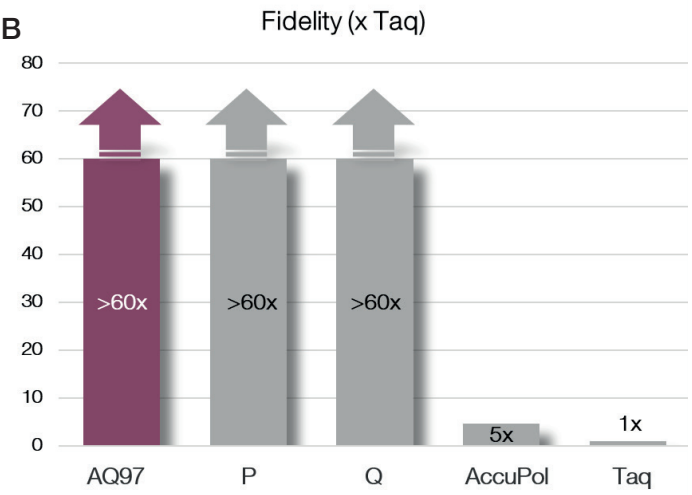

Figure 1. Error rates and corresponding fidelity values.
A: Errors per base per doubling. Standard deviations are given in brackets. B: Fidelity values for AQ97 High Fidelity DNA Polymerase, AccuPol DNA Polymerase and high fidelity DNA polymerases P and Q were compared to the fidelity values of Taq DNA 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 2 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 Q and P. 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 numbers 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 polymerase 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 5).

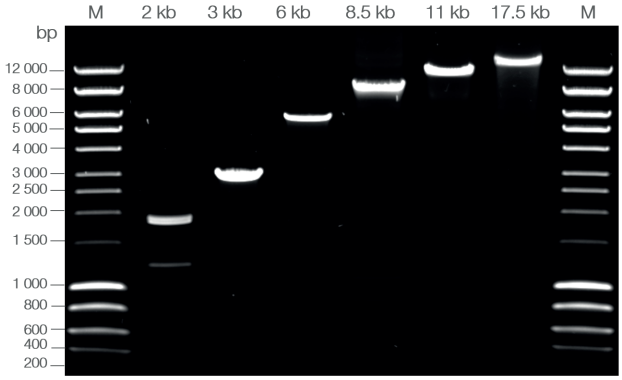

Figure 5: AQ97 enables long range amplification. Six different targets of human genomic DNA ranging from 2 kb and up to 17.5 kb was used in this study. Amplicon sizes are indicated at the top of the gel. Marker M is High Range DNA Ladder from Ampliqon (A610141).

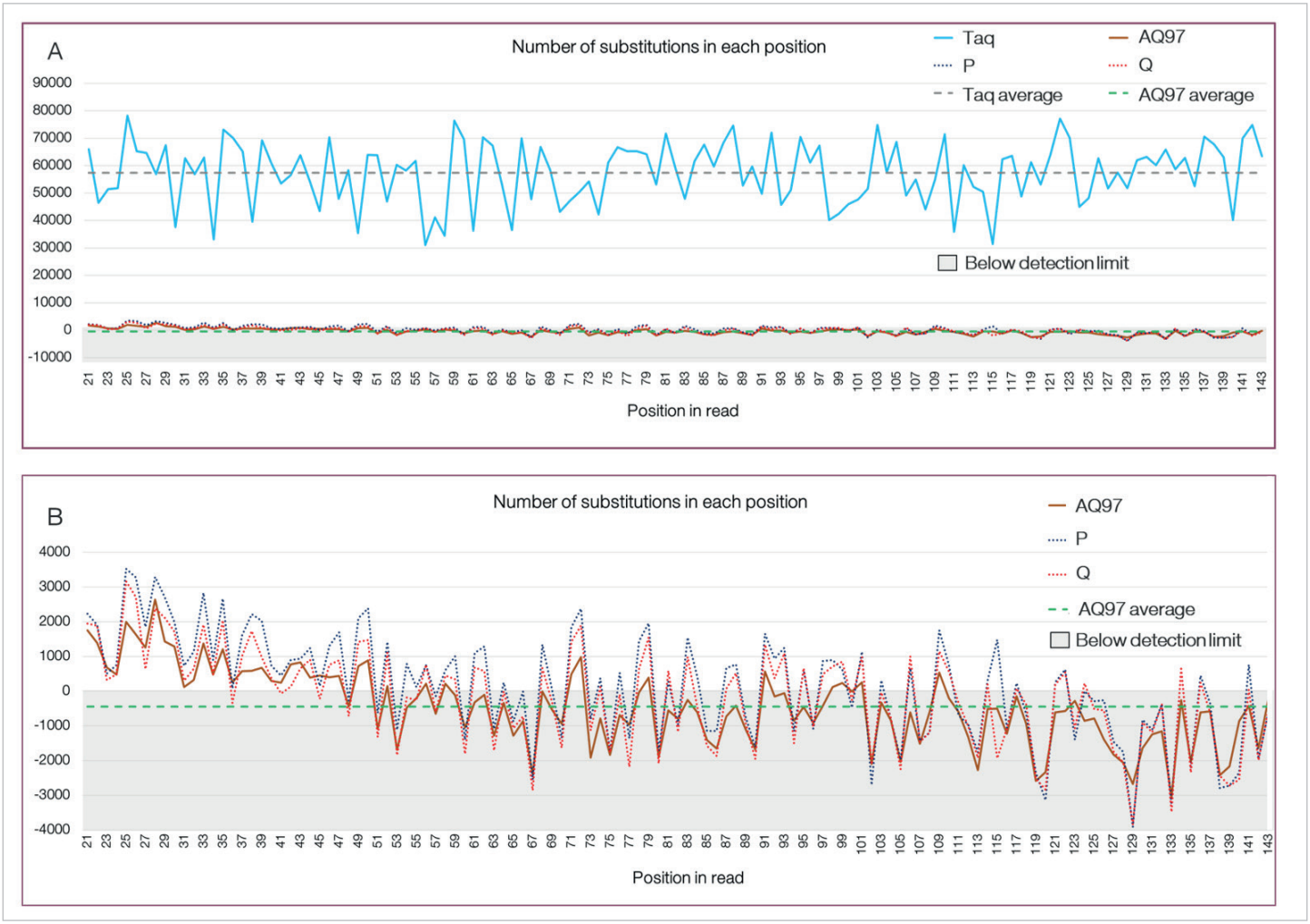

Figure 2. Distribution of substitutions. PCR was performed on a synthetic DNA target, using Taq DNA Polymerase, AQ97 High Fidelity DNA polymerase and the two well-recognized high fidelity DNA polymerase P and Q. The amplified products were purified, NGS-prepped and sequenced. The number of substitutions 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.

AQ97 HIGH FIDELITY DNA POLYMERASE

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

AQ97 DNA High Fidelity DNA Polymerase provides 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.

The PCR performance of AQ97 High Fidelity DNA Polymerase was compared to that of High Fidelity DNA Polymerases from three well-recognized competitors Q, S and P (Figure 3). PCR was performed on eight different human genomic targets, 400 – 800 bp in length and with GC content ranging from 29 – 78 % (Table 1). Robust amplification was observed for all targets using AQ97 High Fidelity DNA Polymerase. High fidelity DNA polymerase Q and S provided results very similar to AQ97 High Fidelity DNA polymerase, except on the last target with the highest GC content of 78%. In contrary, high fidelity DNA polymerase P were not able to provide the same level of robust amplification on the DNA targets with higher GC content, under the conditions tested here.

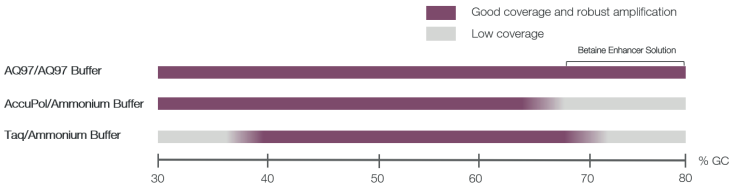

Figure 4. Illustration of the coverage of AQ97 High Fidelity DNA Polymerase. 5x AQ97 Buffer supports robust amplification of DNA targets with a GC content ranging from ~ 30 – 80 %. The addition of 2M Betaine Enhancer solution supports amplification of DNA targets with high GC content. The coverage of AQ97 High Fidelity DNA Polymerase is illustrated against the coverage of AccuPol DNA polymerase and Taq DNA Polymerase when using the 10x Ammonium Buffer.

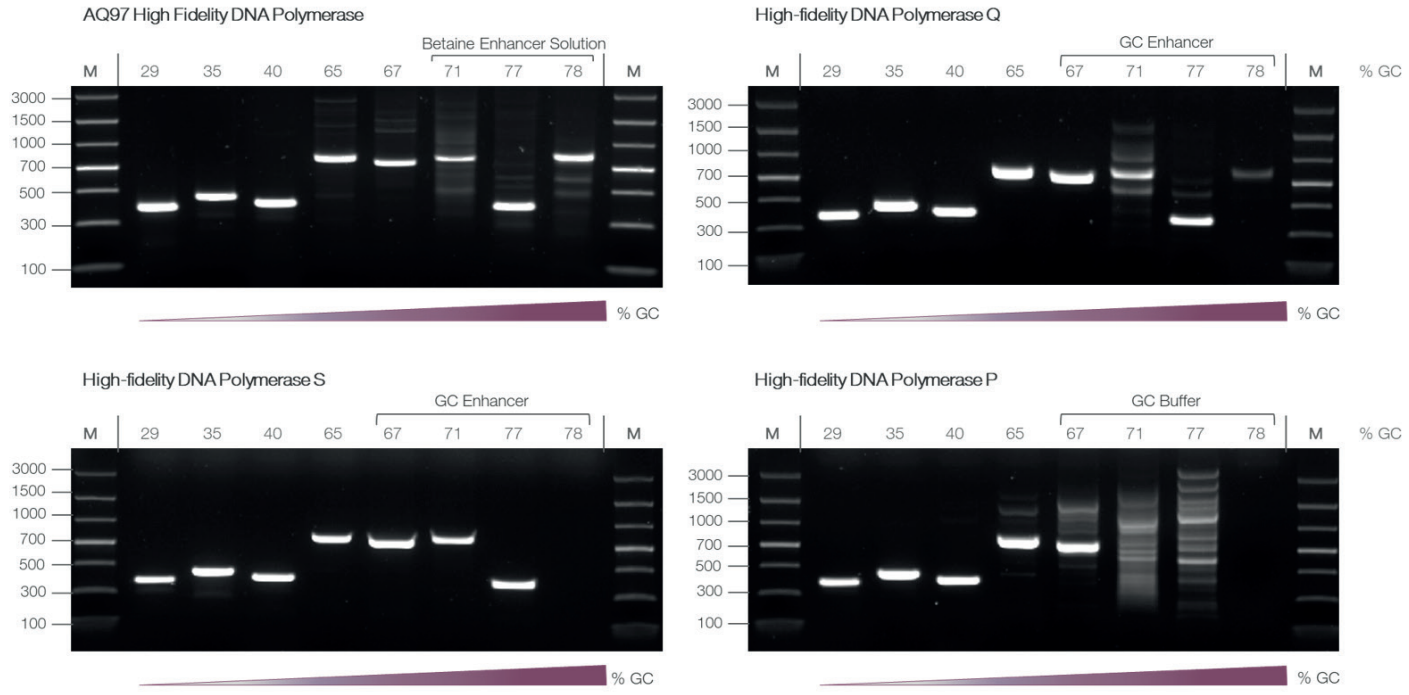

Figure 3. Robust amplification of AQ97 High Fidelity DNA Polymerase. Performance of AQ97 High Fidelity DNA Polymerase was compared to three leading high fidelity DNA Polymerase (Q, S and P). Eight different human genomic DNA targets, 400 – 800 bp in length and with GC content ranging from 29 – 78 %, were amplified. Amplification studies have been set up, as recommended by the manufactures. Tm Calculators of the respective competitors were used to calculate optimal annealing temperature for primers. When amplifying GC-rich targets, 2 M Betaine Enhancer Solution (AQ97High Fidelity DNA Polymerase), GC enhancer (Competitor Q and S) or GC-rich specific PCR Buffer (competitor P) were included in the reaction mix.

% GC	Target	bp
29	CFTR-EX21	396
35	DMD19	459
40	DMD17	416
65	BAIP3	788
67	CEND	737
71	KLF14	777
77	FECH1	381
78	PO3F3	790

Table 1. DNA targets. Overview of the eight genomic DNA targets used for the amplification study in figure 3. This table shows the GC content (% GC), target names and the respective target lenghts (bp).

Applications:

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

AQ97 HIGH FIDELITY DNA POLYMERASE

Ordering information

Product	Size	Cat #
AQ97 High Fidelity DNA Polymerase	100 Units	A767501
	500 Units	A767503
	1000 Units	A767504
	2500 Units	A767506
AQ97 High Fidelity DNA Polymerase 2x Master Mix	100 Reactions	A770101
	500 Reactions	A770103
	2500 Reactions	A770106
	5000 Reactions	A770107
Betaine Enhancer Solution 5 M	5 x 1 ml	A351104

AQ97 High Fidelity DNA Polymerase

AQ97 High Fidelity DNA Polymerase Master Mix

For more information or ordering, please contact Ampliqon.

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