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VWR®
Fast HiFi
DNA Polymerase

VWR®

Fast HiFi DNA Polymerase is a novel proofreading DNA polymerase. Fast HiFi 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, Fast HiFi DNA Polymerase displays a high fidelity ensuring accurate amplification.

Fast HiFi 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'→5' proofreading exonuclease activity

HIGH FIDELITY

Fidelity values for Fast HiFi 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 Fast HiFi 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	5×10^{-4} ($\pm$ $4{,}3 \times 10^{-6}$)
AccuPOL	1.1×10^{-4} ($\pm$ 2.9×10^{-5})
Fast HiFi	Below detection limit[b]
P	Below detection limit[b]
Q	Below detection limit[b]

B

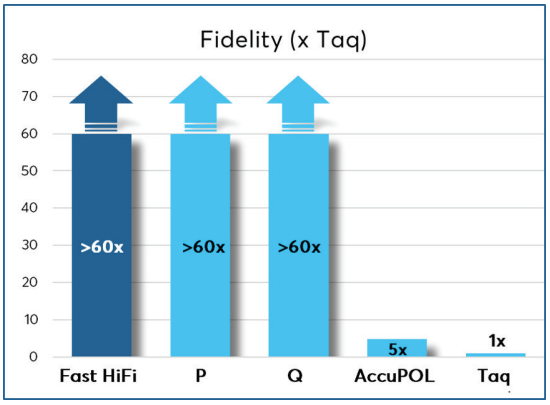

FIGURE 1: **Error rates and corresponding fidelity values.**
A: Errors per base per doubling. Standard deviations are given in brackets. **B:** Fidelity values for Fast HiFi Polymerase, AccuPOL DNA Polymerase and high fidelity DNA polymerases P and Q were compared to the fidelity values of Taq DNA Polymerase (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}.

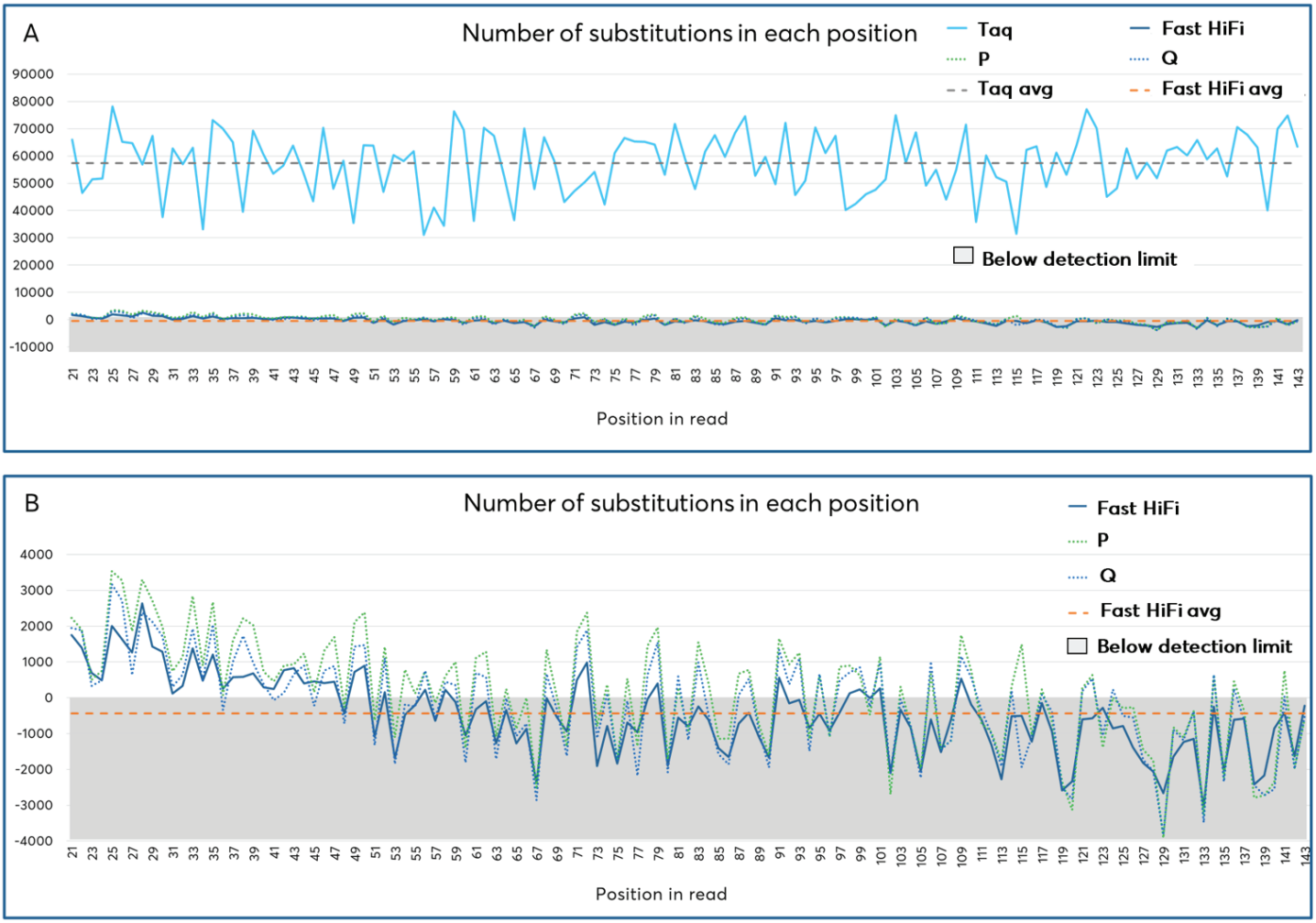

FIGURE 2: **Distribution of substitutions.** PCR was performed on a synthetic DNA target, using Taq DNA Polymerase, Fast HiFi polymerase and the two well-recognized high fidelity DNA polymerase P and Q. The amplified products were purified, NGS-prepped and sequenced. The number of substitutions at each 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 avg) and for Fast HiFi Polymerase (Fast HiFi avg) is also plotted.

Diagram A in Figure 2 displays the distribution profile of the substitution rate across the amplification target for Taq DNA Polymerase, Fast HiFi 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 Fast HiFi Polymerase and the two high fidelity DNA polymerases P and Q. Furthermore, the number of substitutions at each target position for Fast HiFi 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 Fast HiFi DNA Polymerase and the high fidelity DNA polymerase P and Q.

Collectively, these diagrams show that Fast HiFi DNA Polymerase displays an extremely low numbers of substitutions. Furthermore, there is an indication that the substitution pattern of Fast HiFi Polymerase are very similar to both high fidelity DNA polymerase P and Q.

ROBUST AMPLIFICATION ON AT-RICH TO GC-RICH DNA TARGETS

Fast HiFi 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 Fast HiFi 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 Fast HiFi 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 Fast HiFi Polymerase. High fidelity DNA polymerase Q and S provided results very similar to Fast HiFi 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.

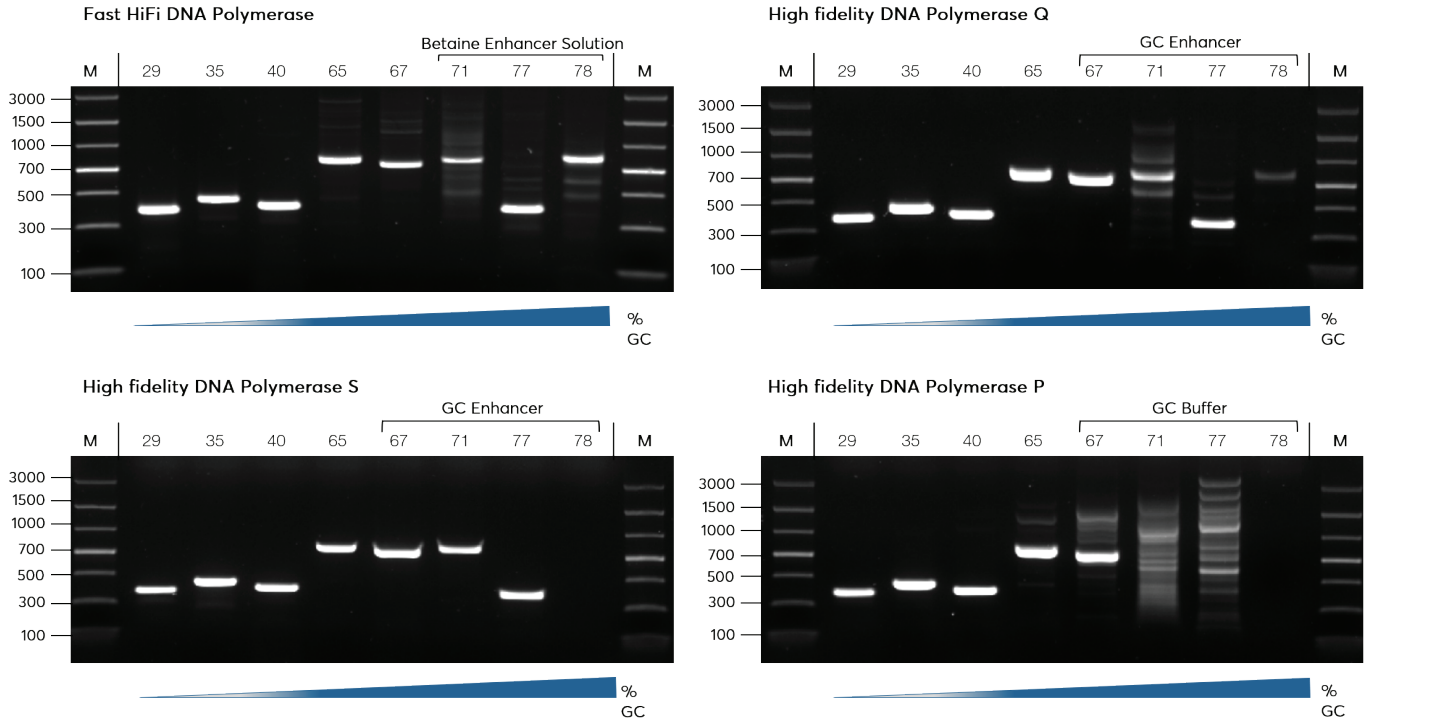

FIGURE 3: **Robust amplification of Fast HiFi DNA Polymerase.** Performance of Fast HiFi DNA Polymerase was compared to three leading high fidelity DNA Polymerase (Q, S and P). Eight different human genomic DNA targets, 400 – 800 bp in length and with GC content ranging from 29 – 78 %, were amplified. 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 (Fast HiFi 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 despicted in figure 4. This table shows the the GC content (% GC), target names and their respective lenghts (bp).

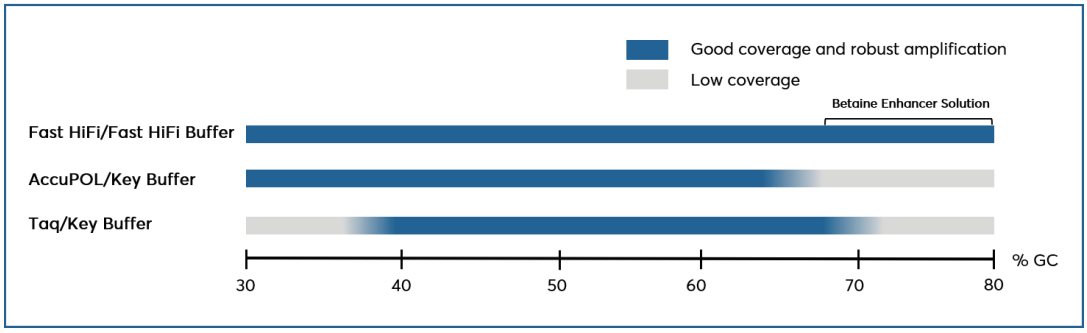

FIGURE 4: **Illustration of the coverage of Fast HiFi DNA Polymerase.** 5x Fast HiFi Buffer supports robust amplification of DNA targets with a GC content ranging from ~ 30 – 80 %. The addition of 2M Betaine Enhancer solution supports amplification of DNA targets with high GC content. The coverage of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

LONG RANGE AMPLIFICATION

Fast HiFi 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).

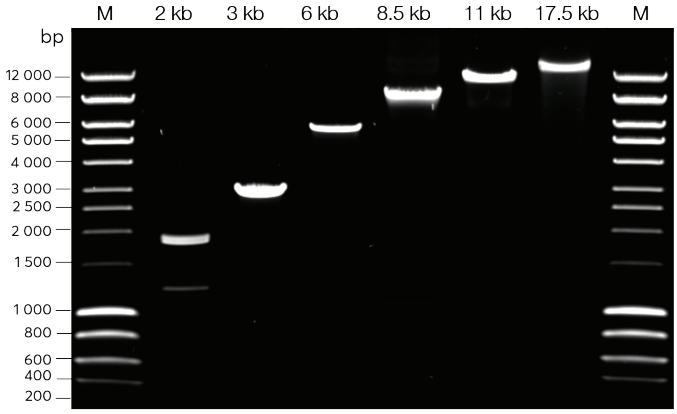

FIGURE 5: **Fast HiFi DNA Polymerase enables long range amplification.** Six different targets of human genomic DNA ranging from 2 kb and up to 17.5 kb was used in this study. Amplicon sizes are indicated at the top of the gel. DNA marker (M).

APPLICATIONS:

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

Ordering information:

Product	Size	Cat. No.
Fast HiFi DNA Polymerase 2 U/µl Incl. 5x Fast HiFi Buffer and 25 mM MgCl2	100 units	733-2880
	500 units	733-2881
	1000 units	733-2882
	2500 units	733-2883
Fast HiFi DNA Polymerase 2X Master Mix 1.5 mM MgCl2 final conc.	100 reactions	733-2884
	500 reactions	733-2885
	2500 reactions	733-2886
	5000 reactions	733-2887

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to create a better world

avantor™ delivered by VWR™

AUSTRIA
VWR International GmbH
Graumanngasse 7
1150 Wien
Tel.: +43 1 97 002 0
info.at@vwr.com

BELGIUM
VWR International bv
Researchpark Haasrode 2020
Geldenaaksebaan 464
3001 Leuven
Tel.: +32 (0) 16 385 011
vwr.be@vwr.com

CZECH REPUBLIC
VWR International s. r. o.
Veetee Business Park
Pražská 442
CZ - 281 67 Stříbrná Skalice
Tel.: +420 321 570 321
info.cz@vwr.com

DENMARK
VWR International A/S
Tobaksvejen 21
2860 Søborg
Tel.: +45 43 86 87 88
info.dk@vwr.com

FINLAND
VWR International Oy
Valimotie 9
00380 Helsinki
Tel.: +358 (0) 9 80 45 51
info.fi@vwr.com

FRANCE
VWR International S.A.S.
Le Périgares – Bâtiment B
201, rue Carnot
94126 Fontenay-sous-Bois cedex
Tel.: 0 825 02 30 30* (national)
Tel.: +33 (0) 1 45 14 85 00 (international)
info.fr@vwr.com
* 0,18 € TTC/min + prix appel

GERMANY
VWR International GmbH
Hilpertstraße 20a
D - 64295 Darmstadt
Tel.: 0800 702 00 07* (national)
Tel.: +49 (0) 6151 3972 0 (international)
info.de@vwr.com
*Freecall

HUNGARY
VWR International Kft.
Simon László u. 4.
4034 Debrecen
Tel.: +36 52 521130
info.hu@vwr.com

IRELAND
VWR International Ltd
Orion Business Campus
Northwest Business Park
Ballycoolin
Dublin 15
Tel.: +353 (0) 1 88 22 222
sales.ie@vwr.com

ITALY
VWR International S.r.l.
Via San Giusto 85
20153 Milano (MI)
Tel.: +39 02 3320311
info.it@vwr.com

THE NETHERLANDS
VWR International B.V.
Postbus 8198
1005 AD Amsterdam
Tel.: +31 (0) 20 4808 400
info.nl@vwr.com

NORWAY
VWR International AS
Brynsalleen 4,
0667 Oslo
Tel.: +47 22 90 00 00
info.no@vwr.com

POLAND
VWR International Sp. z o.o.
Limbowa 5
80-175 Gdansk
Tel.: +48 58 32 38 200
info.pl@vwr.com

PORTUGAL
VWR International - Material de Laboratório, Lda
Centro Empresarial de Alfragide
Rua da Indústria, nº 6
2610-088 Amadora
Tel.: +351 21 3600 770
info.pt@vwr.com

SPAIN
VWR International Eurolab S.L.U.
C/ Tecnología 5-17
A-7 Llinars Park
08450 - Llinars del Vallès
Barcelona
Tel.: +34 902 222 897
info.es@vwr.com

SWEDEN
VWR International AB
Fagerstagatan 18a
163 94 Stockholm
Tel.: +46 (0) 8 621 34 00
kundservice.se@vwr.com

SWITZERLAND
VWR International GmbH
Lerzenstrasse 16/18
8953 Dietikon
Tel.: +41 (0) 44 745 13 13
info.ch@vwr.com

UK
VWR International Ltd
Customer Service Centre
Hunter Boulevard - Magna Park
Lutterworth
Leicestershire
LE17 4XN
Tel.: +44 (0) 800 22 33 44
uksales@vwr.com

CHINA
VWR (Shanghai) Co., Ltd
Bld.No.1, No.3728 Jinke Rd,
Pudong New District
Shanghai, 201203- China
Tel.: 400 821 8006
info_china@vwr.com

ÍNDIA
Avantor Performance Materials India Limited
17th Floor, Building No. 5, Tower C
DLF Cyber City Phase – III
Gurgaon - 122002, Haryana
Tel.: +91-1244-65-6700
help@avantorinc.com

KOREA
VWR International ~
17, Daehak 4-ro, Yeongtong-gu,
Suwon-si, Gyeonggi-do
Tel.: +82 31 645 7256
saleskorea@avantorsciences.com

MIDDLE EAST & AFRICA
VWR International FZ-LLC
Office 203, DSP Lab Complex,
Dubai Science Park,
Dubai, United Arab Emirates
Tel: +971 4 5573271
Info.mea@vwr.com

SINGAPORE
VWR Singapore Pte Ltd
18 Gul Drive
Singapore 629468
Tel: +65 6505 0760
sales.sg@vwr.com

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