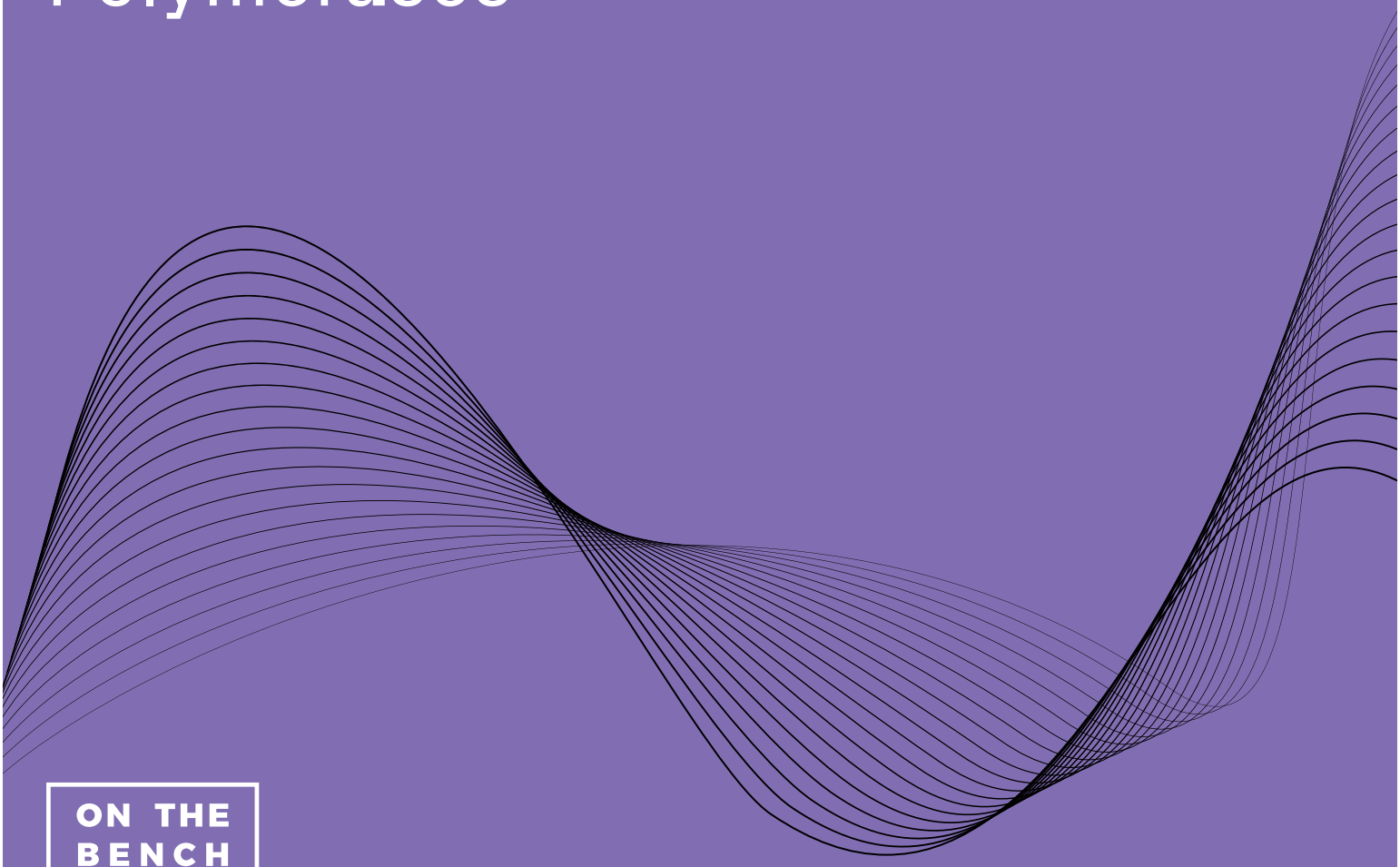
Genomics & Analytical Services

WHITE PAPER

Lab Product Reviews A Matter of *Taq;* Comparing Common PCR DNA Polymerases



ON THE BENCH

Lab Product Reviews

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1.0 Experimental Overview and Conclusions

So many PCR DNA polymerases to choose from, but so little time! How does a bench scientist choose which polymerase is best for their needs? To investigate this common question, Azenta Life Sciences' R&D scientists evaluated the performance of six commercially available DNA polymerases against the following parameters: PCR amplification of (i) a GC-rich template; (ii) a palindromic DNA sequence; (iii) nanogram quantities of human genomic DNA, as well as (iv) the effect of ethanol, (v) template switching rate, and (vi) list price cost per reaction. The enzymes tested were KAPA® HiFi™ HotStart® ReadyMix™ (KAPA; Kapa Biosystems), KOD Hot Start DNA Polymerase (KOD; EMD Millipore®), Phusion® High-Fidelity DNA Polymerase (Phusion; New England Biolabs®), Invitrogen™ Platinum™ SuperFi™ PCR Master Mix (SuperFi; Thermo Fisher Scientfic®), HotStarTaq® DNA Polymerase (HotStarTaq; Qiagen®), and NEBNext® Q5® Hot Start HiFi PCR Master Mix (NEBNextQ5; New England Biolabs).

As expected, there were measurable performance differences between each enzyme tested (Table 1 and Table 2). All enzymes tested yielded fair to good performances when amplifying GC-rich templates. Similarly, all enzymes tested yielded poor to fair results when testing for inhibition by ethanol. Template switching tests yielded results between 30-35%, regardless of the enzyme used. The majority of the enzyme-to-enzyme variability arose in the palindromic DNA amplification test, scarce starting template test, and cost parameters. For in-depth results reporting, please see Table 4 in the Appendix.

Key: Fair Good Excellent

DNA Polymerase Enzyme	KAPA*	KOD*	Phusion	SuperFi*	HotStarTaq	NEBNext* Q5
	Cat. # KK2602	Cat. # 71086-3	Cat. # M0530L	Cat. # 12358010	Cat. # 203205	Cat. # M0530
GC-rich (76%) template						
Palindromic DNA sequence						
Genomic DNA (ng)						
Effect of ethanol						
ŧCost per 20 µl PCR reaction						

Table 1. PCR DNA polymerase testing results – at a glance.

*Calculation was based on the supplier's list price.

DNA Polymerase Enzyme	KAPA*	SuperFi*	NEBNext* Q5	NEB Next High Fidelity	Platinum Taq
	Cat. # KK2602	Cat. # 12358010	Cat. # M0530	Cat. # M0541S	Cat. # 11304011
Template-switching rate					

Table 2. PCR DNA polymerase template-switching results – at a glance.

For the set of parameters tested by Azenta R&D scientists, there are three areas in which the enzymes exhibited performance differences: cost per reaction, ability to amplify palindromic templates, and ability to yield a robust reaction from limited amounts of starting template. If robustness against repetitive sequences and limited starting template mass is of little concern for your experiments, you may see minimal benefit of choosing a more expensive polymerase. However, if you commonly need to start with a low starting template concentration, or deal with repetitive sequences frequently, then choosing a higher-performing polymerase at a higher price may be worth the cost.

2.0 Materials and Methods

All experiments, other than the template strand-switching experiment, were carried out to compare the following commercially-available polymerase enzymes: KAPA HiFi HotStart ReadyMix (KAPA; Kapa Biosystems), KOD Hot Start DNA Polymerase (KOD; EMD Millipore), Phusion High-Fidelity DNA Polymerase (Phusion; New England Biolabs), Invitrogen Platinum SuperFi PCR Master Mix (SuperFi; Thermo Fisher Scientfic), HotStarTaq DNA Polymerase (HotStarTaq; Qiagen), and NEBNext Q5 Hot Start HiFi PCR Master Mix (NEBNextQ5; New England Biolabs).

The template strand-switching experiment was performed independently of the other enzyme experiments and thus examined slightly different enzymes. The template strand switching experiment tested the following enzymes: KAPA HiFi HotStart ReadyMix (Kapa Biosystems Cat. # KK2602), NEBNext High-Fidelity 2x PCR Master Mix, Q5 enzyme (New England Biolabs, Cat # M0541S), Phusion High-Fidelity DNA Polymerase (New England Biolabs, Cat # M0530L), Invitrogen Platinum SuperFi PCR Master Mix* (Thermo Fisher Scientific, Cat. # 12358010), and Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Cat. # 11304011). These results are included in this report as supplementary information that may provide further information to be used when selecting a suitable PCR polymerase.

2.1 PCR Amplification of a GC-Rich Template

A 263 bp portion of the IGF2R gene was selected to be the GC-rich template (76% GC content). It was amplified by each enzyme in parallel per the manufacturers' recommendations. Each enzyme was tested with no additives as well as with one additive to overcome high-GC content. For enzymes that came supplied with a proprietary additive for GC-rich templates, the manufacturer-supplied additive was tested. For enzymes with no manufacturer-supplied additive, a standard 5% (final concentration) DMSO was tested. PCR reactions were set up to amplify a high-GC content, 263 bp sequence of the IGF2R gene using the following primers: Forward (5' CTGCCTCCAACTTTCCCAGAC 3') and Reverse (5' CTTGCCTCCGCTGAACCTCT 3'). PCR cycling conditions followed manufacturers' guidelines and were standardized to a 25-cycle reaction in a 20 µl reaction volume. 10 ng of template was used in each reaction. PCR reactions were visualized on a 1% gel and band intensities were compared visually.

2.2 PCR Amplification of a Palindromic DNA Sequence

To test each enzyme's ability to amplify through hairpin structures, a palindromic sequence forming a 66 bp hairpin structure was amplified by each enzyme in parallel according to the manufacturers' recommendations. PCR reactions were set up to amplify a 378 bp sequence containing the 66-bp hairpin structure. PCR cycling conditions followed manufacturers' guidelines and were standardized to a 25 cycle reaction in a 20 µl reaction volume. 10 ng of template was used in each reaction. PCR reactions were visualized on a 1% agarose gel, and band intensities were compared visually.

2.3 PCR Amplification of Nanogram Quantities of Human Genomic DNA

The ability of each enzyme to amplify small quantities of commercially-purchased human genomic DNA was tested. Three amounts of human genomic DNA were used as input for the PCR reaction: 0.5 ng, 1 ng, and 5 ng. The template used was human genomic DNA, which contains approximately 250 copies in 1 ng. PCR reactions were set up to amplify a ~400 bp sequence of the PTEN gene using the following primers: PTEN_Forward (5' ATTTCCATCCTGCAGAAGAAG 3') and PTEN_Reverse (5' TATAGATAGCCTAAGAAAGCAATCG 3'). PCR cycling conditions followed manufacturers' guidelines and were standardized to a 25 cycle reaction in a 20 µl reaction volume. PCR reactions were visualized on a 1% agarose gel and band intensities were compared visually.

2.4 Effect of Ethanol on the Function of DNA Polymerase Enzymes

Each enzyme's robustness against ethanol inhibition was tested by spiking-in three final concentrations of ethanol into the PCR reaction. PCR reactions were set up using an empty pGEM plasmid as a template and the following primers: Forward (5'TAGCTCTTGATCCGGCAAAC3') and Reverse (5' GGATGGAGGCGGATAAAGTT3'). Ethanol was added at the following final concentrations: 1%, 4%, and 8%. PCR cycling conditions followed manufacturers' guidelines and were standardized to a 25-cycle reaction in a 20 µl reaction volume. The 500 bp amplicon was then visualized on a 1% agarose gel and band intensities were compared visually.

2.5 Comparison of Template Strand-Switching Rates

A pool containing 1 ng of 13 similar sequences was used as a starting template. These constructs were designed to mimic antibody discovery templates and contained sequences in the following format: FR1_CDR1_FR2_CDR2_FR3_CDR3_FR4 of heavy chain and FR1_CDR1_FR2_CDR2_ FR3_CDR3_FR4 of light chain linked together with a common linker. Each template was inserted into a modified pUC57 vector plasmid. The first round of PCR was performed for 25 cycles in a 20 µl reaction system containing polymerase, 4 primers specific to the FR1 motif of heavy chain (5' flanked with Illumina® partial P5 adaptor sequence), and 4 primers specific to the FR4 of light chain (5' flanked with Illumina partial P7 adaptor sequence) at the manufacturer-recommended conditions of each polymerase. The second round of PCR was performed for 6 cycles using Illumina indexed P5 and P7 primers for library generation.

Libraries were purified using AMPure® beads at 0.7x, quantified using Qubit® and qPCR assay, quality checked by Agilent® Bioanalyzer®, and sequenced on the MiSeq® 2×300 bp configuration. The sequence data were further analyzed using BLAST®n and the percentage of switched templates were calculated.

Results

3.1 PCR Amplification of a GC-Rich Template

A DNA template containing greater than 40% of guanine and cytosine bases poses difficulties for PCR amplification due to higher melting temperatures and the possible formation of secondary structures. Often, adjuncts or additives to the PCR reaction are required to disrupt the tight bonding of the GC residues to enable the reaction to proceed. Each enzyme's ability to amplify a high-GC template was tested in the presence and absence of one of these agents (Figure 1).

The KAPA, KOD, Phusion, and NEBNextQ5 enzymes amplified the template with high specificity and yield, even in the absence of DMSO. The activity of NEBNextQ5 was inhibited by 5% DMSO. SuperFi amplified the template only in the presence of a GC-enhancer solution. In the absence of Q-solution, HotStarTaq amplified the template with a relatively low yield but the addition of Q-solution increased the yield.

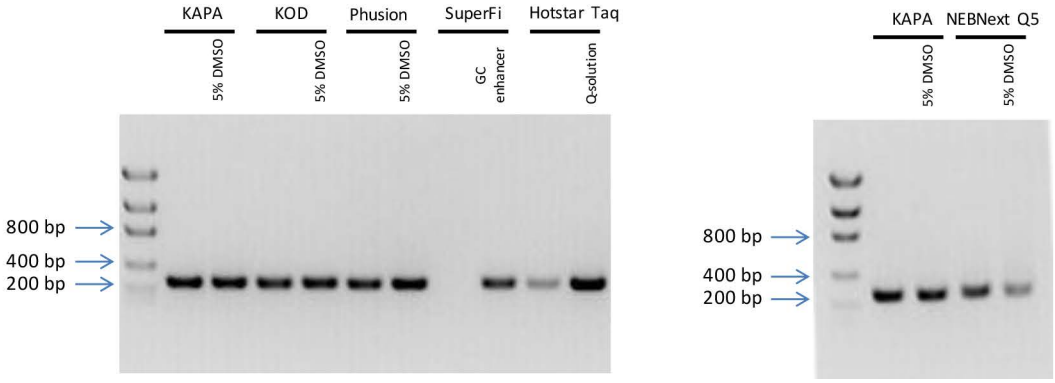

Figure 1. PCR amplification of a GC-rich (76%) template with and without additives. Key: KAPA HiFi HotStart ReadyMix = KAPA; KOD Hot Start DNA Polymerase = KOD; Phusion High-Fidelity DNA Polymerase = Phusion; Invitrogen Platinum SuperFi PCR Master Mix = SuperFi; HotStarTaq DNA Polymerase = HotStarTaq; and NEBNext Q5 Hot Start HiFi PCR Master Mix = NEBNextQ5.

3.2 PCR Amplification of a Palindromic DNA Sequence

DNA templates containing palindromic sequences have a propensity to form intra-strand hairpins, which makes it difficult for direct PCR amplification by DNA polymerases. Figure 2 shows the performance of the DNA polymerases in the amplification of a DNA palindrome. The KAPA enzyme amplified the palindromic DNA template with high yield and specificity. KOD and Phusion enzymes did not amplify the target band. SuperFi and NEBNextQ5 enzymes amplified the template, albeit with a relatively low yield. Although HotStarTaq amplified the DNA template resulting in a high yield, it also produced an extraneous PCR product.

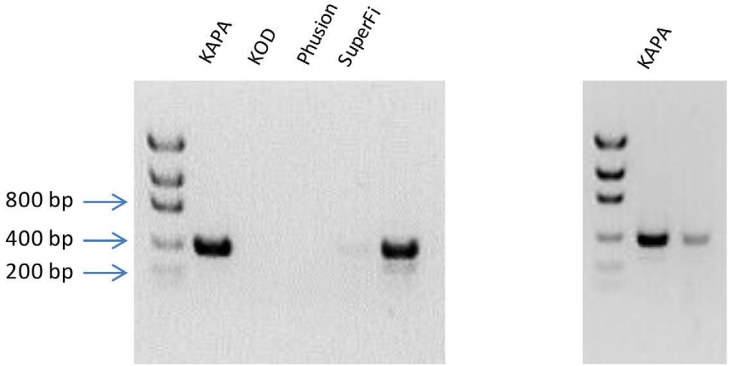

Figure 2. Amplification of a palindromic template. Key: KAPA HiFi HotStart ReadyMix = KAPA; KOD Hot Start DNA Polymerase = KOD; PhusionHigh-Fidelity DNA Polymerase = Phusion; Invitrogen Platinum SuperFi PCR Master Mix = SuperFi; HotStarTaq DNA Polymerase = HotStarTaq; and NEBNext Q5 Hot Start HiFi PCR Master Mix = NEBNextQ5.

3.3 PCR Amplification of Nanogram Quantities of Human Genomic DNA

Starting materials are often available in limited quantities, thus each enzyme's ability to provide robust amplification with low quantities of starting template is a key measurement of enzyme performance. KAPA was the most sensitive enzyme followed by NEBNextQ5 (with smearing), SuperFi, and HotStarTaq enzymes. KOD and Phusion enzymes were unable to generate the target band. Note: The estimated copy number was ~ 250 per 1 ng of human gDNA (Figure 3).

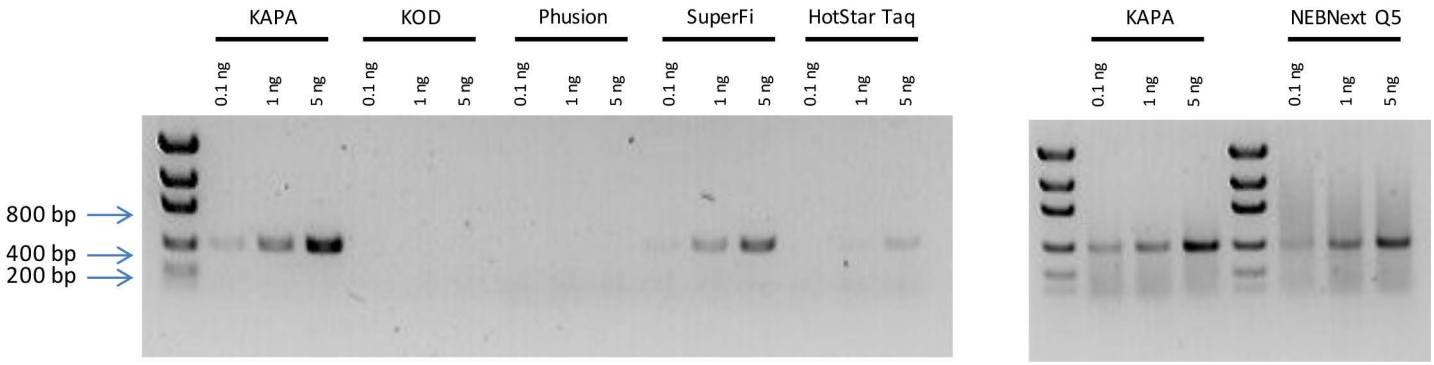

Figure 3. Impact of varying template starting amount on PCR amplification robustness. Key: KAPA HiFi HotStart ReadyMix = KAPA; KOD Hot Start DNA Polymerase = KOD; Phusion High-Fidelity DNA Polymerase = Phusion; Invitrogen Platinum SuperFi PCR Master Mix = SuperFi; HotStarTaq DNA Polymerase = HotStarTaq; and NEBNext Q5 Hot Start HiFi PCR Master Mix = NEBNextQ5.

3.4 Effect of Ethanol on the Function of DNA Polymerase Enzymes

Ethanol precipitation steps are frequently used in the DNA purification process. This often results in the carryover of ethanol, which inhibits PCR, into the reaction. Figure 4 shows the effect of ethanol on the function of the DNA polymerases. KAPA, SuperFi, HotStarTaq, and NEBNextQ5 were more resistant to the effect of ethanol but their efficiencies began to decrease at ethanol concentrations greater than 4%. Inhibition of KOD and Phusion enzymes began at 1% ethanol and above.

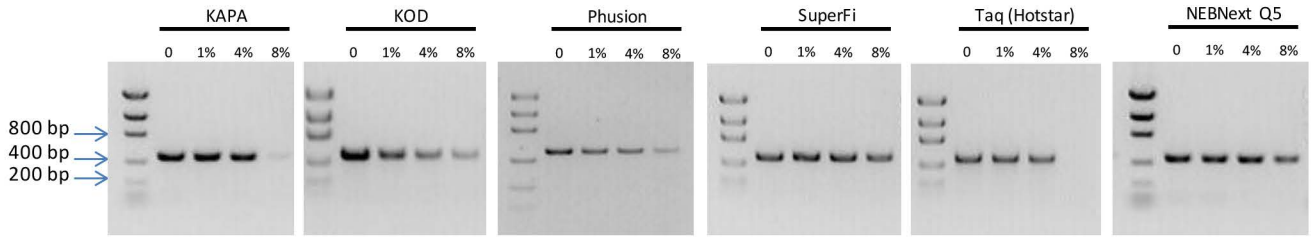

Figure 4. Impact of varying ethanol concentrations on PCR amplification robustness. Key: KAPA HiFi HotStart ReadyMix = KAPA; KOD Hot Start DNA Polymerase = KOD; Phusion High-Fidelity DNA Polymerase = Phusion; Invitrogen Platinum SuperFi PCR Master Mix = SuperFi; HotStarTaq DNA Polymerase = HotStarTaq; and NEBNext Q5 Hot Start HiFi PCR Master Mix = NEBNextQ5.

3.5 Template-Switching Rates of Five DNA Polymerases

Template switching is a phenomenon in which a DNA polymerase in a PCR containing a mixture of similar template sequences switches either between strands of templates or between similar template molecules. This results in the formation of artifacts that can impact downstream application and analysis. Template switching poses a unique challenge to certain NGS applications including antibody repertoire analysis and 16S microbiome analysis. The lower the template-switching rate of a polymerase enzyme, the lower chance of generating PCR artifacts. Table 3 shows the template-switching rate of five DNA polymerases. It should be noted that the template-switching rates all measured between ~30-36%, which, at first analysis, seems like a small range. However, considering the MiSeq platform generated between 100,000 and 180,000 reads per enzyme treatment, the ~6% difference observed between the best performer (lowest switching rate) and the worst performer (highest switching rate) may be significant enough to consider when designing an NGS experiment. Also, it is important to keep in mind that in addition to enzyme performance, the switching rate is also influenced by other reaction conditions such as cycle number, template homology, and template starting amount.

DNA Polymerase	Template-Switching Rate (%)
KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Cat. # KK2602)	29.9
NEBNext High-Fidelity 2x PCR Master Mix (New England Biolabs, Cat. # M0541S)	30.3
Phusion High-Fidelity DNA Polymerase, (New England Biolabs, Cat. # M0530L)	33.7
Invitrogen Platinum SuperFi PCR Master Mix (Thermo Fisher Scientific, Cat. #12358010)	36.4
Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Cat. # 11304011)	36.5

Table 3. Template-switching rate of five PCR DNA polymerases.

Appendix

Key: Fair Good Excellent

DNA Polymerase Enzyme	KAPA*	KOD*	Phusion	SuperFi*	HotStarTaq	NEBNext* Q5
	Cat. # KK2602	**Cat. # 71086-3**	**Cat. # M0530L**	**Cat. # 12358010**	**Cat. # 203205**	**Cat. # M0530**
GC-rich (76%) template	Yes, even without DMSO	Yes, even without DMSO	Yes, even without DMSO	Only in the presence of GC enhancer	Only in the presence of Q-solution	Yes, even without DMSO
Palindromic DNA sequence	Yes, with high yield and specificity	No	No	Yes, with a relatively low yield	Yes, with high yield and a nonspecific product	Yes, with a relatively low yield
Genomic DNA (ng)	Yes. Sensitivity ranking = 1st	Yes. Sensitivity ranking = 2nd	Yes. Sensitivity ranking = 3rd	No	No	Yes. Sensitivity ranking = 4th
Effect of ethanol	Inhibition at > 4%	Inhibition at ≥ 1%	Inhibition at ≥ 1%	Inhibition at > 4%	Inhibition at > 4%	Inhibition at > 4%
ŧCost per 20 µl PCR reaction (USD$)	$0.84	$0.50	$0.34+$0.1 =$0.44	$1.92	$0.25+$0.1 =$0.35	$0.58

Table 4. PCR DNA polymerase testing results – in detail.

*dNTP is included in the kit. ŧCalculation was based on the supplier's list price.

Key: Fair Good Excellent

DNA Polymerase Enzyme	KAPA*	SuperFi*	NEBNext* Q5	NEBNext High Fidelity	Platinum *Taq*
Template-switching rate	29.9%	36.4%	33.7%	30.3%	36.5%

Table 5. Template-switching rate experiment results – in detail.

dNTP	Company	Cat. #	Cost Per 0.4 µl Used in 20 µl PCR Reactions ($USD)
10 mM dNTP	Qiagen	201900	0.1

Table 6. List price calculation for dNTPs.

Disclaimer: In this product review, we mention and discuss several commercially available DNA polymerases and their corresponding suppliers. The inclusion of these proprietary DNA polymerases and vendors is not intended to reflect their importance, nor is it intended as an endorsement by Azenta Life Sciences. The DNA polymerases and suppliers mentioned are provided for informational and noncommercial use only. Any reference to any vendor, process, service or enzyme by trade name, trademark, or manufacturer or otherwise does not constitute or imply the endorsement, recommendation, favoring or approval of Azenta Life Sciences. These experiments represent only a handful of conditions; please note that different products will perform differently when conditions change. These data are intended as starting references only.