Reduced cycling times offer several advantages for routine PCR, but Fast PCR with wild-type enzymes typically saves time at the expense of success rates and consistency.

The novel and highly processive KAPA2G Fast DNA Polymerase and its uniquely formulated reaction buffer have been specifically engineered for Fast PCR. With extension times as short as 1 sec/cycle, PCR time can be reduced by up to 70% without compromising sensitivity, yields, or consistency.

Introduction

Significantly reduced PCR cycling time is an attractive proposition for laboratories performing routine PCR, as it offers increased throughput and shorter turnaround times. Several Fast PCR strategies have been developed in recent years. All of these strategies entail a reduction in the time dedicated to each step of the cycling profile (denaturation, annealing, and extension). In some cases, this has been combined with the improvement of heat transfer during PCR (through the use of significantly reduced reaction volumes and/or specialized thermocyclers and consumables), or by increasing the concentration of PCR reagents (particularly the DNA polymerase).

The amount of time that can be saved in each step of a PCR assay performed with conventional equipment and a wild-type DNA polymerase is limited by the intrinsic extension rate of the enzyme. For this reason, most Fast PCR protocols rely on dramatic reductions in denaturation and annealing times. While this approach may produce acceptable results for a subset of assays and templates, incomplete template denaturation and inefficient primer annealing often leads to reduced sensitivity, low yields of the target amplicon, or inconsistent amplification.

KAPA2G Fast is a second-generation DNA polymerase engineered specifically for Fast PCR through a process of molecular evolution. The improved processivity and specific activity of the enzyme translate to inherently faster extension rates than that of wild-type *Taq*. Fast PCR with KAPA2G Fast is therefore based on significant reductions in extension times, rather than on artificially shortened protocols. Combined with a unique reaction buffer formulated to facilitate primer annealing KAPA2G Fast offers reductions in total PCR cycling times of up to 70% without the risk of compromising performance or the need for specialized equipment or consumables. In addition to speed, KAPA2G Fast offers higher yields and sensitivity across a wide range of targets and template types than conventional (slow) PCR protocols employing wild-type *Taq*. The antibody-mediated HotStart ReadyMix formulation is specifically recommended for routine Fast PCR.

Results

To demonstrate the advantages of KAPA2G Fast in Fast PCR, five amplicons (150 – 626 bp, 42 – 84 % GC) were amplified from human genomic DNA. Results were compared to those obtained with other supplier's hot start *Taq* polymerase using conventional slow protocols. Results are summarized in Figure 1 and Table 1.

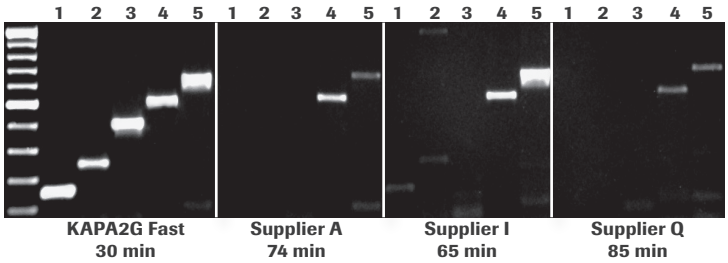

Figure 1. KAPA2G Fast outperforms wild-type *Taq* in routine PCR. Amplification of five human gene fragments using KAPA2G Fast HotStart ReadyMix or other supplier's hot start *Taq* formulations. All reactions (25 µL) contained 5 ng human genomic DNA. For amplicons with a GC content >65% (lanes 2 and 3), 7.5% DMSO was included. KAPA2G Fast reactions were set up as outlined on the next page. Other supplier's reactions were set up according to each manufacturer's instructions. A 3-step cycling profile (35 cycles) with 15 sec denaturation (95°C) and 15 sec annealing (60°C) per cycle was used for all enzymes. The extension time (72°C) was 1 sec/cycle for KAPA2G Fast and 60 sec/cycle for other suppliers. Initial denaturation/enzyme activation and final extension times were as recommended for each enzyme system. A fast-ramping Eppendorf Mastercycler epgradient S was used.

Table 1. PCR success rates and cycling times for KAPA2G Fast and other suppliers

Enzyme system	Success rate	Total cycling time
KAPA2G Fast	100%	30 min
Supplier A	40%	74 min
Supplier I	40 – 60%	65 min
Supplier Q	40%	85 min

Reaction conditions and cycling parameters

For optimal results using KAPA2G Fast it is imperative to follow the recommended reaction setup and cycling parameters given in Tables 2 and 3 and not to use cycling protocols designed for wild-type *Taq*. Recommended extension times (1 sec per cycle for amplicons ≤1 kb and 15 sec/kb per cycle for amplicons >1 kb) must be strictly adhered to. In addition, denaturation and annealing times must be limited to 10 – 15 sec/cycle. Longer annealing and extension times are likely to lead to non-specific amplification and smearing, whereas denaturation and annealing times <10 sec/cycle are likely to compromise performance (Figure 2).

Table 2. KAPA2G Fast HotStart ReadyMix reaction setup for routine Fast PCR

Reaction component	Final conc.	Per 25 µL reaction[1]
PCR grade water	-	Up to 25.0 µL
2X KAPA2G Fast HotStart ReadyMix[2]	1X	12.5 µL
100% DMSO (for amplicons with a GC content >65%)	5.0 – 7.5%	1.25 or 1.875 µL
Forward primer (10 µM)	0.5 µM	1.25 µL
Reverse primer (10 µM)	0.5 µM	1.25 µL
Template DNA[3]	1 – 50 ng	–

[1]For smaller reaction volumes, scale down all volumes proportionally. Do not perform reactions in volumes >25 µL.

[2]Contains $MgCl_2$ at 1X concentration of 1.5 mM. Additional $MgCl_2$ may be added if required for optimal performance with a specific primer-template combination.

[3]Start with 10 ng genomic DNA or 1 ng less complex DNA. Reduce the amount of template to eliminate non-specific amplification or smearing. Increase the amount of template to improve yields and/or sensitivity.

Table 3. KAPA2G Fast cycling parameters for routine Fast PCR

Cycling step	Temperature and time	
Initial denaturation[1]	1 – 3 min at 95°C	1
Denaturation[2]	10 – 15 sec at 95°C	x25 – 40 cycles[4]
Annealing[2]	10 – 15 sec at 60°C[3]	
Extension	1 sec at 72°C for amplicons ≤1 kb 15 sec/kb at 72°C for amplicons >1 kb	
Final extension[5]	0 – 0 min	1

[1]Use 3 min for genomic DNA and GC-rich DNA, and 1 min for less complex templates.

[2]Use 10 sec for slow-ramping cyclers (<1.5°C/sec heating and cooling) or for small reaction volumes (10 µL) and 15 sec for fast-ramping cyclers (≥1.5°C/sec heating and cooling), long (>1 kb) or GC-rich amplicons.

[3]The annealing temperature may be varied between 55° and 65°C to achieve optimal results with specific primer-template combinations.

[4]Start with 35 cycles for genomic DNA or 30 cycles for less complex templates.

[5]Only required if 3'-dA-tailing is essential for fragment analysis or cloning.

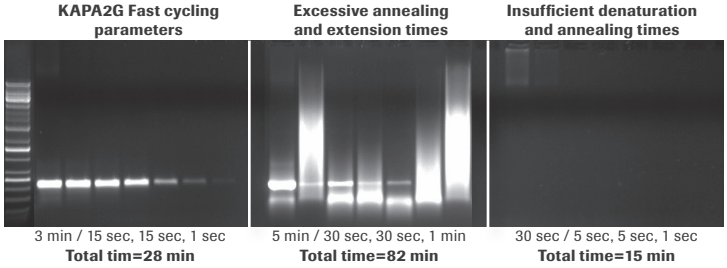

Figure 2. Typical results obtained with inappropriate cycling parameters for KAPA2G Fast. Amplification of a 454 bp fragment of the human cystic fibrosis transmembrane receptor (CFTR) gene from a 5-fold dilution series of human genomic DNA (51.6 ng – 3.3 pg, or 15,625 – 1 copies per 25 µL reaction) with KAPA2G Fast HotStart ReadyMix. Reactions were set up as outlined in Table 2. Three cycling protocols (35 cycles) were used to demonstrate the importance of using the correct cycling parameters for KAPA2G Fast: the correct KAPA2G Fast protocol (left), a protocol typically used for wild-type *Taq* (middle) and an "ultra-fast" protocol, such as those recommended by certain manufacturers of Fast PCR kits. For each protocol, the initial denaturation time is followed by the denaturation, annealing, and extension time used in each cycle. With the correct KAPA2G Fast protocol, the target fragment was successfully amplified from a single template copy. Excessive cycling times ("*Taq* protocol") resulted in inconsistent amplification, poor specificity and smearing. The "ultra-fast" protocol resulted in total reaction failure, primarily as a result of insufficient template denaturation.

Tips for successful Fast PCR

- Follow the reaction setup and cycling parameters recommended for KAPA2G Fast and not those for wild-type *Taq* or other Fast PCR reagents.
- Never anneal for less than 10 sec or more than 15 sec per cycle.
- To reduce non-specific amplification or smearing, reduce the annealing and extension times to those recommended for KAPA2G Fast, reduce the number of cycles, and/or reduce the concentration of template DNA.
- To improve yields of "difficult" short amplicons, increase the extension time to a maximum of 15 sec per cycle, increase the number of cycles and/or increase the concentration of template DNA.
- Do not use reaction volumes >25 µL.
- Sufficient denaturation of template DNA is important, especially for targets with a high GC-content. Although KAPA2G Fast HotStart only needs 30 sec for activation, an initial denaturation of 3 min is recommended for complex genomic templates.
- KAPA2G Buffer A contains $MgCl_2$ at a 1X concentration of 1.5 mM. Some primer-template combinations may require additional $MgCl_2$ for optimal results. Determine the optimal $MgCl_2$ concentration for an assay by performing a $MgCl_2$-gradient PCR.
- Primer and template quality is important for successful Fast PCR. Always store and dilute primers and DNA in 10 mM Tris-HCl, pH 8.0 – 8.5 and not in water.
- Please refer to the Application Note: **Multiplex PCR** for specific recommendations on Fast Multiplex PCR using KAPA2G Fast.

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