



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.

True high-performance, routine Fast PCR requires the combination of an enzyme with intrinsically faster extension rates than wild-type *Taq*, with a state-of-the-art thermal cycler. KAPA2G Fast HotStart ReadyMix, containing the novel and highly processive KAPA2G Fast DNA Polymerase, and the G-Storm range of thermal cyclers offers such a solution.

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).

PCR cycling time is determined by the extension rate of the DNA polymerase used, as well as the ramp (heating and cooling) rates of the thermal cycler. “Fast” PCR performed with conventional equipment and a wild-type DNA polymerase therefore relies on dramatic reductions in denaturation and annealing times. Whilst this 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. Even with this (flawed) approach, it is difficult to reduce the total cycling time for a standard 3-step PCR (35 cycles) to <45 min on a slow ramping cycler.

True high-performance, routine Fast PCR requires the combination of an intrinsically faster enzyme, with a thermal cycler capable of high ramp rates.

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 higher PCR success rates across a wide range of amplicon types in PCR cycling times <50% than those achievable with wild-type *Taq*.

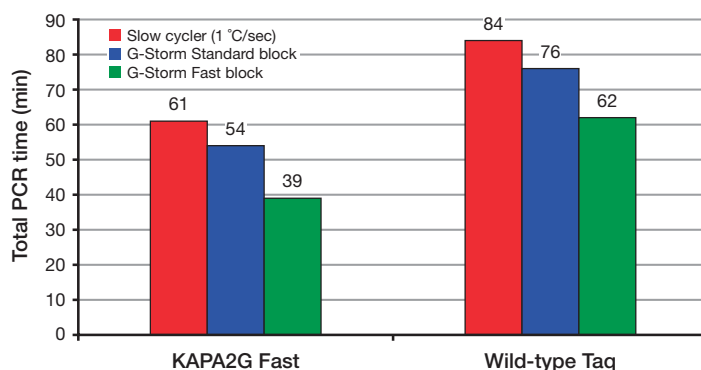


Figure 1. KAPA2G Fast HotStart ReadyMix and G-Storm thermal cyclers offer savings of >50% in total PCR cycling time.

Total PCR cycling times (in min) for KAPA2G Fast HotStart ReadyMix (left) or wild-type *Taq* (right), using recommended cycling parameters and a G-Storm thermal cycler with a fast (green) or standard (blue) block, or a slow ramping (1 °C/sec) PCR instrument. The combination of the engineered KAPA2G Fast DNA Polymerase and the state-of-the-art G-Storm cyclers offers reductions of >50% in the total cycling time achievable with typical PCR equipment and wild-type reagents.

The G-Storm range of thermal cyclers from GRI offers the ideal solution for high-performance Fast PCR. The advanced features of these cyclers include high ramp rates, tight control of thermal uniformity and accuracy using multisensor technology, and ease-of-use. Instruments are available in a range of configurations and block types, with the fast block capable of ramp rates as high as 6 °C/sec. A gradient feature for protocol optimization is a standard feature of all blocks. Active Sample Cooling (ACS) ensures that samples are kept at 4 °C at the start of a protocol – until the heated lid has reached the target temperature. This reduces the risk of spurious primer annealing events that may lead to non-specific amplification or low reaction efficiency. Ease-of-use is ensured by a full-colour, 6.4 inch VGA TFT touch screen display, as well as a USB port, which allows transfer of protocols via a memory stick.

For routine Fast PCR, KAPA2G Fast HotStart ReadyMix and a G-Storm cycler with a fast block offers unrivalled speed, performance and convenience.

Fast PCR: G-Storm Thermal Cyclers

Methods and results

To demonstrate the advantages of KAPA2G Fast HotStart ReadyMix and G-Storm thermal cyclers in high-performance, routine Fast PCR, 24 amplicons (316 – 982 bp, 27 – 67 %GC) were amplified from human genomic DNA, using the recommended Fast PCR protocol. Results were compared to those obtained with hot start formulations of wild-type *Taq* from two competitors, using either a conventional (slow) *Taq* protocol or fast cycling parameters. Results are summarized in **Figure 1** and **Table 1**.

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

Enzyme	Protocol & cycler	Total cycling time	Success rate
KAPA2G Fast HotStart ReadyMix	Fast G-Storm Fast block	39 min	100%
	"Taq" Slow (1 °C/sec)	N/A	N/A
Competitor I (wild-type Taq)	Fast G-Storm Fast block	39 min	55%
	"Taq" Slow (1 °C/sec)	84 min	96%
Competitor Q (wild-type Taq)	Fast, G-Storm Fast block	51 min*	8%
	"Taq" Slow (1 °C/sec)	96 min*	79%

* This hot start formulation requires a 15 min initial re-activation time, as compared to the 3 min used for KAPA2G Fast HotStart ReadyMix and Competitor I.

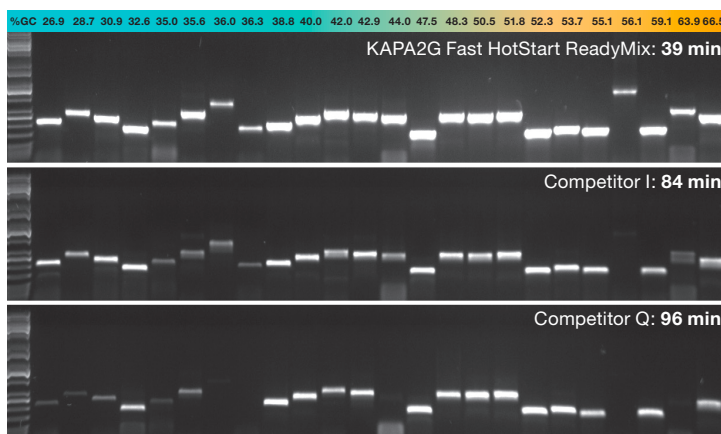


Figure 1: KAPA2G Fast HotStart ReadyMix and G-Storm thermal cyclers offer superior speed and performance in routine Fast PCR.

24 amplicons (316 – 982 bp, 27 – 67% GC) were amplified from human genomic DNA (10 ng per 25 µl reaction), using KAPA2G Fast HotStart ReadyMix (top) or hot start formulations of wild-type *Taq* from Competitors I (middle) and Q (bottom). KAPA2G Fast HotStart reactions were set up as outlined in Table 2. Competitor reactions were set up using their respective reaction buffers, and contained the same final dNTP, MgCl₂ and enzyme concentrations as those supplied by the 2X KAPA2G Fast HotStart ReadyMix (0.2 mM each dNTP, 1.5 mM MgCl₂ and 0.5 U enzyme/25 µl reaction). All reactions contained forward and reverse primers at a final concentration of 0.5 µM. Results shown were obtained with KAPA2G Fast HotStart ReadyMix and G-Storm thermal cycler and fast block (using the recommended cycling parameters outlined in Table 3, 35 cycles). Competitor reactions were performed using a slow instrument (±1 °C/sec ramp rate) and the following slow "Taq" protocol: 3 min (Competitor I) or 15 min (Competitor Q) initial denaturation (95 °C); 35 cycles of 30 sec denaturation (95 °C), 30 sec annealing (60 °C) and 1 min extension (72 °C) per cycle. Half of each reaction product was electrophoresed in a 1% TBE-agarose gel. Amplicons were loaded in order of increasing GC content, from 27% (blue, left) to 67% (orange, right). Reaction products generated with competitor enzymes using the Fast PCR protocol and G-Storm thermal cycler with fast block are not shown due to the poor success rates achieved with the wild-type enzymes in Fast PCR (see Table 1).

Reaction conditions and cycling parameters

The recommended reaction setup for KAPA2G Fast HotStart ReadyMix reactions, and cycling parameters for G-Storm thermal cyclers are given in **Tables 2 and 3**. For more information, please refer to the KAPA2G Fast HotStart ReadyMix Technical Data Sheet or other technical resources available at <http://www.kapabiosystems.com/products/name/kapa2g-fast-pcr-kits>.

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
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 MgCl₂ at 1X concentration of 1.5 mM. Additional MgCl₂ 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 using G-Storm thermal cyclers.

Cycling step	Temperature & time	
Initial denaturation ¹	1 – 3 min at 95 °C	1
Denaturation ²	10 – 15 sec at 95 °C	x 25 – 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 – 10 min at 72 °C	1

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

² Use 10 sec for the standard block or for small reaction volumes (10 µl) and 15 sec for the fast block, and for 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. Primers with a lower optimal annealing temperature (but not lower than 45 °C) may be used, but are not recommended for routine Fast PCR.

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.

For more information on KAPA2G Fast HotStart ReadyMix or other Kapa Biosystems products, please contact:

sales@kapabiosystems.com, or visit
www.kapabiosystems.com

For more information on the G-Storm range of thermal cyclers or other GRI products, please contact:

gri@gri.co.uk, or visit **www.gri.co.uk**