

Reproducible amplification of a broad range of targets currently relies on the optimization of individual assays, which requires multiple PCR protocols and reagents.

KAPA2G Robust HotStart ReadyMix contains a novel DNA polymerase engineered for improved processivity and tolerance to common PCR inhibitors, and offers consistent amplification, high yields and wide coverage of both easy and challenging amplicons. The versatility of the enzyme and its proprietary reaction buffer allows for the simplification of PCR workflows, through the consolidation of reagents and protocols, while increasing success rates and turnaround time.

Introduction

Consistent amplification of a broad range of targets—covering the full spectrum of AT- and GC-rich content—using a single set of reaction conditions and one cycling protocol is the ultimate goal of any lab performing routine PCR. This concept of “Single Protocol PCR” has proven impractical due to the inherent limitations of wild-type *Taq* DNA Polymerase—most notably its inefficient amplification of challenging templates and narrow range of optimal PCR conditions. In order to improve PCR success rates and minimize reaction failures, many laboratories are forced to segregate difficult targets from “easy” targets and optimize individual reactions. The resulting subset of specialized assays disrupts workflows and increases turnaround time and cost by requiring a myriad of PCR reagents, reaction conditions and cycling protocols.

KAPA2G Robust DNA Polymerase is a novel enzyme, engineered for higher processivity and improved tolerance to common PCR inhibitors through a process of molecular evolution. The unique features of the enzyme supports versatile and robust amplification of a wide range of template and amplicon types. Combined with a proprietary reaction buffer that facilitates primer annealing, successful amplification of both AT- and GC-rich amplicons can finally be achieved using a simplified reaction setup and a single, fast cycling protocol that typically requires 20 – 50% less cycling time than protocols designed for wild-type DNA polymerases.

KAPA2G Robust HotStart ReadyMix is a ready-to-use cocktail containing all components for “Single Protocol PCR”, except for primers and template. The 2X ReadyMix contains KAPA2G Robust HotStart DNA Polymerase in its proprietary reaction buffer, dNTPs (0.2 mM each dNTP at 1X), MgCl₂ (2 mM at 1X) and stabilizers. The enzyme is supplied with an antibody-based hot start for improved yield and specificity. Not included in the 2X ReadyMix is DMSO, which should be added to a final concentration of 5% for the amplification of DNA fragments with a GC content >70%.

Methods and results

To illustrate the feasibility of “Single Protocol PCR” and demonstrate the expanded range of targets that can be amplified successfully using KAPA2G Robust HotStart ReadyMix, 96 human primer sets, defining amplicons ≤ 1 kb with a GC content ranging from 25 – 85%, were employed. As shown in Figure 1, an overall success rate of 96% was achieved using the standardized reaction setup and single, fast cycling profile outlined below. Compared to wild-type *Taq*, KAPA2G Robust HotStart ReadyMix achieved:

- a 30% higher overall success rate, in approximately 50% of the total reaction time,
- consistently high success rates across the entire spectrum of GC contents, whereas wild-type *Taq* yielded poor results with AT- and GC-rich amplicons (<30 and >60% GC, respectively),
- higher yields for most amplicons (see Figure 2).

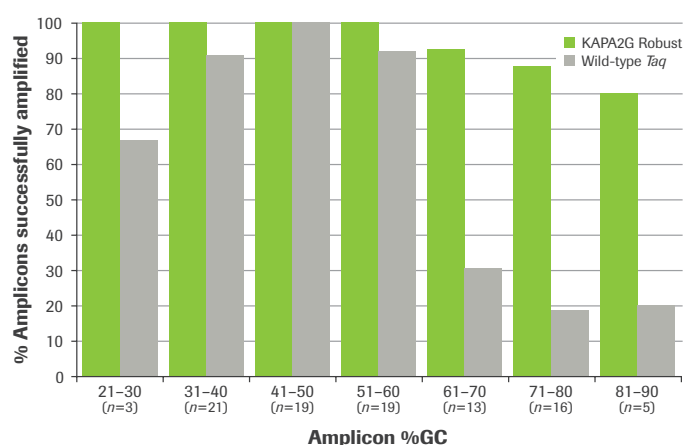


Figure 1. Overview of “Single Protocol PCR” results obtained with KAPA2G Robust HotStart ReadyMix (orange) or wild-type *Taq* (grey). A total of 96 amplicons were amplified from human genomic DNA, using the reaction setup and cycling protocol given on the next page for KAPA2G Robust HotStart ReadyMix, or reaction conditions outlined in Figure 2 for wild-type *Taq*. The overall success rate achieved with KAPA2G Robust HotStart ReadyMix (in a total cycling time of 36 min) was 96%, compared to 66% achieved in >1 hour cycling time with wild-type *Taq*. The data clearly illustrates the expanded amplification range of KAPA2G Robust: high success rates were achieved across the full spectrum of GC contents, whereas wild-type *Taq* showed poor results with AT-rich amplicons and amplicons with a GC content >60%. Numbers in brackets indicate the number of primer sets representing each subset of amplicons (by GC content) in the experiment.

The standard reaction setup for KAPA2G Robust HotStart ReadyMix reactions is given in Table 1. For reactions corresponding to amplicons with a GC content >70%, DMSO should be included at a final concentration of 5%. Cycling conditions are given in Table 2. For optimal results, it is important that templates are properly denatured and that recommended annealing times are not exceeded. For more information about reaction setup and cycling parameters, please refer to the **KAPA2G Robust HotStart ReadyMix Technical Data Sheet**.

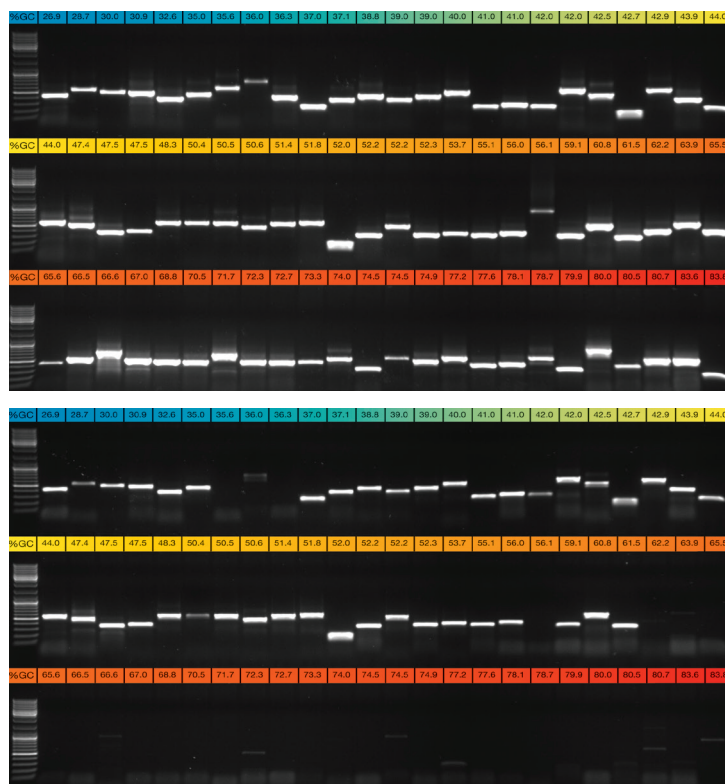


Figure 2: Typical results obtained in “Single Protocol PCR” using KAPA2G Robust HotStart ReadyMix (top) or wild-type *Taq* (bottom). Half of each of the PCR products obtained with 72 of the 96 primer sets used in this study were electrophoresed in a 1% TBE-agarose gel. Amplicons were loaded in order of increasing GC content, with the lowest GC content (27%, blue) at the top left hand side and the highest GC content (84%, red) at the bottom right hand side of each composite gel image. Primers selected for this study had variable primer lengths, sequence composition, theoretical melting temperatures and other design features. Some primers contained 5'-tails for post-PCR sequencing using M13 or other standard sequencing primers. KAPA2G Robust HotStart ReadyMix reactions (25 μ L) were performed as outlined in Tables 1 and 2. Wild-type *Taq* reactions (25 μ L, containing 0.5 U *Taq* per reaction) were performed in *Taq* reaction buffer (1.5 mM MgCl₂ at 1X), using the same final primer and dNTP concentrations as for KAPA2G Robust. All reactions contained 25 ng human genomic DNA. 5% DMSO was included in all reactions (KAPA2G Robust and *Taq*) targeting amplicons with a GC content >70%

Table 1. KAPA2G Robust HotStart ReadyMix reaction setup

| Reaction component | Final conc. | Per 25 µL reaction |
|--|--------------|--------------------|
| PCR grade water | – | Up to 25.0 µL |
| 2X KAPA2G Robust HotStart ReadyMix | 1x | 12.5 µL |
| Forward primer (10 µM) | 0.5 µM | 1.25 µL |
| Reverse primer (10 µM) | 0.5 µM | 1.25 µL |
| 100% DMSO (for amplicons with a GC content >70%) | 5% | 1.25 µL |
| Template DNA | 10 – 100 ng* | – |

*Use 10 ng as a starting point. The amount of template for individual primer-template combinations may be increased or decreased to improve yields and/or specificity.

Table 2. KAPA2G Robust HotStart ReadyMix cycling parameters

| Cycling step | Temperature and time | |
|-----------------------------------|----------------------------------|------------|
| Initial denaturation ¹ | 1 – 3 min at 95°C | |
| Denaturation ² | 10 – 15 sec at 95°C | x35 cycles |
| Annealing ² | 10 – 15 sec at 60°C ³ | |
| Extension ² | 10 – 15 sec at 72°C | |
| Final extension ⁴ | 0 – 10 min | |

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

²Use 15 sec for fast ramping cyclers ($\geq 3^\circ\text{C}/\text{sec}$) and 10 sec for slow ramping cyclers or smaller reaction volumes.

³The annealing temperature may be varied between 55°C and 65°C to achieve optimal yields and specificity with multiple primer sets.

⁴Only required if 3'-dA-tailing is essential for fragment analysis or cloning.

Conclusions

The unique performance characteristics of the second-generation KAPA2G Robust DNA Polymerase offers significant benefits for routine and high-throughput PCR. Existing workflows may be simplified and turnaround times and costs reduced through the replacement of a variety of PCR reagents with a single master mix, and the consolidation of different cycling protocols into a single, fast protocol. The number of primer-template combinations that fail with the above “Single Protocol PCR” approach, and cannot be improved through adjustment of basic reaction parameters (e.g., template concentration or the addition of DMSO), is likely to be <5%. This represents a dramatic reduction in the number of assays to be optimized individually when using wild-type *Taq*. For optimization of recalcitrant assays that fail with the “Single Protocol PCR” approach, KAPA2G Robust HotStart PCR Kits are recommended.

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9115 Hague Road
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sequencing.roche.com

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