Ready-to-use PCR mixes with tracking dye allow for quick, convenient reaction setup and analysis. Mixes containing wild-type *Taq* offer few other advantages in routine end-point PCR.

KAPA2G Fast ReadyMix with dye contains the novel and highly processive KAPA2G Fast DNA Polymerase in a convenient ready-to-use format. The unique properties of this engineered enzyme allow for significantly reduced PCR cycling times without sacrificing performance, providing an industry-leading solution for fast and convenient routine end-point PCR.

Introduction

Routine PCR laboratories—whether commercial, diagnostic, or academic—rely on high success rates and competitive turnaround times. For these reasons, ready-to-use PCR mixes have been widely incorporated in routine PCR workflows. These PCR mixes not only allow for quick and convenient reaction setup, but reduce the likelihood of variation or error during reaction setup that may lead to failures or repeat tests. In workflows where end-point PCR products are analyzed by agarose gel electrophoresis, PCR mixes containing tracking dye(s) additionally reduce the handling of PCR products prior to analysis. PCR master mixes may be prepared in-house from separate reagents and validated. However, the additional costs associated with commercial ready-to-use PCR mixes are easily offset by their guaranteed lot-to-lot consistency and higher performance.

PCR master mixes that contain wild-type enzymes offer few advantages other than quick and convenient reaction setup and analysis, as the performance of these reagents are limited by the inherent characteristics of the wild-type enzyme. To improve performance (yields and sensitivity) and success rates, commercially available ready-to-use PCR mixes containing wild-type enzymes are often formulated with a higher concentration of enzyme than would typically be used in a standard reaction set up from individual components. This offers clear advantages to the end-user. However, some causes of PCR inefficiency cannot be solved by simply increasing the enzyme concentration. High enzyme concentrations also increase the potential for non-specific amplification, which may complicate the interpretation of results.

KAPA2G Fast ReadyMix with dye offers an industry-leading solution for fast and convenient routine end-point PCR. This ready-to-use PCR mix contains the novel and highly processive KAPA2G Fast DNA Polymerase, engineered through a process of molecular evolution for high-performance Fast PCR. The unique properties of the enzyme allow for significantly reduced PCR cycling times (Figure 1). This contributes considerably to improved turnaround times—in addition to the advantages associated with the ready-to-use format with tracking dye. Another unique feature of KAPA2G Fast ReadyMix with dye is its proprietary buffer, designed to improve primer annealing and reaction efficiency. This allows for the successful amplification of a broader range of amplicon types (using a single annealing temperature), and translates to higher yields and sensitivity in selected assays.

KAPA2G Fast ReadyMix with dye contains a dense component for direct loading of PCR products, as well as two tracking dyes that give the PCR mix a green colour. During electrophoresis, the dye mix resolves into a slowly migrating blue and faster migrating orange dye front. In 1% agarose gels, the blue dye co-migrates with dsDNA fragments approximately 5 kb in size, and the orange dye with fragments <100 bp.

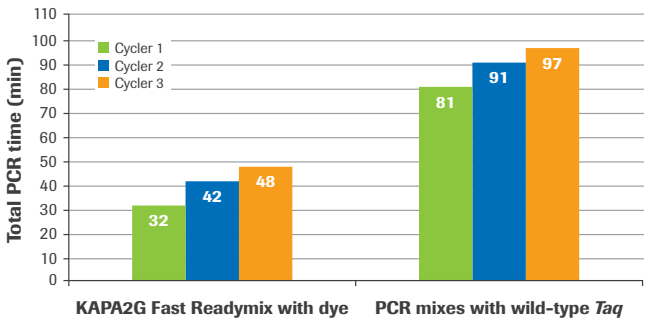

Figure 1. KAPA2G Fast ReadyMix with dye offers savings of >50% in total PCR cycling time in routine end-point PCR. Total PCR cycling times (in min) for the routine amplification of amplicons ≤1 kb from human genomic DNA, using KAPA2G Fast ReadyMix with dye (left) or ready-to-use PCR mixes containing *Taq* DNA polymerase (right), and three fast thermocyclers (with ramp rates ranging from >1.5° to 6°C/sec). The times given are for a standard programme consisting of 3 min intial denaturation (95°C), followed by 35 cycles of 15 sec denaturation (95°C), 15 sec annealing (60°C) and 5 sec extension (72°C), for KAPA2G Fast ReadyMix, or 30 sec denaturation (95°C), 30 sec annealing (60°C) and 1 min extension (72°C) for mixes containing *Taq*. The data shows a reduction of >50% in total PCR cycling time (irrespective of the cycler used), which considerably contributes to improvements in turnaround time and thermocycler capacity.

Methods

To demonstrate the industry-leading performance of KAPA2G Fast ReadyMix with dye in routine end-point PCR, two experiments were conducted.

In the first experiment, 15 human amplicons (222 – 626 bp, GC-contents between 27% and 75%), were amplified using KAPA2G Fast ReadyMix with dye and three competitor products: GoTaq® Green Master Mix (Promega), REDTaq® ReadyMix™ PCR Reaction Mix (Sigma) and DreamTaq™ Green PCR Master Mix (Fermentas). The Promega and Sigma products contain wild-type *Taq* DNA Polymerase, whereas the Fermentas mix contains an "enhanced *Taq*". For all products, reactions (25 µL) contained 1X PCR mix, 10 ng human genomic DNA, and primers at a final concentration of 0.5 µM each. For amplicons with a GC contents >60%, DMSO was included at a final concentration of 5%. Cycling was performed according to the recommended cycling parameters for each product, using a fast ramping cycler. For KAPA2G Fast, an extension time of 5 sec per cycle is recommended for routine fast PCR on such cyclers. For optimal yields and sensitivity, this may be increased to 15 sec per cycle. The latter was used in this case. Half of each reaction product was analyzed directly in a 1% TBE-agarose gel and results visualized by ethidium bromide staining. Results are given in Figure 2. These clearly illustrate that KAPA2G Fast ReadyMix with dye outperformed all competitor PCR mixes, by achieving yields equivalent to those obtained with competitor products, in 54% shorter PCR time. Furthermore, KAPA2G Fast ReadyMix also showed the best specificity of amplification, and was one of only two products that achieved a 100% success rate in this experiment.

In the second experiment, a template dilution series PCR was performed with a human amplicon (327 bp, 52% GC), using KAPA2G Fast ReadyMix with dye and the above mentioned competitor products. Reactions (25 µL) contained 1X PCR mix, primers at a final concentration of 0.5 µM each and human genomic DNA as indicated (six 5-fold serial dilutions, covering the range of 50 ng – 16 pg template per reaction). Cycling was performed according to the recommended cycling parameters for each product, using a fast ramping cycler. For KAPA2G Fast, an extension time of 5 sec per cycle was used. Competitor reactions were performed in duplicate: one set with the recommended cycling parameters, and one set with the KAPA2G Fast protocol. Half of each reaction product was analyzed directly in a 1% TBE-agarose gel and results visualized by ethidium bromide staining. Results (Figure 3) clearly illustrate that KAPA2G Fast ReadyMix with dye is capable of equivalent yields and sensitivity (in less than half of the total reaction time) than competitor products. Additionally, it shows that competitor ready-to-use PCR mixes containing wild-type *Taq* are unable to match the turnaround times offered by the engineered product.

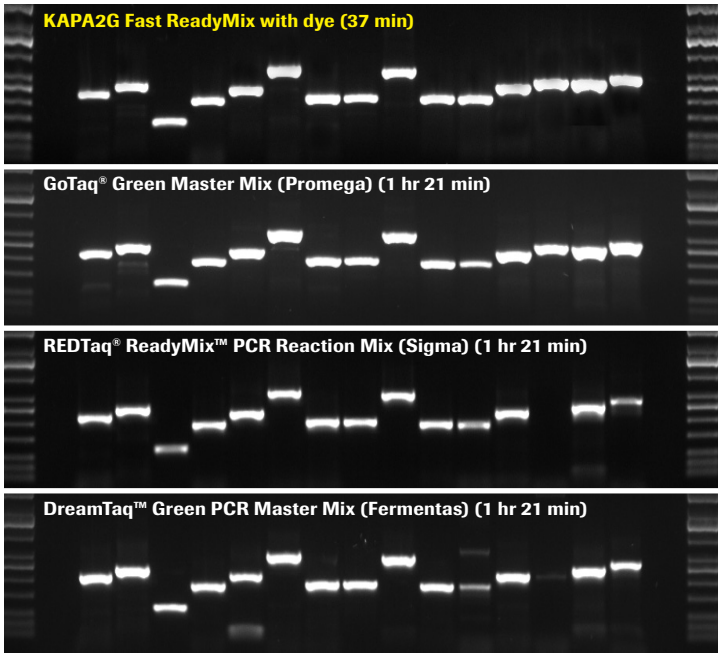

Figure 2: Amplification of 15 human amplicons using KAPA2G Fast ReadyMix with dye or competitor ready-to-use PCR mixes containing *Taq*. Reactions were set up and as described in **Methods**. Cycling protocols consisted of an initial denaturation of 3 min (95°C), followed by 35 cycles of 15 sec denaturation (95°C), 15 sec annealing (60°C) and 15 sec extension (72°C) for KAPA2G Fast ReadyMix with dye, or 30 sec denaturation (95°C), 30 sec annealing (60°C) and 1 min extension (72°C) for competitor products. The total cycling time was 37 min and 1 h 21 min for KAPA2G Fast ReadyMix with dye and competitors, respectively.

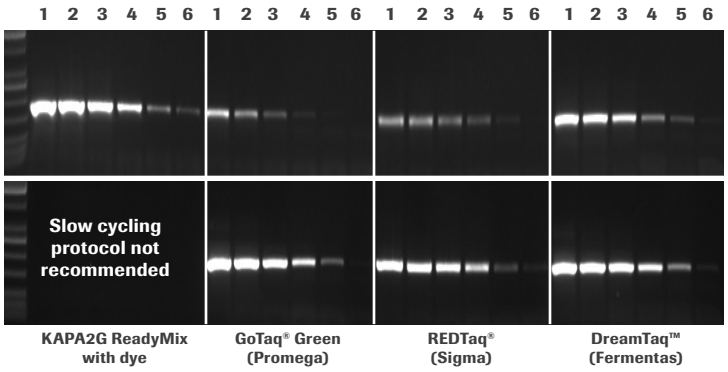

Figure 3: Amplification of a 327bp human amplicon from a 5-fold serial dilution of human genomic DNA, using KAPA2G Fast ReadyMix with dye (fast cycling protocol) and competitor products (standard or fast cycling protocols). Reactions were set up as described in Methods. The amount of template DNA in each reaction was as follows: 1=50 ng, 2=10 ng, 3=2 ng, 4=400 pg, 5=80 pg and 6=16 pg. Cycling protocols consisted of an initial denaturation of 3 min (95°C), followed by 35 cycles of 15 sec denaturation (95°C), 15 sec annealing (60°C) and 5 sec extension (72°C) for the KAPA2G Fast cycling protocol (top), or 30 sec denaturation (95°C), 30 sec annealing (60°C) and 1 min extension (72°C) for the slow, wild-type enzyme protocol (bottom). The total cycling time was 48 min and 1 h 37 min for the fast and slow cycling protocols, respectively.

Conclusion

KAPA2G Fast ReadyMix with dye offers quick and convenient reaction setup and analysis of reaction products. In addition, the engineered KAPA2G Fast DNA Polymerase offers significantly shorter PCR cycling times, thereby offering industry-leading performance in routine end-point PCR over ready-to-use PCR mixes containing wild-type *Taq*.

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