High-throughput qPCR using probe-based chemistries is a demanding application that requires consistent and reproducible results from low-volume, high-speed assays.

The combination of the Eppendorf Mastercycler® ep *realplex*^{4} S real-time PCR instrument and KAPA PROBE FAST qPCR Kits from Kapa Biosystems provides an industry-leading solution for high-performance, fast qPCR without compromising reaction efficiency and reproducibility.

Introduction

In contrast to sequence-nonspecific intercalating dyes that bind indiscriminately to double-stranded DNA (e.g. SYBR® Green I), sequence-specific fluorogenic probe chemistries only quantify the accumulation of specific PCR product. This improved specificity eliminates the need for post-PCR melt curve analysis and permits real-time multiplexing. Common probe chemistries include TaqMan®, FRET probes, scorpion probes, and molecular beacons.

Standard probe-based qPCR cycling protocols take between 1 hr 10 min and 1 hr 30 min, depending on the ramp rate and data acquisition times of the specific qPCR instrument used (**Figure 1**). Faster cycling protocols (~30 min) that do not compromise reaction efficiency typically require instrumentation with increased thermal ramp rates, uniform heating across the block, and specially formulated reagents.

The Eppendorf *realplex*^{4} S real-time PCR system features a super-fast, Peltier-controlled thermoblock that heats up at 6 °C/sec and cools down at 4 °C/sec, which allows completion of a 40-cycle qPCR reaction in less than 1 hour. These Peltier units also ensure accurate temperature control as well as high block homogeneity, which are essential to high-quality qPCR reactions. The optical module comprises an array of 96 LEDs that are pre-selected and equalized so that they excite all wells uniformly. This setup also eliminates the requirement for ROX reference dye that is used to normalize fluorescent signals by many conventional qPCR instruments. The *realplex*^{4} also utilizes the Photo Multiplier Tube (PMT) for signal detection, which is the most sensitive and affordable detection technique currently available. In addition, this four-channel system provides full multiplex flexibility through emission filters corresponding to 520/550/580/605 nm.

KAPA PROBE FAST qPCR Kits from Kapa Biosystems contain a ready-to-use master mix for highly sensitive and accurate real-time PCR using sequence-specific probe chemistries. The kits are compatible with a variety of probe chemistries including TaqMan®, FRET probes, scorpion probes, and molecular beacons. KAPA PROBE FAST qPCR Kits have been specially formulated for high-speed without compromising reaction efficiency and reproducibility. Kits are optimized for versatility and suitable for a broad range of applications including gene expression, SNP genotyping, and multiplexing.

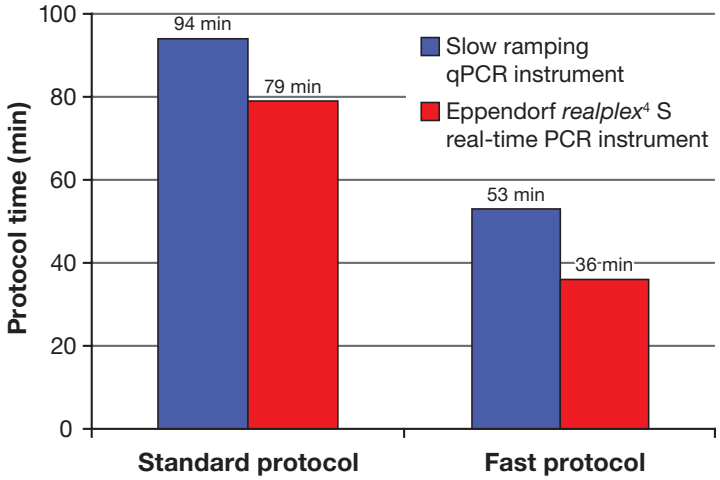

Figure 1: Total qPCR times for either a standard reaction protocol (10 min at 95 °C activation, 40 cycles of 15 sec at 95 °C and 60 sec at 60 °C) or a fast protocol (3 min at 95 °C activation, 40 cycles of 3 sec at 95 °C and 20 sec at 60 °C). Reactions were performed on a slow ramp rate qPCR thermocycler (avg. ramp rate 1°C/sec) and the fast Eppendorf *realplex*^{4} S real time PCR system (6 °C/sec heating and 4 °C/sec cooling).

KAPA PROBE FAST qPCR Kits are supplied with an antibody-based hot start, enabling a short initial activation hold time at 95 °C. PCR protocols using KAPA PROBE FAST are based on reduced extension times that allow for a significant reduction in PCR cycling time without the risk of compromising reaction performance.

Methods

To demonstrate the ability to perform fast qPCR cycling without compromising performance, a set of five 10-fold serial dilutions of human genomic DNA (hgDNA) was amplified using either a standard or fast cycling protocol. Amplification of the 74 bp hApoB100 amplicon was performed in full-skirted Eppendorf Twin.tec plates with optical

clear heat-sealing film using the Eppendorf *realplex*^{4} S real-time PCR system.

The resulting log amplification plots and standard curves for reactions performed using the standard protocol (**Figure 2**) and fast protocol (**Figure 3**) indicate that both the C_T and efficiency of the qPCR reactions have not been compromised as a result of using fast cycling protocols relative to standard cycling protocols (for a description of each protocol see **Figure 1**). The fast protocol results in a time saving of 43 minutes relative to the slow protocol when performed on the Eppendorf *realplex*^{4} S real-time PCR system.

Reduced running costs, particularly in combination with increased throughput, are always desirable. To illustrate the ability to use reduced reagent volumes with comparable results, three different reaction volumes (5 µl, 10 µl and 20 µl) were compared using the fast protocol (**Figure 4**). All three reaction volumes resulted in highly consistent amplification across a set of five 10-fold serial dilutions of hgDNA when cycled using the fast cycling protocol. KAPA PROBE FAST qPCR Kits are formulated to maximize probe signal, delivering high levels of fluorescence even at extremely low reaction volume. In conjunction with the benefit of reduced evaporation during qPCR by using the Eppendorf *realplex*^{4} S real-time PCR system, comparable results were obtained using low-volume qPCR reactions.

Conclusion

High performance, fast qPCR using probe-based chemistries requires a real-time instrument capable of extremely fast ramp rates with excellent uniformity across all samples, and a qPCR reagent that has been formulated for high-speed and sensitivity. The combination of Eppendorf *realplex*^{4} S real-time PCR system and KAPA PROBE FAST qPCR Kits from Kapa Biosystems provides an industry-leading solution for high-performance, fast qPCR without compromising reaction efficiency and reproducibility. In addition, the ability to perform low-volume qPCR can reduce running costs while maintaining high quality results.

References

Lawyer, F.C. *et al.*, High-level expression, purification and enzymatic characterization of full-length *Thermus aquaticus* DNA Polymerase, and a truncated form deficient in 5' to 3' exonuclease activity. PCR Methods Appl. 2, 275-287 (1993).

For more information on the Eppendorf Mastercycler® ep *realplex*^{4} S real-time PCR instrument or Kapa Biosystems KAPA PROBE FAST qPCR Kits please contact your respective local sales representative or visit:
www.eppendorfna.com or **www.kapabiosystems.com**.

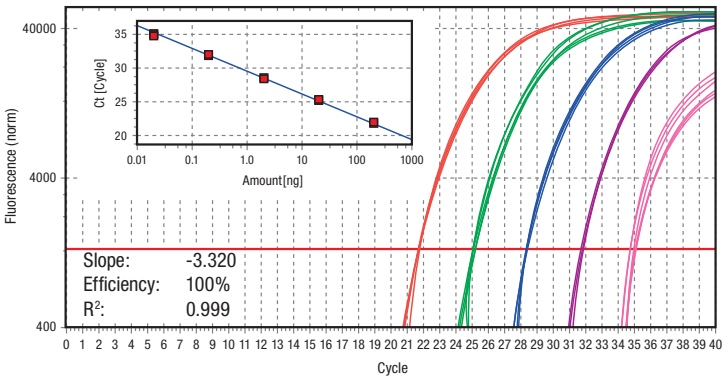

Figure 2: Standard cycling protocol (run time = 79 min). Data showing the log amplification plot across a set of five 10-fold serial dilutions of hgDNA ranging from 200 ng to 20 pg per reaction, using a standard 2-step cycling protocol (10 min at 95 °C activation, 40 cycles of 15 sec at 95 °C and 1 min at 60 °C). 20 µl reactions were performed using KAPA PROBE FAST qPCR Master Mix on the Eppendorf *realplex*^{4} S real-time PCR system. The data represents 7 replicates for each DNA dilution. The hApoB100 assay targets a 74 bp amplicon.

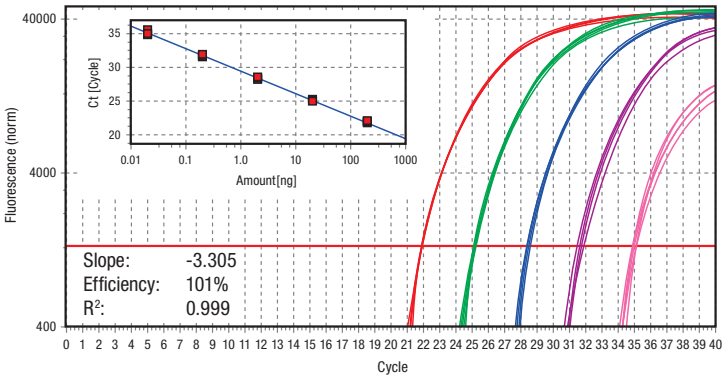

Figure 3: Fast cycling protocol (run time = 36 min). Data showing the log amplification plot across a set of five 10-fold serial dilutions of hgDNA ranging from 200 ng to 20 pg per reaction, using a fast 2-step cycling protocol (3 min at 95 °C activation, 40 cycles of 3 sec at 95 °C and 20 sec at 60 °C). 20 µl reactions were performed using KAPA PROBE FAST qPCR Master Mix on the Eppendorf *realplex*^{4} S real-time PCR system. The data represents 7 replicates for each DNA dilution. The hApoB100 assay targets a 74 bp amplicon.

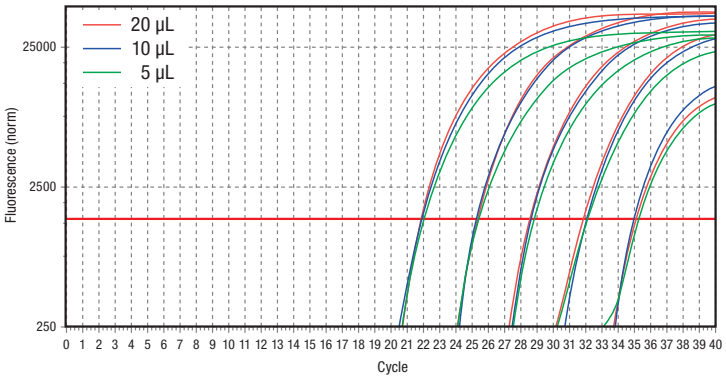

Figure 4: Comparison of different qPCR reaction volumes. Similar results were obtained when either 20 µl, 10 µl or 5 µl reaction volumes were used in the hApoB100 assay across a set of five 10-fold serial dilutions of hgDNA ranging from 200 ng to 20 pg per reaction, using a fast 2-step protocol. Reactions were performed using KAPA PROBE FAST qPCR Master Mix on the Eppendorf *realplex*^{4} S real-time PCR system. The data represents a single reaction at each reaction volume and DNA dilution. The hApoB100 assay targets a 74 bp amplicon.