# Q qPCR Cycler & PerfeCTa SYBR Green FastMix

## Significantly reduce time to results by 34% with no loss in assay efficiency, sensitivity or repeatability

### INTRODUCTION

For this application note, we demonstrate the performance of the PerfeCTa SYBR Green Fastmix on the Q with varying input amounts (10 pg – 1 ng), reaction volumes (5 – 20 µl) and cycling parameters with the goals of identifying peak performance and fast cycling conditions effective for a range of target assays.

### MATERIALS AND METHODS

All trials were conducted on a single Q instrument with PerfeCTa® SYBR® Green Fastmix® (Quantabio cat# 95072). Oligonucleotide primers were obtained from IDT and diluted in low-EDTA TE buffer for final primer concentration of 300 nM each in all reactions. cDNA was generated from 1 µg UHR RNA using qScript cDNA Synthesis Kit (Quantabio cat#95047) and used at the indicated concentrations. Plasmid DNA template containing target sequence was generated in-house as a stock concentration of $10^8$ copies/µl and further diluted over a 5-log range in low-EDTA TE buffer. Data was analyzed using the Q software (v1.0.0).

### RESULTS

Initial performance was evaluated using standard cycling protocols with run times of ~60 minutes. Multiple replicate assays over six distinct runs using a low input amount of 10 pg cDNA showed close agreement among Cq values (Figure 1A).

Assays on 2-fold and 1.2-fold dilution series of cDNA to test quantitative sensitivity yielded amplification curves with the expected Cq differences around 1 cycle and 0.263 cycles, respectively (Figure 1B). These results illustrate the high reliability and repeatability of data generated with PerfeCTa SYBR Green Fastmix on the Q under standard cycling conditions.

We then conducted assays on a 5-log dilution series of plasmid DNA to examine the fast 3-step cycling protocol of the PerfeCTa SYBR Green Fastmix instruction manual and a faster 2-step protocol with a 34% shorter run time. Both trials exhibited good amplification curves with close agreement among replicates and plots of mean Cq vs log input gave similar slopes (Figure 2), indicating that the use of faster cycling conditions does not impact assay efficiency or sensitivity.

To investigate how fast the Q could be run, we assayed a selection of housekeeping genes under increasingly faster cycling conditions with 10 ng and 0.1 ng cDNA input amounts. The results for a GAPDH assay continued to show little variation among replicates under the fastest conditions tested (Figure 3A). The situation was similar for a selection

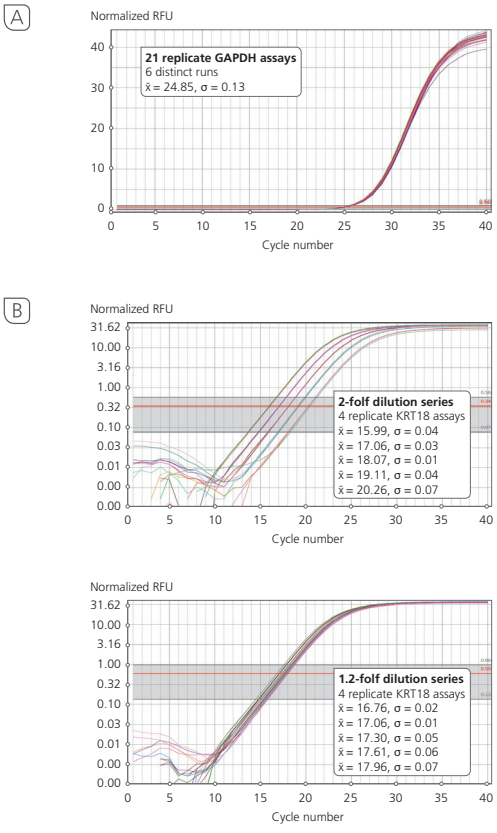


**Figure 1** Repeatability and quantitative sensitivity under standard cycling conditions.

of less abundant non-housekeeping gene assays. Multiple replicate assays of BCL2, for instance, showed a standard deviation from the mean Cq of less than 0.2, proving precise data even under extremely fast cycling conditions (Figure 3B).

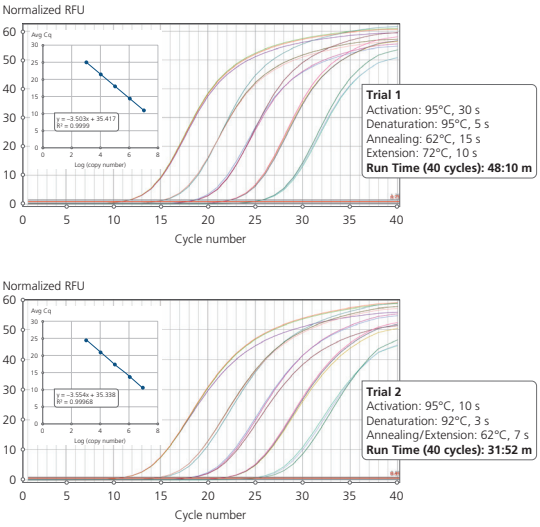


Figure 2 Preservation of assay efficiency and sensitivity under faster cycling conditions.

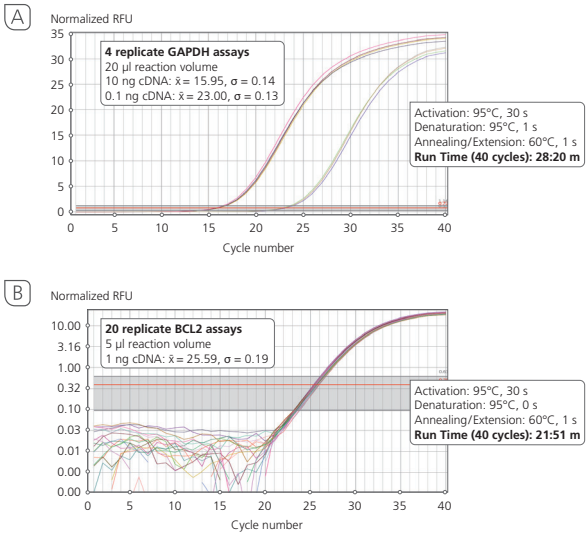


Figure 3 Delivery of precise data under extremely fast cycling conditions.

Having demonstrated peak performance with select assays, multiple gene assays representing a range of abundance levels and GC content were run with incrementally longer denaturation or annealing/extension times to establish fast cycling conditions. Assessments of amplification curves, efficiency values and Cq variability identified conditions suitable for all assays tested (Table 1). Subsequent runs using lower reaction volumes confirmed these conditions (Table 2).

| 10 pg cDNA, 20 µl reaction volume | | | |
|---|---|---|---|
| Activation: 95°C, 30 s<br>Denaturation: 95°C, 5 s<br>Annealing and Extension: 60°C, 10 s | | | |
| BCL2: | $\bar{x}$ = 26.16, σ = 0.04 | IL1RN: | $\bar{x}$ = 29.35, σ = 0.13 |
| CDKN1A: | $\bar{x}$ = 25.82, σ = 0.09 | RPSA: | $\bar{x}$ = 18.63, σ = 0.02 |
| KRT18: | $\bar{x}$ = 20.11, σ = 0.02 | TOP2B: | $\bar{x}$ = 23.13, σ = 0.02 |
| PHLDA2: | $\bar{x}$ = 25.34, σ = 0.10 | SNX4: | $\bar{x}$ = 25.35, σ = 0.14 |

Table 1 Replicate assays

| 1 ng cDNA, 5 µl reaction volume | | | |
|---|---|---|---|
| Activation: 95°C, 30 s<br>Denaturation: 95°C, 5 s<br>Annealing and Extension: 60°C, 10 s | | | |
| BCL2: | $\bar{x}$ = 22.63, σ = 0.11 | IL1RN: | $\bar{x}$ = 25.56, σ = 0.17 |
| CDKN1A: | $\bar{x}$ = 22.27, σ = 0.01 | RPSA: | $\bar{x}$ = 16.35, σ = 0.03 |
| KRT18: | $\bar{x}$ = 17.87, σ = 0.08 | TOP2B: | $\bar{x}$ = 20.57, σ = 0.09 |
| PHLDA2: | $\bar{x}$ = 23.35, σ = 0.05 | SNX4: | $\bar{x}$ = 24.17, σ = 0.05 |

Table 2 Replicate assays

## CONCLUSION

With optimal conditions, a 40-cycle run could be completed in under 25 minutes and possibly further reduced with less cycles. It is possible to distinguish samples from 2-fold and 1.2-fold dilution series. PerfeCTa SYBR Green Fastmix used with Q can facilitate successful completion of a qPCR-based experiment by providing:

- High repeatability
- Shorter run times
- Quantitative sensitivity
- Reaction volume flexibility



MK-AN-0001 REV01 FastMix 0918