Q qPCR Cycler & PerfeCTa NGS Quantification

A simple, reliable and reproducible solution for quantifying NGS libraries in 50 minutes

INTRODUCTION

Knowledge of the number of amplifiable DNA molecules in a library preparation is essential for obtaining the highest quality reads with next generation sequencing (NGS) technologies. The most accurate method uses real-time PCR to amplify DNA fragments possessing the appropriate adapter at each end. Comparison against a range of known DNA standards allows the absolute quantification of unknown library dilutions. We tested how the Q real-time PCR instrument from Quantabio performs for NGS library quantification under different conditions.

MATERIALS AND METHODS

All trials were conducted on a single Q instrument using DNA standards, primers and SYBR® Green SuperMix from the PerfeCTa® NGS Library Quantification Kit for Illumina® Sequencing Platforms (Quantabio cat# 95154). The 426 bp unknown test sample was prepared by pooling an arbitrary amount of each Quantabio DNA standard. DNA libraries were prepared in-house from different microbial DNA sources using the sparQ DNA Frag & Library Prep Kit (Quantabio cat# 95194). Multiple library samples representing a range of GC-contents were pooled and analyzed by Agilent Bioanalyzer to establish the average fragment size value of 450 bp. Data was analyzed using the Q software (v1.0.0).

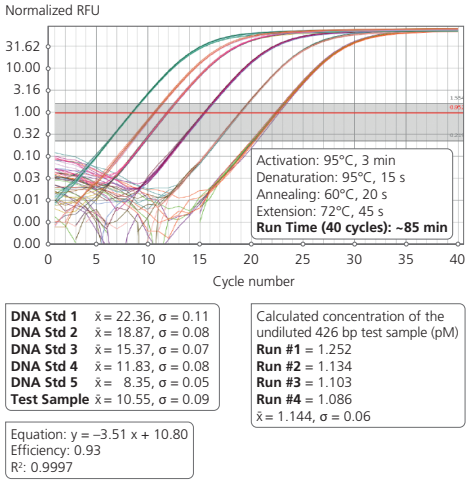


Figure 1 High efficiency amplification and repeatable quantification of a 426 bp fragment under normal cycling conditions.

RESULTS

We first performed assays on a range of stabilized, pre-diluted 426 bp DNA standards using a typical three-step PCR cycling protocol that takes approximately 85 minutes. Results from four distinct trials yielded highly repeatable Cq values for all standards and showed close agreement among multiple measurements of an identically-sized unknown test sample (Figure 1).

Further trials on the test template alone revealed the ability to differentiate samples from 1.5-fold and 1.2-fold dilution series (Figure 2).

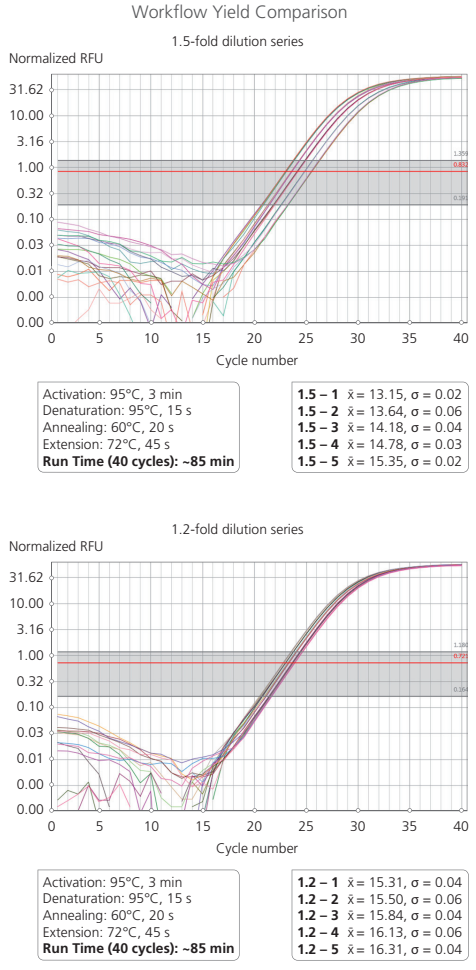
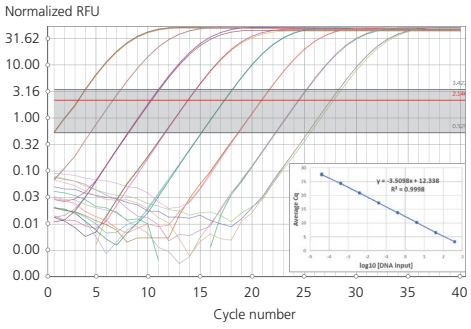


Figure 2 Extreme quantitative precision to differentiate samples in 1.5-fold and 1.2-fold dilution series.

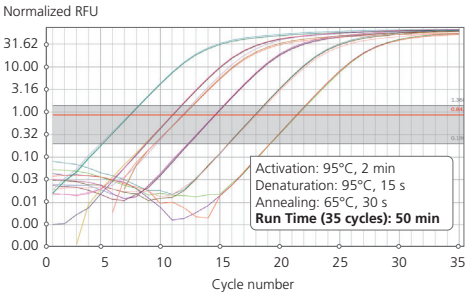
We then examined Q performance using an actual DNA library pool with average fragment size of 450 bp as template. A 10-fold dilution series from the 4 nM stock was prepared and assayed under standard cycling conditions. Analysis of the amplification curves demonstrated high efficiency and sensitivity across an 8-log dynamic range with the Q and SYBR Green SuperMix (Figure 3).



Activation: 95°C, 3 min
Denaturation: 95°C, 15 s
Annealing: 60°C, 20 s
Extension: 72°C, 45 s
Run Time (40 cycles): ~85 min

Figure 3 **Efficiency and sensitivity of NGS library amplification across a broad range of input amounts.**

We next investigated whether the faster heating and cooling capabilities of the Q could be utilized for NGS library quantification. The cycling conditions recommended for amplification of DNA fragments of the sizes typically found in NGS library preparations yield run times between 80 and 90 minutes. Using the 426-bp DNA standards and the identically-sized unknown test sample as templates, high efficiency amplification and accurate quantification was achieved with a 50-minute run time (Figure 4). Preliminary tests indicate that even faster run times may be possible for NGS quantification on the Q depending on DNA fragment size, reaction volume and other parameters.



Activation: 95°C, 2 min
Denaturation: 95°C, 15 s
Annealing: 65°C, 30 s
Run Time (35 cycles): 50 min

DNA Std 1	$\bar{x}$ = 21.33, σ = 0.04
DNA Std 2	$\bar{x}$ = 17.91, σ = 0.07
DNA Std 3	$\bar{x}$ = 14.50, σ = 0.03
DNA Std 4	$\bar{x}$ = 11.00, σ = 0.03
DNA Std 5	$\bar{x}$ = 7.36, σ = 0.04
Test Sample (1:4)	$\bar{x}$ = 11.86, σ = 0.07

Calculated concentration of the undiluted 426 bp test sample (pM) **= 1.252**

Equation: y = –3.48 x + 9.89
Efficiency: 0.94
R^2: 0.9997

Figure 4 **Efficient amplification of 426 bp DNA fragments using the Q under fast cycling conditions.**

CONCLUSION

Together, the results presented clearly establish the suitability of the Q real-time PCR instrument and PerfeCTa NGS Quantification Kit for quantification of NGS libraries of various fragment sizes and GC-contents. The clear benefits provided by the Q include:

- Highly precise measurements across multiple trials
- High efficiency amplifications under varied cycling conditions
- Exceptional quantitative sensitivity for distinguishing down to 1.2-fold differences
- Reliable results and performance from run times shorter than typical cycling protocols

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