Rapid and accurate quantification of Illumina NGS libraries using the Q real-time qPCR Instrument

Achieve 60% faster time to reliable results

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Introduction

Accurate quantification of the number of amplifiable library molecules is a critical factor for obtaining high quality read data with next-generation sequencing technologies. The high sensitivity, broad dynamic range, and specificity of qPCR to quantify library molecules that are suitable for the bridge PCR provide significant advantages over methods for total DNA quantification. However, these advantages are often offset by the time to result, requirement for inclusion of absolute DNA standards in every qPCR run, and errors associated with dilution of libraries so that reportable results are within the linear dynamic range of the technology. Here we describe application of a new real-time quantitative PCR instrument, the Q from Quantabio, to simplify reliable library quantification with faster run times.

Features of the Q Real-Time Quantitative PCR Instrument

Magnetic Induction Technology

- Rapidly heats reactions held in a unique spinning aluminum rotor.

Superior Temperature Uniformity of ± 0.05°C

- Eliminates well position effects associated with traditional peltier block-based real time cyclers.

Ultra-Fast Data Acquisition

- Robust, fixed optical path allows simultaneous acquisition of all channels with no need for reference dyes or crosstalk compensation.

Scalable and Wireless

- Up to 10 Q instruments can be operated from a single workstation wirelessly via Bluetooth, enabling processing of 480 samples simultaneously.

Portable and Compact

- The compact size and 4.5 pound weight of the Q allows easy portability with no need for calibration.
- Occupies 1/4 the bench space required for other cyclers on the market.

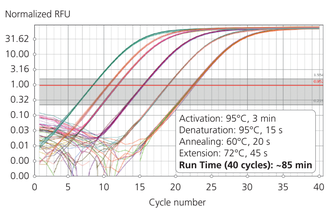
Powerful Software

- User-friendly Q-qPCR software for advanced automated statistical analysis including relative quantification, absolute quantification, genotyping, and allelic discrimination.

Materials and Methods

Most trials were conducted on a single Q using DNA standards, primers, and SYBR Green Supermix from the PerfeCTa NGS Library Quantification Kit for Illumina Sequencing Platforms (Quantabio cat# 95154). PerfeCTa SYBR Green Fastmix (Quantabio cat# 95072) was also used where noted. The 426-bp unknown test sample was prepared by pooling an arbitrary amount of each Quantabio DNA standard. DNA libraries were prepared 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-qPCR software (v1.0.0).

Results



Equation: y = –3.51 x + 10.80
Efficiency: 0.93
R^2: 0.9997

DNA Std 1	x̄ = 22.36, σ = 0.11
DNA Std 2	x̄ = 18.87, σ = 0.08
DNA Std 3	x̄ = 15.37, σ = 0.07
DNA Std 4	x̄ = 11.83, σ = 0.08
DNA Std 5	x̄ = 8.35, σ = 0.05
Test Sample	x̄ = 10.55, σ = 0.09

Pre-diluted 426-bp DNA standards were amplified in a typical three-step cycling protocol that completes in approximately 85 minutes. Results show the highly repeatable Cq values obtained across four distinct trials.

- Reliable results across runs

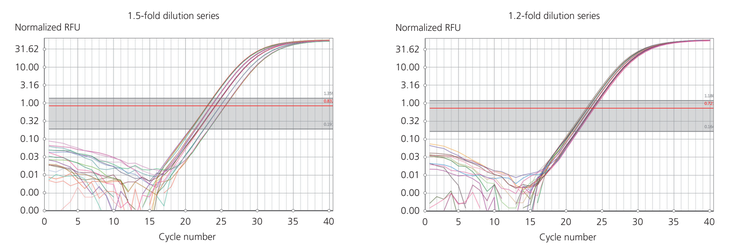
Re-use of Standard Curve for Absolute Quantification Analysis Across Multiple Runs

Run	Standard curve	Standard curve imported			
	of each run	from Run 1	from Run 2	from Run 3	from Run 4
1	x̄ = 1.252, σ = 0.07	–	x̄ = 1.190, σ = 0.07	x̄ = 1.180, σ = 0.07	x̄ = 1.158, σ = 0.07
2	x̄ = 1.134, σ = 0.02	x̄ = 1.192, σ = 0.02	–	x̄ = 1.124, σ = 0.02	x̄ = 1.103, σ = 0.02
3	x̄ = 1.101, σ = 0.02	x̄ = 1.166, σ = 0.02	x̄ = 1.110, σ = 0.02	–	x̄ = 1.080, σ = 0.02
4	x̄ = 1.105, σ = 0.01	x̄ = 1.133, σ = 0.01	x̄ = 1.122, σ = 0.01	x̄ = 1.116, σ = 0.02	–

A 426-bp unknown test sample was amplified alongside known DNA standards in four distinct runs. Concentration values (pM) were obtained using the Absolute Quantification feature of the Q-qPCR software. The chart shows a comparison between values calculated using the standard curves for each run and standard curves imported from the other runs.

- DNA standards are not needed in every run
- Space for more unknown samples

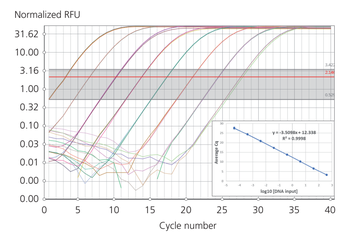
Quantitative Sensitivity of the Q



A range of dilution series were prepared from the 426-bp unknown test sample for qPCR analysis on the Q. Results show the ability of the Q to clearly distinguish samples in both a 1.5-fold and 1.2-fold dilution series.

- Distinguish small concentration differences between samples

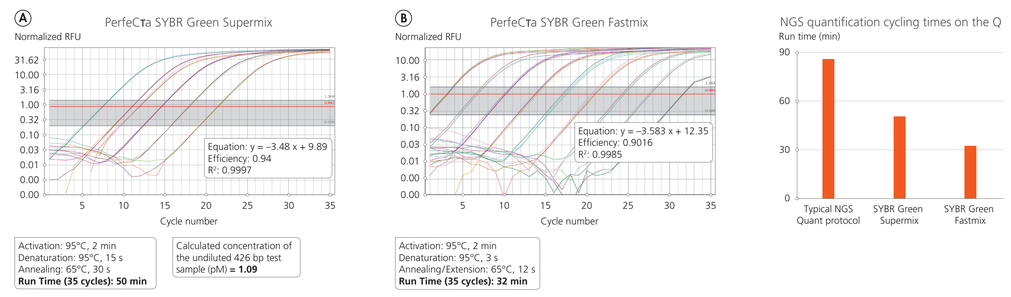
Broad Dynamic Range of the Q



Samples from multiple NGS libraries were pooled into a test sample with average fragment size of 450-bp. A 10-fold dilution series was prepared and assayed on the Q. Analysis of the resulting amplification curves showed high efficiency and sensitivity across an 8-log dynamic range.

- Accurate measurement over a wide range of library dilutions

Accurate Quantification with Rapid Cycling on the Q



Using PerfeCTa SYBR Green Supermix, along with 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 **A**. Furthermore, when we swapped in PerfeCTa SYBR Green Fastmix and included a dilution series of pooled libraries in the tests, we were able to achieve high efficiency amplifications with run times as short as 32 minutes **B**.

- Fast time to results
- 60% shorter run times on the Q

Conclusions

Together, the results presented clearly establish the suitability of the Q real-time PCR instrument and PerfeCTa NGS Quatification Kit for quantification of NGS libraries of various sizes and GC contents.

The clear benefits provided by the Q for NGS Quantification 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 60% shorter than typical cycling protocols