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 ampalible 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
Compact size and 4.5 pound weight of the Q allows easy portability with no need for calibration while occupying 1/4 the bench space of traditional cyclers
Powerful Software
User-friendly Q-qPCR software for advanced automated statistical analysis including relative quantification, absolute quantification, genotyping and allele discrimination

Materials and Methods

Most trials were conducted on a single qPCR 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# 95072). The 426-bp unknown test sample was amplified alongside known DNA standards in four distinct runs. Concentration of the 426-bp unknown test sample was amplified in a typical three-step cycling protocol

Results

Repeatability of NGS Quantification on the Q

Pre-optimized 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.

Accurate Quantification with Rapid Cycling on the Q

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

Conclusions

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 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