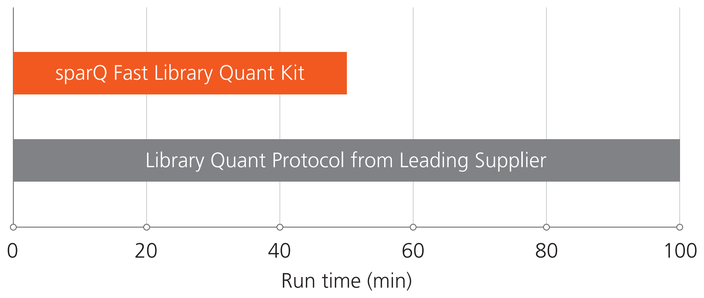
Rapid and accurate quantification of Illumina NGS libraries using sparQ Fast Library Quant Kit on the Q real-time qPCR instrument

Peter Bartholomew, Hongbo Liu, Eleanor Kolossovski, Brian Komorous, and David Schuster
Quantabio, 100 Cummings Center Suite 407J, Beverly, MA 01915

Introduction

Accurate quantification of the number of amplifiable library molecules is a critical factor for obtaining high quality read data with NGS technologies. The high sensitivity, broad dynamic range, and specificity of qPCR to quantify library molecules that are suitable for bridge amplification provide significant advantages over methods involving total DNA quantification. These advantages, however, are often offset by the time to result, complexity of execution, and costs associated with a qPCR run. Here we describe application of the sparQ Fast Library Quant Kit and Q real-time qPCR system to simplify accurate and reproducible library quantification with 50% shorter run times compared to traditional protocols.

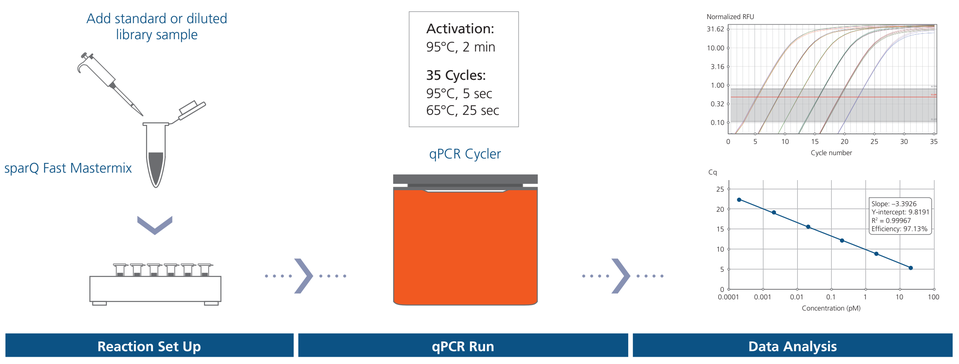
Comparison of NGS Library Quantification Run Time



Comparison of average qPCR run time for library quantification. sparQ Fast Library Quant Kit uses fast cycling protocol, allowing results to be achieved in 40 minutes versus 1 hour and 20 minutes with the traditional cycling protocol.

Materials and Methods

An Overview of sparQ Fast Library Quant Method



sparQ Fast Library Quant Kit

The kit consists of a set of six prediluted standards, a library dilution buffer, and a ready-to-use master mix containing Illumina P5 and P7 primers that is optimized for fast cycling on the Q qPCR system.

Q Compact and Portable qPCR Cycler

Q features a novel magnetic induction technology for rapid heating of reactions and fan-forced air for rapid cooling. Samples are held in a unique spinning aluminum rotor, which allows the Q to maintain superior temperature uniformity of ± 0.05°C and avoid well position effects associated with traditional peltier block-based real-time cyclers.

Experiment Conditions

To test the efficacy of this new library quantification kit and novel qPCR instrument, we measured libraries over multiple trials using a universal fast cycling protocol that completes in under 40 minutes. DNA libraries were prepared from different microbial DNA sources using the sparQ DNA Frag & Library Prep Kit and analyzed by Agilent Bioanalyzer to establish the average fragment sizes. Data was analyzed using the Q-qPCR software (v1.0.0).

Results

- The sparQ Fast Mastermix and Q facilitated efficient amplification over a broad dynamic range of library concentrations (Figure 1)
- Multiple distinct runs showed little variation of amplification efficiency or quantification values (Figure 2).
- Quantitative sensitivity was demonstrated by the ability to distinguish and quantitate samples from as little as a 1.5-fold dilution series (Figure 3).
- Measurements on libraries of varying GC content and size established that results obtained using the sparQ Fast Library Quant Kit and Q with fast cycling were highly correlated with values obtained using a leading library quantification kit and the manufacturer's recommended protocol (Figure 4).

Results

Efficient Amplification Over a Broad Dynamic Range

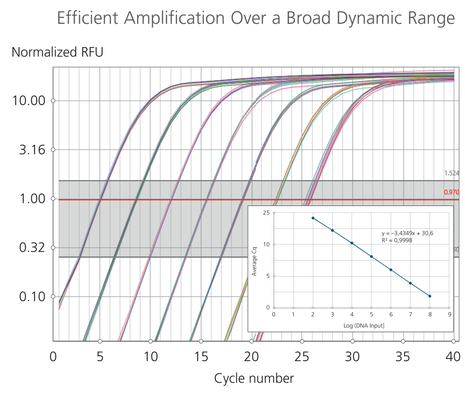


Figure 1 A 10-fold dilution series of a GC-rich library with average fragment size of 470 bp was amplified using the sparQ Fast Mastermix and the Q under fast cycling conditions. Analysis of the resulting amplification curves showed high efficiency across a 7-log dynamic range.

Outstanding Repeatability of Amplification and Quantification

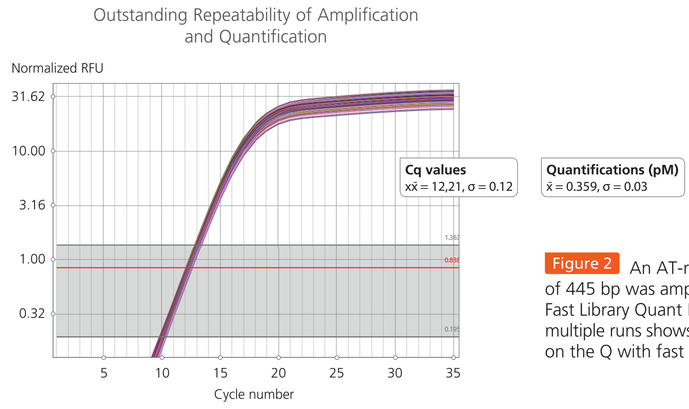
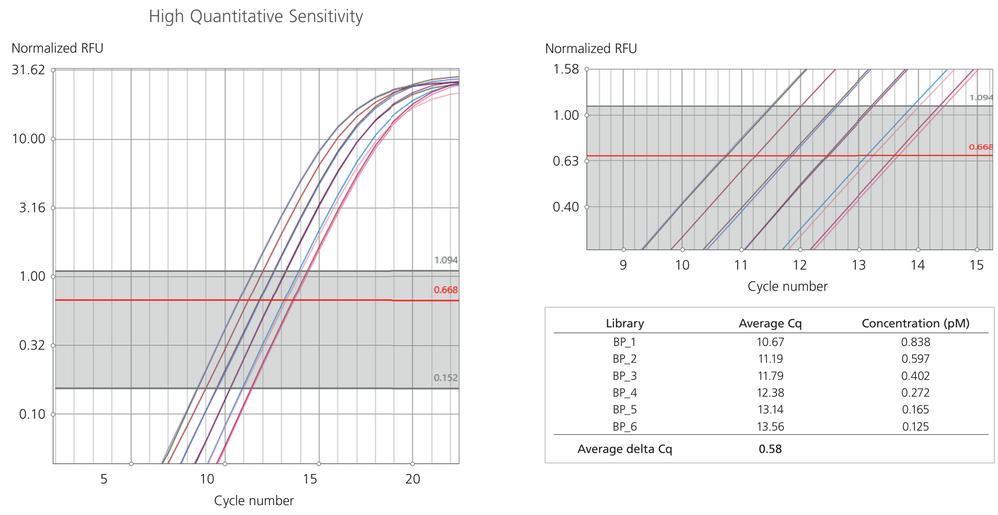


Figure 2 An AT-rich library with average fragment size of 445 bp was amplified and quantitated using the sparQ Fast Library Quant Kit. Results from 122 reactions across multiple runs shows the high repeatability of data generated on the Q with fast cycling conditions.

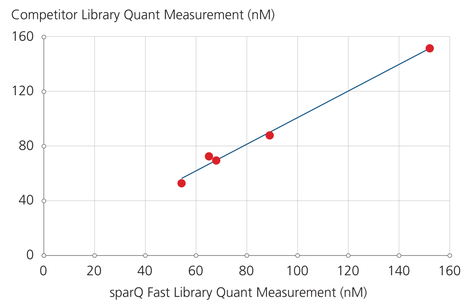
High Quantitative Sensitivity



Library	Average Cq	Concentration (pM)
BP_1	10.67	0.838
BP_2	11.19	0.597
BP_3	11.79	0.402
BP_4	12.38	0.272
BP_5	13.14	0.165
BP_6	13.56	0.125
Average delta Cq	0.58	

Figure 3 A 1.5-fold dilution series of a GC-rich library with average fragment size of 470 bp was amplified and quantitated using the sparQ Fast Library Quant kit and Q real-time cycler with fast cycling conditions. The delta Cqs and concentration measurements closely correlated with theoretically expected values.

Accurate Quantification of Diverse NGS Libraries



	Calculated Concentrations (nM)	
	sparQ Fast Library Quant Kit	Competitor's NGS Quant Kit
RNA Seq Library 1	89,63	87,52
RNA Seq Library 2	65,61	72,09
RNA Seq Library 3	153,09	151,07
RNA Seq Library 4	68,39	69,59
RNA Seq Library 5	64,66	52,61

Figure 4 Five libraries prepared for RNA-Seq were quantified using either the sparQ Fast Library Quant kit or a leading competitor's NGS quant kit. Fast cycling on the Q was used for the sparQ kit (runtime < 40 min). Cycling on the Q according to manufacturer instructions was used for the competitor kit (runtime > 85 min). Data presented is from 18 distinct quantification reactions for each library.

Conclusions

Together, the results presented clearly demonstrate the outstanding performance of the sparQ Fast Library Quant Kit and Q real time cycler for rapid, sensitive, and accurate quantification of NGS libraries of various sizes and GC contents. The streamlined reaction assembly, fast cycling protocol, and highly intuitive software significantly shorten the time to result compared to other combinations of reagents, instruments, and protocols.