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

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Introduction

Accurate quantification of the number of amenable 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

<table>
<thead>
<tr>
<th>Library Quant Protocol from Leading Supplier</th>
<th>sparQ Fast Library Quant Kit</th>
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<tr>
<td>0</td>
<td>3</td>
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<tr>
<td>20</td>
<td>9</td>
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<tr>
<td>60</td>
<td>26</td>
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<tr>
<td>80</td>
<td>51</td>
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<tr>
<td>100</td>
<td>75</td>
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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 cycler.

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 multiple runs shows the high repeatability of data generated on the Q with fast cycling conditions.

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).

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.