Improved library quantitation for a broad range of library types using the NEBNext® Library Quant Kit for Illumina®

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Introduction

Accurate quantitation of a next generation sequencing (NGS) library is essential for maximizing data output and quality from each sequencing run. qPCR is widely accepted as the most effective method for library quantitation, as it measures only sequenceable library fragments, with a high level of accuracy and consistency. The NEBNext Library Quant Kit for Illumina offers a simple, robust, qPCR-based method for the quantitation of libraries to be sequenced on the Illumina platform.

Here we demonstrate the effectiveness of the NEBNext Library Quant Kit for a broad range of library types and sizes, while also highlighting the advantages offered by qPCR quantitation for obtaining optimal cluster density and performance consistency.

General Protocol

The NEBNext Library Quantitation Workflow is shown in Figure 1, with a more detailed view of the protocol in Figure 2. The full protocol can be found in the product manual, which can be downloaded at www.neb.com/e7630. This kit can be used to quantitate any Illumina library containing the P5 and P7 sequences, and the kit quantitates only molecules with an adaptor on each end (e.g., only sequenceable molecules).

Figure 1. NEBNext Library Quant Kit workflow

Kit (Time)	Reagent Preparation	Library Dilution	Set Up	qPCR	Data Analysis	Workflow Time
Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	51 min.
Total	5 min.	10 min.	20 min.	60 min.	10 min.	1 hr. 45 min.

The NEBNext Library Quant Kit uses 4 pre-diluted DNA standards and Illumina adaptor-specific primers to quantitate diluted library samples of interest. The use of 4 standards maximizes the number of libraries that can be quantitated without sacrificing performance.

Materials

- NEBNext Library Quant Kit for Illumina (NEB #E7630)
- Nuclease-free water
- qPCR machine
- qPCR plates and seals
- PCR strip tubes or microcentrifuge tubes
- Conical centrifuge tubes

(see other side)

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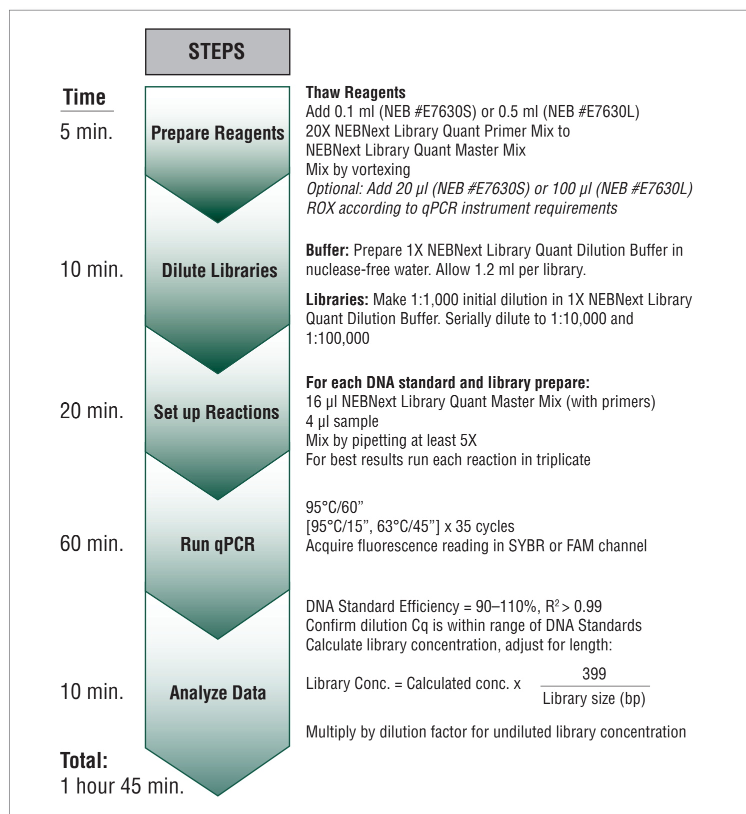
Figure 2. NEBNext Library Quant Kit workflow



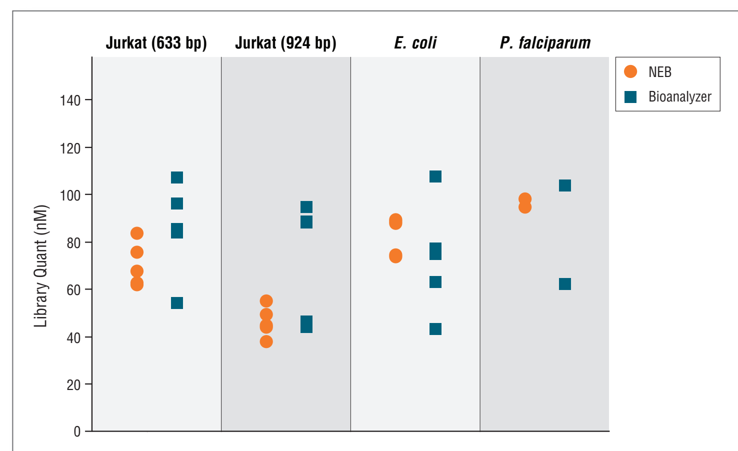
Results

For a method or kit to be a trusted way to quantitate libraries, the values obtained must not only be accurate but also consistent, both between libraries and between users.

Electrophoretic quantitation methods such as the Agilent Bioanalyzer® instrument can provide information on library size, but for quantitation this method can be less accurate and less consistent. Also, electrophoretic methods quantitate all DNA molecules present in a library, in contrast to qPCR which quantitates only molecules with an adaptor ligated to each end.

In this experiment, quantitation values were obtained for multiple replicates of libraries of different size and GC content (Figure 3). Concentrations of 4 libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured by the Bioanalyzer (blue). Compared to NEBNext qPCR, Bioanalyzer concentrations displayed a greater level of variation. This finding demonstrates the benefits of qPCR for library quantitation.

Figure 3. qPCR provides more consistent library quantitation results than Bioanalyzer analysis

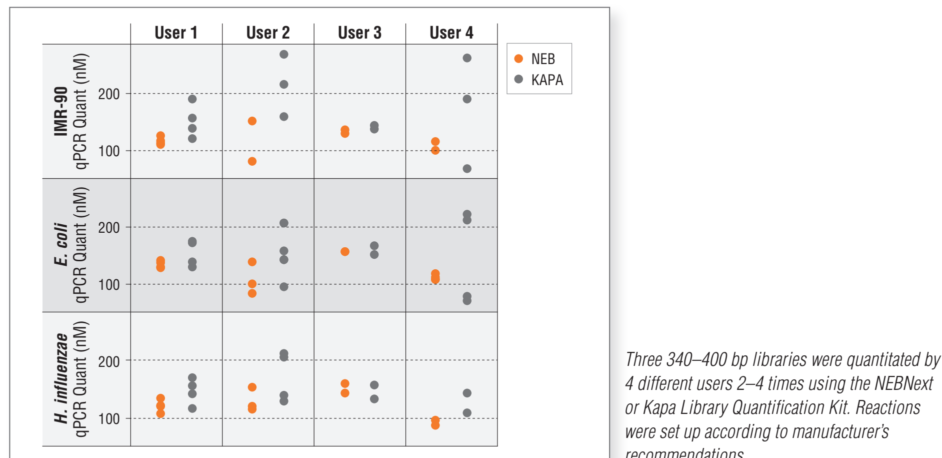


Concentrations of 4 libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured using the Aglient Bioanalyzer (blue).

Results (continued)

In addition to the method itself, consistency of the reagents involved and ease-of-use of a protocol both minimize variability. In this experiment, replicates of 340–400 bp libraries from *E. coli*, *H. influenzae* and human (IMR-90) genomic DNA were quantitated by four different users with the NEBNext or Kapa™ Library Quant Kit (Universal). While both kits were able to successfully quantitate the various sample types, a marked improvement in quantitation consistency was observed using the NEBNext Library Quant Kit (Figure 4).

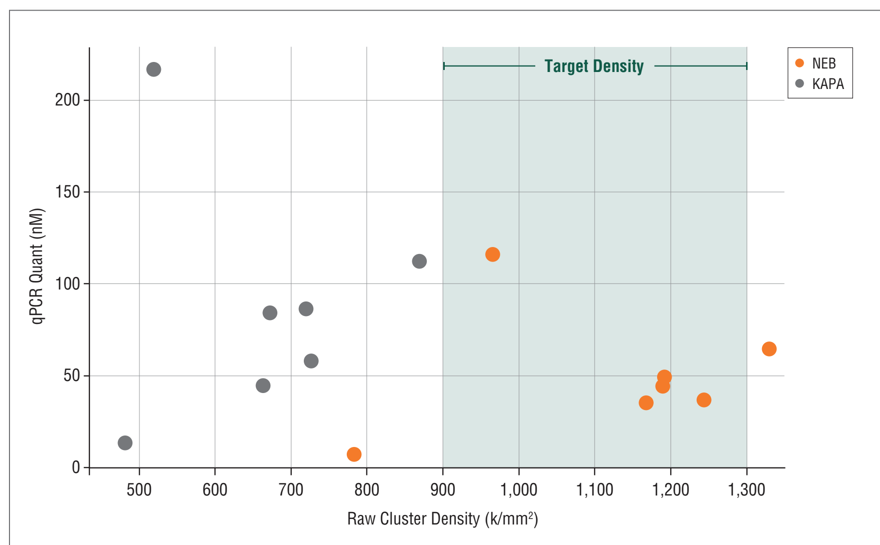
Figure 4. NEBNext Library Quant Kit delivers reproducible quantitation for a variety of sample types



Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using the NEBNext or Kapa Library Quantification Kit. Reactions were set up according to manufacturer's recommendations.

The most relevant measure of accuracy of library quantitation is the density of clusters achieved after loading the recommended amount of library. If the quantitation value is too low, more library than desired will be loaded, and over-clustering will result. In contrast, if the quantitation value is too high, less library will be loaded, resulting in under-clustering. In this experiment, seven different libraries at a range of concentrations were quantitated using the NEBNext Library Quant Kit or the Kapa Library Quant Kit (Universal), then diluted to 8 pM and loaded into cluster generation. We observed a raw cluster density average of 1160 k/mm^2 for libraries prepared with the NEBNext Kit. This falls directly in the optimal range of 900–1300 k/mm^2 (Figure 5). In contrast, libraries loaded based on the Kapa quantitation averaged only 660 k/mm^2, which is sub-optimal.

Figure 5. NEBNext Library Quant Kit delivers improved accuracy in quantitation



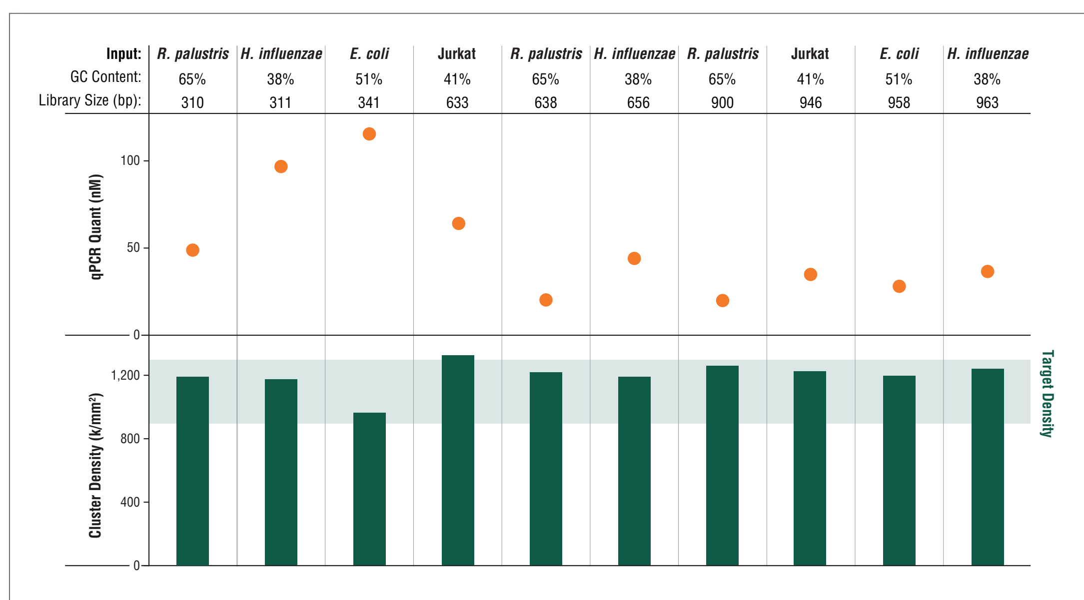
Seven different libraries were quantitated using the NEBNext Library Quant Kit. Undiluted library concentrations ranged from 2–200 nM. Libraries were diluted to 8 pM and loaded onto a MiSeq® instrument (v2 chemistry; MCS v2.4.1.3). Reactions were set up according to manufacturer's recommendations.

Results (continued)

The consistent and reliable performance of a method or kit with a variety of libraries, in terms of GC content and insert size, is critical for practical utility. The ability of the NEBNext Library Quant Kit to accurately quantitate a wide range of library types was tested by using libraries from 10 different sources, including human and microbial DNA, of high GC and high AT content, and a broad range of library sizes (150–963 bp) (Figure 6). In all cases, optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit.

Furthermore, the NEBNext Library Quant Kit has been used to successfully quantitate libraries from 20–70% GC, with a broad range of sizes, made with several library prep kits including NEBNext, Illumina TruSeq® Nano and Kapa Hyper library prep kits (data not shown).

Figure 6. NEBNext Library Quant Kit delivers accurate quantitation for a variety of sample types and sizes



Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit.

Conclusion

The NEBNext Library Quant Kit provides accurate and reliable qPCR-based library quantitation of Illumina libraries. This is shown by the production of optimal cluster densities. The NEBNext kit demonstrates improved reproducibility and consistency when compared to alternative methods and kits. Furthermore, this kit can successfully quantitate samples from a wide variety of sample types, as well as a broad range of sizes and GC-content.

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