Benefits of Illumina® Library Quantitation with NEBNext® Library Quant Kit

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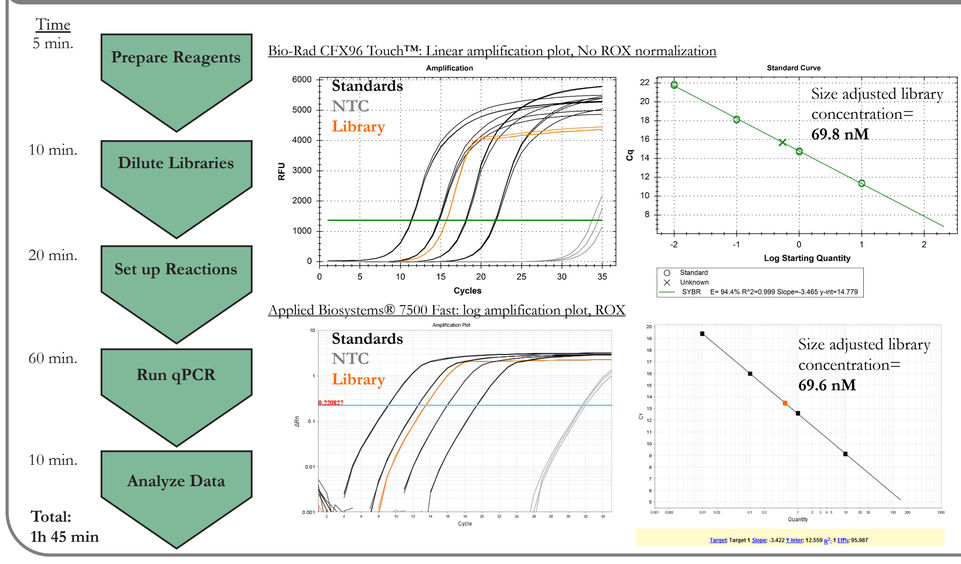
1: Introduction

Accurate quantitation of a next-generation sequencing library is essential to maximizing data output and quality from each instrument run. qPCR is widely accepted as the most effective method for library quantitation by both users and manufacturers[1], as qPCR methods measure only sequenceable library fragments with a high level of accuracy and consistency. The NEBNext Library Quant Kit for Illumina presents a simple, robust method for quantitation of Illumina libraries. Here we demonstrate the effectiveness of the Kit for a broad range of library types and sizes as well as advantages offered by qPCR quantitation for obtaining optimal cluster density and user-to-user consistency. The NEBNext Quant Kit offers an efficient and cost-effective qPCR library quantitation workflow for users looking to optimize both sequencing yield and throughput.

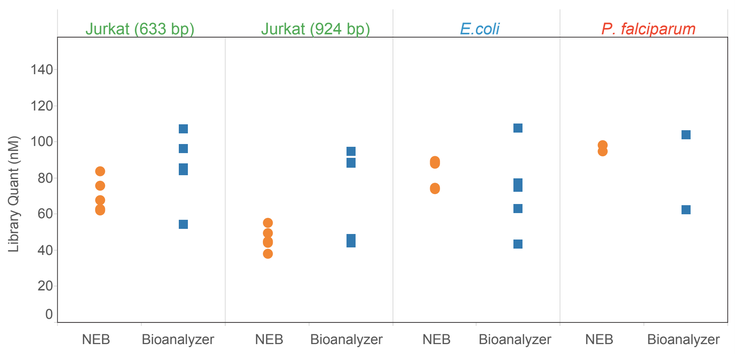
2: Quant Kit Workflow and qPCR Data

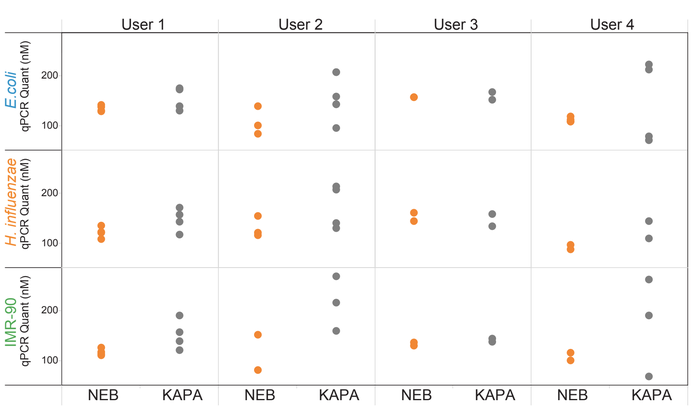


The NEBNext Quant Kit uses 4 pre-diluted DNA standards and Illumina adaptor-specific primers to quantitate diluted library samples of interest. The use of 4 standards maximizes the number of libraries that can be quantitated without sacrificing performance. Example data from the same library quantitated with two different qPCR instruments are shown, with a consistent determination of library concentration.

3: Library Quantitation Consistency

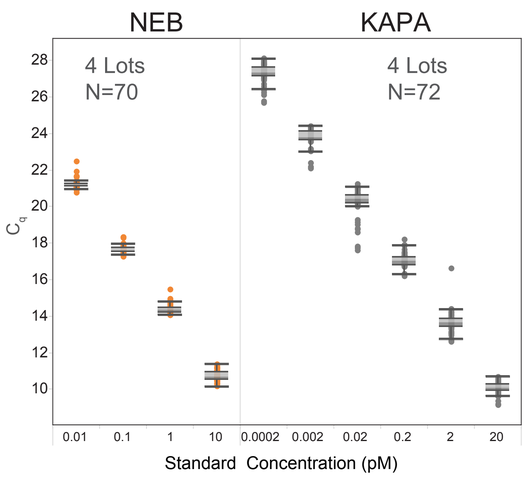
qPCR v. Bioanalyzer™
Concentrations of 4 libraries were determined by NEBNext Library Quant Kit (orange) and compared to values measured by Bioanalyzer (blue). Compared to NEBNext qPCR, Bioanalyzer concentrations displayed a greater level of variation. This finding demonstrates the benefits of qPCR for library quantitation.

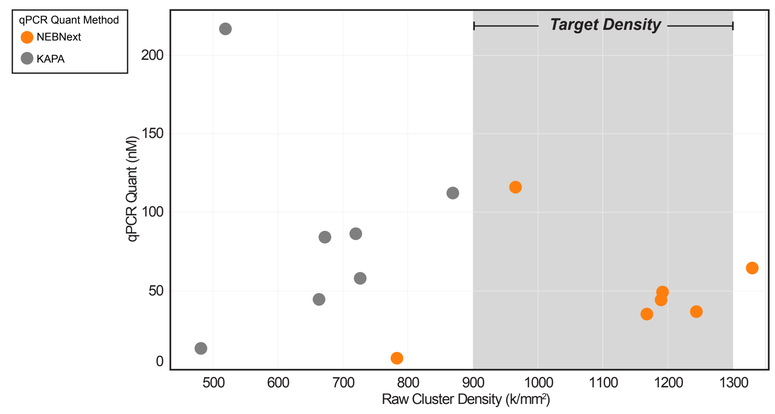




Quant Reproducibility
Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or KAPA™ Library Quant Kit. A marked improvement in quantitation consistency was observed for concentrations determined by the NEBNext Library Quant Kit (orange) versus those from the KAPA kit (gray).

4: Accurate Library Quantitation Produces Optimal Cluster Density



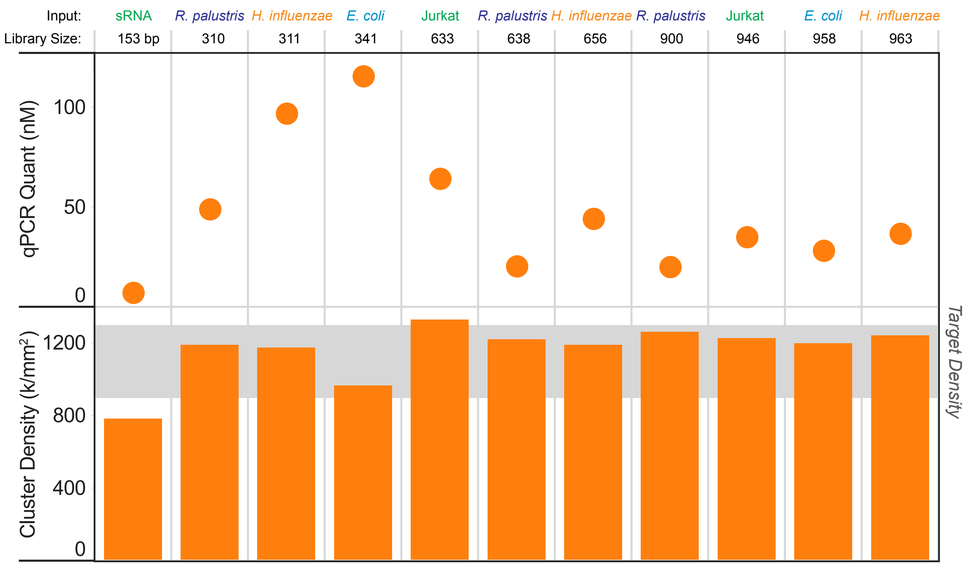


Standards Lot-to-lot Consistency
Accurate qPCR quantitation requires the use of high-quality DNA standards with known concentration. The NEBNext Library Quant Kit contains 4 standards produced with a high level of both quantitation accuracy and consistency. Above is data from 70+ total runs of 4 lots of both NEB and KAPA standards, with all C_q plotted. Box and whiskers indicate mean and quartiles. The NEBNext Library Standards displayed much lower variation in C_q, resulting in more consistent quantitation performance.

Sequencing Cluster Density using Quant Kits
Seven different Illumina libraries were quantitated using either the NEBNext (orange) or KAPA (grey) library quantitation kit. Undiluted library concentration ranged from 2–200 nM, and libraries were diluted to 8 pM and loaded onto a MiSeq™ instrument (v2 chemistry; MCS v2.4.1.3). Libraries quantitiated with the NEBNext kit resulted in a raw cluster density average of 1160 k/mm², directly in the optimal range of 900–1300 k/mm². In contrast, libraries loaded based on the KAPA quantitation averaged only 660 k/mm².

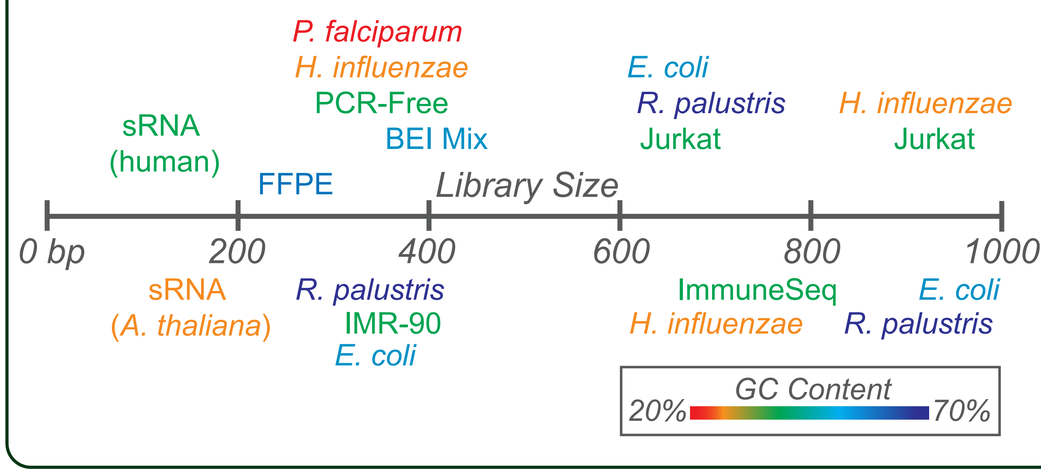
NEBNext Library Quant Kit Performance Across Library Type and Size

5: Cluster Density from Various Quantitated Libraries



Libraries from 150–963 bp from indicated genomic input were first 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 2–140 nM, but resulting raw cluster density for all libraries was 790–1300 k/mm² (ave. =1160). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes and gDNA or sRNA inputs.

6: Range of Library Sizes and Types Quantitated



A selection of libraries successfully quantitated with the NEBNext Library Quant Kit. Libraries are plotted by size, ranging from smaller libraries (sRNA, FFPE) at 150–230 bp to largest libraries at 980 bp. Various source gDNA were used (e.g. human from FFPE, PCR-Free, ImmuneSeq, IMR-90, Jurkat), ranging from 20–70% GC content as indicated by text color. Libraries were prepared with NEBNext, Illumina TruSeq™, and KAPA library prep kits. No dependence on size or GC content was observed in library quantitation.

7: Summary

Quant Method

	NEBNext qPCR	KAPA qPCR	Bioanalyzer
# Libraries/run	13-27	12-25	11-12
Workflow Time	1h 45min	2h	1h
Ease of Use	++*	+	++
Reproducibility	+++	++	+
Cluster Density	+++	+	++

* NEBNext Library Quant Kit contains a provided Library Dilution Buffer, uses 4 standards (vs. 6 in KAPA), and does not require additional pipetting of water to reaction mixes.

- NEBNext Library Quant Kit provides reliable qPCR library quantitation of Illumina libraries
- Library quants are more reproducible and consistent vs. Bioanalyzer, KAPA
- Accurate library quant produces optimal sequencing cluster density
- Easy-to-use quant tool at NEBiocalculator.neb.com
- NEBNext Library Quant Kit accommodates libraries 150–1000 bp, 20–70% GC, various prep methods

www.NEBNext.com

1: Illumina (2015). Diagnosing and Preventing Flow Cell Overclustering on the MiSeq System, p.8. (http://support.illumina.com/content/dam/illumina-marketing/documents/products/other/miseq-overclustering-primer-770-2014-038.pdf)