



KAPA Library Quantification Kits

Next-generation qPCR meets next-generation sequencing

Accurate and reproducible quantification of NGS libraries prior to pooling and sequencing is critical for optimal and cost-effective use of sequencing capacity. KAPA Library Quantification Kits provide a proven, qPCR-based solution for the quantification of libraries prepared for sequencing on Illumina® platforms.

KAPA Library Quantification Kits contain all the reagents needed for NGS library amplification using absolute, qPCR-based quantification. This includes KAPA SYBR® FAST qPCR Master Mix (formulated with different passive reference dyes for different qPCR instruments), a platform-specific library quantification primer premix, and a pre-diluted set of DNA standards.

- Reliable and sensitive quantification of all sequencing-competent library molecules
- Accurate and reproducible quantitation across a wide range of library types, concentrations, fragment length distributions, and GC content
- More accurate, equimolar pooling for multiplexed sequencing
- Flexibility to support manual and automated, high-throughput pipelines; as well as PCR-free workflows

Reagent Preparation

Sample Dilution

Prepare and Dispense
Master Mixes

Add Samples and Controls

Perform qPCR

Data Analysis



The complete library quantification solution

- Only/all sequencing-competent library fragments are quantified, irrespective of over-amplification—whereas other methods under- or over-estimate library concentrations
- Standard curves are designed to support all library construction workflows, including PCR-free methods
- Kits contain all the reagents needed for absolute, qPCR-based quantification of individual NGS libraries or indexed library pools
- Data analysis templates and assistance are available from sequencing.roche.com/techsupport

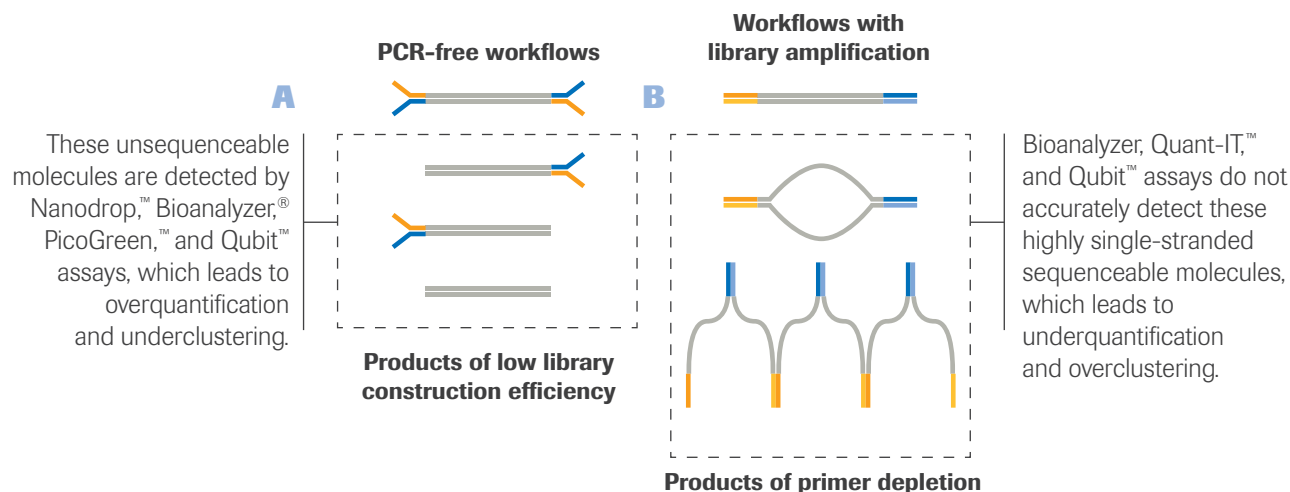


Figure 1. qPCR quantifies all sequenceable molecules. (A) The ligation of adapters to insert DNA results in a mixed population of molecules, some of which do not support cluster amplification and sequencing. qPCR measures only those molecules with the correct adapter configuration, which is especially critical in PCR-free workflows. Spectrophotometric, fluorometric, and electrophoretic methods, measure all double-stranded molecules, irrespective of adapter configuration. (B) Excessive library amplification leads to primer depletion. Subsequent rounds of denaturation and annealing produce imperfectly annealed, partially double-stranded DNA heteroduplexes. Electrophoretic and fluorometric assays that employ dsDNA-binding dyes are designed to detect double-stranded DNA fragments only, leading to under-quantification of over-amplified libraries. Data on file.

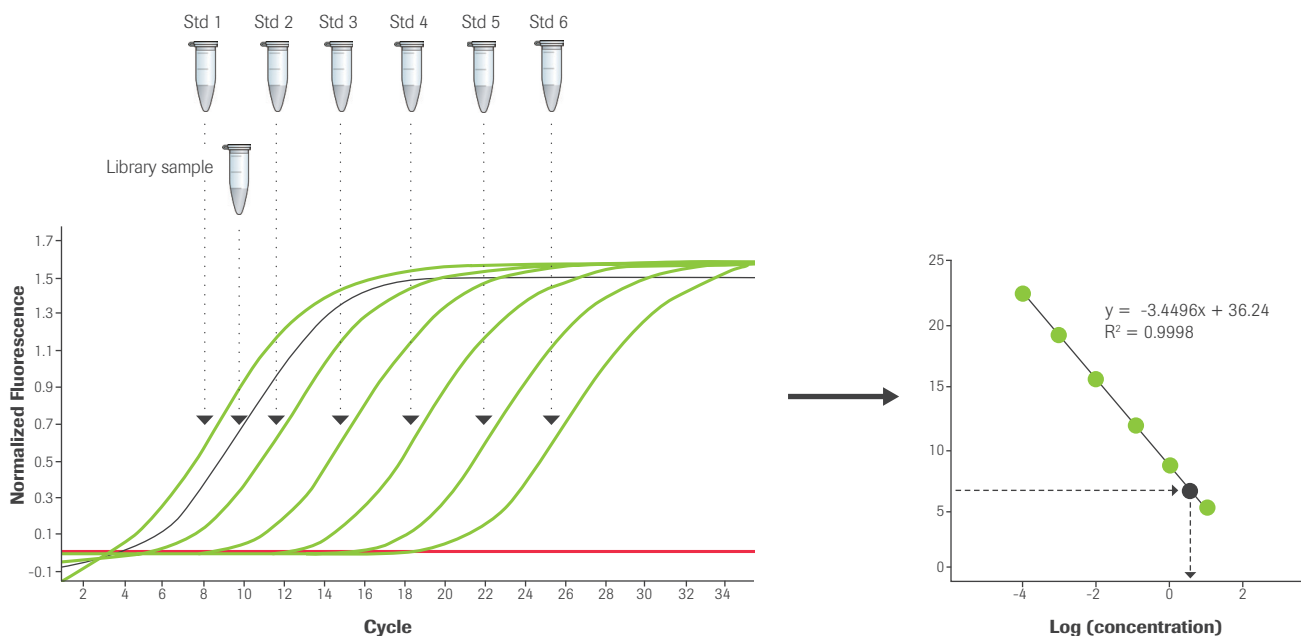


Figure 2. The principle of the KAPA Library Quantification Kit. Six pre-diluted DNA Standards and appropriately diluted NGS libraries are amplified using qPCR primers that target Illumina adapter-specific sequences. The average Cq value for each DNA Standard is plotted against its known concentration to generate a standard curve. The standard curve is used to convert the average Cq values for diluted libraries to concentration, from which the working concentration of each library is calculated.

The complete library quantification solution

The engineered KAPA SYBR[®] FAST DNA Polymerase is the key to efficient library quantification

- KAPA SYBR FAST was engineered to amplify diverse DNA fragments with similar efficiency
- Library quantification kits containing wild-type DNA polymerases count only “easy” library molecules

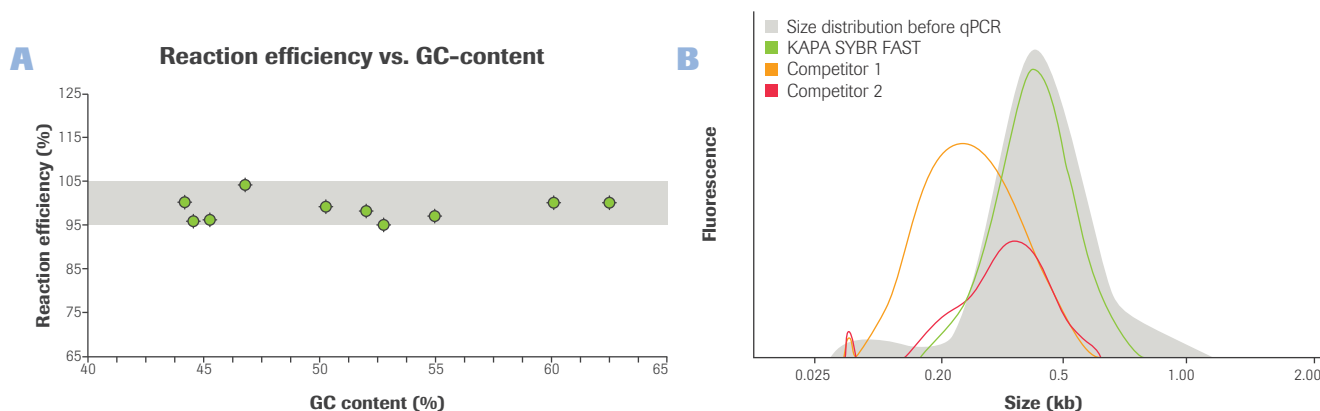


Figure 3. KAPA SYBR FAST is engineered for high-efficiency library quantification. (A) Amplification efficiencies achieved with the KAPA SYBR FAST qPCR Mix for ten diverse amplicons, plotted against GC content. All ten amplicons were amplified with similar efficiency (within the optimal range of 95% – 105%). This confirms that the KAPA SYBR FAST enzyme is ideal for the amplification of heterogeneous populations of DNA fragments, such as NGS libraries. (B) DNA fragment size distributions for an NGS library before (grey fill) and after qPCR amplification with the KAPA SYBR FAST qPCR Kit and two competitor kits containing wild-type Taq polymerase. Reactions were performed with the following cycling protocol: 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, and 60°C for 45 sec. The KAPA SYBR FAST quantification product is representative of the template DNA (NGS library), whereas the quantification products generated with wild-type enzymes are not.

A proven track record for product quality and consistency

- The properties of KAPA SYBR FAST enable the use of a single DNA Standard for all library types.
- Pre-diluted standards are produced with very high lot-to-lot consistency

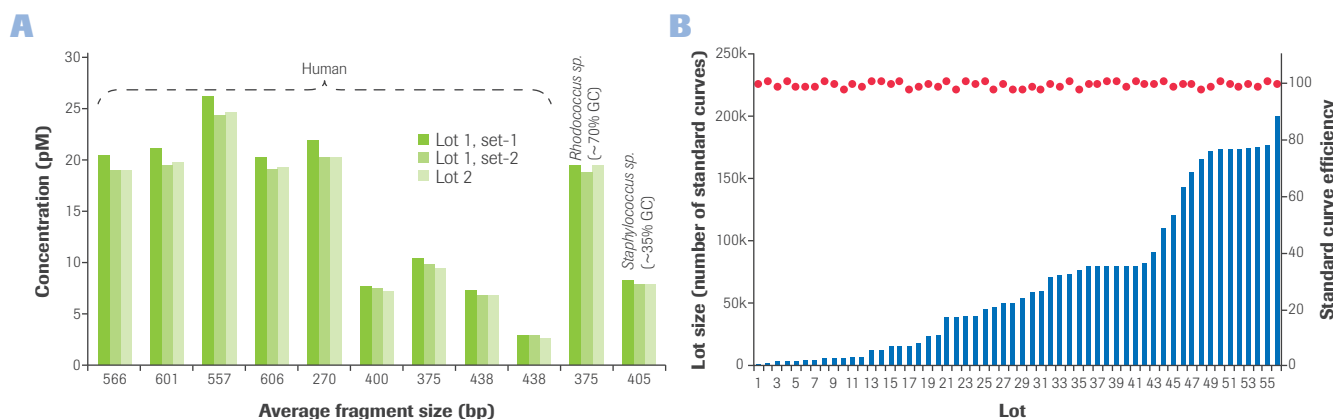


Figure 4. High consistency across different library types and across hundreds of production lots. (A) Nine human WGS libraries (41% GC) and two microbial WGS libraries (*Rhodococcus* sp., 70% GC and *Staphylococcus* sp., 35% GC) were quantified with distinct lots (Lots 1 and 2), and distinct sets of reagents from the same lot (Set 1 and 2) of KAPA Library Quantification Kit for the Illumina[®] platform. (B) Amplification efficiencies for 55 consecutive lots of KAPA DNA Standards released since September 2009.

Reproducible cluster density and improved pooling for multiplexed sequencing from samples of variable quality

- Improved library quantification enables optimal and more predictable cluster densities, to maximize sequencing capacity and throughput
- Accurate and sensitive quantification facilitates equimolar pooling of indexed libraries, irrespective of pre-pooling library concentrations
- Uniform distribution of reads across all the libraries in a pool optimizes sequencing costs
- Reliable results are achievable for all library types, including those prepared from low-quality FFPE DNA

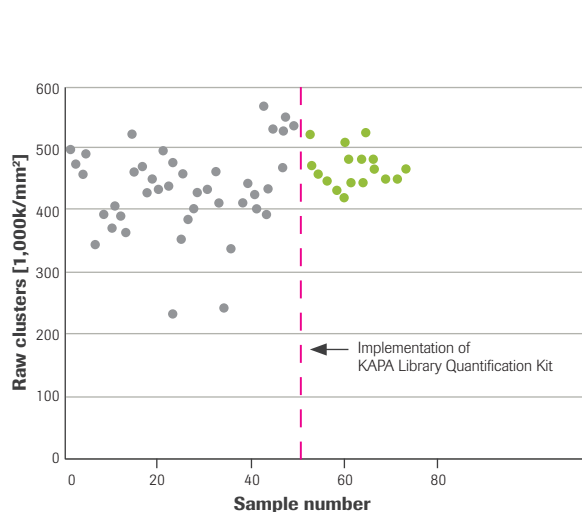


Figure 5. Accurate and reproducible library quantification enables improved and consistent cluster densities. The implementation of KAPA Library Quantification Kit into the Illumina® GA sequencing workflow at The Broad Institute (Cambridge, MA, USA) significantly reduced cluster density variability and eliminated the need for titrations. Average number of clusters per tile are shown for consecutive libraries.

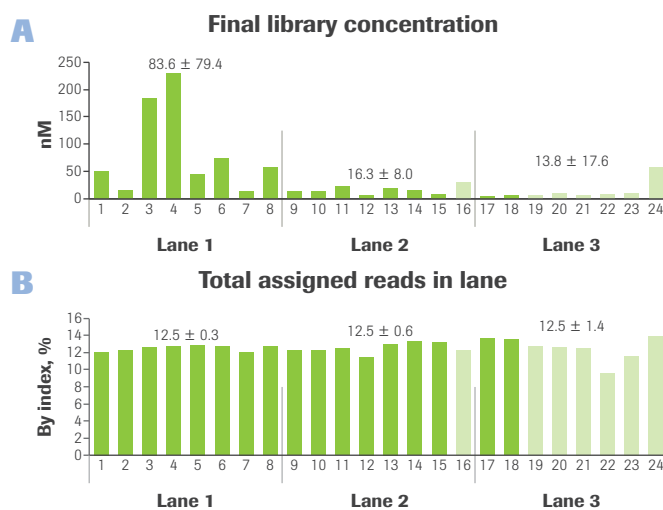


Figure 6. Accurate and sensitive quantification enables a uniform distribution of reads in multiplexed sequencing pools. Twenty-four indexed libraries were quantified by qPCR using the KAPA Library Quantification Kit (for Illumina platforms) and combined to create three sequencing pools of equimolar concentration. Each pool was sequenced in a different lane of the same flow cell on a HiSeq® 2500 instrument. **(A)** The twenty-four individual libraries represented a ~44-fold range of pre-pooling concentrations (5.2 nM to 229.8 nM), whereas the sequencing read distribution **(B)** only varied between 9.6% and 13.9%. The coefficient of variation (CV) for pre-pooling library concentration was 94.9%, 49.4%, and 127.9% for the libraries pooled for sequencing in lanes 1, 2, and 3, respectively. The KAPA Library Quantification Kit enabled accurate equimolar pooling, reducing the CV to 2.5%, 4.8%, and 11.2% after normalization.

Ordering information

Roche cat. no.	KAPA code	Description	qPCR instrument/Reference dye
07960140001	KK4824	KAPA Library Quantification Kit – Illumina	Universal
07960204001	KK4835	KAPA Library Quantification Kit – Illumina	ABI Prism®
07960255001	KK4844	KAPA Library Quantification Kit – Illumina	Bio-Rad iCycler™
07960298001	KK4854	KAPA Library Quantification Kit – Illumina	Roche LightCycler® 480
07960336001	KK4873	KAPA Library Quantification Kit – Illumina	ROX Low

*All kits contain 5 mL KAPA SYBR® FAST qPCR Master Mix (2X), 1 mL Primer Premix, and 6 x 80 µL DNA Quantification Standards. Kits contain primers, DNA standards, and qPCR reagents specific for both DNA sequencing platform and qPCR instrument. Primer Premix and DNA Quantification Standards are also sold separately.

Published by:

Roche Sequencing and Life Science
9115 Hague Road
Indianapolis, IN 46256

sequencing.roche.com

go.roche.com/KAPA-LQK

For Research Use Only. Not for use in diagnostic procedures.

LIGHTCYCLER and KAPA are trademarks of Roche. All other product names and trademarks are the property of their respective owners.

© 2022 Roche Sequencing and Life Science. All rights reserved.

MC-US-10927

SS104001

A520

7/22