# Optimize Capacity

## 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.

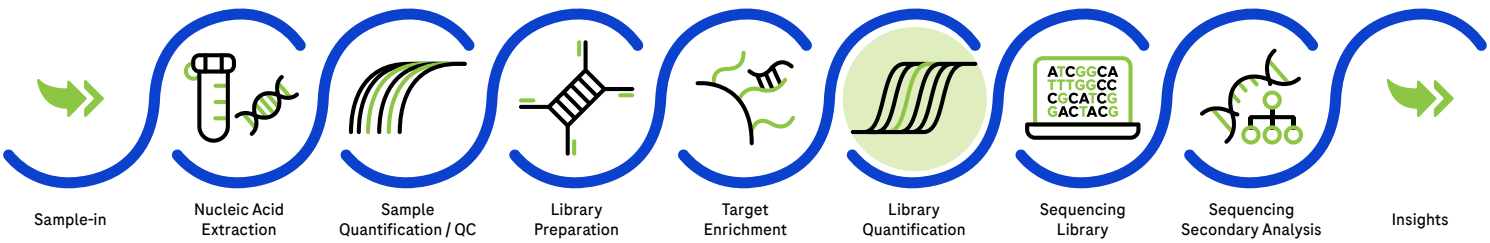
KAPA Library Quantification Kits contain all the reagents needed for NGS library amplification using absolute, qPCR-based quantification. This includes KAPA SYBR® FAST qPCR Master Mix (formulated with different passive reference dyes for different qPCR instruments), a platform-specific library quantification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

- Regent preparation
- Sample dilution
- Prepare and dispense master mixes
- Add samples and controls
- Perform qPCR
- Data Analysis

## Benefits

| | |
|---|---|
| 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 |

**Constantly evolving, efficient, and complete solutions**

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## The complete library quantification solution

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

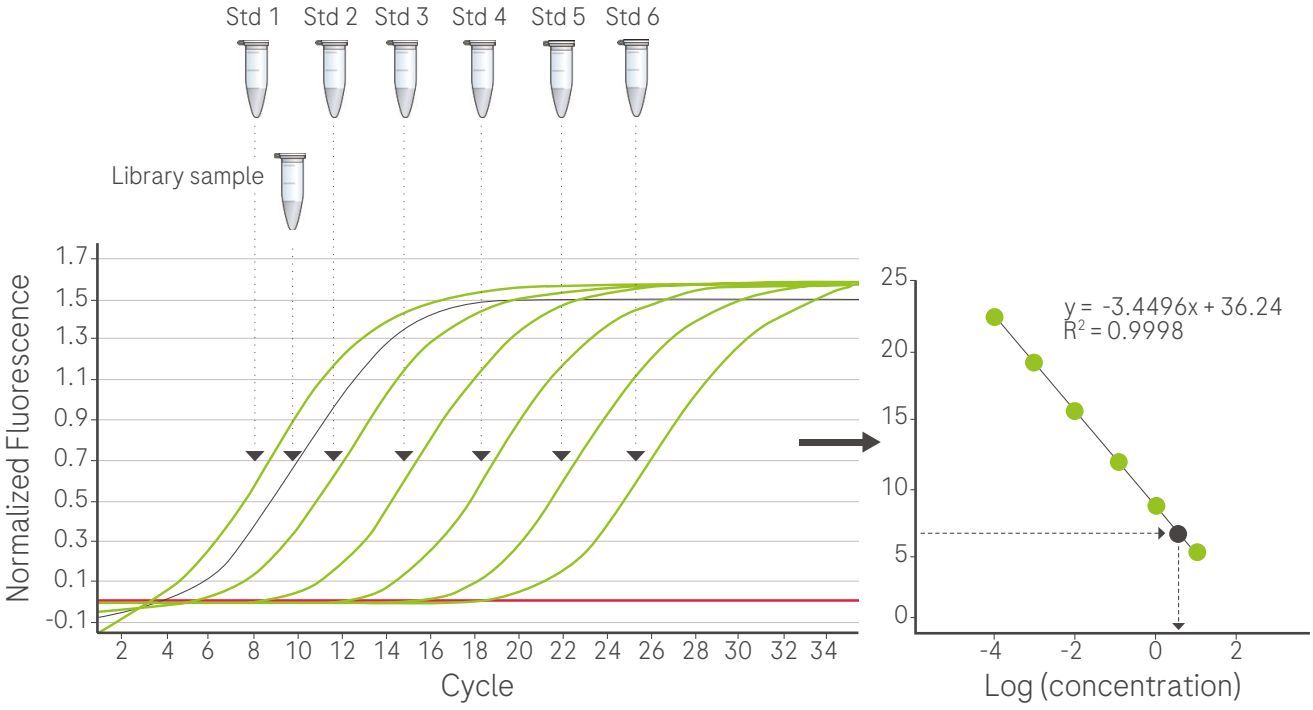


**Figure 1.** The principle of the KAPA Library Quantification Kit. Six pre-diluted DNA Standards and appropriately diluted NGS libraries are amplified using platform-specific qPCR primers that target adapter sequences. The average Cq value for each DNA Standard is plotted against its known concentration 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.

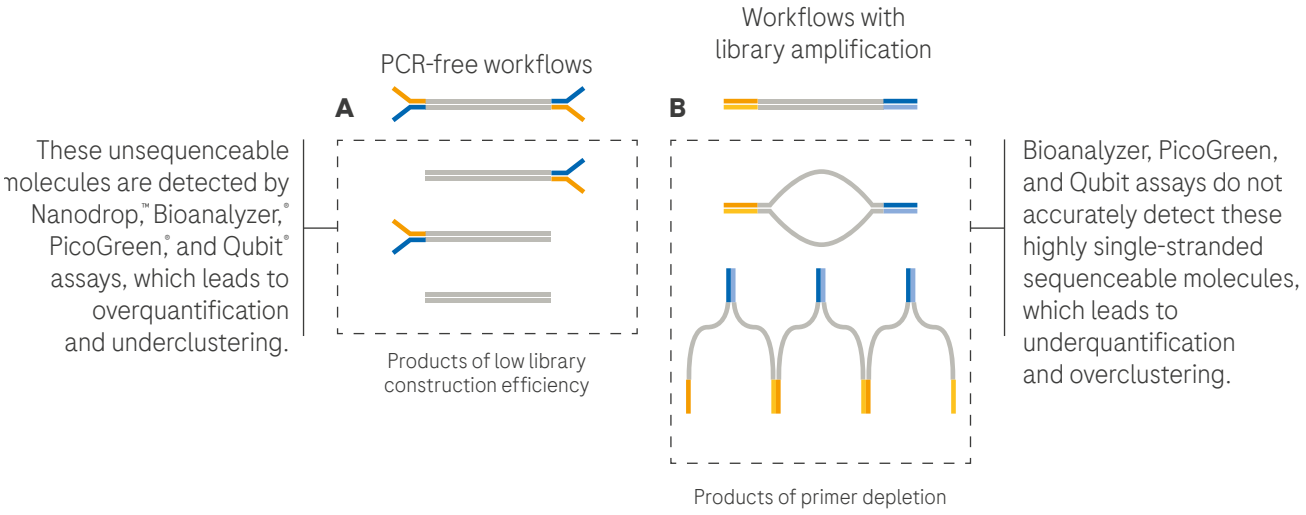


**Figure 2.** qPCR quantifies all sequenceable molecules. (A) The ligation of adapters to insert DNA results in a mixed population of molecules, some of which do not support cluster amplification and sequencing. qPCR measures only those molecules with the correct adapter configuration, which is especially 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 produces 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.

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## 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 only count “easy” library molecules

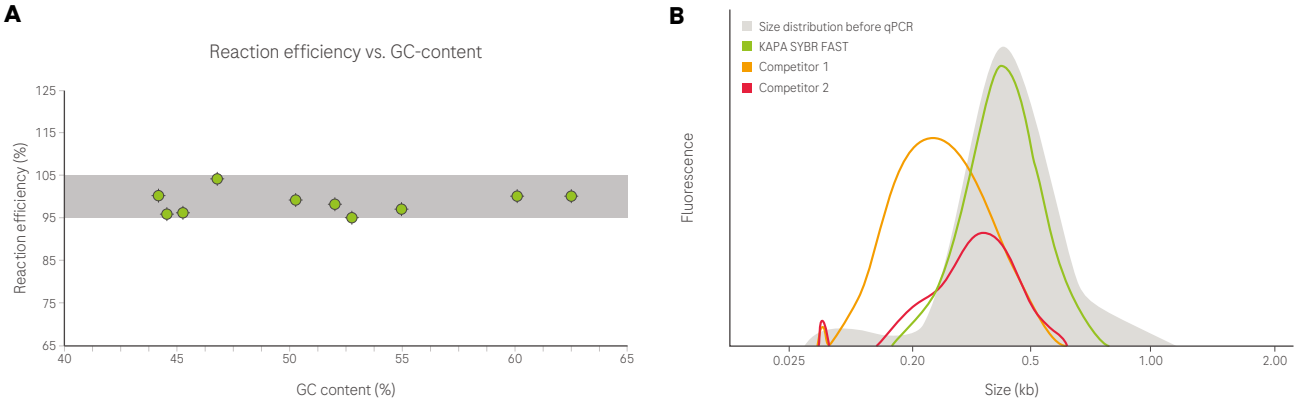


**Figure 3.** KAPA SYBR FAST is engineered for high-efficiency library quantification. (A) Amplification efficiencies achieved with the KAPA SYBR FAST qPCR Mix for ten diverse amplicons, plotted against GC content. All ten amplicons were amplified with similar efficiency (within the optimal range of 95% – 105%). This confirms that the KAPA SYBR FAST enzyme is ideal for the amplification of heterogenous 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

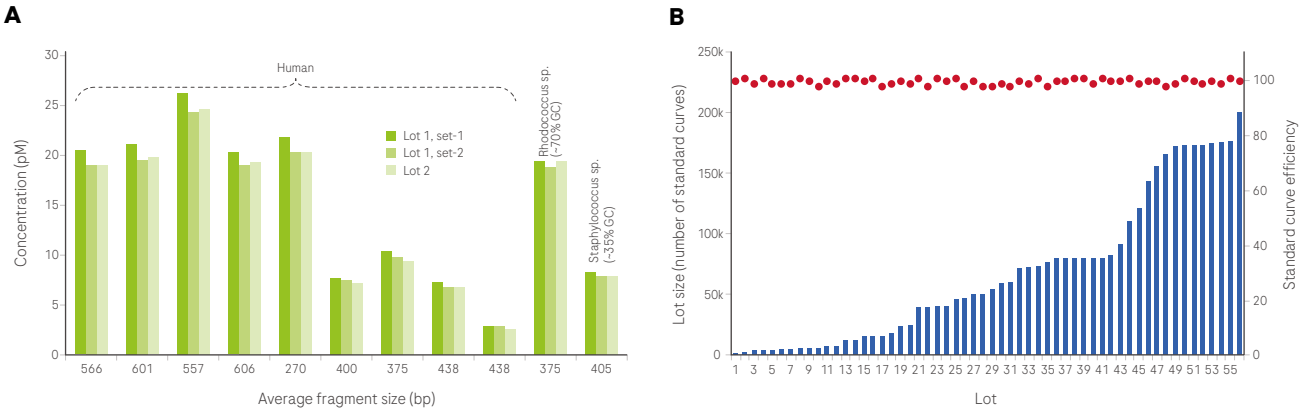


**Figure 4.** High consistency across different library types and across hundreds of production lots. (A) Nine human WGS libraries (41% GC) and two microbial WGS libraries (*Rhodococcus sp.*, 70% GC and *Staphylococcus sp.*, 35% GC) were quantified with distinct lots (Lots 1 and 2), and distinct sets 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.

†Compared to earlier KAPA DNA Library Prep chemistries. Data on file with Roche.
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## 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

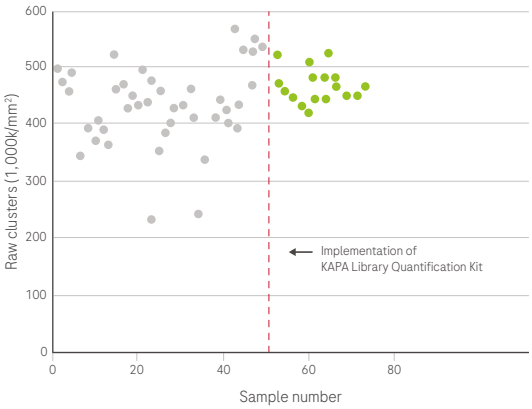


**Figure 5.** Accurate and reproducible library quantification enables improved and consistent cluster densities. The implementation of KAPA Library Quantification Kit into the Illumina® GA sequencing workflow at The Broad Institute (Cambridge, MA, USA) significantly reduced cluster density variability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

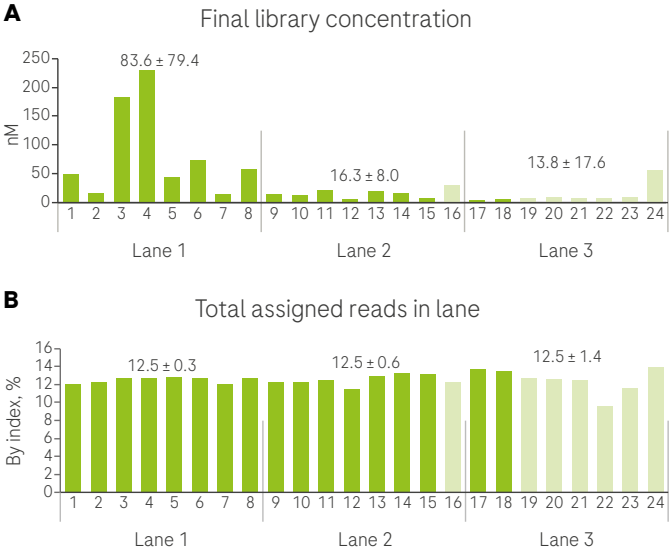


**Figure 6.** Accurate and sensitive quantification enables a uniform distribution of reads in muliplexed sequencing pools. Twenty-four indexed libraries were quantified by qPCR using the KAPA Library Quantification Kit (for Illumina® platforms) and combined to create three sequencing pools of equimolar 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.

Data on file with Roche.

### Ordering Information

| Roche cat. no. | KAPA code | Description | Kit size |
|---|---|---|---|
| 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.


## Learn more at sequencing.roche.com

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