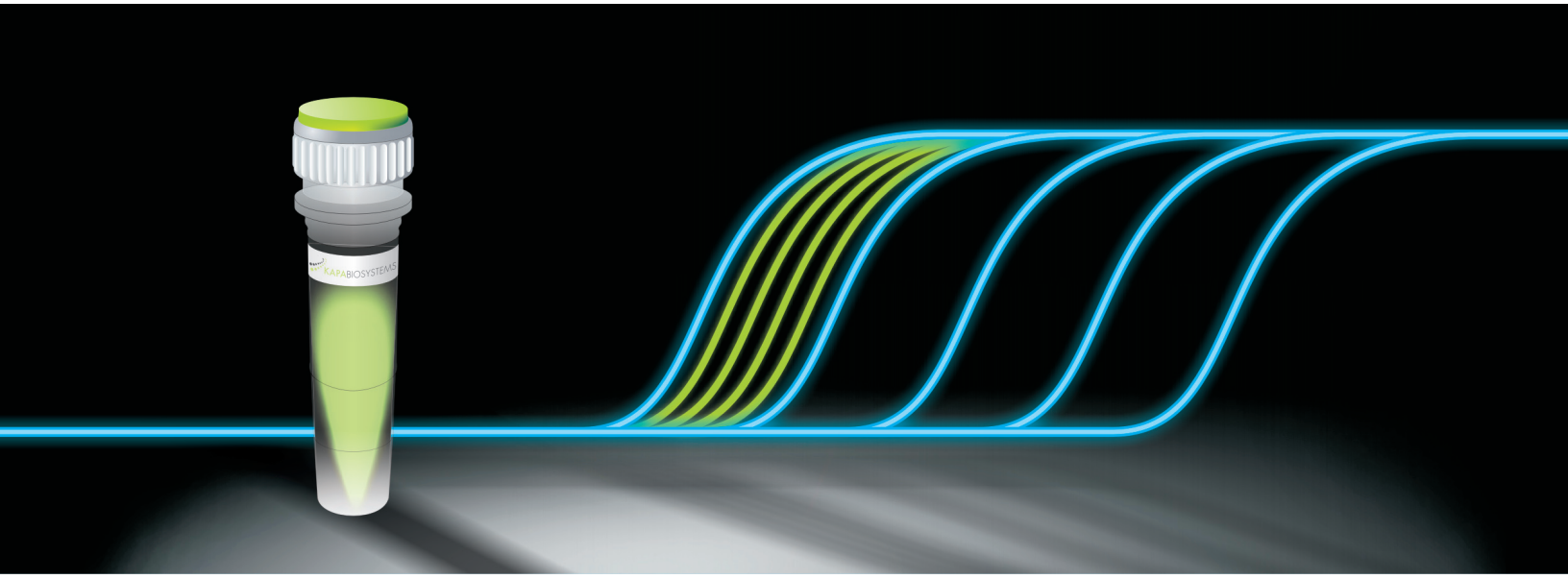
Next generation DNA sequencing meets next generation qPCR.



Eliminate time-consuming and expensive titrations and reduce variability in cluster density or template-to-bead ratio with a high performance qPCR solution for library quantification.

KAPA Library Quantification Kits

KAPABIOSYSTEMS

next generation thinking
in enzyme technology

Current standard protocols for commercial next generation sequencing platforms employ laborious, costly, and unreliable methods for quantifying DNA libraries.

Accurate quantification of PCR-competent sequencing templates is crucial for reliable clonal amplification via either emulsion PCR (emPCR) or bridge PCR (bPCR) - underestimation results in non-clonality, while overestimation leads to inefficiency via poor yields of clonally amplified templates.

Standard methods for quantifying NGS libraries have a number of important disadvantages. Electrophoresis and spectrophotometry measure *total* nucleic acid concentrations, whereas optimal cluster density or template-to-bead ratio depend on the appropriate concentration of *PCR-amplifiable* DNA molecules. These methods also have low sensitivity, consuming nanograms of precious samples, and are not suitable for high-throughput workflows.

Quantitative PCR (qPCR) is inherently well-suited for next-generation sequencing library quantification:

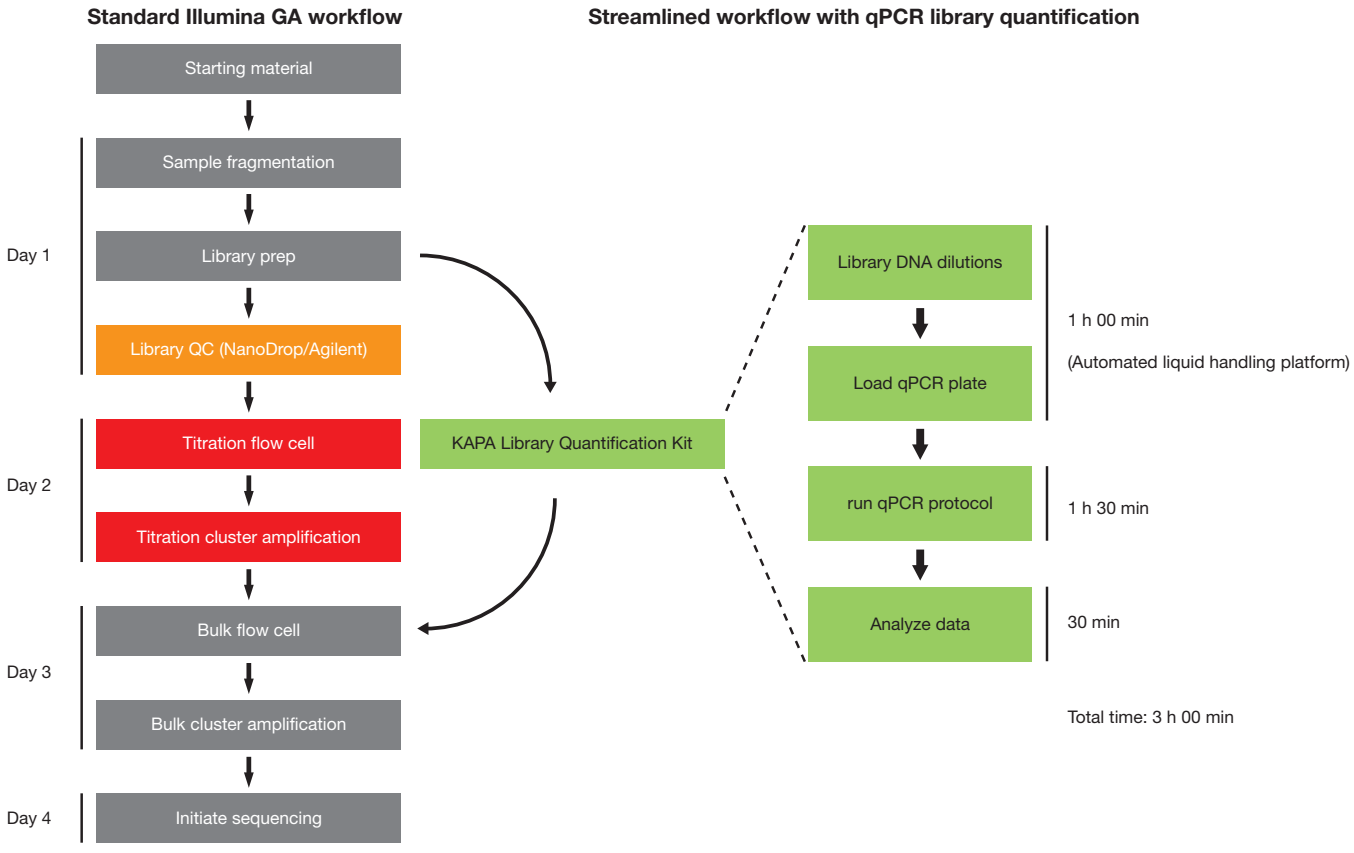
- qPCR specifically quantifies only PCR-competent DNA molecules,
- is highly sensitive allowing accurate quantification of low concentration libraries,
- is amenable to automated liquid handling.

KAPA Library Quantification Kits are optimized for the Illumina Genome Analyzer, Roche 454 Titanium series, and Roche 454 FLX series platforms and include defined, reliable DNA concentration standards and state-of-the-art qPCR reagents, containing a DNA polymerase engineered for SYBR® Green-based qPCR through a process of molecular evolution.

Library quantification

qPCR library quantification results in streamlined workflows

KAPA Library Quantification Kits eliminate the need for time-consuming and expensive titrations and provide a conducive format for streamlining high-throughput workflows.



>> KAPA Library Quantification Kits

Reliable quantification results in consistent cluster density

"Before qPCR was adopted for library quantification, cluster density was extremely variable. Implementation of the KAPA Library Quantification Kit in our sequencing workflows resulted in a significant reduction in variability across multiple libraries, negating the need for cluster amplification titration runs."

- Broad Institute, Cambridge, MA U.S.A.

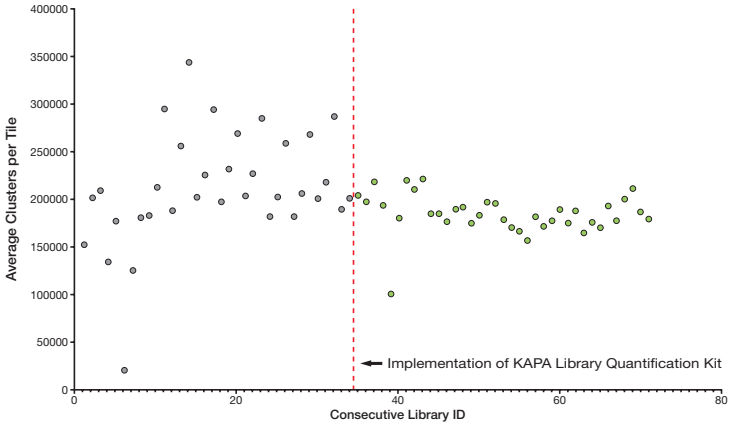


Fig.1 Cluster density before and after implementation of the KAPA Library Quantification Kit (right). The implementation of KAPA Library Quantification Kits into the Illumina GA sequencing workflow at the Broad Institute significantly reduced cluster density variability and eliminated the need for titrations. Average number of clusters per tile are shown for consecutive libraries.

Efficient amplification of a wide range of templates during qPCR

Traditional qPCR reagents are optimized for short amplification targets; longer targets, unbalanced GC-content, and problematic secondary structures may result in low amplification efficiency and unreliable quantification of some library molecules. To address the demands of quantifying complex DNA libraries, Kapa Biosystems has engineered a DNA polymerase specifically for SYBR® Green-based qPCR, enabling efficient amplification of targets that present a challenge to wild-type enzymes. KAPA Library Quantification Kits contain this engineered polymerase to ensure robust amplification of longer fragments, across a broad range of GC-content, required for accurate library quantification.

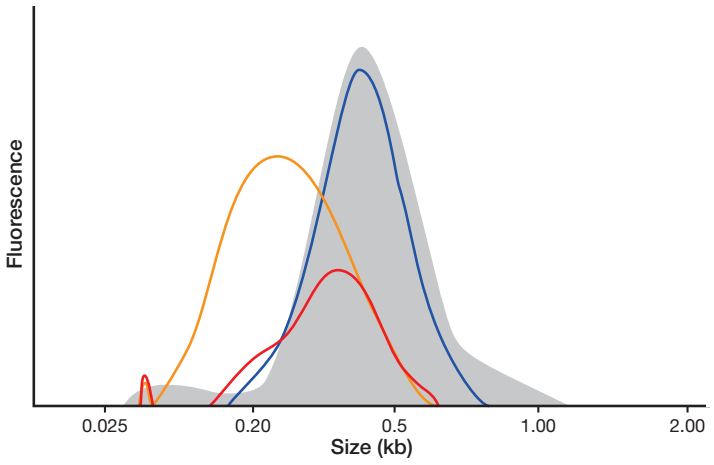


Fig.2 Fragment size distributions before and after qPCR. Fragment size distributions before (grey fill) and after qPCR amplification using three commercial qPCR master mixes (KAPA SYBR® FAST (blue), Competitor S (red), and Competitor F (orange)). Competitor kits contained wild-type Taq polymerase. Reactions were performed with the following cycling protocol: 95 ºC for 10 min followed by 40 cycles of 95 ºC for 10 sec and 60 ºC for 45 sec.

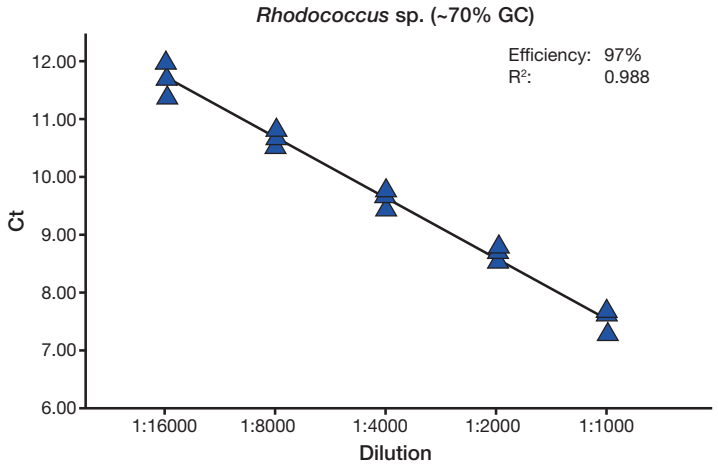


Fig.3 Robust amplification translates into accurate qPCR quantification of diverse libraries. The KAPA Libary Quantificaiton Kit was used to determine the concentration of two Illumina GA libraries with unusual GC content (*Rhodococcus* sp.; ~70% GC - shown above, *Staphylococcus* sp.; ~35% GC - not shown). Both libraries amplified with efficiency >95%. Two-fold dilution series (1:1000 through 1:16000) were prepared in triplicate, and qPCR performed according to the recommendations in the product technical data sheet.

>> KAPA Library Quantification Kits

Reliable DNA quantification standards with minimal variability from lot-to-lot

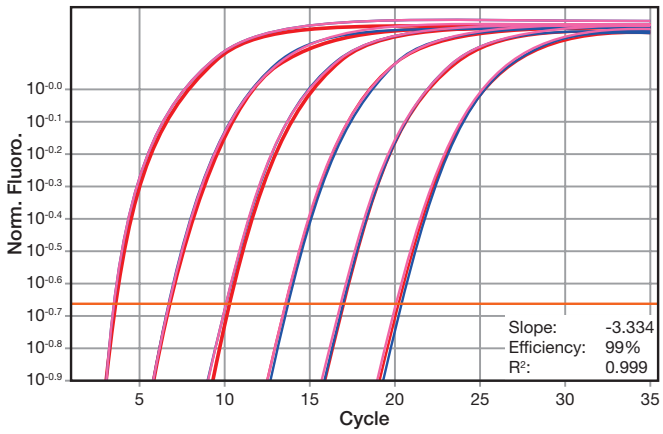


Fig. 4 Lot-to-lot variability of the KAPA Library Quantification Kit for the Roche Titanium series platform. Three distinct lots (red, pink, blue) were compared by analyzing amplification plots of each set of quantification standards. Triplicates of each data point were averaged.

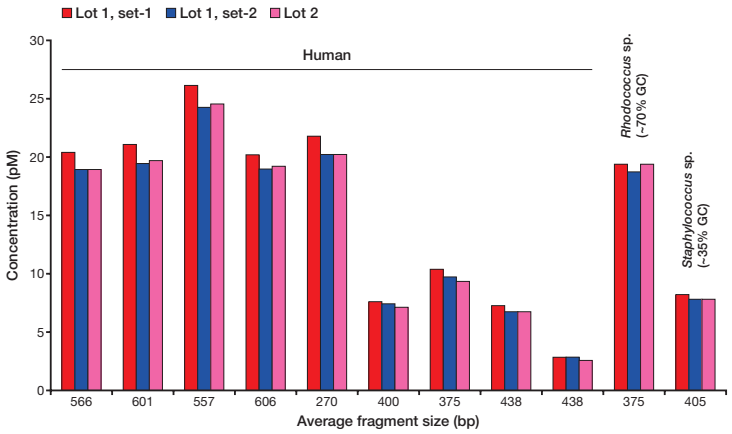


Fig. 5 Minimal lot-to-lot and kit-to-kit variability. 9 human DNA libraries and two microbial DNA libraries were used to compare quantification results obtained with distinct lots ("Lot 1" and "Lot 2"), and distinct sets of reagents from the same lot ("set 1" and "set 2") of KAPA Library Quantification Kits for the Illumina GA platform.

ORDERING INFORMATION

Description*	qPCR instrument	Code
KAPA Library Quantification Kit - Illumina GA	Universal	KK4824
KAPA Library Quantification Kit - Illumina GA	ABI Prism®	KK4835
KAPA Library Quantification Kit - Illumina GA	Bio-Rad iCycler™	KK4844
KAPA Library Quantification Kit - Illumina GA	Roche LightCycler® 480	KK4854
KAPA Library Quantification Kit - Roche 454 Titanium	Universal	KK4821
KAPA Library Quantification Kit - Roche 454 Titanium	ABI Prism®	KK4831
KAPA Library Quantification Kit - Roche 454 Titanium	Bio-Rad iCycler™	KK4841
KAPA Library Quantification Kit - Roche 454 Titanium	Roche LightCycler® 480	KK4851
KAPA Library Quantification Kit - Roche 454 FLX	Universal	KK4820
KAPA Library Quantification Kit - Roche 454 FLX	ABI Prism®	KK4830
KAPA Library Quantification Kit - Roche 454 FLX	Bio-Rad iCycler™	KK4840
KAPA Library Quantification Kit - Roche 454 FLX	Roche LightCycler® 480	KK4850

*All kits contain 5 mL KAPA SYBR® FAST qPCR Master Mix (2X), 1 mL Primer Premix, and 6 x 80 uL 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.

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For more information please contact **sales@kapabiosystems.com** or your local representative.

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