KAPABIOSYSTEMS

NEXT GENERATION SEQUENCING

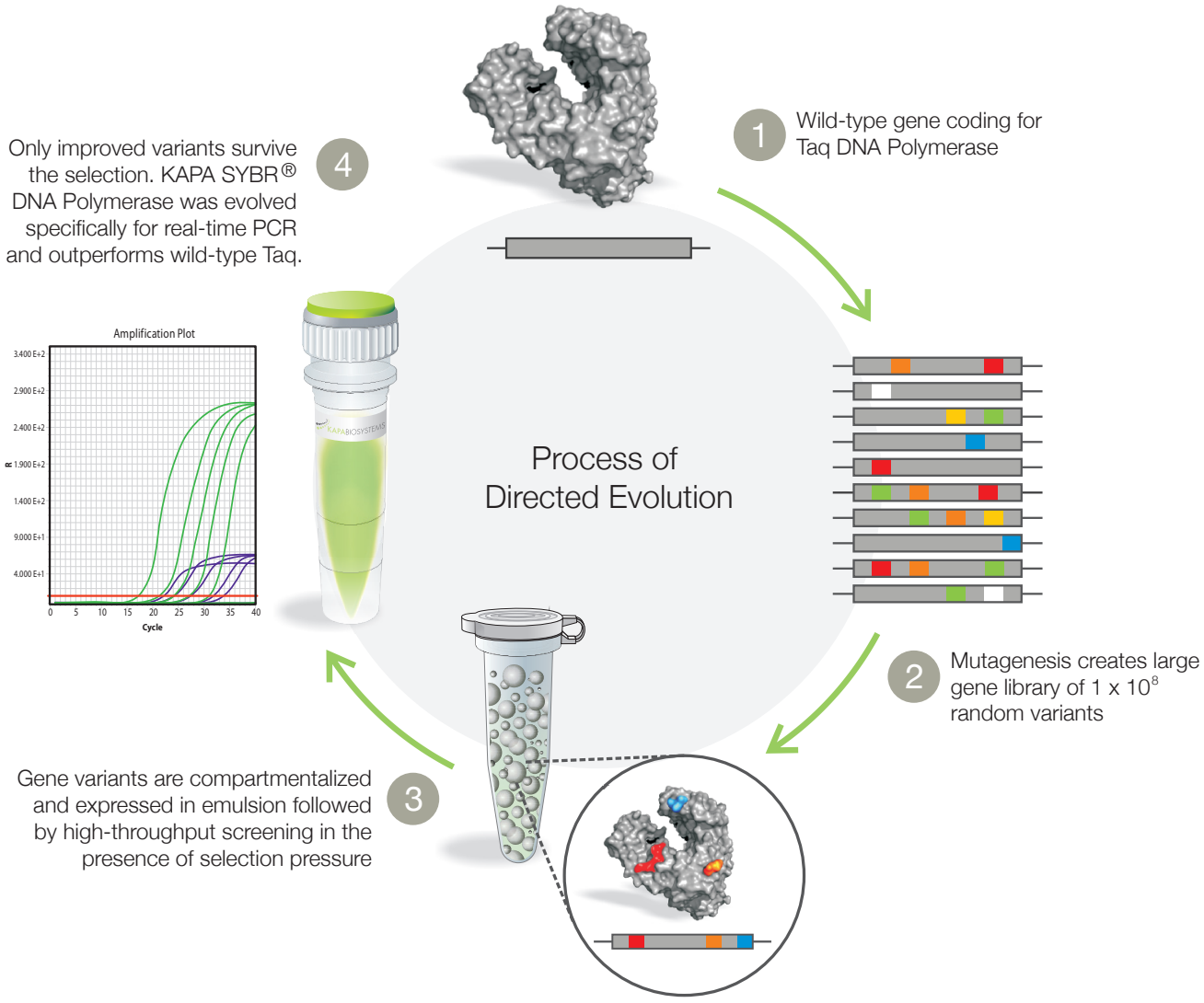
Innovative solutions for library preparation and quantification.

NEXT GENERATION SOLUTIONS

Dramatic improvements to commercial next generation sequencing (NGS) platforms have resulted in spectacular reductions in the cost-per-base of DNA sequencing. Until relatively recently, the primary focus for innovation has been on the core sequencing technologies, with optimization of library preparation playing a secondary role. The exponential gains in sequencing capacity have simultaneously led to growing sample throughput, increasing the demand for streamlined, efficient, and cost-effective library preparation protocols for multiplexed sample sequencing.

Kapa Biosystems is using novel enzymes engineered via *in vitro* molecular evolution to develop a suite of innovative products for next generation sequencing library preparation, amplification, and quantification.

Kits include: KAPA hgDNA Quantification and QC Kits, KAPA Library Preparation Kits, KAPA Library Amplification Kits, KAPA Real-Time Library Amplification Kits, and KAPA Library Quantification Kits.



KAPA Human Genomic DNA Quantification and QC Kits

The KAPA hgDNA Quantification and QC Kit is designed for the reliable quantification and quality assessment of human genomic DNA samples prior to library construction. Benefits of the KAPA hgDNA Quantification and QC Kits include:

- Reliable quantification of low concentration DNA samples
- QC variable quality DNA such as FFPE
- Predict success of library construction, post-amplification yield and mean insert size

KAPA Library Preparation Kits

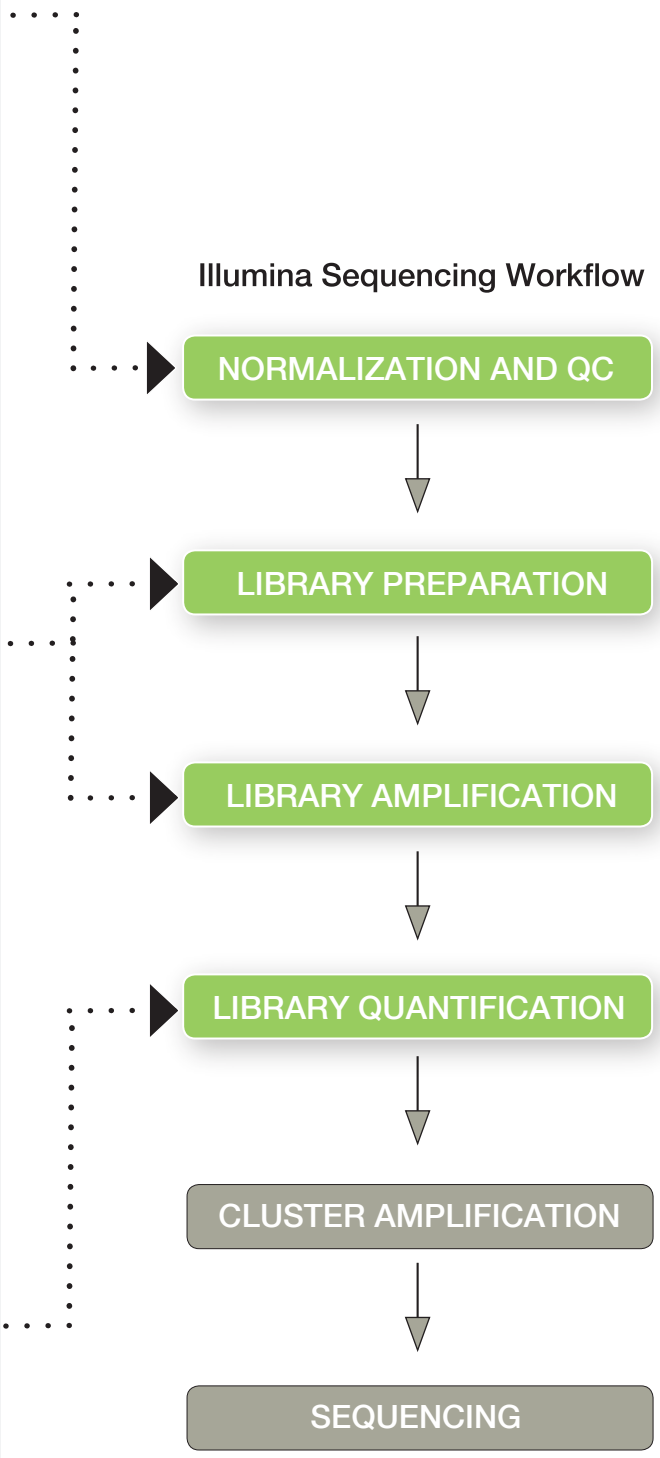
KAPA Library Preparation Kits provide all of the enzymes and reaction buffers required for constructing libraries from fragmented dsDNA and include the following modules: End Repair, A-Tailing, Ligation, and Amplification. Benefits of the KAPA Library Preparation Kits include:

- Manual and automation-friendly formats
- Improved DNA recovery accommodates lower input
- Cost of SPRI beads reduced by 60%
- Higher yields of adaptor-ligated library molecules
- Reduced PCR bias resulting in improved sequencing coverage

KAPA Library Quantification Kits

KAPA Library Quantification Kits quantify PCR-amplifiable library molecules with extreme sensitivity and reproducibility, in a format that is amenable to high sample throughput. Benefits of the KAPA Library Quantification Kits include:

- Quantification of only PCR-amplifiable library molecules
- Broad dynamic range allows quantification of low concentration libraries
- Format amenable to automated liquid handling



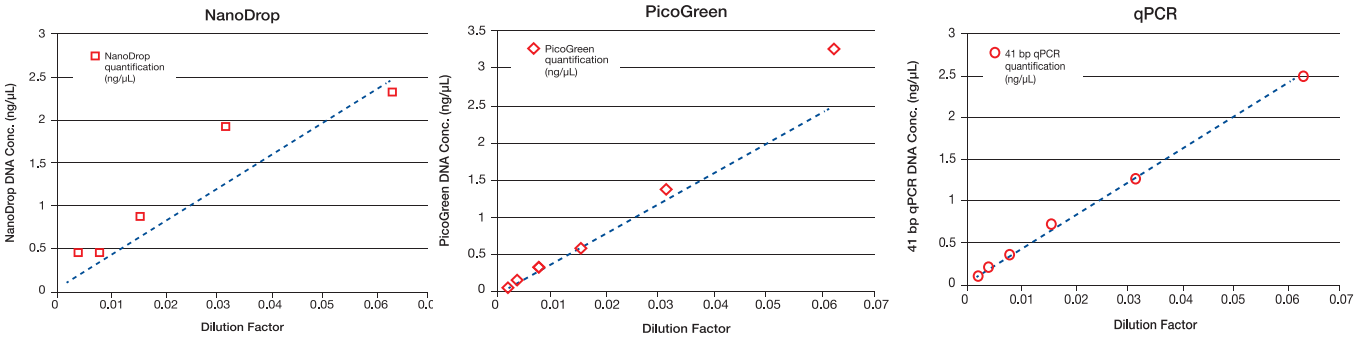
KAPA hgDNA Quantification and QC Kits

The KAPA Human Genomic DNA Quantification and QC Kit is designed for the reliable quantification and quality assessment of hgDNA samples prior to NGS library construction. These include:

- Formalin-fixed paraffin-embedded (FFPE) tissue samples, which are notorious for variable DNA quality
- Samples obtained by laser-capture microdissection of fresh, frozen or FFPE tissue
- DNA extracted from cells collected by flow cytometry
- Free circulating DNA from plasma or serum
- Forensic samples
- Any other low concentration or precious clinical sample

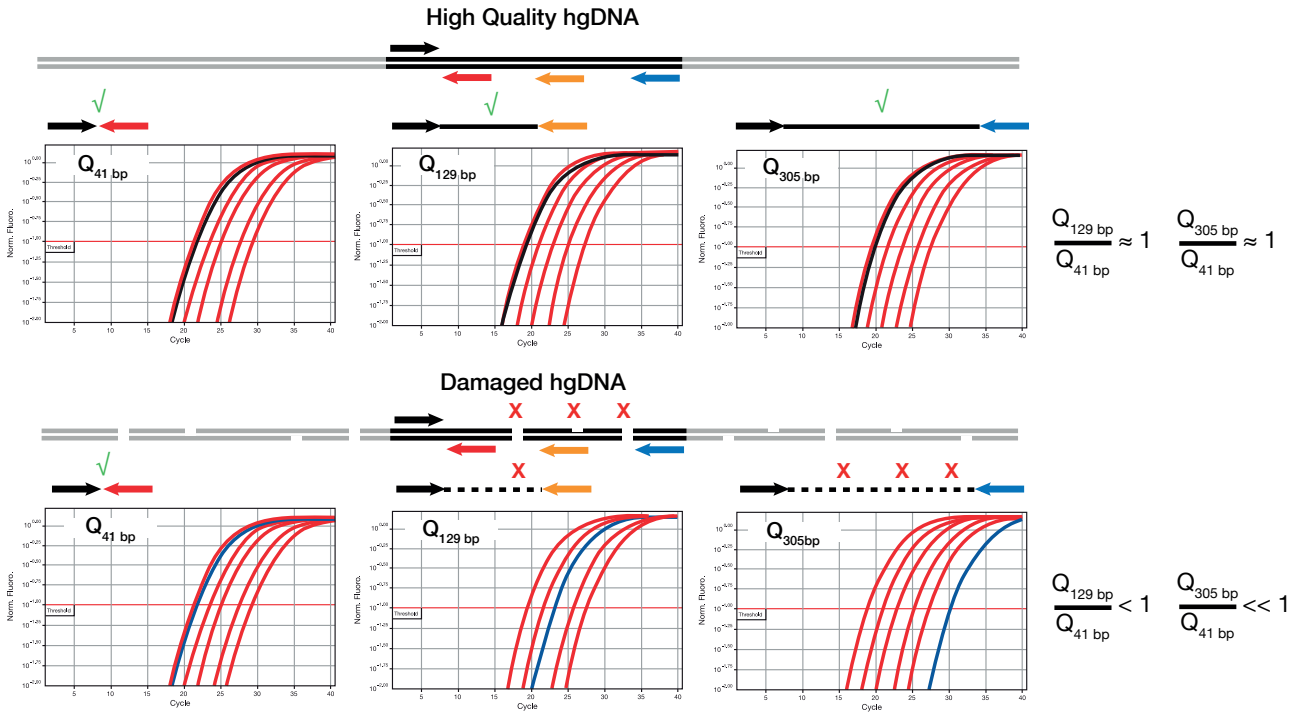
Accurate quantification at low DNA concentrations

Quantification methods that rely on spectrophotometry or electrophoresis (e.g. those employing a NanoDrop™, Qubit® or Bioanalyzer) have significant limitations for assessing input DNA, and provide poor predictions of library construction success. Such limitations include: poor accuracy in the quantification of very dilute samples, an inability to discriminate between damaged DNA and template material suitable for PCR-based processes such as library amplification, qPCR-based library quantification, cluster amplification and sequencing, and sensitivity to contaminants, which can lead to significant over- or underestimation of DNA concentrations.



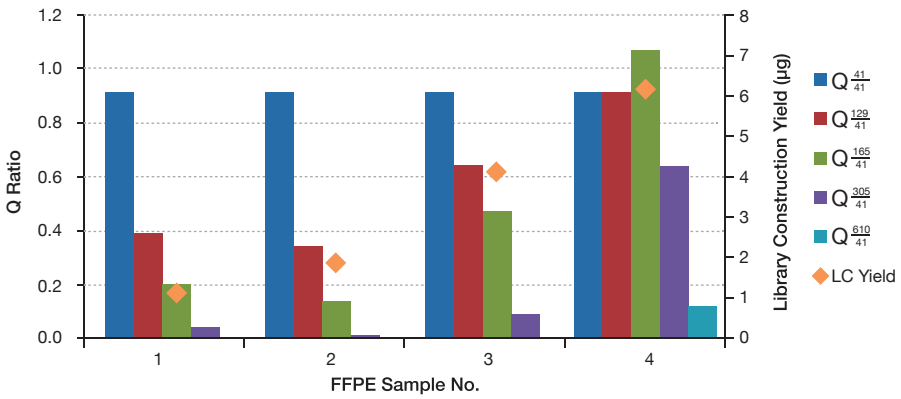
qPCR is accurate across a wide range of low DNA concentrations. Six 2-fold dilutions of intact DNA from 2.5 ng/µL to 0.09 ng/µL were quantified using the NanoDrop, PicoGreen® assay and 41 bp amplicon. Sample concentrations determined with each method are plotted against the dilution factors of the samples.

Using Q-ratios to assess DNA quality



Principle of the hgDNA Quantification and QC assay. A single set of DNA Standards is used to generate up to three standard curves, using three different primer pairs that amplify targets of 41 bp, 129 bp or 305 bp within a conserved, single-copy human locus. The 41 bp assay is used for absolute quantification of DNA samples. For an assessment of DNA quality, standard curves are generated and samples assayed with the 129 bp and/or 305 bp primer premix(es). Since poor DNA quality has a greater impact on the amplification of longer targets, the relative quality of a DNA sample can be inferred by normalizing the concentration obtained using the 129 bp or 305 bp assay against the concentration obtained from the 41 bp assay. This normalization generates a "Q-ratio" (with a value between 0 and 1) that can be used as a relative measure of DNA quality.

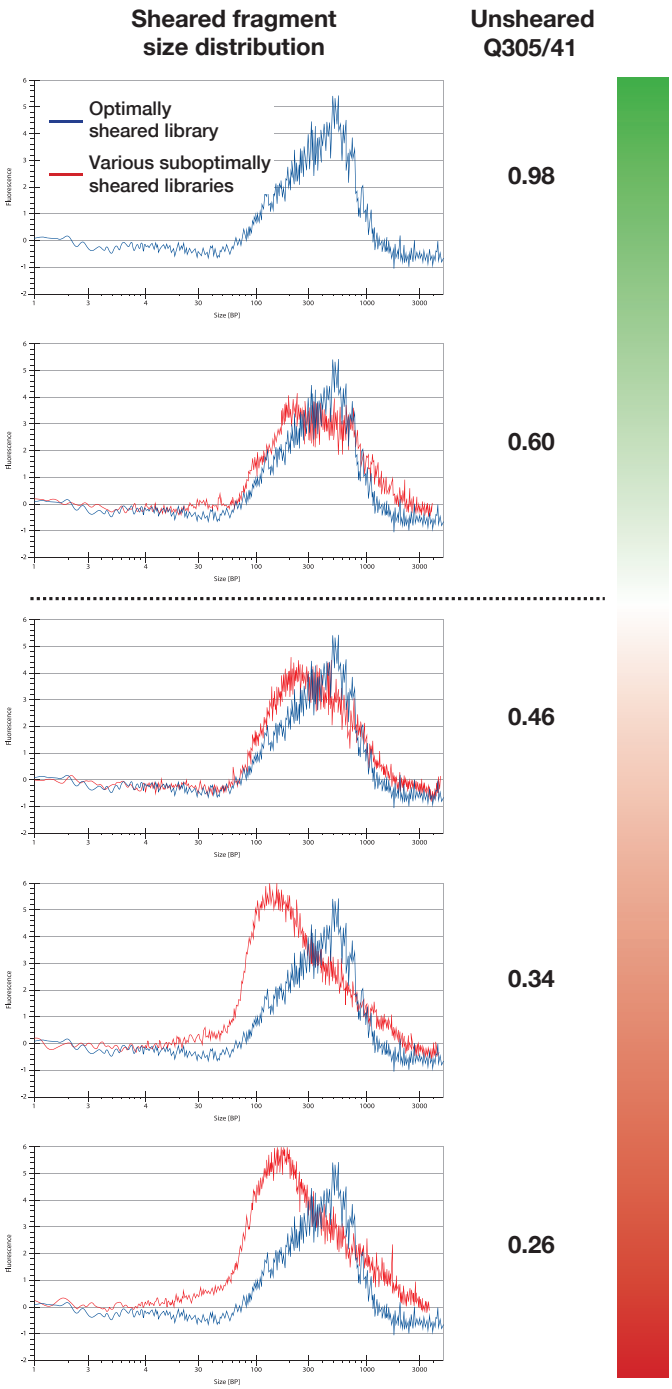
Q-ratios predict successful FFPE library construction



Q-ratios of FFPE samples correlate with library construction yields.

Q-ratios were determined for five human FFPE DNA samples of variable quality. These Q-ratios correlated well with library construction success. Data courtesy of Mirna Jarosz and Frank Juhn, Foundation Medicine, Cambridge, MA, USA.

Q-ratios predict mean insert size and sequencing quality



Ten hgDNA samples were selected from a whole-genome sequencing (WGS) production set that included some samples showing fragment size distributions smaller than expected (~bp average) after shearing.

All samples were processed through the standard WGS library protocol using 100 ng input DNA. Size selection was carried out using the Sage Science Pippin Prep, and comprised a tight cut centered at 500 bp.

Descending Q305/41 values for the unsheared sample correlated with:

- lower average fragment size after shearing, and
- increasing amounts of small inserts in the sequenced libraries (data not shown).

Abundant small library fragments indicated by low Q-ratio's may affect library quality despite stringent size selection. Data is presented for five representative samples of those tested.

Data courtesy of Kathleen Steinmann, Sharon Kim and Maura Costello at The Broad Institute, Cambridge, MA, USA.

KAPA DNA Library Preparation Kits

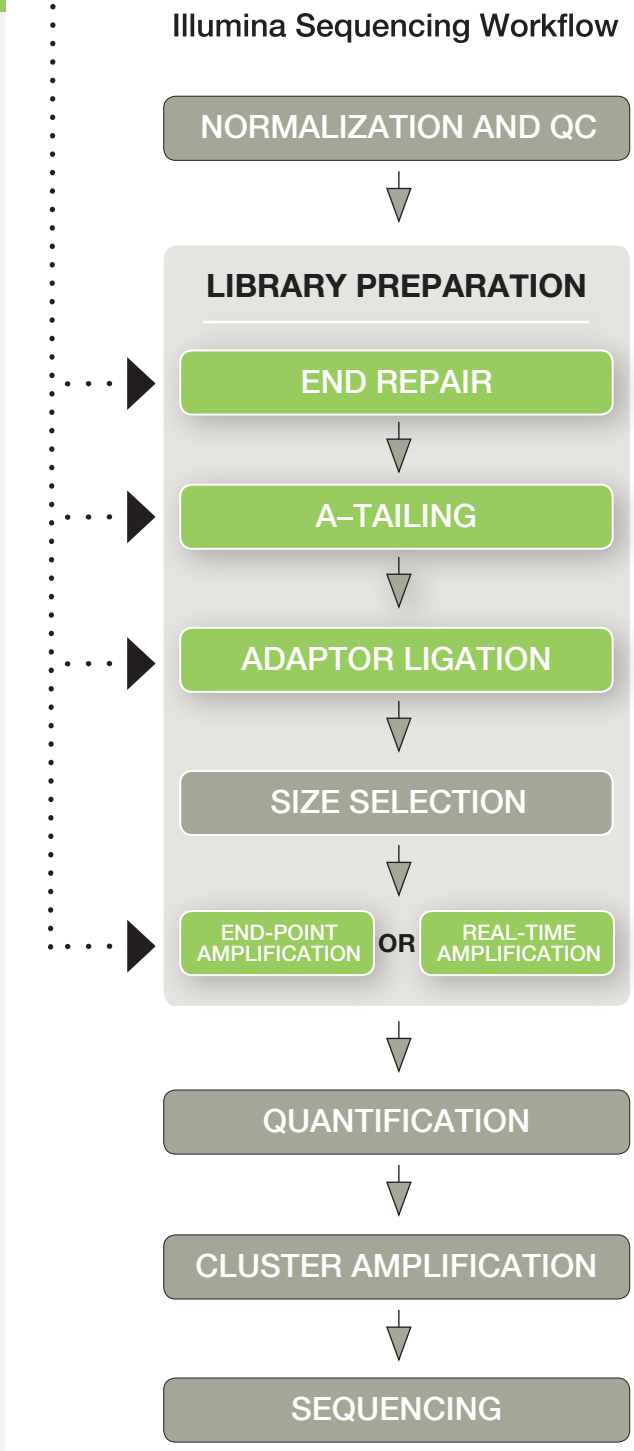
KAPA Library Preparation Kits are available in both low-throughput (LTP) and high-throughput (HTP) formats and provide all of the enzymes and reaction buffers required for constructing Illumina libraries from 100 ng - 5 µg of fragmented dsDNA via the following steps: End Repair, A-Tailing, Adaptor Ligation, and Library Amplification.

Reaction buffers are supplied in convenient, concentrated master mix formats comprising all of the required reaction components except oligonucleotide adaptors or PCR primers.

The KAPA HTP Library Preparation Kit has been tailored for high-throughput workflows and automated liquid handling. Each kit contains sufficient reaction buffer and enzyme to process 96 samples, with generous excesses to accommodate the dead volumes required for automated liquid handling.

Efficient, cost-effective, and automation-friendly reaction cleanups are achieved through implementation of the "with-bead" strategy developed at The Broad Institute of MIT & Harvard and Foundation Medicine, and kits include PEG/NaCl SPRI (Solid Phase Reversible Immobilization) solution for this purpose.

In order to maximize sequence coverage uniformity, it is critical that library amplification bias be kept to a minimum. KAPA HiFi DNA Polymerase has been designed for low-bias, high fidelity PCR, and is the reagent of choice for NGS library amplification. KAPA Library Preparation Kits include KAPA HiFi HotStart ReadyMix (2X), a ready-to-use cocktail comprising all components for PCR except primers and template. Kits without an amplification module (KK8231 and KK8233) are available for PCR-free workflows. These kits can also be combined with KAPA HiFi Real-Time Library Amplification Kits (KK2701, KK2702) or with KAPA HiFi Uracil+ ReadyMix (KK2801, KK2802) for the amplification of libraries constructed from bisulfite-treated DNA.



KAPA Library Preparation Kits contain the following reaction modules:

End Repair

The End Repair module produces blunt-ended, 5'-phosphorylated DNA fragments.

- End Repair Enzyme Mix containing T4 DNA Polymerase and T4 Polynucleotide Kinase (LTP: 50 µL or 250 µL, HTP: 600 µL)
- 10X End Repair Buffer with dNTPs (LTP: 100 µL or 500 µL, HTP: 1.2 mL)

A-Tailing

The A-Tailing module adds dAMP to the 3'-ends of the dsDNA fragments.

- A-Tailing Enzyme: Klenow Fragment 3'- 5' exo- (LTP: 30 µL or 150 µL, HTP: 360 µL)
- 10X A-Tailing Buffer (LTP: 50 µL or 250 µL, HTP: 650 µL)

Adaptor Ligation

The Ligation module ligates dsDNA adaptors with 3'-dTMP overhangs to library fragments.

- DNA Ligase (LTP: 50 µL or 250 µL, HTP: 600 µL)
- 5X Ligation Buffer (LTP: 100 µL or 500 µL, HTP: 1.3 mL)

Amplification

KAPA Library Preparation Kits are supplied with and without a Standard PCR Library Amplification module. Kits not supplied with a Library Amplification module (KK8231, KK8233, KK8235) can be used for PCR-free workflows or combined with the Real-Time PCR Library Amplification module (KK2701, KK2702) for optimizing PCR cycle number, or KAPA HiFi HotStart Uracil+ ReadyMix (KK2801, KK2802) for amplifying bisulfite-treated DNA libraries.

End-Point PCR

- 2X KAPA HiFi HotStart ReadyMix (LTP: 250 µL or 1250 µL, HTP: 3 mL)

OR

Real-Time PCR

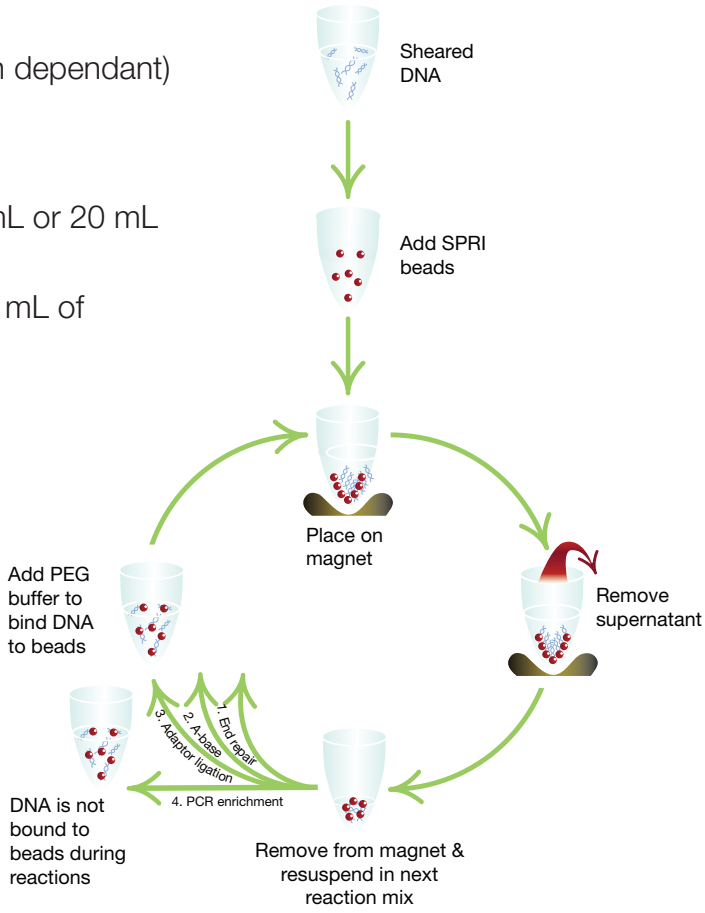
- 2X KAPA HiFi HotStart Real-Time PCR Master Mix (LTP only: 250 µL or 1250 µL)
- 4 x Fluorescent Standards (LTP only: 1500 µL each)

Efficient, cost-effective reaction cleanups and higher recoveries are achieved using “with-bead” strategy

Benefits of the “with-bead” protocol

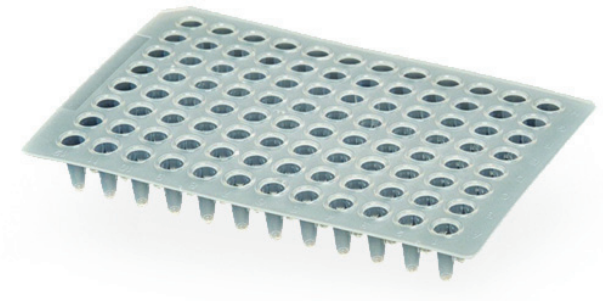
- Improved DNA recovery
- Input DNA as low as 1 ng - 100 ng (application dependant)
- Reduced cost of SPRI beads by 60%
- Streamlined workflow
- KAPA LTP Library Preparation Kits contain 5 mL or 20 mL of KAPA PEG/NaCl SPRI Solution
- KAPA HTP Library Preparation Kits contain 40 mL of KAPA PEG/NaCl SPRI Solution

“With-bead” workflow



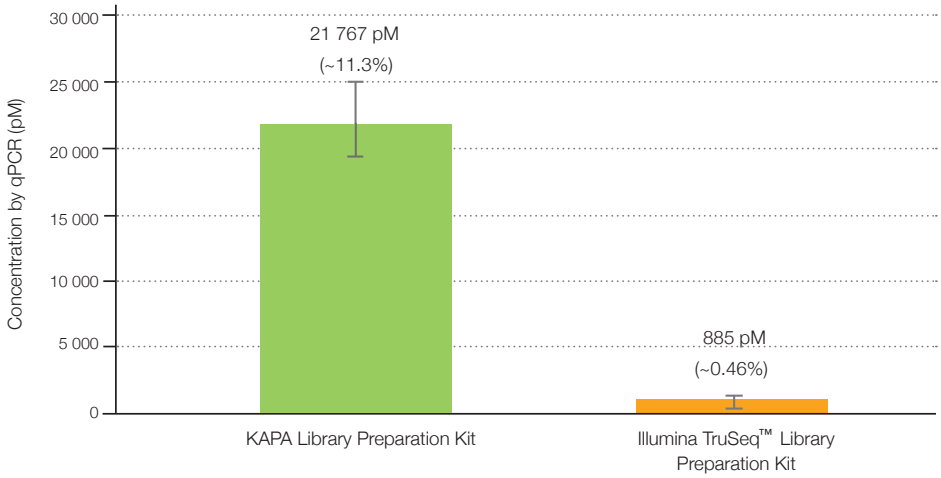
Adapted from Fisher, S. et al. Genome Biology 12, R1 (2011)

KAPA HTP Library Preparation Kit is formatted for automation



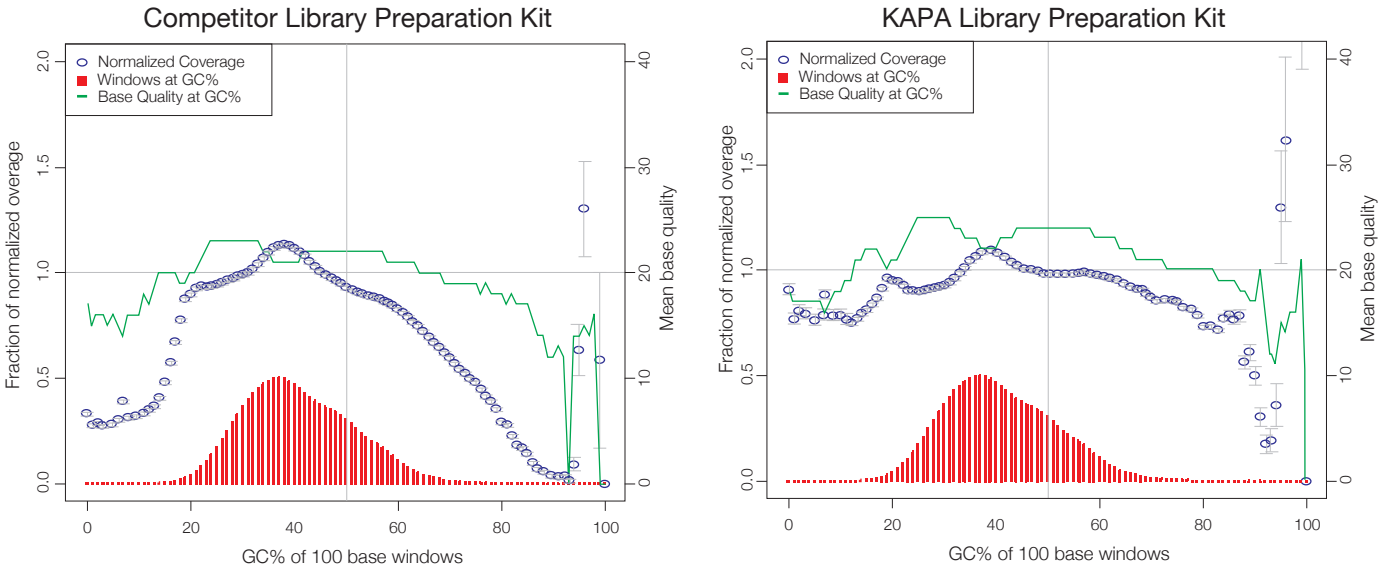
- Sufficient reagents to accommodate “dead volumes”
- Plate-friendly reaction volumes
- Robot-friendly pipetting volumes
- Fewer pipetting steps for automated reaction setup saves time and plasticware

Ultra pure, high quality library construction reagents result in higher yield of adaptor-ligated library molecules



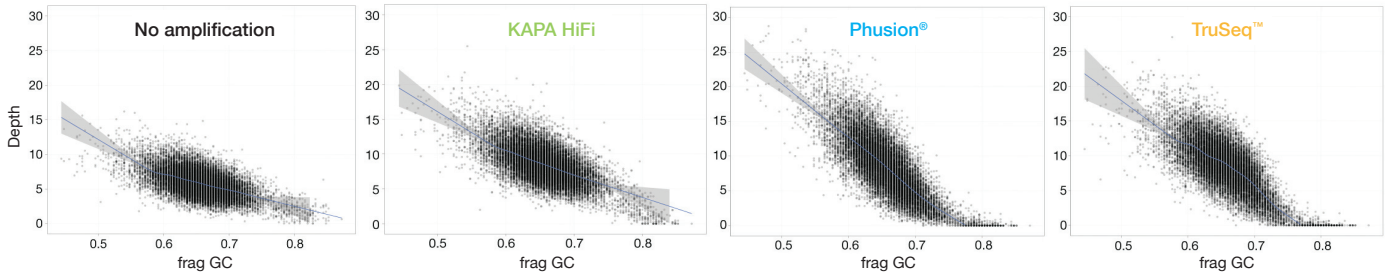
Sheared genomic DNA from three organisms (*S. aureus, E. coli, or M.tuberculosis*) was prepared in bulk by nebulization and 1 µg of identical starting material was used for each library. Libraries were constructed using the KAPA Library Preparation Kit and recommended protocol (green; 9 libraries), or using the Illumina TruSeq™ DNA Sample Prep Kit and Low-Throughput Protocol (orange; 15 libraries). Libraries were quantified by qPCR before size selection using the KAPA Library Quantification Kit according to recommended protocol. The estimated percentage of starting material that was converted to useful, adaptor-ligated (PCR-amplifiable) library molecules is provided. The KAPA Library Preparation Kit and protocol produced ~25-fold more adaptor-ligated library fragments from the same amount of starting material than did the standard Illumina Truseq™ DNA Sample Prep Kit.

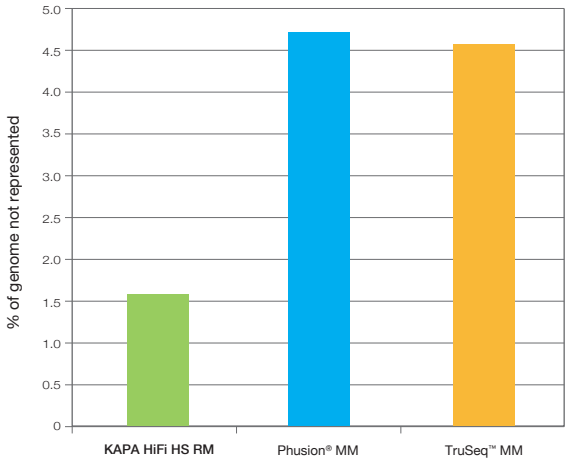
An engineered, high fidelity DNA polymerase reduces amplification bias and improves sequencing coverage



Indexed libraries were constructed and amplified from 100 ng human genomic DNA using either the KAPA Library Preparation Kit (right) or the standard library preparation reagents and protocol in use at The Broad Institute (left). Individual libraries were quantified using qPCR, pooled prior to denaturation, and sequenced (pair-ended, 2 x 25 bp) on an Illumina HiSeq 2000. Libraries prepared using the KAPA Library Preparation Kit resulted in more uniform coverage distribution across the range of GC-content. Data courtesy of The Broad Institute.

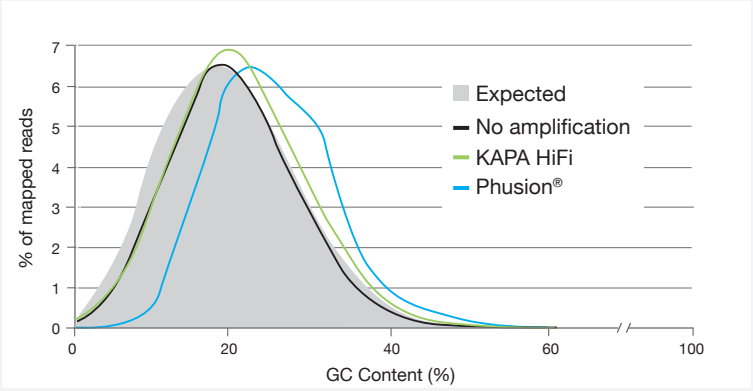
KAPA HiFi DNA Polymerase reduces amplification bias of GC- and AT-rich genomes

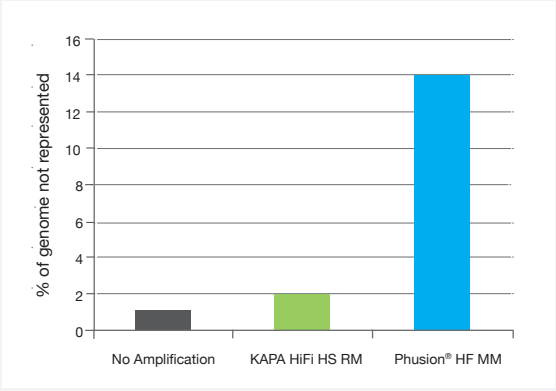




Effect of high-GC content on coverage depth for libraries amplified using common proof-reading (B-family) polymerases.

Indexed Illumina TruSeq™ libraries prepared from identical sheared *M. tuberculosis* (65% GC) gDNA were amplified using the indicated PCR reagents, and compared to an equivalent unamplified library by paired-end sequencing (2 x 75 bp). After filtering and aligning read pairs to reference sequences, 250 000 read pairs were randomly sampled for each genome, and scatter plots of mean sequence coverage depth vs. GC content were generated by analyzing 250 bp windows. GC-rich sequences were under-represented following library amplification using Phusion® HF Master Mix or Illumina TruSeq™ PCR Master Mix. In contrast, library amplification with KAPA HiFi HotStart ReadyMix resulted in coverage distribution across the range of GC-content that is almost indistinguishable from that of the unamplified control.





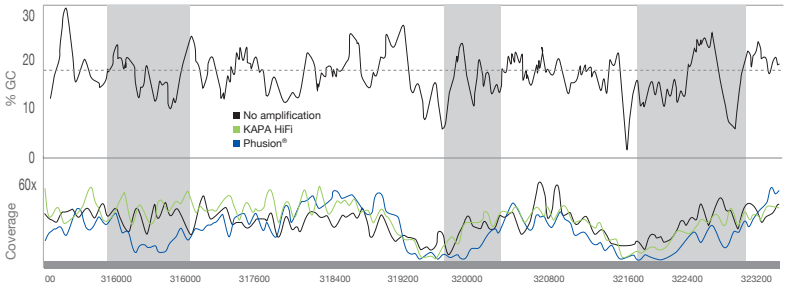
Low GC-content libraries result in variable bias depending on the polymerase used for amplification.

Libraries prepared from identical sheared *P. falciparum* (19% GC) gDNA were amplified using the indicated PCR reagents, and compared to an equivalent unamplified library. Observed frequencies of GC-content for reads are plotted for each condition tested. The expected frequency distribution of reads is indicated by the grey shaded area.

The unamplified library tracked the expected frequency distribution. Amplification with KAPA HiFi showed minimal bias while amplification with Phusion® resulted in a dramatic bias against reads with low GC-content. Average read depth for each library was 16.0x (unamplified control); 16.5x (KAPA HiFi); 18.8x (Phusion®). Data courtesy of Dr. Michael A. Quail, The Wellcome Trust Sanger Institute.

Library amplification can dramatically affect coverage uniformity

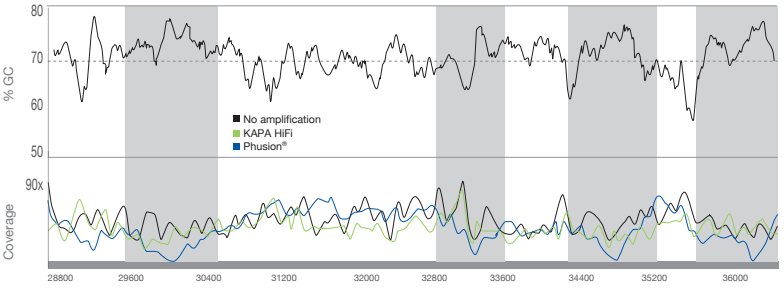
The following Artemis screen captures depict examples of coverage bias in libraries amplified with either KAPA HiFi HotStart Master Mix or Phusion® HF Master Mix, compared to an unamplified control library. In short stretches of either high or low-GC content, the degree of coverage bias varies according to the method used to amplify the library.



Data courtesy of Dr. Michael A. Quail, The Wellcome Trust Sanger Insitute

Coverage depth and GC content across a ~7 kb region of the *P. falciparum* genome.

Within this region of the genome there are 3 locations where high-AT sequences (>80%) lead to coverage bias (grey bars). In all three regions coverage depth drops significantly after amplification with Phusion® (blue), while the library amplified using KAPA HiFi (green) shows more uniform coverage depth which tracks that of the unamplified control library (black).

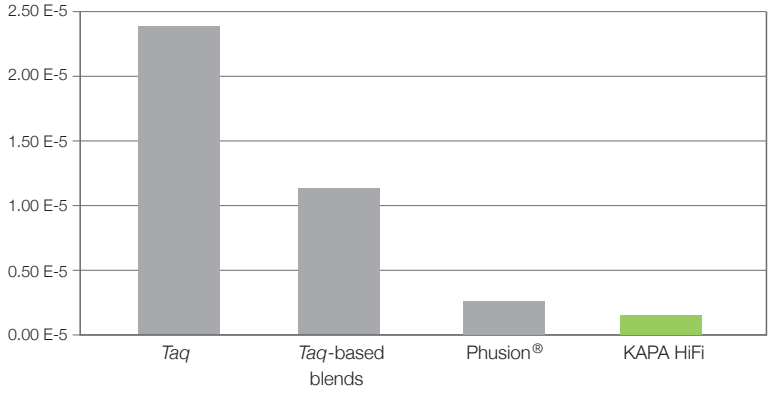


Data courtesy of Dr. Michael A. Quail, The Wellcome Trust Sanger Insitute

Coverage depth and GC content across a ~7 kb region of the *B. pertussis* genome.

Within this region of the genome there are 4 distinct locations of high-GC sequence (>75%) that lead to sequence coverage bias (grey bars). In these regions the library amplified using Phusion® (blue) exhibits lower depth of coverage compared to the unamplified control. In contrast, the library amplified with KAPA HiFi (green) exhibits more even coverage depth, similar to the control library (black).

Superior accuracy for high fidelity library amplification



Error rate of DNA polymerases and blends.

The error rate of KAPA HiFi is calculated at 1 error in 3.54 x 10^6 bases covered (2.82 x 10^{-7}). The error rate of KAPA HiFi is 100X lower than *Taq* polymerase, 40X lower than polymerase blends and 2X lower than Phusion®.

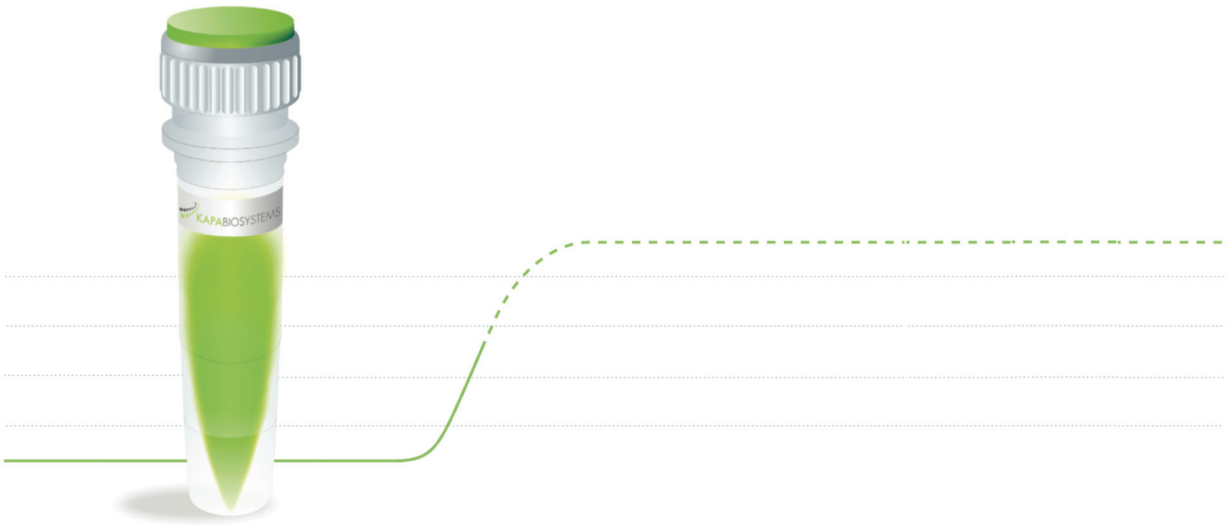
Real-time PCR alternative to standard library amplification

High fidelity PCR is used to selectively enrich library fragments carrying appropriate adaptor sequences and to amplify the amount of DNA prior to sequencing. During PCR enrichment of libraries, minimizing amplification bias is critical to ensure uniform coverage. Introduction of amplification bias requires more total sequencing in order to obtain sufficient coverage in regions of the genome that are underrepresented, increasing sequencing costs.

Amplification bias occurs when a DNA polymerase is unable to amplify all targets within a complex population of library DNA with equal efficiency. Bias is further exacerbated when libraries are over-amplified.

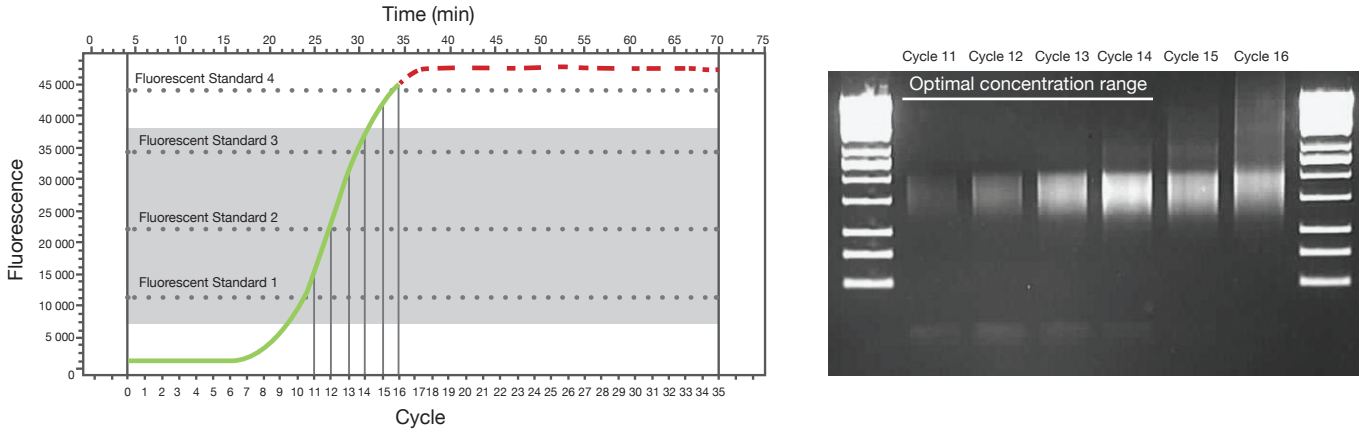
KAPA Library Preparation Kits containing the Real-Time PCR Library Amplification module are designed to address both sources of PCR-induced bias. The novel KAPA HiFi DNA Polymerase, engineered for high fidelity and processivity, is capable of balanced amplification of complex library DNA. Real-time monitoring of library amplification provides additional information required to minimize over-amplification. Benefits of performing high fidelity, real-time PCR for next-generation sequencing library amplification include:

- Real-time monitoring of amplification allows precise control over the optimal number of PCR cycles, reducing the uncertainty of when to terminate enrichment PCR.
- Real-time amplification workflows are amenable to automation because post-PCR DNA electrophoresis is not required.
- Real-time amplification plots provide quality metrics for individual enriched libraries, eliminating expensive and time-consuming post-enrichment gel electrophoresis and identifying inconsistencies in library preparation.
- Real-time library amplification integrates seamlessly with qPCR-based KAPA Library Quantification Kits. Termination of enrichment PCR in real-time enables tight control over the yield of enriched library DNA prior to quantification, requiring fewer library dilutions for accurate DNA quantification.

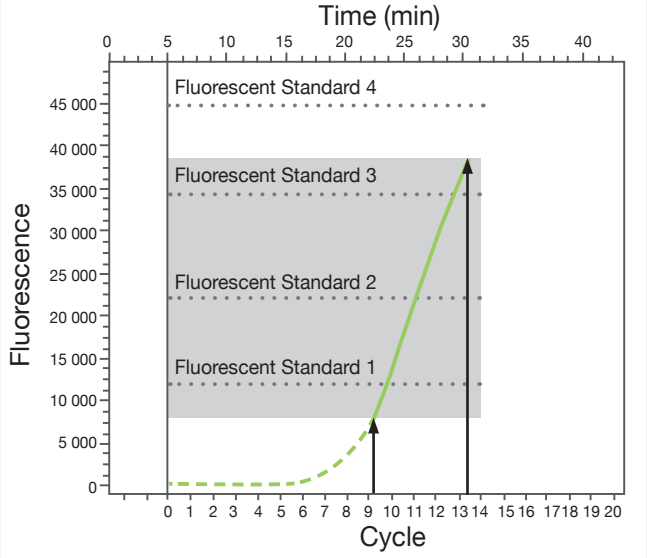


Real-time, high fidelity amplification of next-generation DNA sequencing libraries

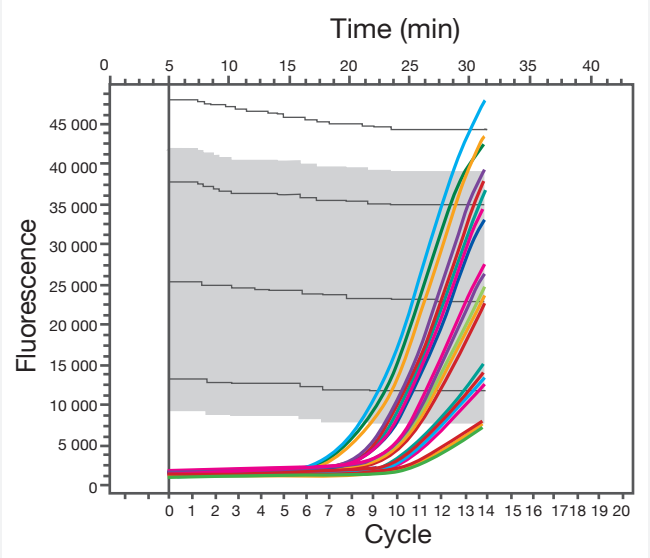
Enzyme bias – along with other artifacts such as PCR-induced errors, adaptor dimers, PCR duplicates, and chimeras – is exacerbated by over-amplification, while under-amplification results in insufficient yields. Inherent uncertainty in the outcome of end-point PCR often demands downstream validation of library quality by electrophoresis. KAPA Real-Time PCR Library Amplification Kits provide a high fidelity, real-time PCR method for rapid and convenient amplification of libraries. Precise control over the number of cycles required for optimal amplification eliminates expensive and time-consuming post-enrichment gel electrophoresis.



Libraries are amplified using a SYBR® Green I-based real-time, high fidelity PCR master mix (left panel). Four triplicate wells of the PCR plate contain fluorescent reference standards representing a range of distinct DNA concentrations. Reactions terminated between standards 1 and 3 represent the optimal library amplification range (grey box), depicted here from cycle 10- 14. Gel image illustrating the enrichment of a typical library stopped at different amplification cycles relative to the fluorescent standards supplied in the KAPA Real-Time Library Amplification Kit (right panel). Low and high molecular weight artifacts increase progressively with additional cycles.



Superimposed amplification plots for reactions terminated at the lower bound (hashed line, cycle 10) or upper bound (solid line, cycle 14) of the targeted concentration range (grey box). Library amplification reactions should ideally be terminated anywhere within the indicated target concentration range.



Example of real-time, high fidelity amplification of multiple libraries. 20 libraries, spanning a ~64-fold concentration range (6 cycles), were simultaneously amplified and terminated after 14 cycles. 14 of the 20 libraries fall within the targeted amplification range. The remaining 6 libraries could either be used as is, noting that they may be outside the optimal concentration range, or they could be re-amplified individually or in high- or low-concentration groups.

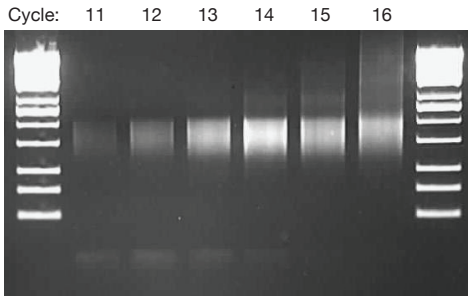
KAPA Real-Time PCR Library Amplification Kit workflow

TruSeq™ Cycle Number Optimization Protocol

SET UP AND INITIATE SMALL-SCALE PCR

REMOVE SMALL-SCALE PCR REACTIONS AT DESIRED PCR CYCLES

DETERMINE IDEAL CYCLE NUMBER BY PERFORMING GEL ELECTROPHORESIS



Run an aliquot of each small-scale sample on an agarose gel or Bioanalyzer chip to determine the optimal cycle number to be used for preparative PCR.

SET UP AND INITIATE PREPARATIVE PCR WITH PREDEFINED CYCLE NUMBER

CLEAN UP PCR REACTION

QUANTIFY LIBRARY USING ILLUMINA QUANTIFICATION PROTOCOL GUIDE

Total time: 3 h 30 min

Disadvantages

- Wastage of library material required for cycle number optimization leads to loss of library diversity.
- Inconsistencies in reaction volume scale-up can cause variable results.
- Longer protocol involves more time and labor.
- Gel electrophoresis steps are not amenable to automation.

TruSeq™ Fixed Cycle Number Amplification Protocol

SET UP AND INITIATE PREPARATIVE HIGH FIDELITY PCR

TERMINATE HIGH FIDELITY PCR AT PREDEFINED CYCLE NUMBER

?

No information provided as to whether cycle number was optimal for library amplification.

- Even if prior cycle number optimization is performed for similar sample types, variability in library preparation and sample handling may result in over- or under-amplified libraries.
- Post cleanup electrophoresis may be required for quality control to confirm that libraries were neither over- nor under-amplified.

CLEAN UP PCR REACTION

QUANTIFY AMPLIFIED LIBRARY USING ILLUMINA QUANTIFICATION PROTOCOL GUIDE

Total time: 1 h 45 min

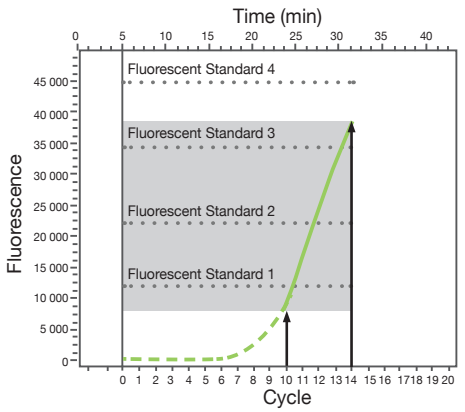
Disadvantages

- Variability in the nature and/or concentration of the input DNA can result in under- or over-amplification, exacerbating amplification bias and artifacts.
- Post-amplification electrophoresis QC is not amenable to automation.
- Ideally, libraries should be minimally amplified to a low final concentration (~3.5 µM), not detectable via standard gel electrophoresis.

KAPA Real-Time PCR Library Amplification Protocol

SET UP AND INITIATE PREPARATIVE HIGH FIDELITY REAL-TIME PCR

TERMINATE HIGH FIDELITY REAL-TIME PCR



Terminate real-time PCR reaction when the linear amplification plots of samples fall between fluorescent standards 1 - 3 or within targeted concentration range. This is a 4 cycle termination window and enables single plate amplification of libraries with up to a 16-fold difference in initial concentration.

CLEAN UP REAL-TIME PCR REACTION

QUANTIFY LIBRARY USING KAPA LIBRARY QUANTIFICATION KIT

Total time: 1 h 45 min

Advantages

- Built-in real-time quality metrics (concentration range) for each amplified DNA library.
- Real-time PCR is amenable to automation.
- Precise control over PCR cycle number required for optimal amplification.
- Seamless integration with qPCR-based library quantification.
- KAPA HiFi DNA Polymerase is less prone to amplification bias due to high- or low-GC content.

KAPA Library Quantification Kits

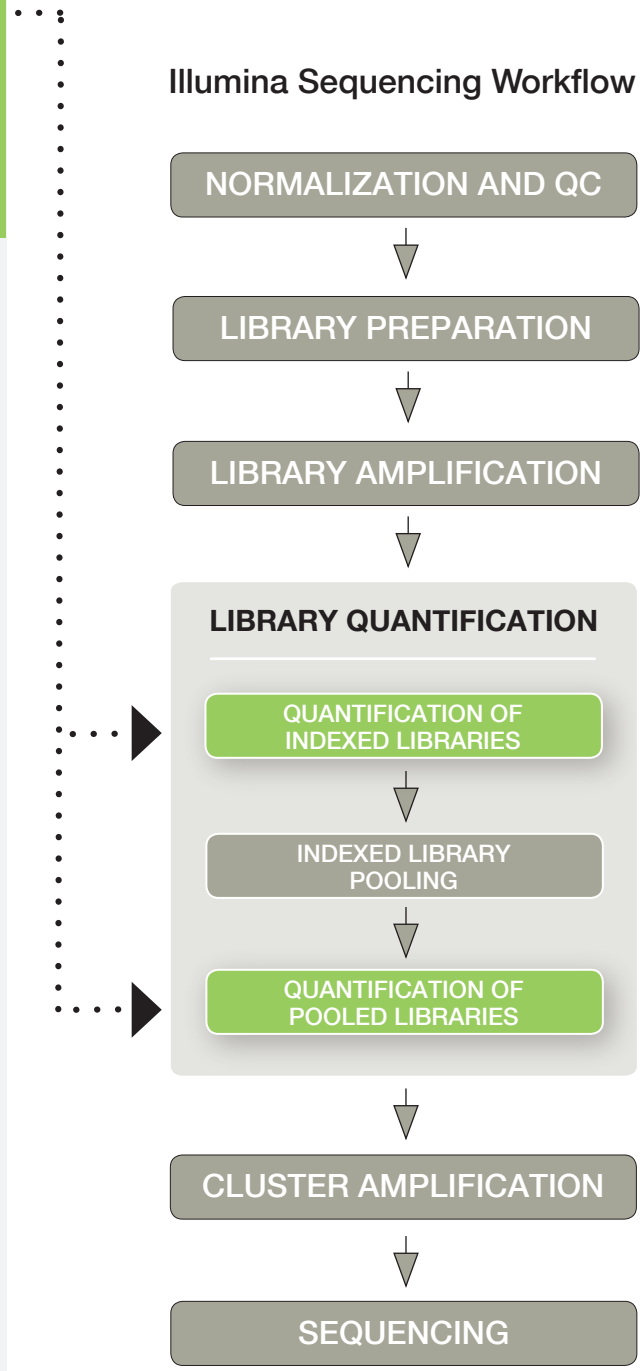
Accurate quantification of amplifiable library molecules is critical for the efficient use of next generation sequencing (NGS) platforms – underestimation results in non-clonality, while overestimation leads to inefficiency via poor yields of clonally amplified templates. Accurate library quantification is equally important when pooling indexed libraries for multiplexed sequencing to ensure equal representation of each library.

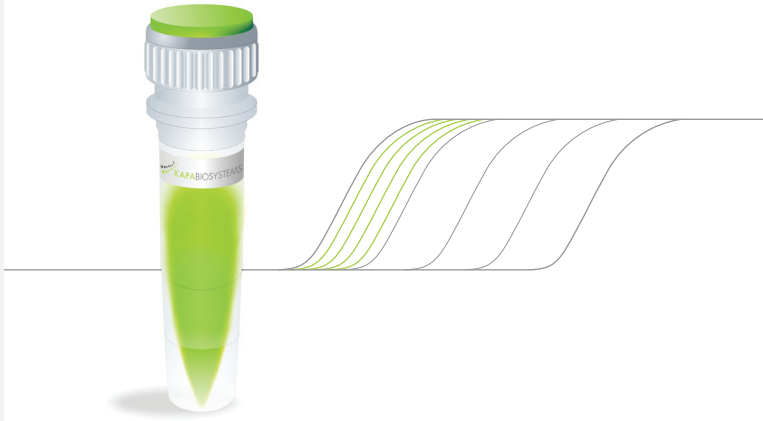
Standard methods for quantifying NGS libraries have a number of important disadvantages. Electrophoresis and spectrophotometry measure total nucleic acid concentrations, whereas optimal cluster density or template-to-bead ratio depends on the appropriate concentration of PCR-amplifiable DNA molecules.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

qPCR is inherently well-suited to NGS library quantification, and overcomes many of the difficulties posed by alternative approaches:

- qPCR quantifies only the PCR-amplifiable library molecules;
- is exceptionally sensitive and accurate across an extremely broad dynamic range;
- is amenable to high sample throughput and automated liquid handling.

The extreme sensitivity of qPCR enables accurate quantification of very dilute libraries, minimizing the need for PCR amplification of libraries and the associated problematic biases.





KAPA Library Quantification Kit workflow

KAPA Library Quantification Kits comprise DNA Standards (six 10-fold dilutions) and 10X Primer Premix, paired with KAPA SYBR® FAST qPCR Kits to accurately quantify the number of amplifiable molecules in an NGS library. The KAPA DNA Standards consist of a linear DNA fragment flanked by qPCR primer binding sites. Quantification is achieved by inference from a standard curve generated using the six DNA Standards.

1. Prepare qPCR/Primer Premix. Add 1 ml of Primer Premix to 5 ml bottle of KAPA SYBR® FAST qPCR Master Mix (2X).

2. Make 1:1000 dilution of dsDNA library. Dilute library DNA in 10 mM Tris-HCl. Optional additional library dilutions: 1:2000, 1:4000 and 1:8000.

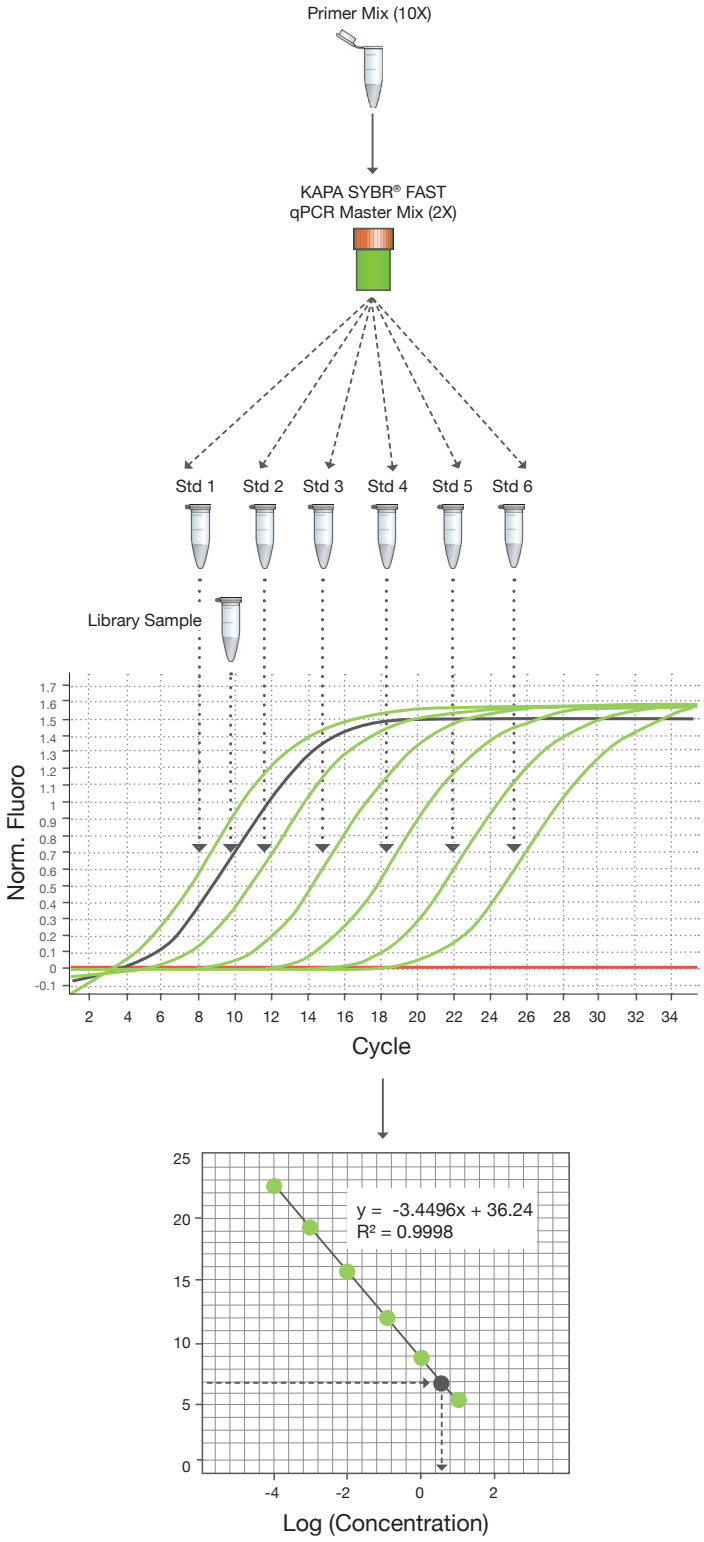
3. Prepare qPCR plate. For 20 µl reactions use 12 µl KAPA SYBR® FAST qPCR Master Mix containing Primer Premix, 4 µl PCR-grade water, and 4 µl diluted library DNA or DNA Standard (1 - 6).

4. Run qPCR protocol. Initial denaturation for 5 min at 95 ºC followed by 35 cycles of denaturation for 30 sec at 95 ºC and combined annealing and extension for 45 sec at 60 ºC.

5. Analyze data. Confirm 90 - 110% reaction efficiency for DNA Standards and library.

6. Calculate library concentration. Use absolute quantification against the DNA Standard provided.

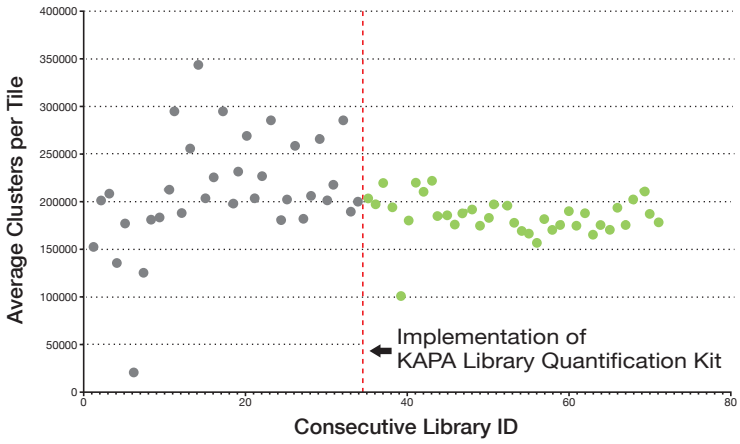
7. Use the calculated concentration of the undiluted library to prepare appropriate dilution of the library and proceed with cluster amplification or emPCR.



Reliable quantification leads to 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."

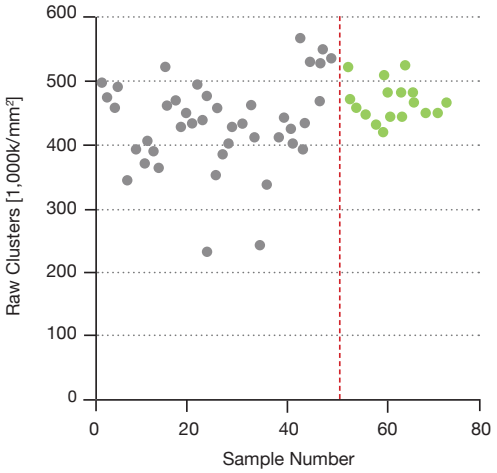
- The Broad Institute, Cambridge, MA U.S.A.



Cluster density before and after implementation of the KAPA Library Quantification Kit.

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.

qPCR only quantifies PCR-amplifiable library molecules



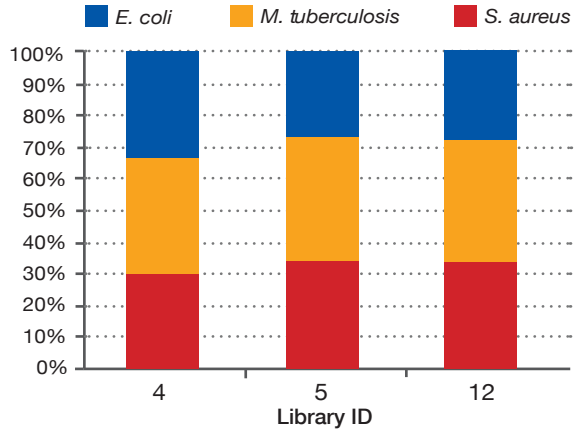
qPCR-based KAPA Library Quantification Kits reduce variability in cluster density compared to Agilent Bioanalyzer.

Human exome samples were prepared using Nimblegen solution-based capture. All preparations were performed in a 96-well plate format on a liquid handling system. Average number of clusters per tile are shown for paired-end, 76-bp Illumina GAIIx runs. Data courtesy University of Washington.

Compared to spectrophotometric, fluorimetric, and electrophoretic methods, qPCR only quantifies adaptor-ligated and amplifiable library molecules.

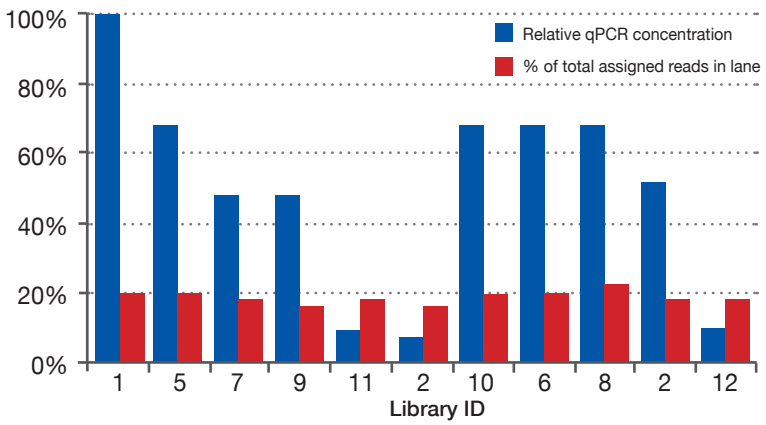
The ligation of adaptor sequences (orange and blue) to library DNA molecules (grey) results in a mixed population of molecules without the correct adaptor configuration. Electrophoresis and spectrophotometry measure total nucleic acid concentrations, whereas optimal cluster density or template-to-bead ratio depends on the appropriate concentration of PCR-amplifiable DNA molecules. Only qPCR-based library quantification methods count library molecules with the correct adaptor sequence configuration.

qPCR quantification enables equal representation of intra- and inter-indexed libraries for multiplex sequencing



Equal representation of different sample types within indexed libraries.

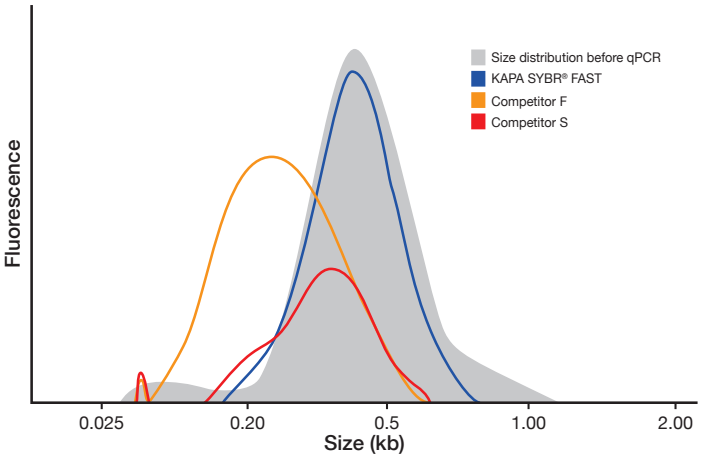
Three separate libraries (*S. aureus*, 33% HGC; *E. coli*, 51% GC; and *M. tuberculosis*, 65% GC) were constructed for each index (TruSeq™ 4, 5, and 12). Individual libraries were quantified using the KAPA Library Quantification Kit and for each index the libraries were pooled to achieve equimolar representation for each genome. The results indicate that quantification is reliable for samples with a wide range of GC content.



Equimolar pooling of indexed libraries.

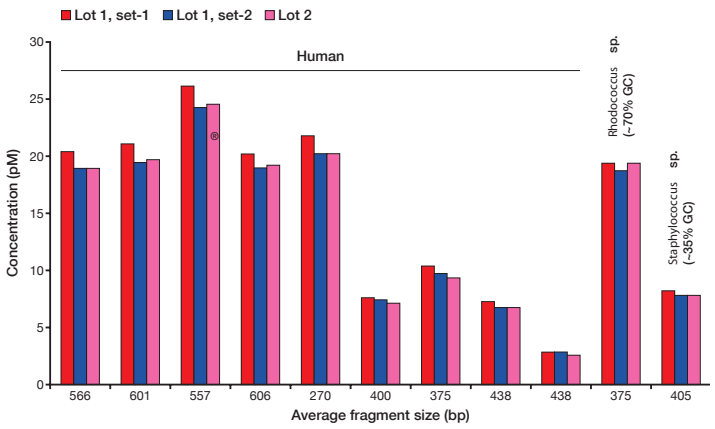
Eleven indexed Illumina TruSeq™ libraries were quantified by qPCR using the KAPA Library Quantification Kit, and then combined to achieve equal final concentrations in two separate pools for multiplexed sequencing on different flow-cell lanes. The eleven libraries ranged ~11-fold in concentration from 0.67 pM to 7.65 pM, while representation of each index varied between 90% and 127% of expected assigned reads per lane.

Engineered DNA polymerase and reliable DNA quantification standards with minimal variability from lot-to-lot



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, Competitor S, and Competitor F). Competitor kits contained 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.



Standards with high lot-to-lot consistency.

9 human DNA libraries and two microbial DNA libraries (*Rhodococcus sp.* ~70% GC and *Staphylococcus sp.* ~35% GC) 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

KAPA Library Preparation Kits	Contents	Code
KAPA LTP Library Preparation Kit with Standard Library Amplification	10 libraries	KK8230
KAPA LTP Library Preparation Kit with Standard Library Amplification	50 libraries	KK8232
KAPA Library Preparation Kit with Real-Time Library Amplification	10 libraries	KK8220
KAPA Library Preparation Kit with Real-Time Library Amplification	50 libraries	KK8221
KAPA LTP Library Preparation Kit without Library Amplification	10 libraries	KK8231
KAPA LTP Library Preparation Kit without Library Amplification	50 libraries	KK8233
KAPA HTP Library Preparation Kit with Standard Library Amplification	96 libraries	KK8234
KAPA HTP Library Preparation Kit without Library Amplification	96 libraries	KK8235

KAPA Library Amplification Kits	Contents	Code
KAPA Library Amplification Kit - Standard PCR	50 x 50 µl rxn	KK2611
KAPA Library Amplification Kit - Standard PCR	250 x 50 µl rxn	KK2612
KAPA Library Amplification Kit - Real-Time PCR	50 x 50 µl rxn	KK2701
KAPA Library Amplification Kit - Real-Time PCR	250 x 50 µl rxn	KK2702
KAPA HiFi Uracil+ HotStart ReadyMix for Bisulfite Sequencing	50 x 50 µl rxn	KK2801
KAPA HiFi Uracil+ HotStart ReadyMix for Bisulfite Sequencing	250 x 50 µl rxn	KK2802

KAPA Library Quantification Kits	qPCR Instrument	Code
KAPA Library Quantification Kit - Illumina	Universal	KK4824
KAPA Library Quantification Kit - Illumina	ABI Prism®	KK4835
KAPA Library Quantification Kit - Illumina	Bio-Rad iCycler™	KK4844
KAPA Library Quantification Kit - Illumina	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
KAPA Library Quantification Kit - ABI SOLiD	Universal	KK4823
KAPA Library Quantification Kit - ABI SOLiD	ABI Prism®	KK4833
KAPA Library Quantification Kit - ABI SOLiD	Bio-Rad iCycler™	KK4843
KAPA Library Quantification Kit - ABI SOLiD	Roche LightCycler® 480	KK4853
KAPA Library Quantification Kit - Ion Torrent PGM/Proton	Universal	KK4827
KAPA Library Quantification Kit - Ion Torrent PGM/Proton	ABI Prism®	KK4838
KAPA Library Quantification Kit - Ion Torrent PGM/Proton	Bio-Rad iCycler™	KK4847
KAPA Library Quantification Kit - Ion Torrent PGM/Proton	Roche LightCycler® 480	KK4857

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

For more information on our complete range of innovative products please visit **www.kapabiosystems.com**

KAPABIOSYSTEMS