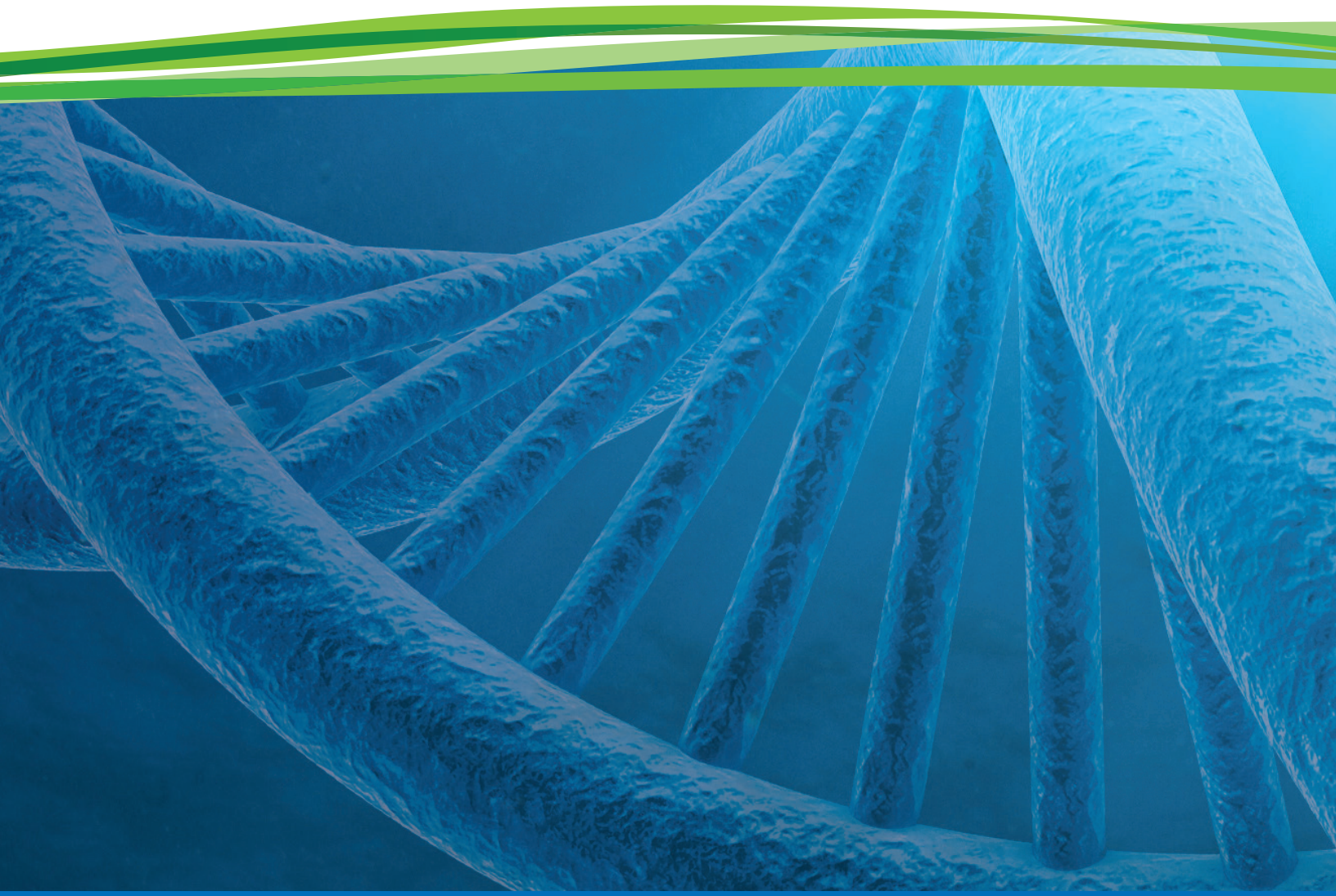
Application Highlight

Whole-Exome Sequencing

Seq success with every sample



WHOLE-EXOME SEQUENCING

Every sample is precious

Whole Exome Sequencing (WES) enables in-depth, targeted interrogation of genomic coding regions while conserving sequencing resources compared to whole genome sequencing (WGS). Rely on Roche Sequencing workflows—from automated DNA extraction through library preparation, exome enrichment, and accurate library quantification—to provide you with high-quality exome results on Illumina® sequencing platforms.

From sample to sequencing, with support at every step

- Automated DNA extraction in as little as 30 minutes
- Streamlined, single-tube, PCR-free library construction with high conversion rates
- Enrichment of exonic regions with KAPA HyperExome
- Accurate, sensitive quantification for reliable library pooling and clustering
- Integrated service and support throughout the workflow

Nucleic Acid Extraction

DNA EXTRACTION

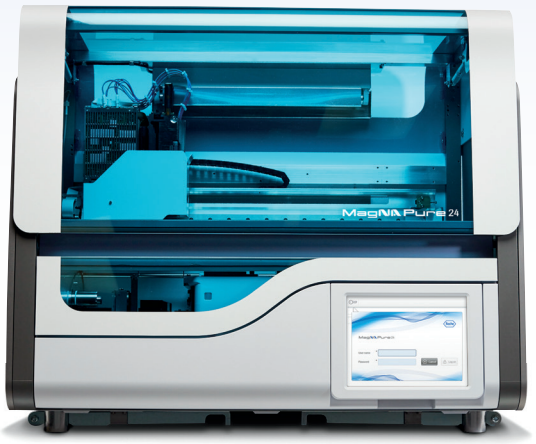
MagNA Pure® 24 and MagNA Pure 96 Systems

Why do extraction methods matter?

High-quality starting material leads to sequencing success. High-molecular-weight input DNA is essential for the creation of libraries with the 350 – 650 bp inserts required for sequencing whole human genomes on Illumina® HiSeq® and NovaSeq™ instruments.

Obtain high-quality, high-molecular-weight DNA for direct use in sequencing with the **MagNA Pure 24 and MagNA Pure 96 Systems**. These fully automated nucleic acid extraction instruments provide walkaway automation, require less user intervention, and minimize variability between extractions.

- Reliable DNA extraction from as little as 200 µL whole blood (Figure 1)
- Scalable extraction for low-, mid-, or high-throughput levels
- Optimized protocols for NGS workflows with blood or plasma samples



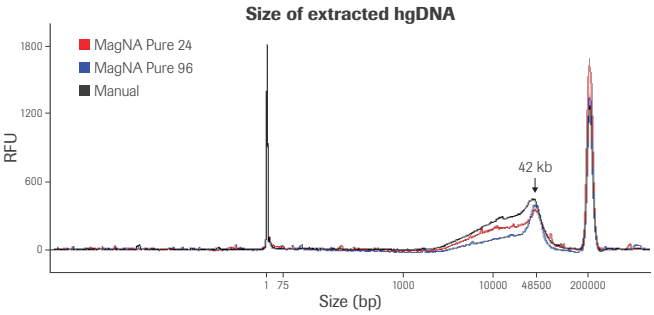
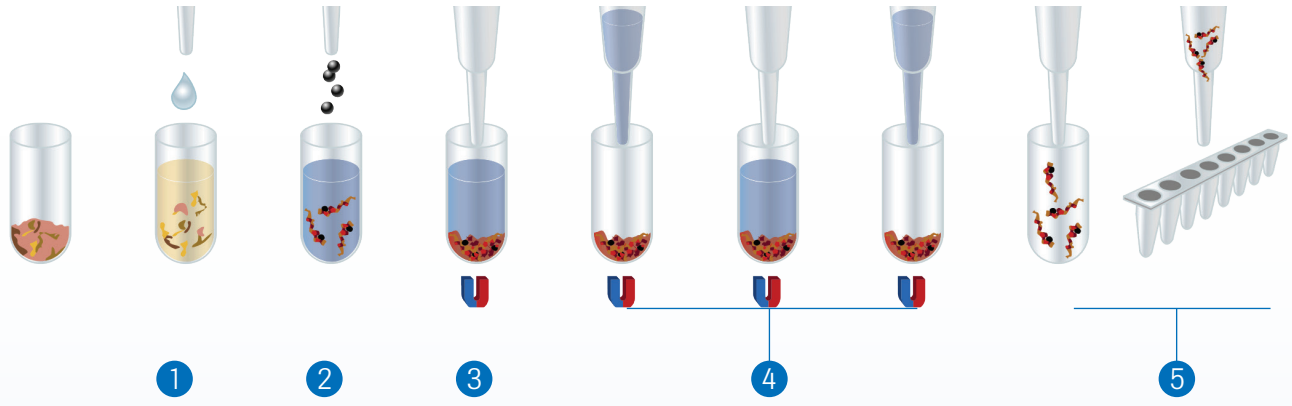
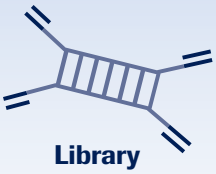


Figure 1. hgDNA extracted from 200 µL whole blood in a final elution volume of 100 µL. All three extraction methods yield similar DNA profiles. (Fragment Analyzer™, using the Genomic DNA 50 kbp Analysis Kit; Advanced Analytical).



1. Sample lysis, inactivation of nucleases, and release of nucleic acids
2. Magnetic glass particle (MGP) addition and binding of nucleic acids to MGPs
3. Magnetic separation of MGP/nucleic acid-complexes from the lysed sample
4. Multiple washing steps
5. Elution of purified nucleic acids from MGPs

Figure 2: Overview of the MagNA Pure System nucleic acid extraction process.



Library Preparation

DNA LIBRARY PREPARATION

Versatile, streamlined options for DNA shearing and library preparation

Two automation-friendly options for library preparation

Ensure comprehensive exome capture with high library conversion rates. The robust chemistries of KAPA HyperPrep and KAPA HyperPlus Kits lead to greater conversion of input DNA into adapter-ligated molecules, improving target coverage following enrichment and reducing duplicate reads (Figure 4)

Generate libraries from a wide variety of DNA input types. KAPA HyperPrep and KAPA HyperPlus produce high-quality libraries from diverse inputs, including challenging samples such as FFPE DNA and samples with GC- or AT-rich content.

Choose from mechanical or enzymatic methods for fragmenting input DNA. The choice of fragmentation methods offers additional flexibility to meet the needs of each experiment (Figure 3).

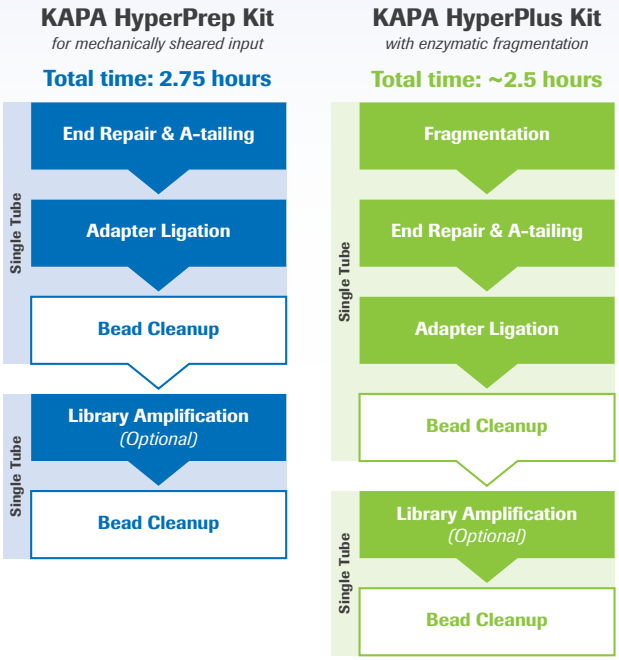


Figure 3. Summary of KAPA HyperPrep and KAPA HyperPlus workflows. Both KAPA HyperPrep and KAPA HyperPlus Library Preparation Kits offer fast, streamlined workflows that are easily completed in under 3 hours. In target enrichment workflows, such as HyperCap v3, a double-sided size selection step using KAPA HyperPure Beads may be included after the post-ligation cleanup step.

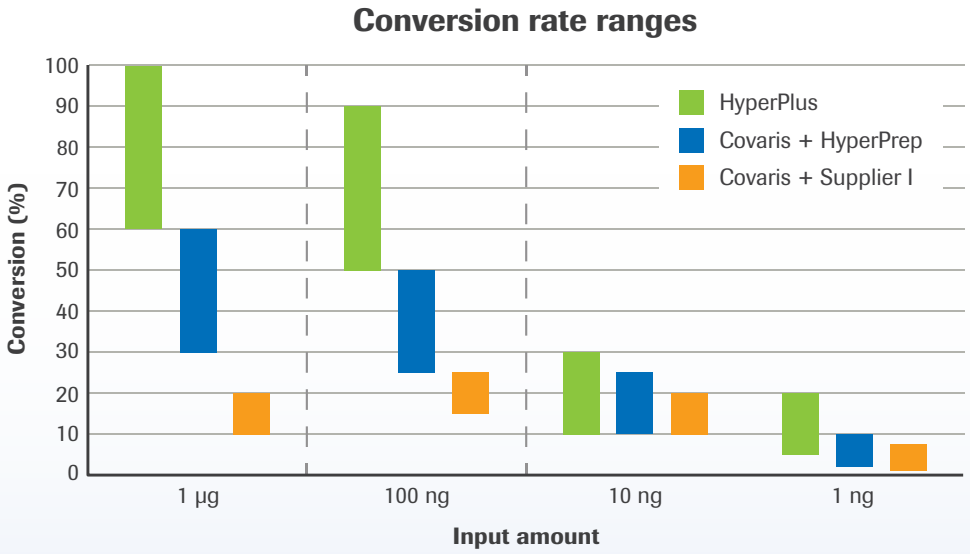
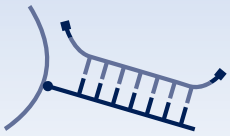


Figure 4. The KAPA HyperPlus and HyperPrep Kits demonstrate superior conversion of input DNA into adapter-ligated, sequenceable molecules. Conversion rates are highest for the KAPA HyperPlus Kit (with enzymatic fragmentation) for both high- and low-input applications. KAPA HyperPrep, which uses Covaris-sheared DNA as input, also outperforms the Supplier I kit.



Target Enrichment

TARGET ENRICHMENT

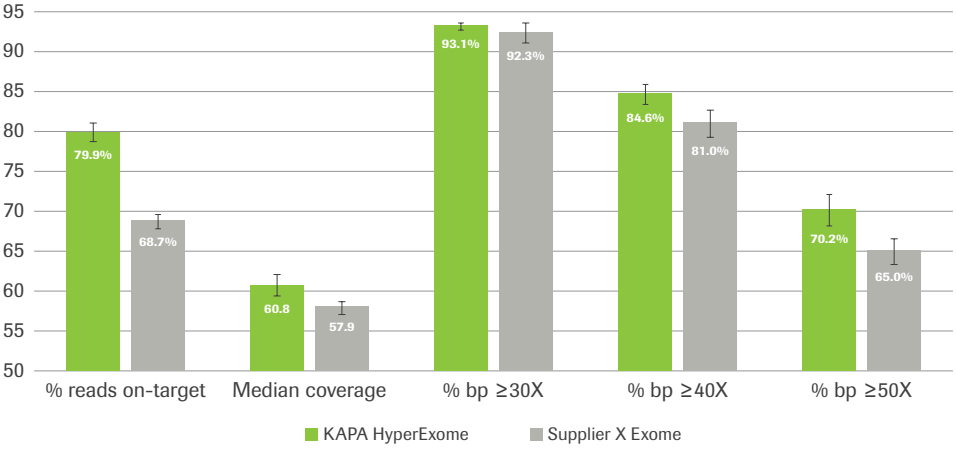
KAPA HyperExome panel

Better by Design

KAPA HyperExome offers comprehensive coverage and enhanced uniformity with low sequencing requirements. Based on the GRCh38/hg38 human genome assembly, the comprehensive-yet-compact panel (~43 Mb) efficiently covers content in CCDS (97.8%), RefSeq (97.4%), ENSEMBL (97.4%) and ClinVar (97% of pathogenic regions).

- **Reduce costs and save time** through superior capture uniformity that enables the detection of rare variants with less sequencing
- **Identify variants in medically relevant exonic regions**, including previously inaccessible regions and regions of high or low GC content
- **Ensure accurate sample identification with 387 tracking SNPs**, eliminating the need for tracking spike-ins and reducing human error
- **Streamline the entire WES workflow** by incorporating KAPA HyperExome into the HyperCap v3 workflow

A



- Reliably enrich challenging, previously inaccessible exonic regions
- Improve coverage of key research genes in important genomic databases including ACMG59, CCDS, and ClinVar
- Discover more variants with the comprehensive-yet-compact KAPA HyperExome panel

B

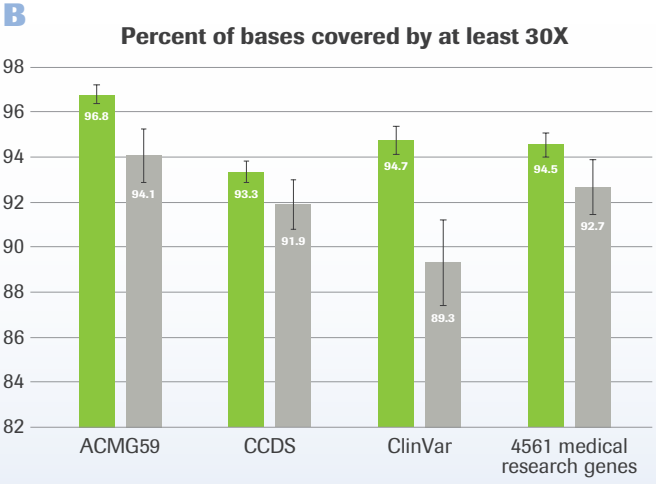


Figure 5. (A) KAPA HyperExome yields greater % reads-on-target, deeper median coverage, and broader target coverage compared to the Supplier X exome. **(B)** KAPA HyperExome provides better coverage of important genomic databases compared to the Supplier X exome.

For both the KAPA HyperExome and Supplier X exome workflows: DNA from 16 cell lines was processed in triplicate (48 total libraries per workflow); input DNA was enzymatically sheared; samples were pre-capture multiplexed in sets of 8 and hybridized for 16 hours; final post-capture libraries were amplified with 8 PCR cycles; and libraries were sequenced (2 x 100 bp) on an Illumina® NovaSeq™ sequencer. For the KAPA HyperExome samples, libraries were prepared from 100 ng of DNA with KAPA HyperPlus Kits using KAPA Universal Adapter and KAPA UDI Primer Mixes; hybridization and washes were carried out at 55ºC following 8 pre-capture PCR cycles. Supplier X samples were prepared according the manufacturer's instructions from 50 ng of genomic DNA. For analysis, sequencing data was subsampled proportionally to exome panel size to achieve the same targeted average depth of coverage.

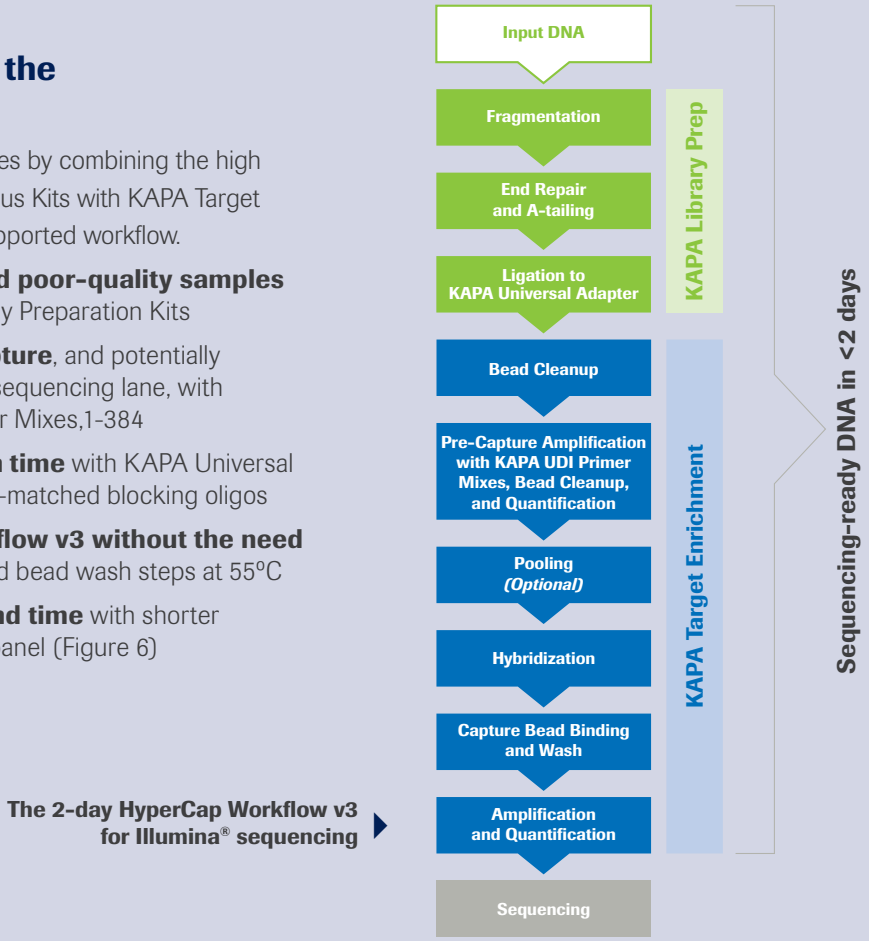
Workflow focus:

KAPA HyperCap Workflow v3 featuring KAPA Target Enrichment probes

Streamline target enrichment with the KAPA HyperCap Workflow v3

KAPA HyperCap Workflow v3 delivers complex libraries by combining the high conversion rate of KAPA HyperPrep or KAPA HyperPlus Kits with KAPA Target Enrichment, creating a streamlined, single-vendor-supported workflow.

- **Achieve greater success with low-input and poor-quality samples** with KAPA HyperPrep and KAPA HyperPlus Library Preparation Kits
- **Multiplex up to 16 samples in the same capture**, and potentially post-capture multiplex more samples in the same sequencing lane, with KAPA Unique Dual-Indexed Adapters (UDI) Primer Mixes,1-384
- **Reduce workflow complexity and hands-on time** with KAPA Universal Enhancing Oligos, eliminating the need for adapter-matched blocking oligos
- **Automate the entire KAPA HyperCap Workflow v3 without the need for a SpeedVac™**—now with all hybridization and bead wash steps at 55°C
- **Explore options to further reduce turnaround time** with shorter hybridization steps using our KAPA HyperExome panel (Figure 6)



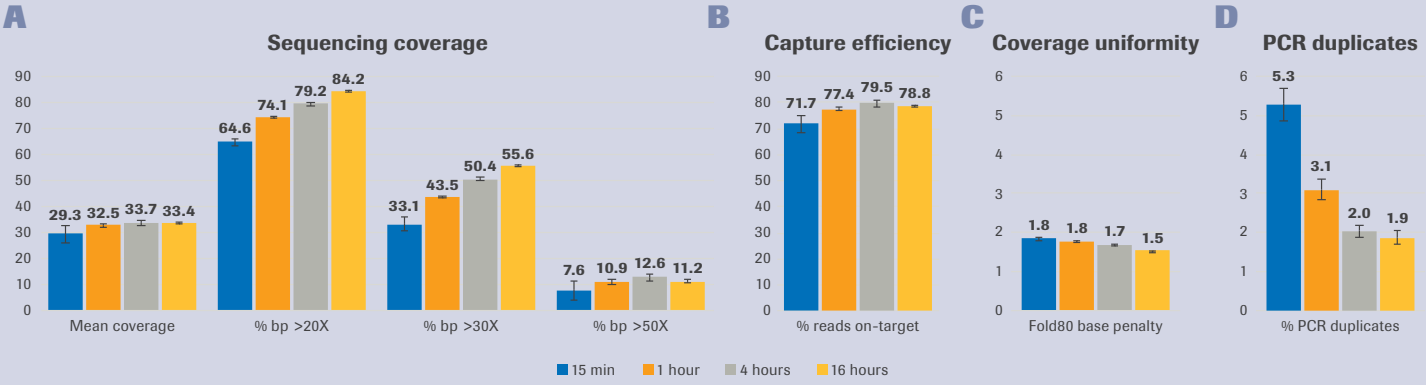


Figure 6. KAPA HyperExome yields high-quality results with hybridization times as short as 1 hour. (A) Sequencing coverage **(B)** Capture efficiency, presented as % reads on-target (the percent of mapped, non-duplicate reads overlapping the target region by at least 1 base). **(C)** Coverage uniformity, presented as Fold-80 base penalty. **(D)** PCR duplicates, a measure of library complexity (fewer % PCR duplicates=greater library complexity). **METHOD:** Target-enriched libraries were generated using the HyperCap Workflow v3.0, with the KAPA HyperPrep Kit and KAPA HyperExome Probes. Single-plex hybridization reactions were carried out at 55°C with 1 µg library and KAPA HyperExome probes, for the following durations: 15 minutes, 1 hour, 4 hours, and 16 hours (standard hybridization is 16 – 20 hours). Normalized, pooled libraries were sequenced on an Illumina NextSeq 500 instrument using the NextSeq High Output kit (2 x 75 bp). Data was down-sampled to 50X raw coverage. For all charts, bars represent the mean from triplicate libraries and error bars indicate the standard deviation. Note: this protocol is still in development and has not yet been fully validated.

Library Quantification

LIBRARY QUANTIFICATION

KAPA Library Quant and Roche LightCycler®

Why is qPCR-based library quantification preferred for library QC?

Sequencing capacity is maximized when sequencing-competent molecules are accurately measured with qPCR, enabling libraries to be pooled at the desired ratios.

Clustering can be optimized by quantification of library pools, further improving sequencing results.

KAPA Library Quantification Kits contain all reagents needed for qPCR-based quantification of NGS libraries for Illumina® sequencing.

- Accurate quantification of sequencing-competent libraries (Figure 7)
- Better accuracy when pooling libraries
- Automation-friendly workflow for increased throughput

Roche LightCycler® 96 and LightCycler® 480 Instruments ensure reproducible, reliable, accurate data.

- Scalable instrument options
- Dependable temperature accuracy and homogeneity
- Ideal for use with KAPA Library Quantification Kits

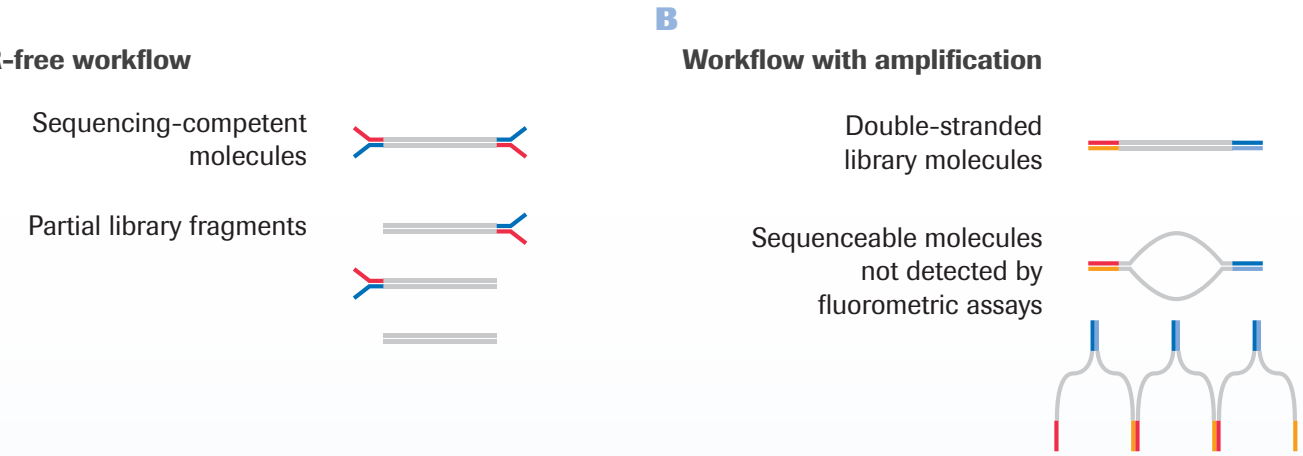


Figure 7. Library quantification via qPCR-based methods, such as the KAPA Library Quantification Kit, enables accurate sample pooling and optimal clustering.

(A) Libraries prepared with PCR-free workflows can contain partial library fragments that are not sequenceable. qPCR-based library quantification methods detect only the sequencing-competent molecules. In contrast, other assays detect fragments that are not sequenceable, leading to *underclustering* on the sequencing flow cell.

(B) Libraries prepared using methods with PCR amplification can include sequencing-competent single-stranded configurations. qPCR-based library quantification data counts these molecules. In contrast, other methods do not detect these molecules, leading to *overclustering* on the sequencing flow cell.

ORDERING INFORMATION

	Roche cat. no.	KAPA code	Description	Kit size
Automated Nucleic Acid Purification	07290519001		MagNA Pure® 24 System	1 Instrument
	06541089001		MagNA Pure 96 System	1 Instrument
Sample Quantification/QC	07960590001	KK4960	KAPA hgDNA Quantification and QC Kit - qPCR Master Mix (Universal)	300 x 20 µL rxns
	07960603001	KK4961	KAPA hgDNA Quantification and QC Kit - qPCR Master Mix (ABI Prism®)	300 x 20 µL rxns
	07960611001	KK4962	KAPA hgDNA Quantification and QC Kit - qPCR Master Mix (Bio-Rad®)	300 x 20 µL rxns
	07960689001	KK4969	KAPA hgDNA Quantification and QC Kit - qPCR Master Mix (ROX Low)	300 x 20 µL rxns
	07960620001	KK4963	KAPA hgDNA Quantification and QC Kit - qPCR Master Mix (Bio-Rad)	300 x 20 µL rxns
Library Prep	07962312001	KK8500	KAPA HyperPrep Kit with KAPA Library Amplification Primer Mix (10X)	8 rxns
	07962347001	KK8502	KAPA HyperPrep Kit with KAPA Library Amplification Primer Mix (10X)	24 rxns
	07962363001	KK8504	KAPA HyperPrep Kit with KAPA Library Amplification Primer Mix (10X)	96 rxns
	07962380001	KK8510	KAPA HyperPlus Kit with Library Amplification	8 rxns
	07962401001	KK8512	KAPA HyperPlus Kit with Library Amplification	24 rxns
	07962428001	KK8514	KAPA HyperPlus Kit with Library Amplification	96 rxns
	08963835001	KK8007	KAPA HyperPure Beads	5 mL
	08963843001	KK8008	KAPA HyperPure Beads	30 mL
	08963851001	KK8009	KAPA HyperPure Beads	60mL
	08963878001	KK8011	KAPA HyperPure Beads	4 x 60 mL
	08963860001	KK8010	KAPA HyperPure Beads	450 mL
Adapters and Primers	09063781001		KAPA Universal Adapter, 15 µM	960 µL
	09063790001		KAPA Universal Adapter, 15 µM	4 x 960 µL
	09134336001		KAPA Unique Dual-Indexed Primer Mix 1-96	96 rxns
	09329838001		KAPA UDI Primer Mixes, 97-192	96 rxns
	09329846001		KAPA UDI Primer Mixes, 193-288	96 rxns
	09329854001		KAPA UDI Primer Mixes, 289-384	96 rxns
Enrichment Reagents	09075810001		KAPA HyperCapture Reagent Kit	24 rxns
	09075828001		KAPA HyperCapture Reagent Kit	96 rxns
	09075917001		KAPA HyperCapture Reagent Kit	4 x 96 rxns
	09075879001		KAPA Probes Resuspension Buffer	1 mL
	09075887001		KAPA Probes Resuspension Buffer	2 mL
	09075763001		KAPA Hybrid-Enhancer Reagent	1 mL
	09075836001		Roche Universal Enchancing Oligos	24 rxns
	09075852001		Roche Universal Enchancing Oligos	96 rxns
	09075895001		Roche Universal Enchancing Oligos	4 x 96 rxns
Beads	09075780001		KAPA HyperCapture Bead Kit	24 rxns
	09075798001		KAPA HyperCapture Bead Kit	96 rxns
	09075909001		KAPA HyperCapture Bead Kit	4 x 96 rxns
Probes	09062548001		KAPA HyperExome Probes	12 rxns
	09062556001		KAPA HyperExome Probes	24 rxns
	09062564001		KAPA HyperExome Probes	48 rxns
	09062572001		KAPA HyperExome Probes	96 rxns
	09062599001		KAPA HyperExome Probes	192 rxns
	09062602001		KAPA HyperExome Probes	384 rxns
	09062629001		KAPA HyperExome Probes	768 rxns
	09062637001		KAPA HyperExome Probes	1152 rxns
	09062645001		KAPA HyperExome Probes	1536 rxns
KAPA HyperExome Kits	09107592001		KAPA HyperExome Prep Kit (for mechanically sheared DNA)	192 8-plex rxns
	09107606001		KAPA HyperExome Plus Kit (with enzymatic shearing)	192 8-plex rxns
Real-Time PCR	05815916001		LightCycler® 96	1 Instrument
	05015278001		LightCycler® 480 (96-well)	1 Instrument
	05015243001		LightCycler® 480 (384-well)	1 Instrument
	07960298001	KK4854	KAPA Library Quantification Kit for Illumina® Platforms: LC480 qPCR Master Mix	500 rxns
	07960140001	KK4824	KAPA Library Quantification Kit for Illumina Platforms: Universal Master Mix	500 rxns

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For more information, please visit
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