KAPA RNA HyperPrep Kits for RNA-sequencing

Rapid, robust, and reliable

Explore transcriptomics with RNA-seq and unlock discoveries with your research

Start the RNA-seq process off the right way by making high-quality libraries through the streamlined, single-day workflow of the KAPA RNA HyperPrep Kits. Combine these flexible kits with KAPA Dual-Indexed Adapters in an automation-friendly workflow that is compatible with mRNA capture, ribosomal depletion, and globin depletion.

- Construct libraries in a **single day**, inclusive of RNA enrichment
- Save steps and **reduce hands-on time** with streamlined protocols
- Achieve **robust performance** across different sample types and low-input amounts, including degraded samples
- Rely on Roche Support throughout the entire workflow, including custom depletion

Data on file.
For Research Use Only. Not for use in diagnostic procedures.

Choose from flexible workflow options for a broad range of applications

- Generate libraries from a variety of RNA sample types and input amounts, including both high-quality and degraded samples (Table 1)
- Rely on streamlined workflows (Figure 1) for stranded library construction when sequencing non-coding and coding transcripts

Table 1. Roche Sample Prep Solutions for RNA-seq.

	KAPA RNA HyperPrep Kits	KAPA RNA HyperPrep Kits with RiboErase (HMR)	KAPA RNA HyperPrep Kits with RiboErase (HMR) Globin	KAPA mRNA HyperPrep Kits
RNA Enrichment	None	rRNA Depletion	rRNA and Globin Depletion	Poly(A) Selection
Sample Type	High-quality total RNA Degraded or FFPE total RNA Previously enriched RNA	High-quality total RNA Degraded or FFPE total RNA	Blood-derived RNA High-quality total RNA Degraded or FFPE total RNA	High-quality total RNA
Species	Eukaryotic (animal, plant, etc.) Prokaryotic (bacterial, etc.)	Human, mouse, and rat*	Human, mouse, and rat*	Eukaryotic (animal, plant, etc.)
Differentiating Applications	Analysis of specific transcripts, including those of low abundance, when paired with target enrichment	Whole transcriptome analysis, including non-coding RNA profiling	Whole transcriptome analysis, including non-coding RNA profiling	mRNA sequencing for coding transcriptome analysis
Shared Applications	Gene expression analysis; detection of gene fusions, isoforms, and other structural variants; SNV discovery			

*Custom depletion protocol support available for other organisms or transcripts. *See Featured Application Note.*

KAPA RNA HyperPrep Kits with RiboErase (HMR)

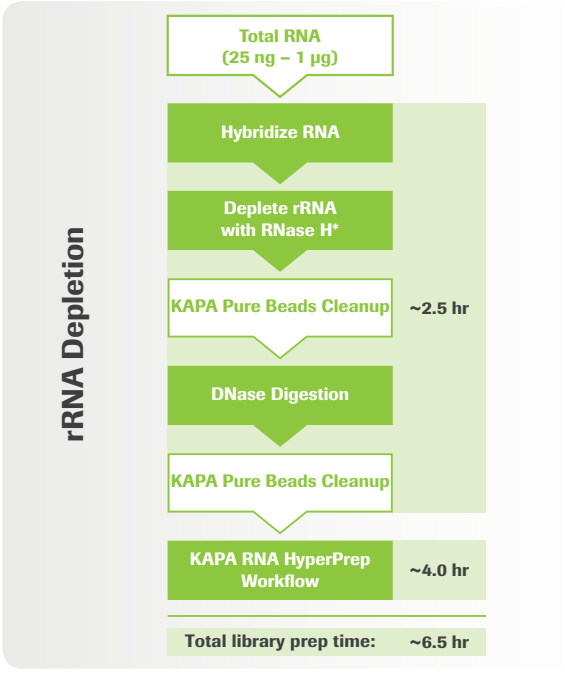


Figure 1A. KAPA RiboErase (HMR) workflow. Sequencing of rRNA-depleted total RNA samples provides a more comprehensive representation of the whole transcriptome. rRNA is targeted and depleted enzymatically using DNA probes and RNase H, resulting in improved coverage of transcripts of interest, including precursor mRNAs and important regulatory species such as non-coding RNAs.

*KAPA RiboErase (HMR) Globin is available for globin mRNA depletion from blood-derived samples. Custom depletion protocol support is available for other non-HMR organisms and other transcript species.

KAPA mRNA HyperPrep Kits

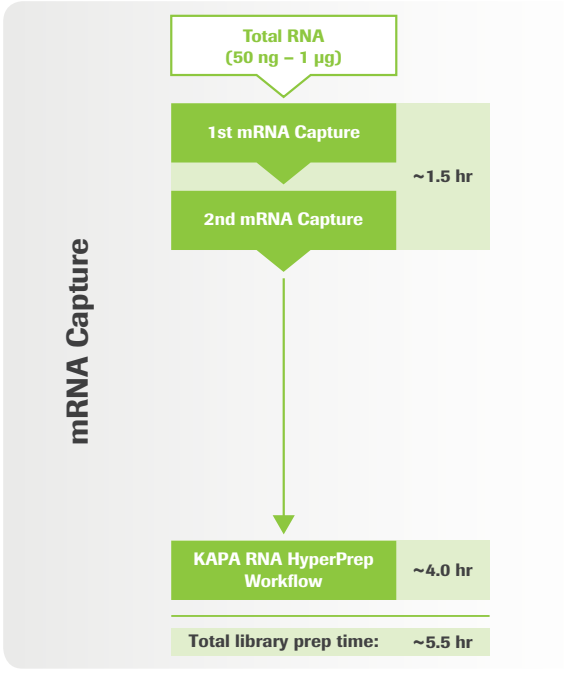


Figure 1B. mRNA Capture workflow. Sequencing of mRNA-enriched samples provides a focused view of the protein-coding regions in the transcriptome. mRNA capture beads are used prior to library preparation with the KAPA RNA HyperPrep workflow, which enriches for mRNA over non-polyadenylated species such as ribosomal, precursor, and non-coding RNAs.

Perform single-day, single-tube library prep

- Reduce hands-on time and overall turnaround time with fewer enzymatic and cleanup steps (Figure 2)
- Produce strand-specific libraries from input RNA in approximately 4 hours
- Complete the entire workflow, inclusive of upfront RNA enrichment, in a standard work day
- Achieve high-throughput processing and consistency with automation-friendly workflows

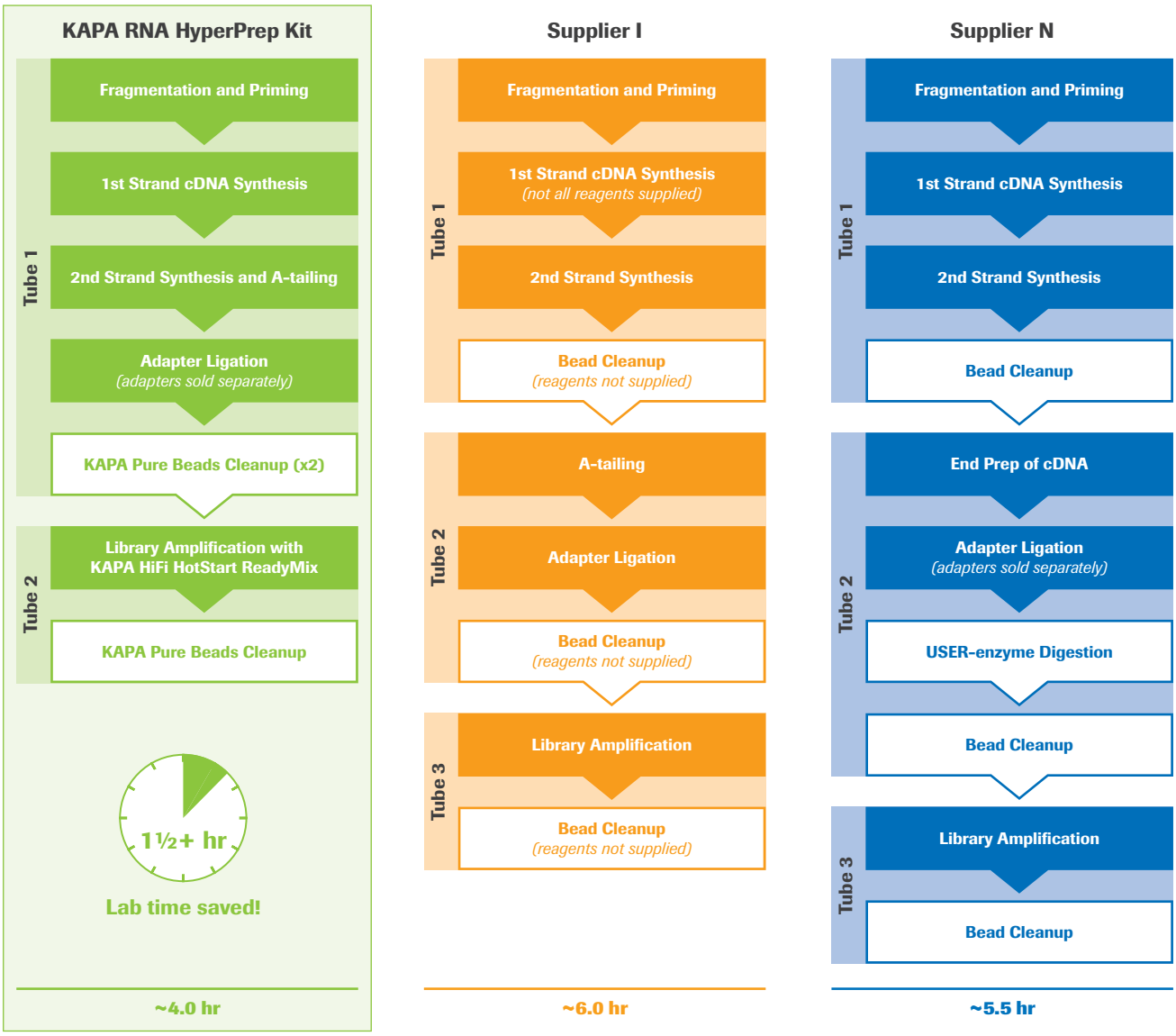


Figure 2. Streamlined, strand-specific library construction. The novel chemistry employed in KAPA RNA HyperPrep Kits allows for fewer and shorter enzymatic steps, reducing hands-on time and overall library prep time. rRNA depletion with KAPA RiboErase (HMR) or KAPA RiboErase (HMR) Globin Kits adds approximately 2.5 hours to the overall workflow time, whereas mRNA capture adds approximately 1.5 hours. The entire workflow, from input RNA to sequencing-ready library, can easily be completed in a standard workday. All KAPA RNA HyperPrep library construction workflows are automation friendly.

Sequence what matters

- Waste fewer reads by reducing rRNA carryover and PCR duplicates (Figures 3A and 3C)
- Identify more unique transcripts with equivalent sequencing (Figures 3B and 3D)

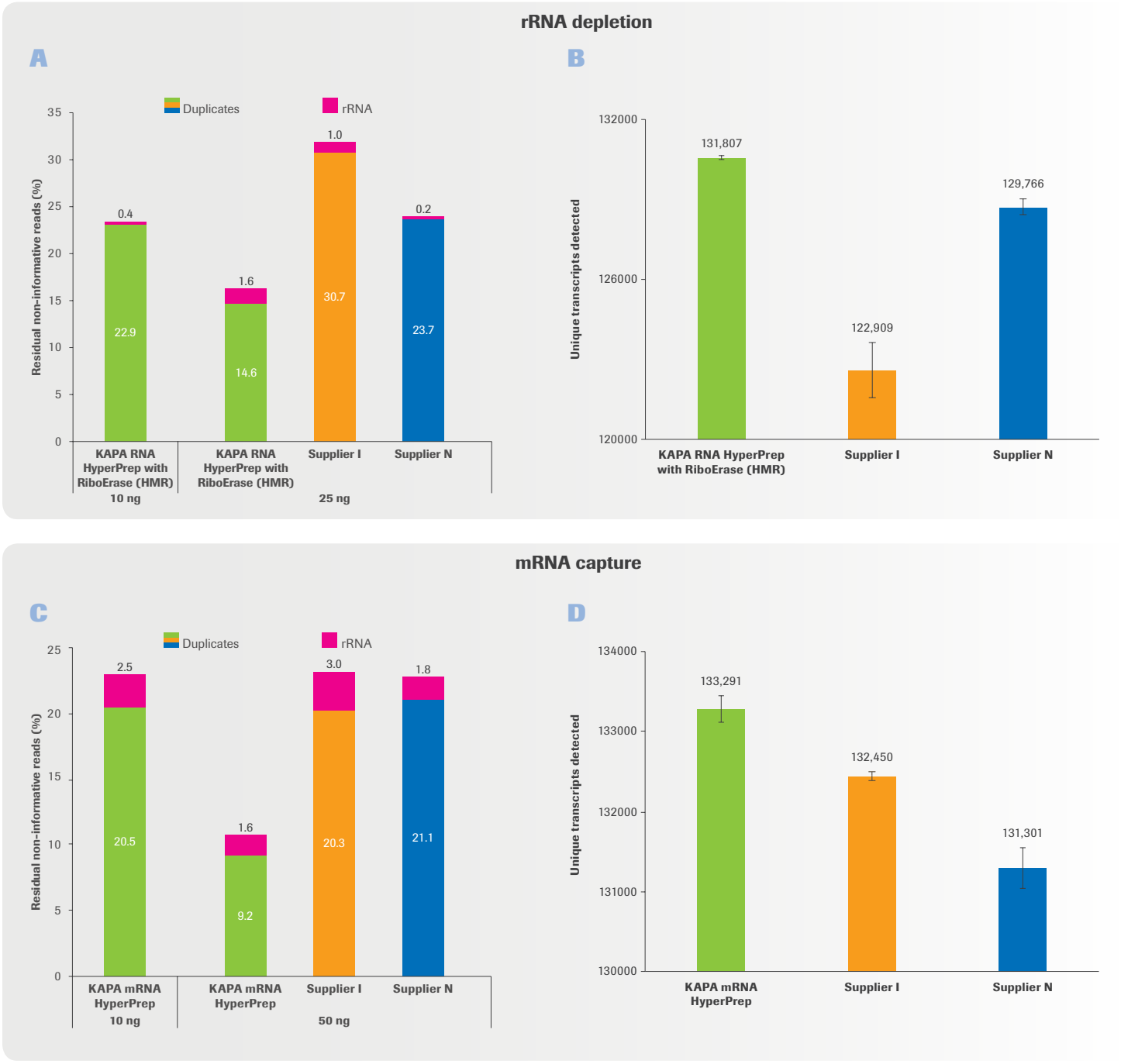


Figure 3. Highly efficient library prep enables better utilization of sequencing resources. KAPA RNA HyperPrep Kits (green) enable highly efficient conversion of input RNA (enriched by either rRNA depletion or mRNA capture) to adapter-ligated library. Because fewer reads are associated with unwanted content—PCR duplicates and residual rRNA **(A and C)**—a larger proportion of sequencing data is associated with unique transcript identification **(B and D)**.

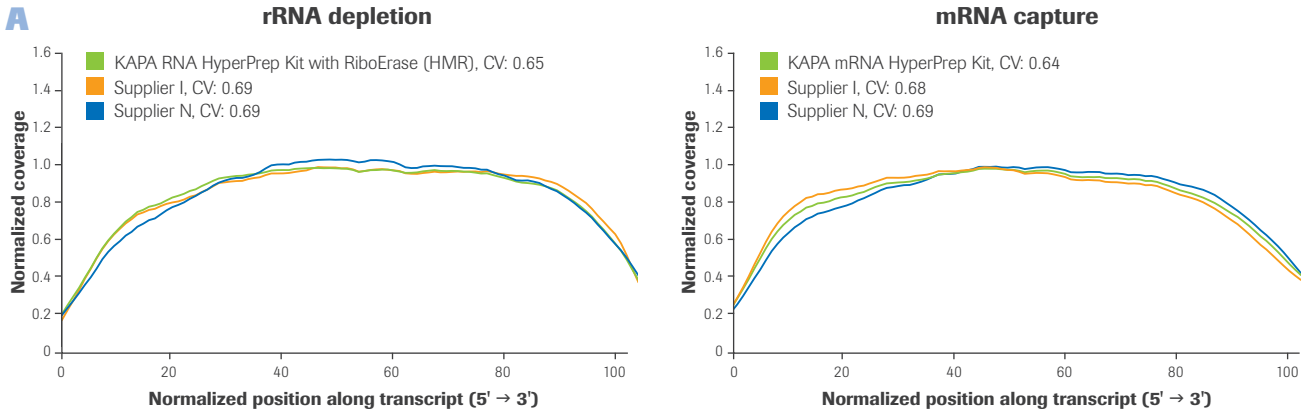
Libraries were generated in quadruplicate, using variable inputs of Universal Human Reference (UHR) RNA (Agilent Technologies) with either an rRNA depletion (top) or mRNA capture (bottom) prior to library construction. The lowest input for each workflow (10 ng) is lower than the validated minimum input for both KAPA RNA HyperPrep workflows. Where present, error bars represent the standard deviation.

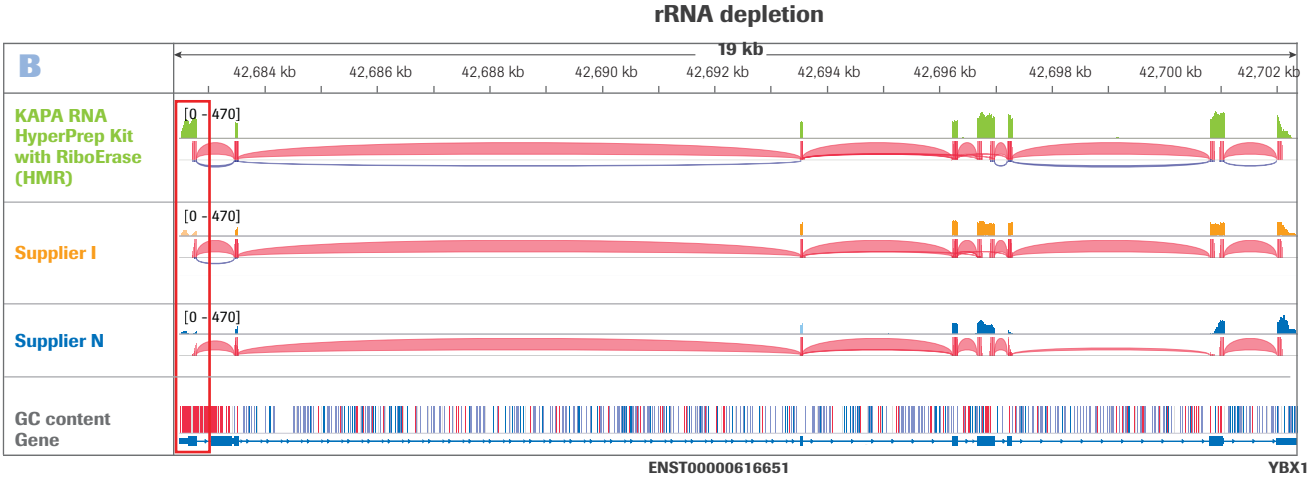
For 25 ng and 50 ng samples, paired end (2 x 100 bp) sequencing was performed using an Illumina® HiSeq® 2500 instrument. Reads aligning to rRNA were removed, and reads were randomly subsampled to 14 M for comparative analyses. Transcripts were quantified using RNA-SeQC.

For 10 ng samples, paired end (2 x 75 bp) sequencing was performed using an Illumina NextSeq 500 instrument. Reads were randomly subsampled to 14 M for comparative analysis prior to removing reads aligning to rRNA and subsequent marking of duplicates. Transcripts were quantified using Kallisto (data not plotted due to analysis and sequencing depth differences).

Cover your bases with higher uniformity

- Efficient RNA enrichment and library construction processes result in more even coverage along the entire transcript (Figure 4A)
- Library amplification with KAPA HiFi HotStart ReadyMix enables better coverage of difficult GC-rich regions (Figures 4B and 4C)





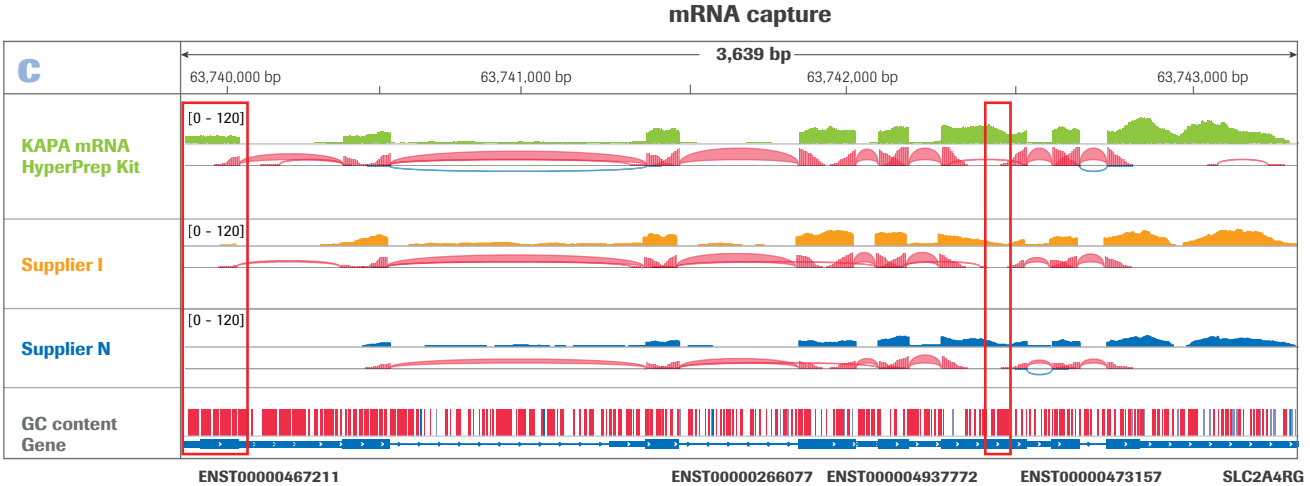


Figure 4. Improved coverage uniformity. Libraries were generated from 25 ng (rRNA depletion) or 50 ng (mRNA capture) of high-quality UHR RNA, using the manufacturers' standard recommendations for each workflow where possible. For the 1000 most highly expressed transcripts, Roche workflows resulted in more even coverage across the entire transcript length, as compared to the workflows from two other suppliers **(A)**. This is evident both from normalized coverage plots and the coverage coefficient of variation (CV). KAPA RNA HyperPrep workflows, employing KAPA HiFi HotStart ReadyMix for library amplification, also better preserve difficult GC-rich regions (outlined in red), as highlighted in IGV plots of select regions of the YBX1 **(B)** and SLC2A4RG **(C)** genes.

Perform convenient, effective library construction from blood samples

- Effectively co-deplete cytoplasmic rRNA, mitochondrial rRNA, and globin mRNA (Figure 5) in an integrated, single-day workflow
- Rely on an automation-friendly RNase H-based depletion that offers high reproducibility and minimal off-target depletion (Figure 5D)

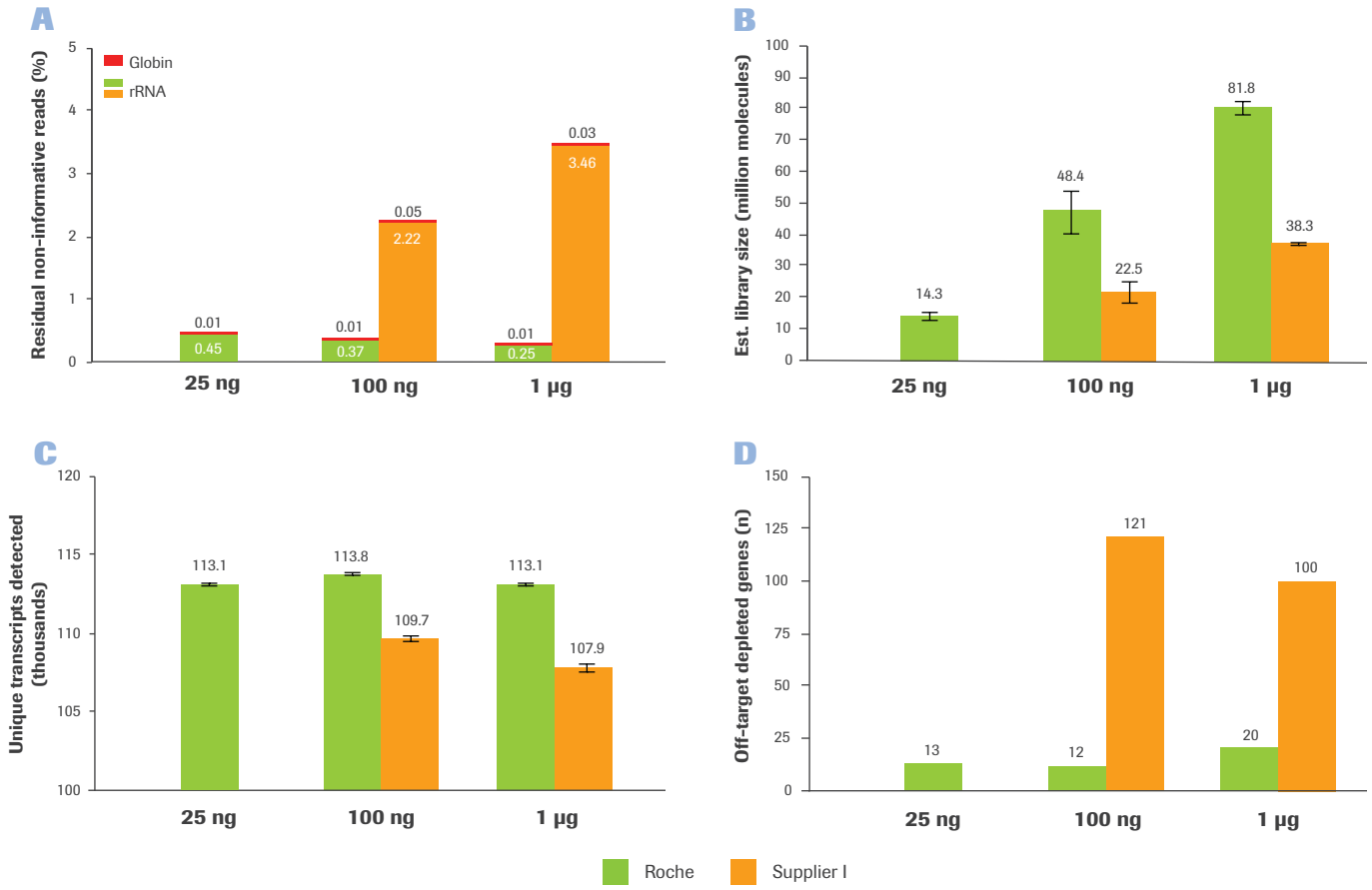


Figure 5. Effective library construction from blood-derived RNA. KAPA RNA HyperPrep Kits with RiboErase (HMR) Globin (green) allow for the simultaneous depletion of rRNA and globin transcripts from blood-derived RNA. Effective RNase H-based depletion and highly efficient library construction result in fewer non-informative reads **(A)** and PCR duplicates (not shown) as compared to the workflow from Supplier I, which employs bead-based depletion (orange). This translates to more complex libraries **(B)** and a larger number of sequencing reads associated with transcripts of interest **(C)**. In addition, the Roche workflow results in much lower levels of off-target depletion **(D)**.

Libraries were prepared from different inputs of RNA extracted from human blood, as indicated on the x-axis of each graph. The 25 ng input is lower than the recommended minimum input for the Supplier I workflow. Paired-end (2 x 125 bp) sequencing was performed on an Illumina® HiSeq® 2500 instrument. Data were sub-sampled to 17 M reads per sample for analysis. Each bar represents the average of three technical replicates. Transcript abundance was quantified using Kallisto. To assess off-target depletion, transcript abundances were aggregated at the gene level and TMM-normalized prior to differential expression analysis. The expression profiles of libraries generated with or without globin depletion were compared to assess off-target depletion for each workflow.

Featured Application Notes:

- KAPA RNA HyperPrep: A streamlined library preparation workflow that enables robust gene expression profiling using RNA-sequencing
- KAPA RNA HyperPrep Workflow: Recommendations and expectations for RNA-sequencing using degraded inputs
- High-efficiency species-specific ribosomal RNA depletion with the KAPA RNA HyperPrep Kit
- KAPA RNA HyperPrep Kits and the Genialis™ NGS data analytics platform: a qualified, streamlined RNA-seq solution for gene expression analysis
- KAPA RiboDesigner—A custom probe design solution for rRNA depletion from single- and multi-species bacterial samples

go.roche.com/SeqResources

Generate high-quality libraries from degraded samples

- Input as little as 25 ng with FFPE samples, depending on total RNA quality
- Achieve low duplication rates and highly efficient, reproducible rRNA removal with degraded samples (Figure 7)
- Identify more unique transcripts with equivalent sequencing (Figure 7B)

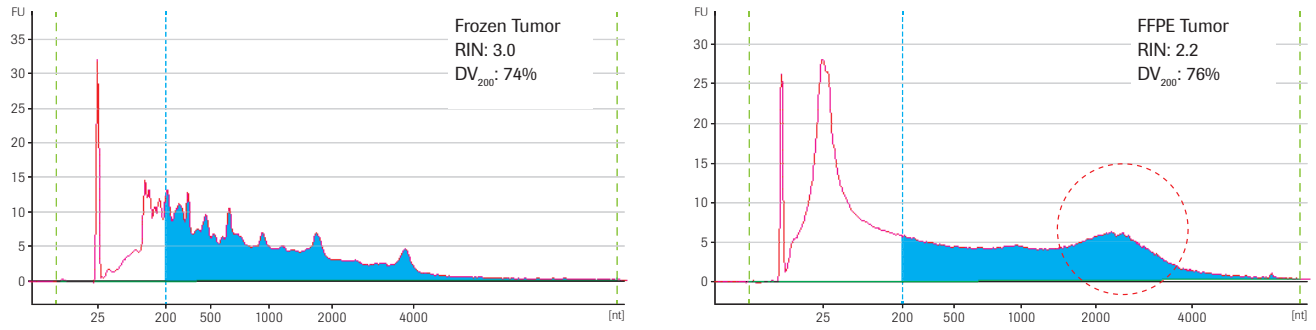


Figure 6. Quality assessment of input RNA from a paired frozen and FFPE breast tumor sample set. Blue shading highlights RNA fragments >200 nt. The region circled in red indicates a high-molecular-weight peak that is likely the result of crosslinking or inefficient deparrafinization and not intact material that could support library construction. Electropherograms were generated using an Agilent Bioanalyzer with an Agilent RNA 6000 Pico Kit.

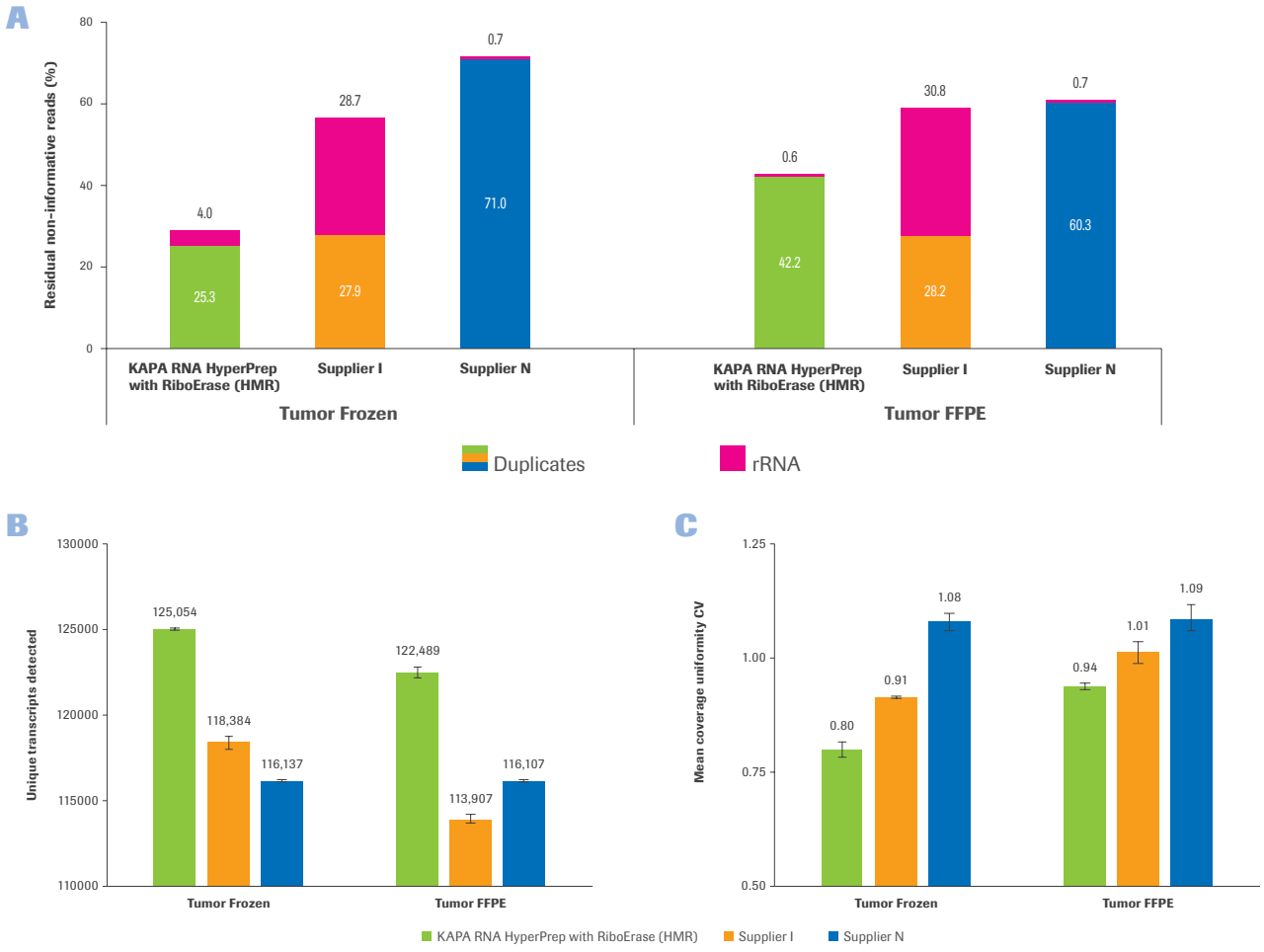


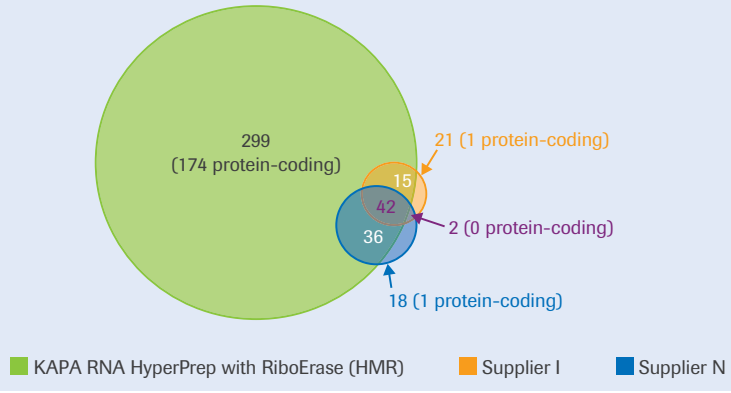
Figure 7. Robust and reproducible results from degraded samples. For both the degraded frozen and FFPE breast tumor samples (Figure 6), KAPA RNA HyperPrep with RiboErase (HMR) reduces the total number of reads wasted on PCR duplicates and residual rRNA in comparison to alternative workflows **(A)**. Fewer reads associated with undesired content enables increased identification of unique transcripts **(B; also see Figure 8)**. Additionally, the KAPA workflow more evenly covers transcripts, as indicated by a lower mean coverage uniformity CV **(C)**. *See Featured Application Note.*

Libraries were generated in triplicate using 25 ng of total RNA. Where present, error bars represent the standard deviation. Paired end (2 x 100 bp) sequencing was performed using an Illumina® HiSeq® 2500 instrument. Reads aligning to rRNA were removed, and reads were randomly downsampled to 14 M for comparative analyses. Transcripts were quantified using RNA-SeQC.

Application highlight:

Tumor profiling

Identify more differentially expressed transcripts.
KAPA RNA HyperPrep with RiboErase (HMR) identifies more differentially expressed transcripts in comparison to alternative workflows. Using 100 ng of matched breast tumor and adjacent normal RNA, libraries were prepared in duplicate. Overlap analysis revealed that the KAPA kit identified the majority of transcripts detected by other workflows, plus an additional 299, of which 174 were protein-coding. In contrast, only 2 of the transcripts not identified by the KAPA kit were protein-coding. Results were independently verified for a subset of transcripts using an orthogonal qPCR assay (data not shown), indicating that differentially expressed transcripts identified only by the KAPA kit reflect measurable changes in gene expression. *See Featured Application Note.*



Ordering information for KAPA RNA HyperPrep Kits

Roche cat. no.	KAPA code	Description*	Kit size
08098093702	KK8540	KAPA RNA HyperPrep Kit	24 rxn
08098107702	KK8541	KAPA RNA HyperPrep Kit	96 rxn
08098131702	KK8560	KAPA RNA HyperPrep Kit with RiboErase (HMR)	24 rxn
08098140702	KK8561	KAPA RNA HyperPrep Kit with RiboErase (HMR)	96 rxn
08308314702	KK8562	KAPA RNA HyperPrep Kit with RiboErase (HMR) Globin	24 rxn
08308241702	KK8563	KAPA RNA HyperPrep Kit with RiboErase (HMR) Globin	96 rxn
08098115702	KK8580	KAPA mRNA HyperPrep Kit	24 rxn
08098123702	KK8581	KAPA mRNA HyperPrep Kit	96 rxn

*All KAPA RNA HyperPrep Kits contain KAPA Pure Beads for reaction cleanups

Ordering information for KAPA Dual-Indexed Adapters

Roche cat. no.	KAPA code	Description	Kit size
08278555702	KK8722	KAPA Dual-Indexed Adapters Kit (15 µM)**	96 adapters x 20 µL each
08278539001	KK8721	KAPA Adapter Dilution Buffer	25 mL
08861919702	KK8727	KAPA Unique Dual-Indexed Adapter Kit (15 µM, incl. KAPA Adapter Dilution Buffer)	96 adapters x 20 µL each

**Contains KAPA Adapter Dilution Buffer, as well as three additional sealing films to support multiple use

For more information on Roche RNA-sequencing solutions, visit: **sequencing.roche.com/RNA-seq**

Roche is your trusted partner in Next-Generation Sequencing

Consult our dedicated Support & Applications Scientists

- Discuss product selection and project-specific workflow considerations
- Consult and collaborate to integrate new applications into your lab
- Develop and install Roche-demonstrated methods on automated liquid handlers
- Request wet-lab and remote product training
- Troubleshoot product-related challenges

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Email: **support.seqls@roche.com**
Phone: 855-527-2246 (M-F 8:30am-5pm EST)
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