

Optimize RESOURCES

Roche Sample Prep Solutions for RNA-Seq: Sequence what matters.

In the NGS workflow continuum, sample prep holds the key to unlocking the potential of every sample. Because NGS samples are precious, the best methods are needed to process more samples successfully, obtain more information from every sample, and optimize sequencing resources. Roche Sample Prep Solutions offer workflows for different sample types and RNA-Seq applications that are proven, simple, and complete.

Benefits

Single-day library construction	inclusive of RNA enrichment	
Streamlined	automation-friendly RNA enrichment and library prep protocols	
Robust and reliable	performance across different sample types and input amounts	
Higher success rates	with lower input and degraded samples	
Integrated service and support	for the entire workflow from RNA to sequencing-ready library	





Flexible workflow options for a variety of applications

- Stranded library construction solutions for the sequencing of both coding and non-coding transcripts
- Compatible with a variety of sample types and input amounts, of both high-quality and degraded RNA



Figure 1. Roche Sample Prep Solutions for RNA-Seq. KAPA RNA HyperPrep Kits provide a streamlined, versatile core library construction solution, that may be combined with various enrichment options, either before or after library preparation. Depending on the sample type and experimental design, unwanted rRNA and/or other transcripts (e.g., globin in blood samples) may be enzymatically depleted using KAPA RiboErase (HMR) Kits; or poly(A)-tailed mRNA may be selected with KAPA mRNA Capture Kits prior to library construction. Alternatively, total RNA libraries may be prepared and coding or non-coding content selected by hybridization capture, using KAPA HyperCap reagents.

HMR: human, mouse, and rat. User-supplied probe sets may be used to deplete additional transcripts from these species, or transcripts from other species. All KAPA HyperPrep Kits contain KAPA Pure Beads for reaction cleanups.

Single-day, single-tube library prep

- Reduce hands-on and overall turnaround time with fewer enzymatic and cleanup steps
- · 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 and consistency with automation-friendly workflows



Figure 2. Single-day, single-tube library prep. Streamlined, strand-specific library construction. The novel chemistry employed in KAPA RNA HyperPrep Kits allows for fewer enzymatic and cleanup steps, which reduces hands-on and overall library prep time. RNA 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

 Effective RNA enrichment (rRNA depletion or mRNA capture), combined with highly efficient library construction, reduces the number of reads associated with unwanted content and PCR duplicates

• More unique transcripts and genes are detected from a fixed amount of sequencing



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 rRNA depletion or mRNA capture) to adapter-ligated library. Higher final library yields (**A**) are therefore achieved with fewer cycles of amplification, as compared to using the workflow from a different supplier (gray). Because fewer reads are associated with unwanted content (PCR duplicates; (**B**)); and residual rRNA (**C**), a bigger proportion of sequencing data is associated with transcripts of interest (**D**).

Libraries were prepared from different inputs of Universal Human Reference (UHR) RNA (Agilent Technologies), as indicted on the x-axis of each graph, using either an rRNA depletion workflow (left) or mRNA capture (right) prior to library construction. The lowest input for the mRNA capture workflow (10 ng) is five-fold lower than the recommended minimum input for the KAPA mRNA capture workflows. Paired-end (2 x 100 bp) sequencing was performed on an Illumina[®] HiSeq[®] 2500 instrument. Data were sub-sampled to 15 million reads per sample for analysis. Each bar represents the average of three technical replicates. Transcript abundance was quantified using Kallisto.

Cover your bases with higher uniformity

- · Efficient RNA enrichment and library construction processes result in more even coverage along transcript length
- Library amplification with KAPA HiFi HotStart ReadyMix enables better coverage of difficult GC-rich regions



rRNA Depletion





mRNA Capture

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. (A) 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. 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.

Convenient, effective library construction from blood samples

- Effectively co-deplete both cytoplasmic and mitochondrial rRNA, as well as globin transcripts in an integrated, single-day workflow
- Automation-friendly RNase H-based depletion offers high reproducibility and minimal off-target depletion



Figure 5. Effective library construction from blood-derived RNA. KAPA RNA HyperPrep Kits with RiboErase (HMR) Globin (green) allows for the simultaneous depletion of rRNA and globin transcripts from blood-derived RNA. Effective RNase H-based depletion and highly efficient library construction results in fewer unwanted transcripts (A) and PCR duplicates (not shown) as compared to the workflow from a different supplier, 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 A workflow. Paired-end (2 x 125 bp) sequencing was performed on an Illumina[®] HiSeq[®] 2500 instrument. Data were sub-sampled to 17 million 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, TMM-normalized and differential expression calculated. The expression profiles of libraries generated with or without globin depletion were subsequently compared to assess off-target depletion for each workflow.

Robust results from FFPE samples

- · Construct high-quality libraries from FFPE-derived RNA of variable quality
- · High reproducibility between replicates and different input amounts; and high transcript expression correlation between paired freshfrozen and FFPE samples confer confidence in sequencing results









Reproducibility between quantities







Figure 6. Robust and reproducible results from FFPE samples. Top: Libraries were constructed with the KAPA RNA HyperPrep Kit with RiboErase (HMR), from three different FFPE-derived RNA samples, and a fresh-frozen (FF) sample originating from the same biological specimen as the breast tumor FFPE sample. RIN and DV_2000 values are different quality scores, determined with the Agilent 2100 Bioanalyzer, RNA 6000 Pico Kit and Agilent Expert software. The breast tumor FFPE sample likely contains a significant amount of cross-linked material (circled), which artificially inflated the DV200 value.

Bottom: Libraries were prepared from 25 ng or 100 ng inputs of the respective samples. Paired-end (2 x 100 bp) sequencing was performed on an Illumina® HiSeq® 2500 instrument. After removal of residual rRNA and duplicate reads, data were sub-sampled to 14 million reads per sample. Pearson correlation plots are shown for replicates of the same sample and input (A, B and C), different inputs of the same sample (D), and between the fresh-frozen and FFPE breast tumor samples, for both the 25 and 100 ng inputs (E and F).

Process more samples successfully, get more information from every sample, and optimize your sequencing resources with solutions that are **proven**, **simple**, and **complete**.

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

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