An even shorter and more streamlined KAPA RNA HyperPrep with RiboErase (HMR) workflow

The gene expression patterns detected with the shorter workflow were highly similar to data generated with the standard workflow.

Since the advent of RNA sequencing, a major hurdle for laboratories and sequencing organizations has been the immense time investment required for ribosomal RNA (rRNA) depletion and RNA library preparation. However, many workflows that reduce the length of these steps have a negative effect on important quality metrics, such as the % residual rRNA sequence and overall transcript detection. The KAPA RNA HyperPrep with RiboErase (HMR) workflow utilizes robust enzymes developed through directed evolution for maximal efficiency, enabling us to shorten our workflow without modifying our kits. The result is a shorter, more streamlined workflow that still provides high-quality, ribodepleted libraries and sequencing metrics—while also reducing the consumption of reagents and plastics.

Highlights

- Save over an hour compared to the standard KAPA RNA HyperPrep with RiboErase (HMR) workflow*
- When the turn-around time is reduced by >1 hour, the robust data quality of KAPA RNA HyperPrep with RiboErase (HMR) is retained
- Elimination of one bead cleanup leads to a reduction in hands-on time, reagents, and plastics usage

**Roche fully supports the standard protocols detailed in our Instructions for Use. To help customers seeking a faster workflow, we have created a shorter protocol that reduces the RNA library preparation process to 5 hours and 15 min across the rRNA depletion and library prep steps.*

Data on File.
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The shorter workflow yields the same data quality and key sequencing metrics produced by the standard for KAPA RNA HyperPrep with RiboErase (HMR) workflow

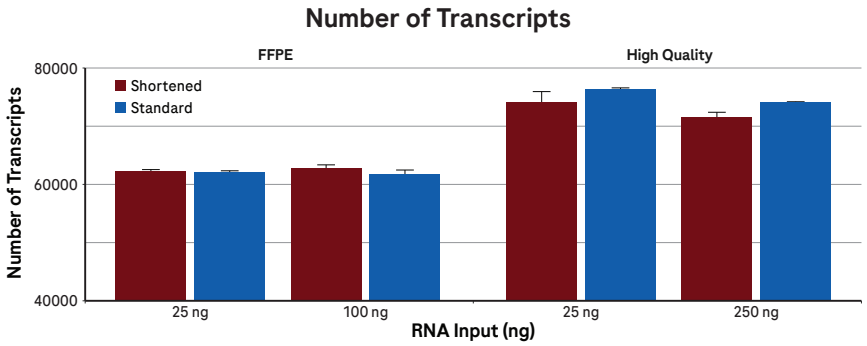

Figure 1. An equivalent number of unique transcripts were identified with both shortened and standard KAPA RNA HyperPrep with RiboErase (HMR) workflows for each RNA input.

Table 1. Comparison of gene expression results between the two workflows

Type of input RNA	Mass of input RNA (ng)	% of total genes with significant differential expression for each input
FFPE	25	0.005%
	100	0.026%
High-quality	25	0.000%
	250	0.015%

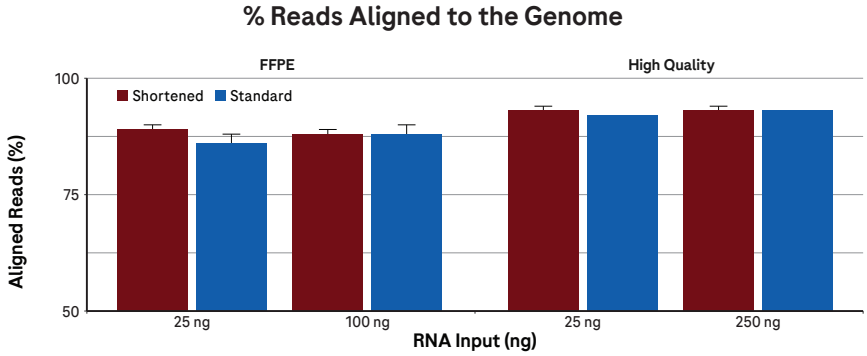

Figure 2. The % aligned reads is similar with both workflows.

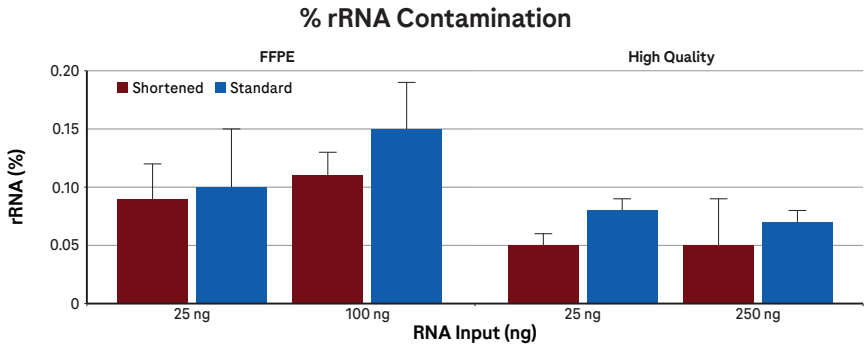

Figure 3. The % of reads that represent residual rRNA remains very low with both workflows.

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Reducing the adapter concentration for lower-input samples has little impact on adapter contamination in the data.

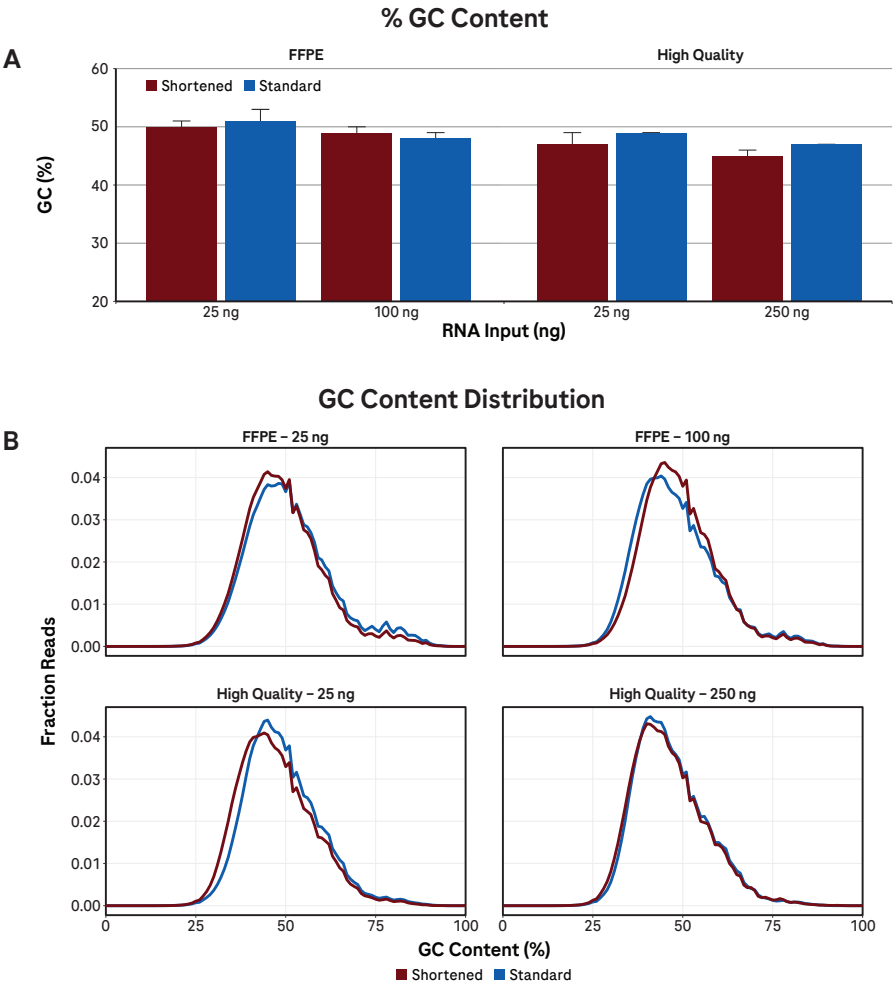

Figure 4. The %GC content and GC distribution metrics are similar with both workflows. **(A)** %GC content for all four input samples. **(B)** GC distribution for all four input samples. Each chart section shows the GC content distribution for a single input type processed using both the shortened and standard workflows.

Table 2. Adapter concentrations used for each input sample for the shortened and standard KAPA RNA HyperPrep with RiboErase (HMR) workflows

Type of input RNA	Mass of input RNA (ng)	Adapter stock concentration shortened workflow (µM)	Adapter stock concentration standard workflow (µM)
FFPE	25	0.15	1.5
	100	1.5	1.5
High Quality	25	0.15	1.5
	250	1.5	1.5

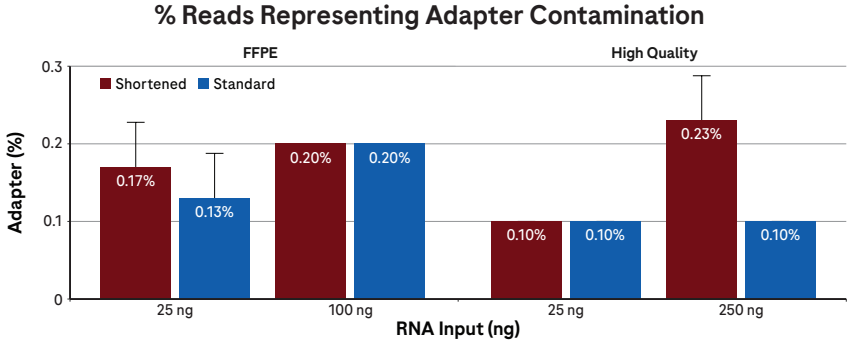

Figure 5. Residual adapter sequences remain very low with the shortened workflow, ensuring that few sequencing reads are wasted on adapter artifacts. Adapter inputs were adjusted for lower-input samples in the shorter workflow as shown in Table 2; this adjustment reduces the amount of adapters used per library.

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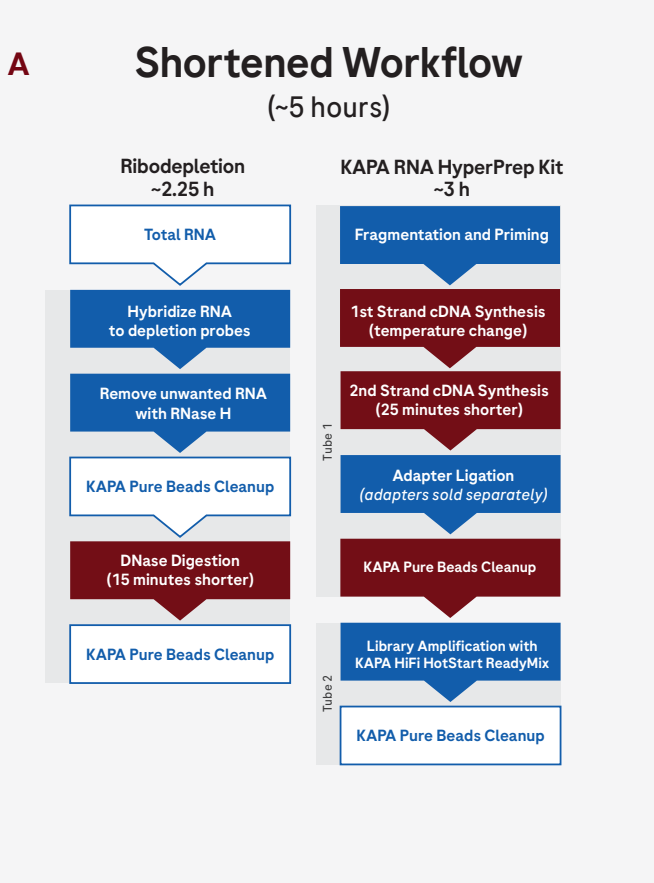
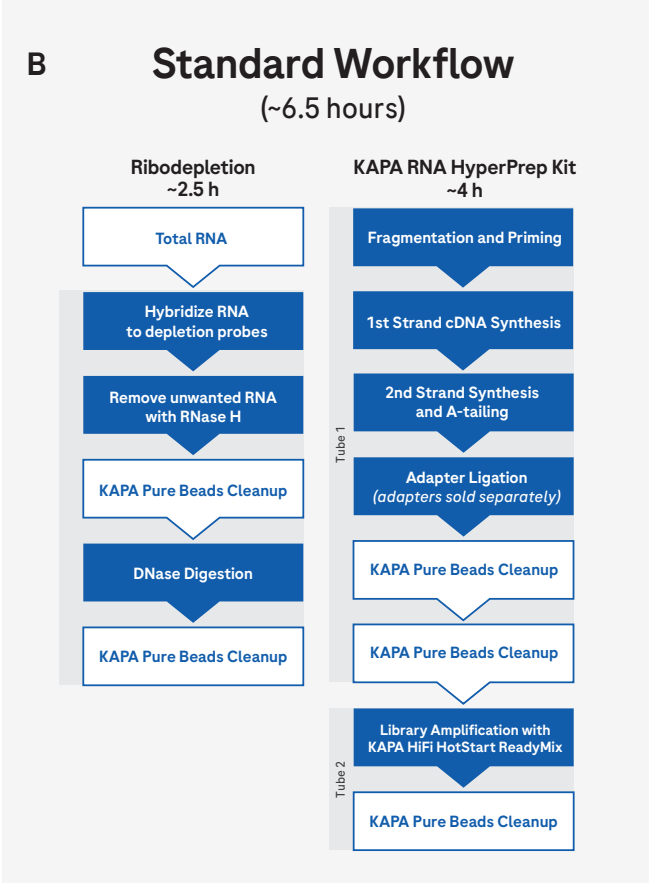
Several steps in the KAPA RNA HyperPrep with RiboErase (HMR) workflow were modified to achieve the shortened workflow:

- rRNA depletion step (shorter DNAse 1 digestion)
- cDNA synthesis step (shorter incubation)
- Bead cleanup (one cleanup eliminated).





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