Biomek i7 Hybrid Automated KAPA RNA HyperPrep Kit with RiboErase (HMR) Workflow

Introduction

The KAPA RNA HyperPrep Kit with RiboErase (HMR) allows users to prepare stranded RNA-Seq libraries compatible with Illumina® sequencing platforms from intact total RNA (25 ng – 1 µg). The kit depletes both cytoplasmic (5S, 5.8S, 18S, and 28S), and mitochondrial (12S and 16S) rRNA species using Roche KAPA's RiboErase chemistry, allowing for the retention of both polyadenylated and non-polyadenylated RNAs while removing high abundance transcripts. The protocol is applicable to a wide range of RNA-Seq applications including: gene expression analysis of high- and low-quality RNA samples (e.g., extracted from FFPE tissue), single nucleotide variation (SNV) discovery, splice junction and gene fusion identification, and characterization of both polyadenylated and non-polyadenylated RNAs including noncoding and immature RNAs. The KAPA RNA HyperPrep Kit with RiboErase (HMR) is compatible with dual or single-indexing strategies and can process up to 96 samples.

In comparison to manual pipetting, automation of the KAPA RNA HyperPrep Kit with RiboErase (HMR) on Biomek platforms provides:

- Reduced hands-on time and increased throughput
- Minimized potential pipetting errors
- Standardized workflow for improved results
- Quick implementation with ready-to-install methods
- Knowledgeable support

In this flyer, the automated performance of the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Biomek i7 Hybrid Genomics Workstation is demonstrated through successful library preparation and sequencing.

Spotlight

Biomek i7 Hybrid (Multichannel 96, Span-8) Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time. Features of the Biomek i7 used to demonstrate this method include:

- 1200 µL Multichannel head with 1 - 1200 µL pipetting capability including Enhanced Selective Tip pipetting to transfer a custom array of samples
- Span-8 pod with disposable tips for enzyme deployment
- Independent 360˚ rotating grippers with offset fingers
- High deck capacity with up to 45 positions
- Orbital Shaker, Static Peltier with flat adapter for reagent cooling, and Shaking Peltier* with PCR adapter for sample mixing and thermal control
- Span-8 (passive) and MC-96 (active) channel tip washing stations
- Optional integrated thermocycler for enzymatic incubations and PCR

*Replaceable with a second static peltier if desired. Thermally controlled shaking is not required for this automation method.

Automated method

Figure 1. KAPA RNA HyperPrep Kit with RiboErase (HMR) automated method workflow on the Biomek i7 Hybrid Genomics Workstation

Automation provides increased efficiency by reducing hands-on time (Table 1). The complete method (Figure 1) can be run with full walk-away capability but does include logical start and stop points assigned based on the KAPA RNA HyperPrep Kit with RiboErase (HMR) Technical Data Sheet. Approved stop points provide users flexibility in workflow scheduling, allowing laboratories to address their individual requirements for sample processing and throughput. Each section of the workflow shown above with a Method Start Location icon can serve as a place to begin for error-recovery purposes. The automated method also offers additional workflow options based on Roche's recommendations, including the ability to use either full-length Indexed Adapters or a Universal Adapter with Index Primers and the ability to select between RiboErase, RiboErase Globin, or a Custom Depletion option.

Metric	KAPA RNA HyperPrep with RiboErase Complete Workflow	KAPA RiboErase RNA Enrichment Only	KAPA RNA HyperPrep Only
Sample Throughput	96	96	96
Hands-On Time	1 hour	45 minutes	45 minutes
Biomek Run Time	8 hours, 20 minutes	3 hours, 20 minutes	5 hours
Total Run Time	9 hours, 20 minutes	4 hours, 5 minutes	5 hours, 45 minutes
Number of User Interactions (aside from initial setup with integrated thermal cycler)	0	0	0

Table 1. Estimated run times for the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Biomek i7 Hybrid Genomics Workstation. Timing estimates are based on the standard workflow as outlined in the KAPA RNA HyperPrep Kit with RiboErase (HMR) Technical Data Sheet with 9 cycles of library amplification.

The KAPA RNA HyperPrep Kit with RiboErase (HMR) automated method for the Biomek i7 Hybrid Genomics Workstation offers several user-friendly software features to guide the user through the setup process including:

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without affecting method integrity. It allows users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed.

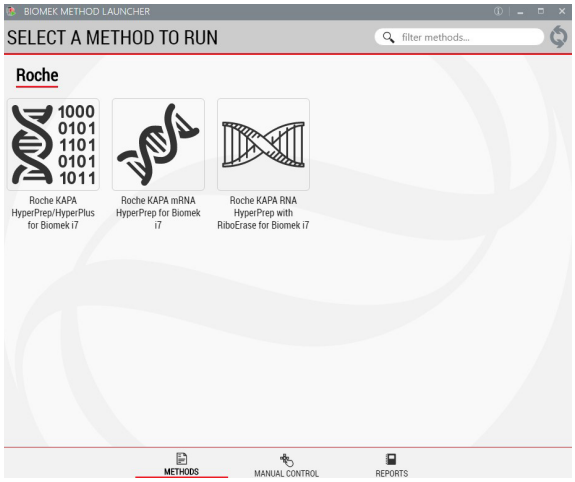


Figure 2. Biomek Method Launcher provides an easy interface to launch the method.

2. Method Options Selector (MOS)

The MOS enables selection of workflow and sample number options to maximize flexibility, adaptability and the ease of method execution.

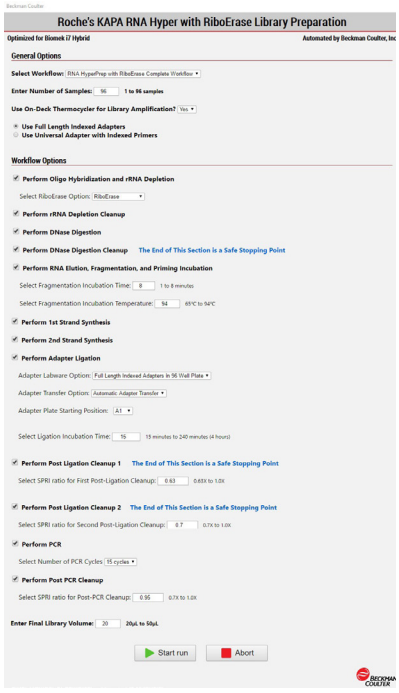


Figure 3. The KAPA RNA HyperPrep Kit with RiboErase (HMR) MOS enables users to select the desired workflow, sample number, and a variety of workflow customization options.

3. Guided Labware Setup (GLS)

The GLS is generated based on options selected in the MOS, and provides the user specific graphical setup instructions with reagent volume calculation and step-by-step instructions to prepare reagents.

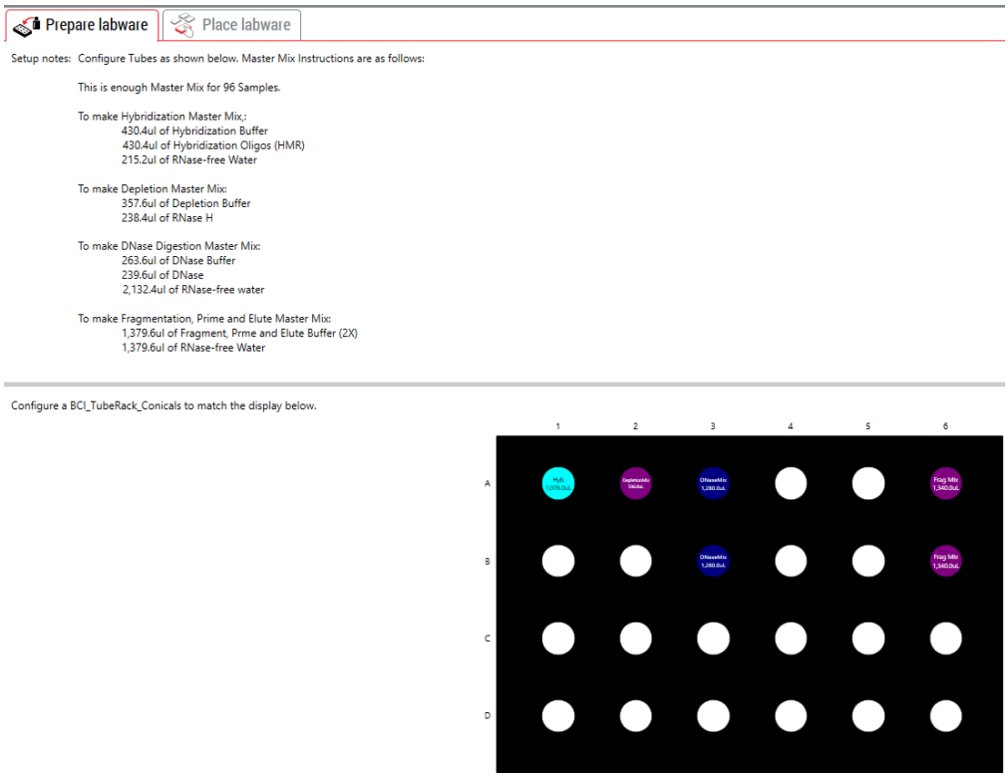


Figure 4. The GLS indicates reagent volumes and guides the user for correct deck setup.

Experimental Design

Two experiments were conducted to demonstrate the automated method. In Experiment 1, Invitrogen™ Universal Human Reference (UHR) RNA was used as the input sample for a KAPA RNA HyperPrep Kit with RiboErase (HMR) run of 12 samples, testing the input range of the workflow and comparing the results of manual versus automated library construction. Eight 250 ng replicate and four 1000 ng replicate automated libraries were created along with a set of four 250 ng replicate and four 1000 ng replicate manual libraries for comparison. Input RNA was checked for quality using the Agilent 2100 Bioanalyzer with RNA 6000 Pico reagents and was shown to have an RNA Integrity Number (RIN) of 7.7. Total RNA was depleted using the KAPA RiboErase (HMR) kit proceeding into the KAPA RNA HyperPrep kit. Depleted samples were fragmented for 8 minutes at 94°C. All samples were adapter ligated with KAPA Unique Dual-Indexed (UDI) adapters at 1.5 µM concentration. Library amplification was performed on all samples for a total of 9 cycles.

The manual libraries were prepared according to Roche specifications as noted in the KAPA RNA HyperPrep Kit with RiboErase (HMR) Technical Data Sheet, while the automated libraries deviated from the standard workflow slightly, using a more stringent post-amplification cleanup (0.95X SPRI ratio). For libraries produced on the Biomek i7, all incubations and library amplification were performed on-deck using the Life Technologies Automated ThermoCycler (ATC) while manually prepared libraries were prepared using an off-deck thermal cycler. Final library size distributions were assayed on the Agilent 2100 Bioanalyzer using the High Sensitivity DNA assay. Post-amplification yields were assessed using the KAPA Library Quantification Kit. Four library replicates of each condition were selected for sequencing on the Illumina® NextSeq™ 550 System. A 2 x 75 bp paired-end run was performed using the High Output flow cell and raw reads were down-sampled to 9 million per sample after sequencing.

In Experiment 2, a full 96-well plate was processed using the automated method to assay for the presence of plate effects when using the automated method. A single RNA input amount was used (250 ng UHR RNA). All library preparation and automation conditions were kept consistent with Experiment 1. For a selection of libraries distributed across the plate (Figure 5), final library yields were measured using the Qubit™ dsDNA HS assay. Library size distribution was assessed for a subset of libraries using the High Sensitivity DNA assay on the Agilent 2100 Bioanalyzer. Libraries were subsequently sequenced on the Illumina® NextSeq™ 550 System. A 2 x 75 bp paired-end run was performed using the High Output flow cell and raw reads were down-sampled to 9 million per sample after sequencing.

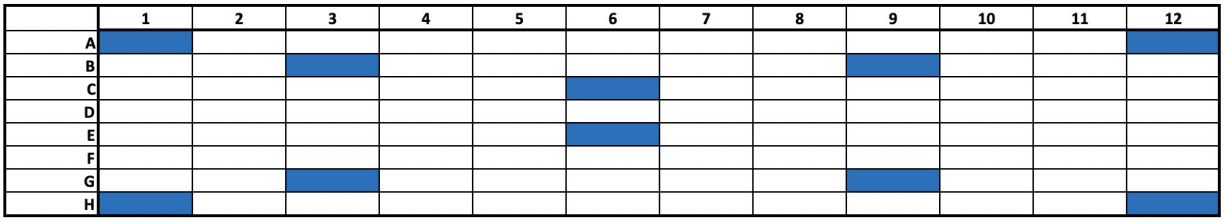


Figure 5. Visual representation of libraries selected for quality assessment.

Results and Discussion

Experiment 1 results showed equivalent post-amplification yields between the automation and manually prepared libraries across the two input amounts (Figure 6). This suggests equivalency with respect to library complexity, as well as amplification efficiency.

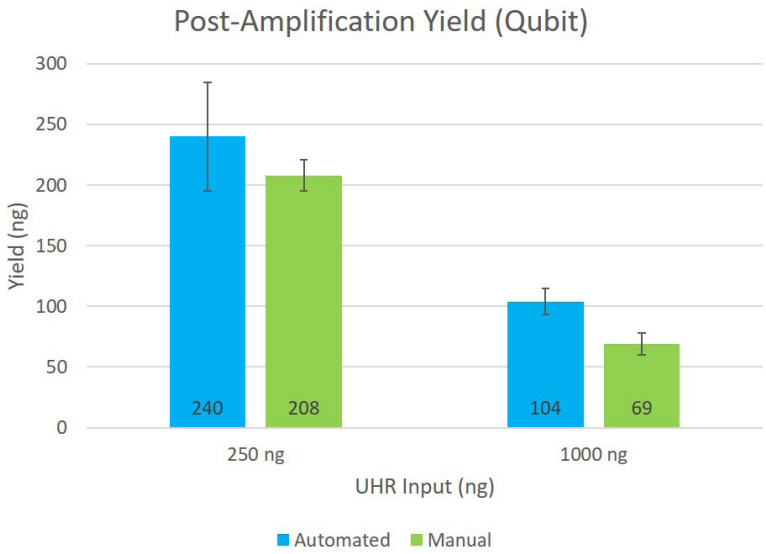
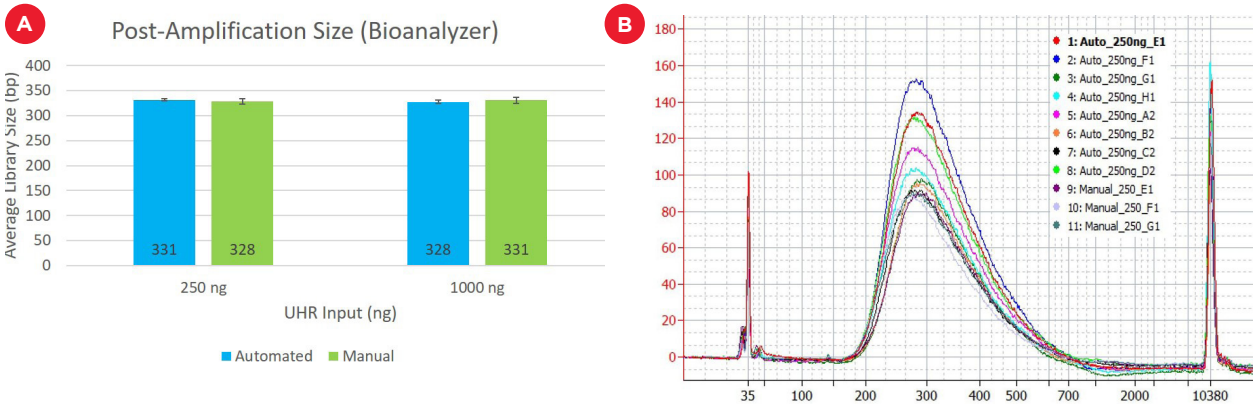


Figure 6. Post-amplification yields indicate the automated method produces libraries with comparable yields to manually prepared samples.

Average final library size was comparable between automated and manual preparations across the two inputs (Figure 7a). Additionally, an overlay electropherogram of the 250 ng inputs (Figure 7b) shows comparable library size distributions between both preparations.



Figures 7a and 7b. Average post-amplification sizes (a) indicate the automated method produces final libraries with comparable sizes to manually prepped samples. Representative library traces (b) of the 250 ng samples show consistent size distribution between automated and manual libraries, with an expected average size of between 220 bp and 320 bp.

Four replicates of each condition were selected for sequencing, after subsampling the percent mapped to genome for all samples was first assessed. All samples showed mapping rates of greater than 97% (Figure 8a). The percent mapped to genome metric can be used as a global indicator of the overall sequencing accuracy and presence of contamination.

Next assessed was duplication rate, for which all samples showed less than 10% duplicates and rates were comparable between both preparations and across input amounts (Figure 8b). Mean CV, or the coefficient of variation in base coverage across transcript length, was also comparable between manual and automated samples for both inputs (Figure 8c). For this metric, a smaller number reflects less variation in base coverage, and thus, better coverage uniformity.

Next shown, Figure 8d, are the total number of unique transcripts identified, which were greater than 78,000 for all samples sequenced. Sequencing reads were then binned to assess overall mapping rates between exonic, intronic and intergenic reads. RNA depletion intentionally biases the read distribution toward non-intergenic regions, as shown for the 250 ng inputs in Figure 8e, and these mapping rates are comparable across both preparations. Finally, the percent of ribosomal RNA (rRNA) reads is calculated to ensure an efficient RNA depletion process with minimal rRNA carryover into library preparation. Automated samples are comparable to manual samples for both inputs, with all samples showing less than 0.5% rRNA reads (Figure 8f).

Figure 8. Comparable sequencing metrics indicate equivalent performance between automated and manual samples for both input amounts. After subsampling, data was analyzed to quantify the (a) Percent Mapped Reads (b) Percent Duplicate Reads (c) Mean Coverage Uniformity (d) Total Unique Transcripts Identified (e) Read Distribution (exonic, intronic, v. intergenic) and (f) Percent rRNA Content.

Experiment 2 final library yields showed consistent results across the plate in terms of average size (Figure 9). Sequencing results show low amounts of rRNA content and a consistent distribution of exonic, intronic and intergenic mapping rates (Figure 10). Overall, results do not indicate the presence of significant plate effects.

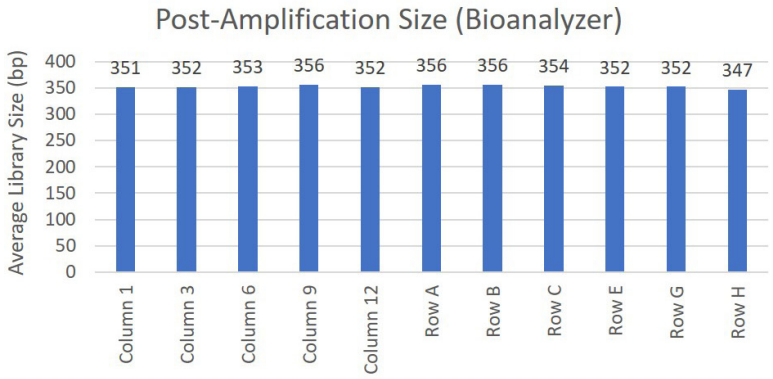


Figure 9. Average size of libraries created during Experiment 2 analyzed by row and column.

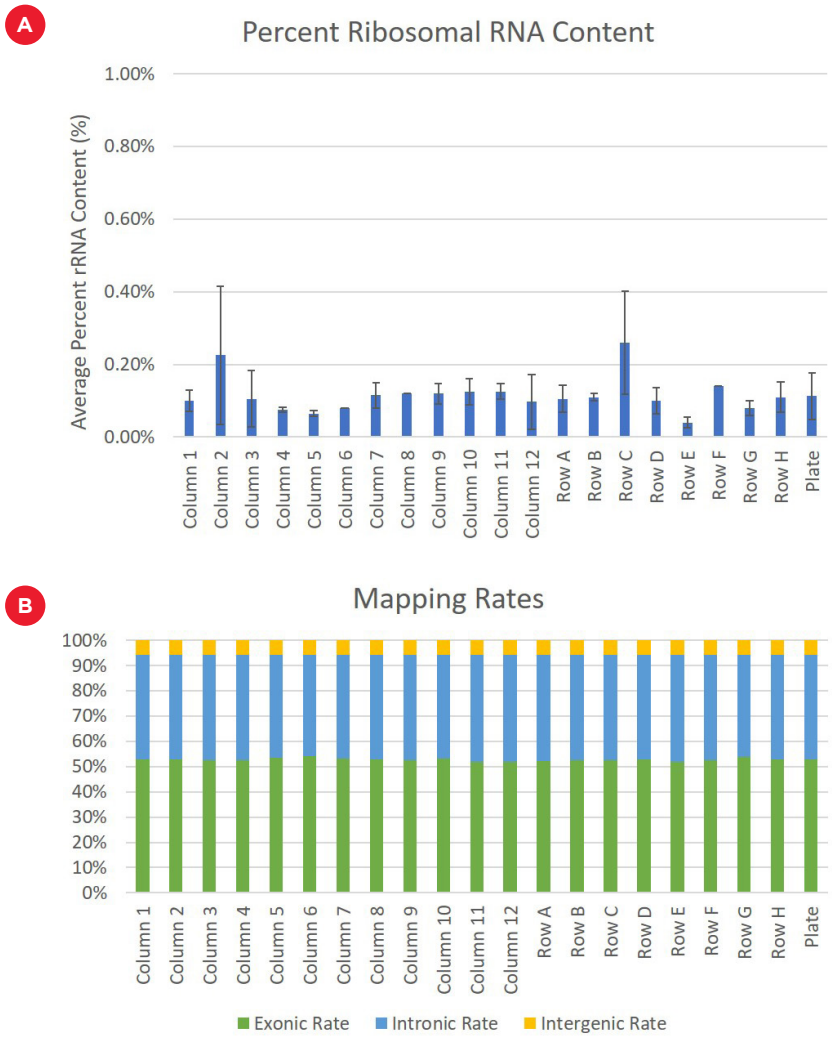


Figure 10. Sequencing analysis of the Experiment 2 libraries including Percent Ribosomal Content (a) and Mapping Rates (b) examined by rows and columns. In both analyses, results from Experiment 2 are consistent with that of Experiment 1, indicating consistency across the 96 well plate experiment.

Conclusion

NGS library preparation for RNA-Seq can be a time-consuming and tedious process. Automation of these workflows can save valuable time and money, providing researchers with more flexibility, efficiency and reliability.

With the included dataset, performance of the KAPA RNA HyperPrep Kit with RiboErase (HMR) method on the Biomek i7 Hybrid Genomics Workstation has been demonstrated. Results show that the method delivers high-quality libraries on par with those prepared manually. The automated solution offers full walk-away capability and scalability thus providing fast, efficient and reliable RNA-Seq library construction.

References

1. KAPA RNA HyperPrep Kit with RiboErase (HMR) KR1351 – v3.20

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