AUTOMATED RNA SEQUENCING LIBRARY PREPARATION FOR WHOLE-TRANSCRIPTOME SEQUENCING USING THE BECKMAN COULTER BIOMEK i7

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INTRODUCTION

RNA sequencing (RNA-seq) is a powerful tool for transcriptome-wide analysis of differential gene expression, as well as for the identification of novel and alternatively-spliced transcripts. However, RNA-seq library preparation protocols are complex and require high levels of precision, posing a challenge to scaling and reproducibility—especially for high-volume studies.

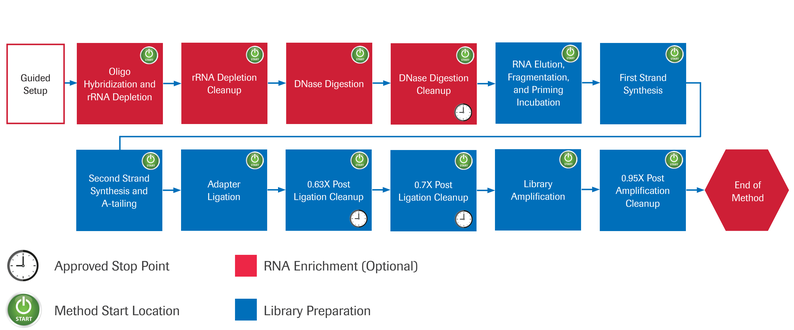
To help improve the scalability and reproducibility of RNA-seq library preparation, we developed an automated protocol for the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Beckman Coulter Biomek i7 Hybrid liquid handling platform. This kit uses Ribosomal RNA (rRNA) specific probes and enzymatic depletion to remove human, mouse, or rat rRNA from the input RNA. Custom-designed probes are also compatible with this method and can be used to remove other targets, such as globin or rRNA from other species.

To verify the automated protocol, RNA-seq libraries were prepared in parallel using the Biomek i7 and manual methods; pre- and post-sequencing metrics were then compared. To benchmark the reproducibility of the automated protocol, 96 additional libraries were prepared in a single automated run and compared.

The results show that libraries prepared on the Beckman Coulter Biomek i7 were comparable to libraries prepared manually on all pre- and post-sequencing metrics. Similarly, libraries prepared simultaneously in a single automated run were highly consistent across multiple metrics.

WORKFLOW, MATERIALS, AND METHODS

Input RNA: Commercially available Universal Human Reference RNA (Total RNA pooled from 10 human cell line extracts) with average RIN ~ 8 was used as input material.



Metric	KAPA RNA HyperPrep Kit with RiboErase Complete Workflow	KAPA RiboErase Only	KAPA RNA HyperPrep Only
Sample Throughput	96	96	96
Hands-On Time	1 hr	45 min	45 min
Biomek Run Time	8 hr, 20 min	3 hr, 20 min	5 hr
Total Time	9 hr, 20 min	4 hr, 5 min	5 hr, 45 min
Number of User Interactions (aside from initial setup)	0	0	0

Figure 1: Schematic of the RNA-seq library preparation workflow using the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Biomek i7. RNA Enrichment (Optional) Start and stop points available on the method. Estimated run times of the workflow are provided in the table.

PRE-SEQUENCING METRICS ARE SIMILAR FOR BIOMEK AND MANUAL METHODS

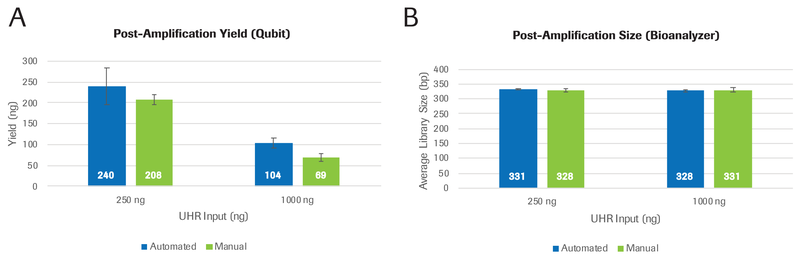


Figure 2: Pre-sequencing library metrics are similar between the two library preparation methods. The slightly higher yield **(A)** resulting from automated preparation may be the result of more thorough mixing during bead cleanups. Library fragment sizes **(B)** are very similar between methods.

Method: 250 ng and 1000 ng of UHR were used as input (n=3) into automated and manual library preparation. Post-amplification library yield and library size were then compared between the two methodologies.

POST-SEQUENCING METRICS ARE SIMILAR FOR BIOMEK AND MANUAL METHODS

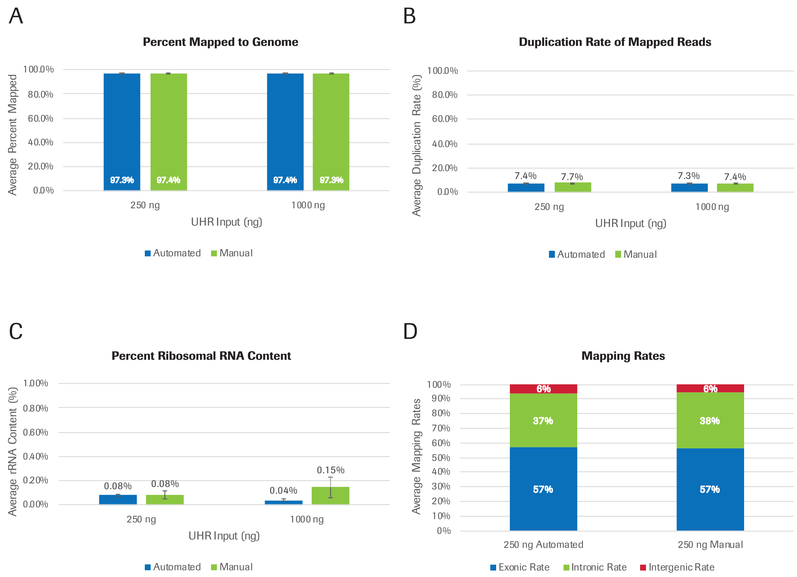


Figure 3: Post-sequencing library metrics are similar between the two library preparation methods. The percent mapped reads **(A)** were very high (>97%) for all libraries and duplication rates **(B)** were similar between methodologies. rRNA depletion **(C)** was more consistent and more efficient using automated workflows compared to the manual method. Mapping rates **(D)** were similar between methodologies.

Method: Libraries were normalized and then sequenced on the Illumina® NextSeq 550 using a high-output kit. Raw reads were downsampled to 9 million reads and then aligned to hg38 genome build. Subsequent RNA-seq analysis was performed using an in-house RNA-seq pipeline.

A HIGH-THROUGHPUT 96-SAMPLE AUTOMATED RUN YIELDED EFFICIENT DEPLETION AND CONSISTENT MAPPING RATES

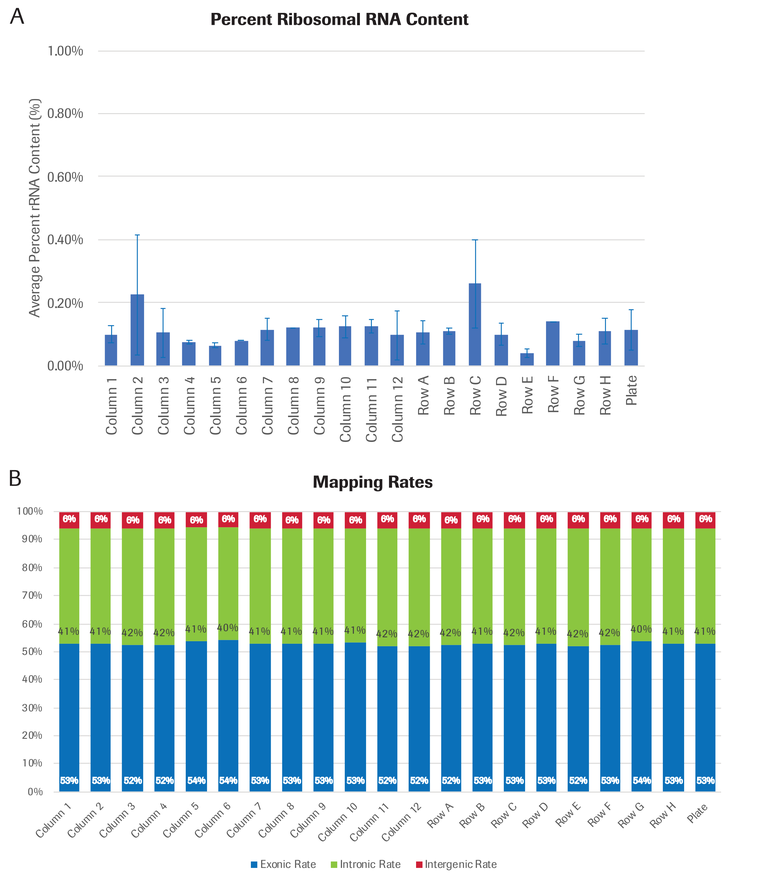


Figure 4: A high-throughput 96-sample automated run yielded highly consistent libraries. Less than 1% residual rRNA was detected in all samples **(A)**, indicating efficient rRNA depletion across the plate. The exonic, intronic, and intergenic mapping rates were consistent across all samples **(B)**, indicating that this library preparation method is highly reproducible.

Method: 250 ng of UHR was used as input for the high-throughput automated run. Libraries were normalized and then sequenced on the Illumina NextSeq 550 using a high-output kit. Raw reads were downsampled to 9 million reads and then aligned to hg38 genome. Subsequent RNA-seq analysis was performed using an in-house RNA-seq pipeline.

CONCLUSION

- The comparison of RNA-seq libraries prepared using automated and manual methods showed that the automated library method performed similarly to manual methods. One notable exception is that rRNA depletion was more efficient and consistent using the automated method, indicating that the automated method may out-perform manual methods in some cases.
- The high-throughput 96-sample experiment showed that this automated method provides highly efficient rRNA depletion and consistent library preparation, thus offering a robust solution for high-throughput workflows using the KAPA RNA HyperPrep Kit with RiboErase (HMR) on the Biomek i7.

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