THE TECAN FREEDOM EVO® NGS WORKSTATION YIELDS HIGH-QUALITY mRNA-ENRICHED LIBRARIES AND RIBODEPLETED LIBRARIES USING A SINGLE AUTOMATED RNA-SEQ LIBRARY PREPARATION METHOD

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OVERVIEW

- Many RNA-seq applications benefit from removing ribosomal RNA (rRNA) molecules from the input RNA prior to library preparation and sequencing.
- rRNA removal approaches utilize either mRNA capture or ribodepletion.
- The mRNA capture and ribodepletion modules for KAPA RNA HyperPrep Kits have been automated on the Tecan Freedom EVO® NGS Workstation using a single method.
- In this study, libraries were created using both the KAPA mRNA HyperPrep Kit and the KAPA RNA HyperPrep Kit with RiboErase (HMR); libraries for each workflow were prepared using manual and automated methods, and all libraries were compared.
- Pre- and post-sequencing metrics demonstrate that the performance of the automated method on the Tecan Freedom EVO® NGS Workstation is comparable to manual preparations for both RNA-seq library preparation

INTRODUCTION

RNA-seq is a powerful tool for investigating cellular physiology in many applications such as comparative transcriptomics, *de novo* transcriptome assembly, epigenetics, splice variation and transcription regulation. For most of these applications, the RNA of interest is enriched over the abundant rRNA molecules prior to library construction using either rRNA depletion or mRNA capture methods.

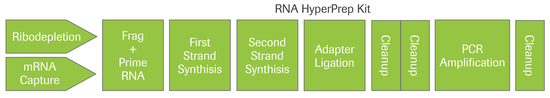
The automation-friendly KAPA RNA HyperPrep Kits offer workflows for both RNA-enrichment approaches:

- **KAPA RNA HyperPrep Kit with RiboErase (HMR):** an RNase H-based ribodepletion module
- **KAPA mRNA HyperPrep Kit:** a bead-based poly(A) mRNA enrichment module

While both modules significantly reduce rRNA reads and generate high-quality libraries, method selection depends upon the experimental question, the sample type, and logistical parameters.

KAPA RNA HyperPrep Kits have been automated on the Tecan Freedom EVO® NGS Workstation. A single method enables either mRNA capture (with the KAPA mRNA HyperPrep Kit) or ribodepletion (with the RNA HyperPrep Kit with RiboErase (HMR). Ribodepleted and mRNA-enriched libraries were generated using this automated method and were evaluated against manually prepped libraries generated with the same kits using pre- and post-sequencing metrics.

METHODS



Library Prep:

- Automated and manual RNA-seq libraries were prepared using the KAPA mRNA HyperPrep Kit and the KAPA RNA HyperPrep Kit with RiboErase (HMR) following the parameters in **Table 1**. n=8 for each method.
- Library fragment size distribution was analyzed using the High Sensitivity DNA assay on the Agilent 2100 Bioanalyzer.

Sequencing and Analysis:

- Three libraries generated using each method were sequenced on an Illumina® NextSeq® 500 (2x75 bp).

Table 1: Parameters for the preparation of ribodepleted and mRNA-enriched RNA-seq libraries using both manual and automated methods.

Parameters	KAPA RNA HyperPrep Kit with RiboErase (HMR)	KAPA mRNA HyperPrep Kit
Samples	100 ng Universal Human Reference (UHR) control RNA	
Replicates	8 Automated 8 Manual	8 Automated 8 Manual
Fragmentation	8 minutes at 94°C (targeting 100-200 bp insert)	
Indexed adapters	KAPA Dual-Indexed Adapters at [1.5 µM]	
Amplification cycles	12	13

RESULTS

PRE-SEQUENCING QUALITY METRICS WERE SIMILAR FOR LIBRARIES GENERATED USING ALL FOUR METHODS

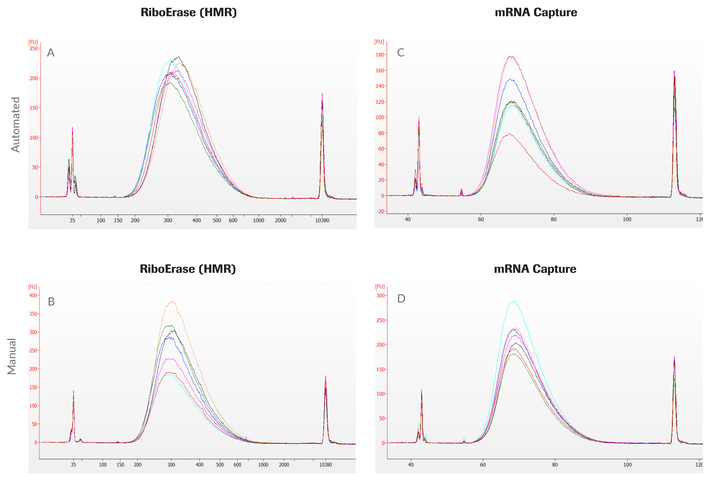


Figure 1: Comparison of fragment distributions generated between manual and automated workflows. Libraries generated using the automated workflow **(A and C)** showed similar fragment distributions compared to manually generated libraries **(B and D)**. Variation observed between replicates was also similar between automated and manual preparations. There was a minor increase in the presence of adapter-dimers in the automated mRNA capture libraries **(C)**. For all conditions, n=8 libraries prepared.

POST-SEQUENCING QUALITY METRICS WERE COMPARABLE FOR LIBRARIES GENERATED USING ALL FOUR METHODS

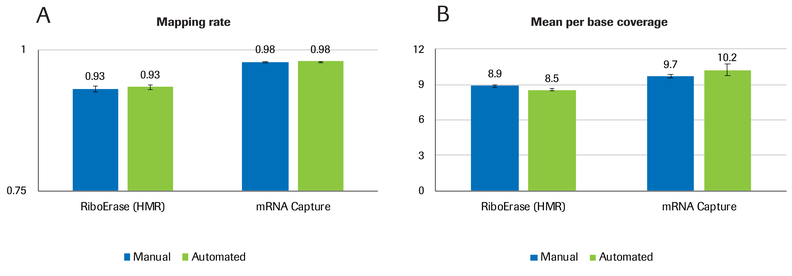


Figure 2: Analysis of sequencing reads mapped to the reference genome. The percentage of reads mapping to the reference genome **(A)** and the average per-base sequencing coverage across the genome **(B)** were comparable between automated and manual libraries generated with each kit. Data and error bars reflect the mean and standard deviation respectively. n=3 libraries sequenced.

LIBRARY COMPOSITION WAS SIMILAR FOR ALL METHODS

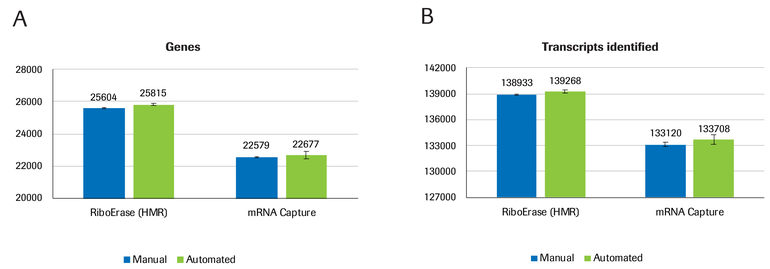


Figure 3: Unique genes and transcripts identified using all four library preparation methods
Automated libraries yielded similar numbers of identified genes **(A)** and transcripts **(B)** compared to manually-prepared libraries. Data and error bars reflect the mean and standard deviation respectively.
n=3 libraries sequenced.

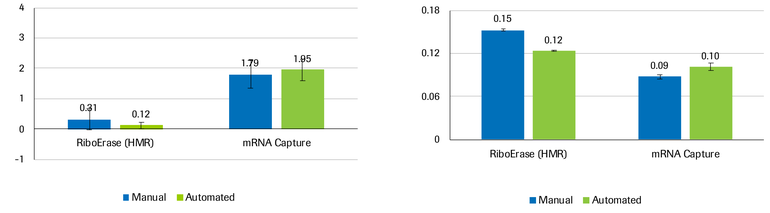


Figure 4: Proportions of uninformative reads in sequencing data across all four library preparation methods. The consistency of the residual rRNA reads **(A)** between automated and manual preparations using each KAPA RNA HyperPrep Kit indicated that the efficacy of ribosomal removal was maintained using both manual and automated methods. The percentage of duplicate reads **(B)** also demonstrated that the read complexity remains consistent. Data and error bars reflect the mean and standard deviation respectively. n=3 libraries sequenced.

CONCLUSIONS

Automation of the KAPA RNA HyperPrep Kit with RiboErase(HMR) and the KAPA mRNA HyperPrep Kit on the Tecan Freedom EVO® NGS Workstation generates high-quality RNA-seq libraries.

When compared with manually prepared libraries, these libraries demonstrate:

- comparable fragment size distribution
- similar mapping rates, coverage, and read complexity, and read complexity, and
- comparable mRNA enrichment and ribodepletion efficacy

Data on file.

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