High-quality RNA libraries generated on the Agilent Bravo NGS workstation with a new, shorter RNA library prep workflow with rRNA depletion

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INTRODUCTION

RNA sequencing (RNA-seq) with ribosomal RNA (rRNA) depletion has become an important tool for studying gene expression. In order to reduce the workflow complexity, processing time, and amount of user intervention, Roche has created a shorter RNA-seq library preparation workflow with its on-market RNA-seq kits. This workflow has been automated on the Bravo NGS workstation to allow for higher throughput capacity and reduced hands-on time. This study demonstrates that the new automated method produces results comparable to both manual and automated preparations of the standard workflow.

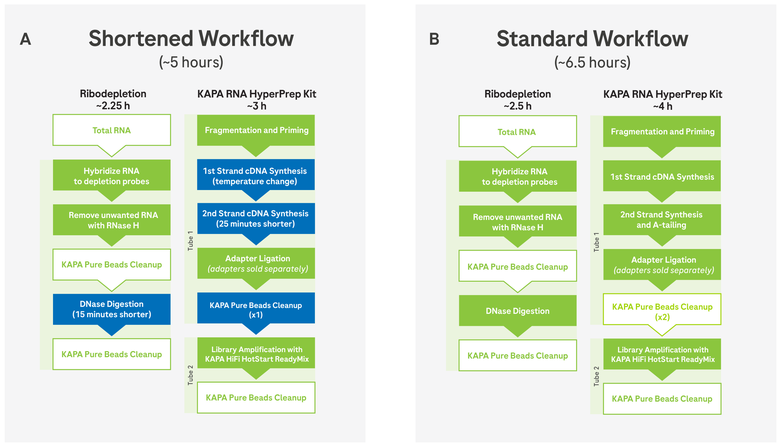

Figure 1. Workflow comparison. This figure compares the new shortened workflow for RNA-seq library preparation using KAPA RNA HyperPrep Kit with RiboErase (HMR) (A) to the Standard workflow (B). Steps that have been modified are colored blue. Note that the shortened workflow takes less processing time and has 1 fewer bead cleanup steps.

EXPERIMENT DESIGN

Ribodepleted RNA HyperPrep libraries were created using the KAPA RNA HyperPrep Kit with RiboErase (HMR) under the following 3 conditions:

Table 1. Experiment parameters. Other than number of replicates, all three conditions shared the same experimental parameters.

Condition	Chemistry	Input	Replicates	Fragmentation	Indexing Strategy	PCR Cycles
Shortened workflow on Bravo NGS workstation	KAPA RNA HyperPrep Kit with RiboErase (HMR)	250 ng Universal Human Reference RNA (UHR) (Thermo Fisher Scientific)	4	8 min @ 94°C	UDI Adapters	9
Full length workflow on Bravo NGS workstation			3			
Full length workflow prepared manually			3			

Figure 2. Experiment workflow. All 3 conditions followed the same general steps. Data was generated from the post amplification QC and sequencing steps. See table 2 for QC and sequencing details.

RESULTS

QC AND SEQUENCING METHODS

Table 2. Metrics analyzed.

Metric	Assay
Post Amplification Yield	Invitrogen™ Qubit™ - 1X dsDNA HS
Post Amplification Library Size	The LabChip® GX Touch™ - DNA NGS 3K
% rRNA % Aligned to genome Number of transcripts detected Number of genes detected	Sequencing on Illumina NextSeq 550 • 150 cycle High-Output 2x76 • RNA-Seq Analysis • Downsampled to 9 M read pairs per sample

LIBRARY QC: PRE-SEQUENCING RESULTS

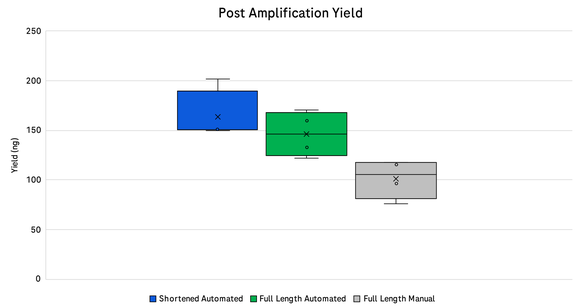

Figure 3. Post-amplification yields. Yields measured by Qubit were similar between the shortened and full length workflows when automated. Both automated methods outperformed the full-length manual preparation.

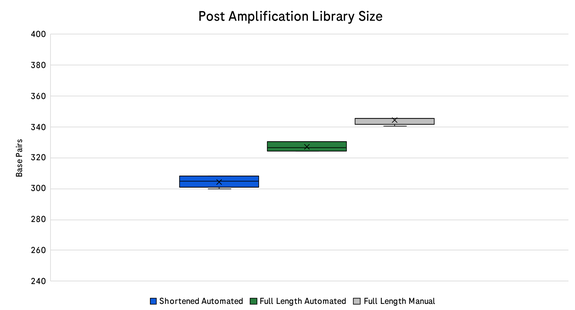

Figure 4. Post-amplification library sizes. The shortened automated workflow yielded smaller library sizes than both of the full length workflows. If necessary, library sizes can be fine-tuned by adjusting fragmentation parameters.

RESULTS (CONTINUED)

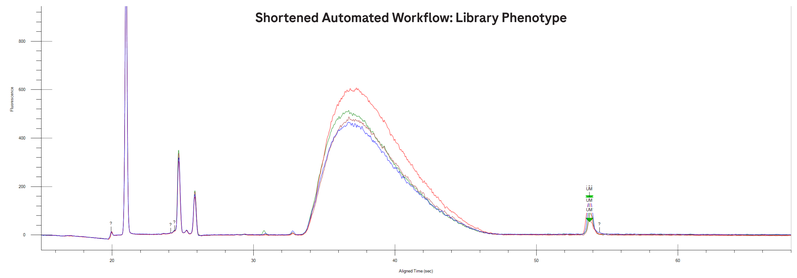

Figure 5. Post-amplification library size distribution. This figure shows the post-amplification library size distribution for the shortened automated workflow. The resulting libraries display the desired phenotype with consistent library size distributions.

SEQUENCING ANALYSIS: RNA-SEQ RESULTS

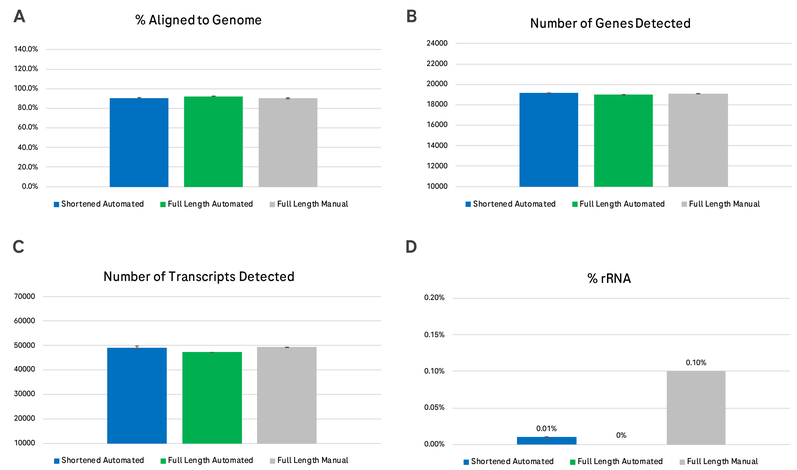

Figure 6. Sequencing Metrics. All three workflows achieved similar alignment rate to the genome (A), number of genes detected (B), and number of transcripts detected (C), with minimal to no rRNA contamination (D). Bars represent the mean and error bars reflect the standard deviation amongst replicates.

CONCLUSION

The automated shortened KAPA RNA HyperPrep with RiboErase method on the Bravo NGS workstation:

- Produces libraries that are comparable to those produced using the full-length workflow, whether they were prepared by hand or automated on the Bravo NGS workstation
- Reduces processing time when compared to the full-length workflow
- Reduces hands-on time when compared to manual preparation
- Increases sample throughput when compared to manual preparation.

Data on file.
Project: Bravo NGS workstation Shortened RNA with RiboErase
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