Automated RNA-seq Library Preparation on the Beckman Coulter Life Sciences Biomek i7 Hybrid Workstation Using a Shortened KAPA RNA HyperPrep Workflow

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INTRODUCTION

As RNA sequencing (RNA-seq) has become more important to our understanding of disease and to the development of new pharmaceuticals, the need for faster, scalable, reproducible workflows has also grown. However, RNA-seq library preparation methods are complex and require high levels of precision, posing a challenge to the required scaling and reproducibility.

In this study, a two-fold approach was taken to overcome these challenges:

1. a new, shorter RNA-seq library prep workflow was developed using on-market kits without the need for additional reagents, and
2. this shorter workflow was automated on a liquid handler that is frequently used for NGS library preparation.

WORKFLOW MODIFICATIONS

From the original KAPA RNA HyperPrep Kit

- Shorter DNase digestion incubation (from 30 min to 15 min)
- Lower temperature for the 1st strand synthesis primer extension (20°C vs 25°C).
- Shorter 2nd strand synthesis incubation (42°C for 5 min, instead of 62°C for 30 min)
- Reduced adapter concentrations with lower input amounts, to minimize primer dimer formation
- Fewer 0.7X post-ligation cleanups (one, instead of two)

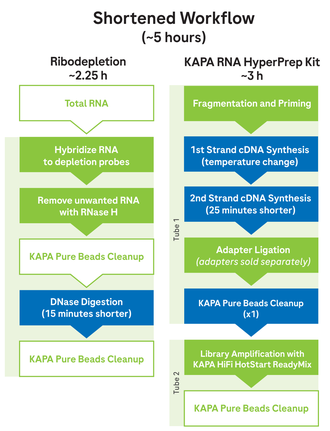

Figure 1. Shortened KAPA RNA HyperPrep Workflow, including ribodepletion.

METHODS TESTED

A new, shorter workflow for the KAPA RNA HyperPrep Kit with RiboErase (HMR) was automated on the Beckman Coulter Life Sciences Biomek i7 Hybrid Automated Workstation. KAPA Unique Dual-Indexed Adapters (full-length) were used.

High-quality universal human reference (UHR) RNA was used as input across a 40-fold range of input amounts (25 ng, 250 ng, and 1000 ng); see table below for corresponding adapter concentrations used. For 25 ng input samples, n=8 for each workflow. For all other samples, n=4 for each workflow.

Input Amount (ng)	Adapter Concentration (µM)
25	0.15
250	1.5
1000	7

For comparison, RNA-seq libraries were also prepared manually using the full-length standard workflow. User guide recommended adapter concentrations and PCR cycles were used. Libraries were run on an Illumina NextSeq500 system. Error bars on charts represent standard deviation.

PRE-SEQUENCING METRICS

Total average library yield (ng) and average size (bp) of libraries were comparable between the standard full-length KAPA RNA HyperPrep with RiboErase (HMR) manual workflow and the automated shortened workflow run on the Beckman Coulter Life Sciences Biomek i7 Workstation.

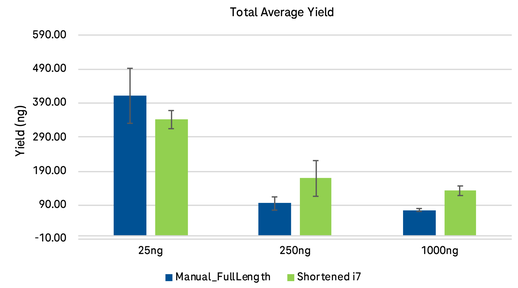

Figure 2. Total average library yield using the original workflow and the shorter, automated workflow. Evaluated using Qubit.

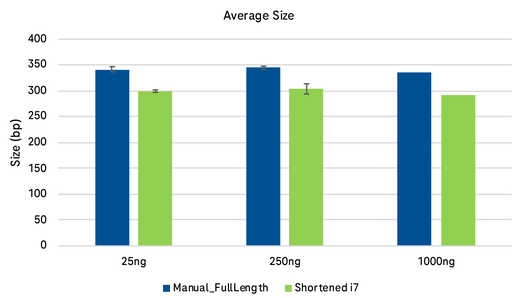

Figure 3. Average library size using the original workflow and the shorter, automated workflow. Evaluated using a BioAnalyzer.

SEQUENCING RESULTS

The sequencing results demonstrate that the shortened automated RNA-seq workflow generates high-quality libraries as effectively as the full-length manual workflow.

Both methods identify the equivalent number of genes and unique transcripts.

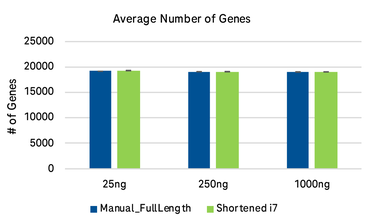

Figure 4. Average number of genes identified.

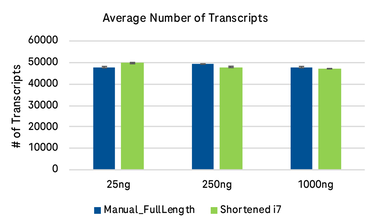

Figure 5. Average number of transcripts identified.

SEQUENCING RESULTS (Continued)

Residual rRNA reads and duplication rates were low for the shortened, automated workflow, and comparable to the original full-length manual workflow.

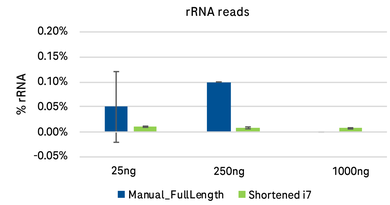

Figure 6. Residual rRNA reads (%).

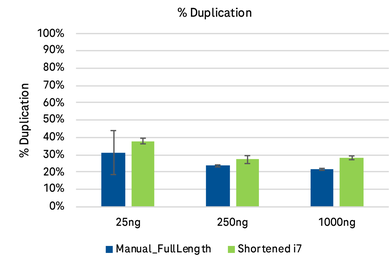

Figure 7. Duplicate reads (%).

The average insert size determined by sequence analysis is consistent with the average library size measured during QC of libraries (values shown here do not include adapter sequences).

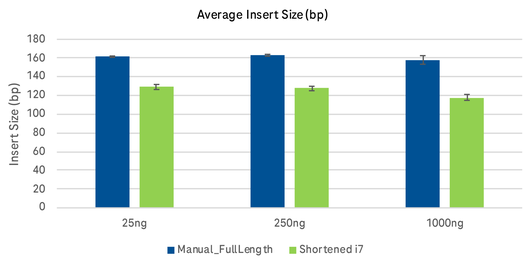

Figure 8. Average Insert Size (ng).

CONCLUSION

This faster, shortened, automated method for KAPA RNA HyperPrep with RiboErase (HMR) increases the speed and reproducibility of RNA-seq library prep without impacting the accuracy or efficiency of the original KAPA RNA HyperPrep with RiboErase (HMR) Kit.

Overall, this new workflow:

- reduces hands-on time
- improves reproducibility, and
- enables higher sample throughput for RNA library preparation.

Data on file.
Project: BioMek i7 Shortened RNA
For Research Use Only. Not for use in diagnostic procedures.

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