Evaluating a Simple, Automatable, and Sensitive Solution for Rapid RNA-Seq

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1 Introduction

Whole Transcriptome Sequencing (WTS) has become an indispensable tool for investigating gene expression, alternative splicing, and transcriptional dynamics across the genome. It provides high-resolution insights into cellular RNA, enabling researchers to explore gene function, regulatory mechanisms, and cellular responses with unprecedented detail. As the adoption of RNA-Seq technology expands, there is an increasing demand for efficient, scalable workflows that can process clinically relevant sample types, including formalin-fixed paraffin-embedded (FFPE) and low input RNA. Automation of RNA-Seq sample preparation is essential to addressing these challenges by improving throughput, minimizing technical variability, reducing human error, and lowering costs - all while maintaining robust and reproducible results. Here we're demonstrating the simple and sensitive Watchmaker RNA Library Prep Kit with Polaris™ Depletion automated on the Revvity Sciclone™ G3 NGSx workstation.

2 Efficient RNA Library Preparation

The Watchmaker RNA Library Prep Kit with Polaris™ Depletion offers an efficient, simplified method for preparing stranded whole transcriptome sequencing (WTS) libraries from 1 ng to 1 µg of total RNA. It delivers high library complexity with minimal coverage bias. The workflow supports human, mouse, and rat species, and is suitable for both high- and low-quality samples, including FFPE material. The kit includes simple-to-prepare master mixes that can be swiftly implemented on the Revvity Sciclone™ G3 NGSx workstation, enabling rapid high-throughput sample processing.

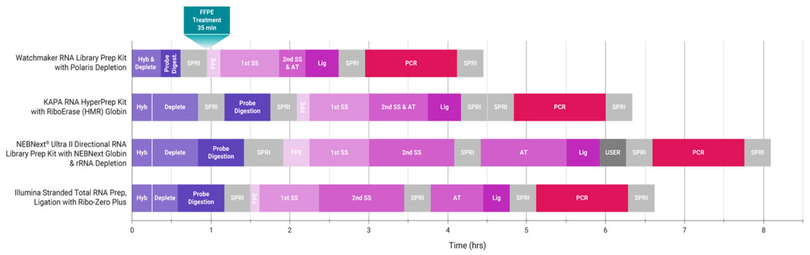

Figure 1: The Watchmaker solution streamlines enzymatic processes and minimizes bead purification steps compared to alternative commercial kits. This enables a fully automatable workflow, drastically cutting down hands-on time (up to one hour per 96 plate) and reducing consumable usage (up to 1,000 pipette tips per 96 plate).

3 Flexible and Fast Automated Method

Rapid 7-Hour Processing for 96 Libraries:
The system processes up to 96 libraries in just 7 hours, ensuring fast turnaround times for both small and large projects. This rapid processing reduces hands-on time and the risk of human error.

Supports FFPE, High-Quality, and Degraded RNA:
It efficiently handles RNA from FFPE tissues, high-quality samples, and degraded RNA, making it versatile for a range of sequencing applications. This flexibility is ideal for clinical and routine RNA sequencing, where sample integrity may vary.

Dynamic Bead Ratios for SPRI Cleanups:
Dynamic bead ratios during SPRI cleanups ensure optimal purification by adjusting based on sample quality. This technique effectively removes contaminants while preserving RNA fragments, even from degraded samples.

Choice of Adapters:
Users can choose between stubby and full-length adapters to meet the specific needs of their experiment. This flexibility allows for tailored adapter lengths, whether for minimal adapter sequences or longer ones for targeted sequencing.

Scalable for 8 to 96 Samples:
The system is scalable, supporting library preparation from 8 to 96 samples in a single run. This adaptability makes it suitable for both small-scale studies and large, high-throughput sequencing projects.

Simplified Setup:
The method includes user-friendly interfaces and straightforward prompts to assist inexperienced users, complemented by streamlined workbooks that simplify reagent loading.

4 Dynamic User Interface

The Revvity Sciclone™ NGSx workstation is an automated liquid handler that features an open-deck design configured with a 96-channel pipetting head, a Sciclone gripper, on-deck microplate shaker, four temperature controlled Cold Plate Air Cooled (CPAC) positions, and two magnetic separation positions. The Watchmaker RNA Library Prep Kit with Polaris™ Depletion automated method was designed with a variety of dynamic run-time options and user-friendly reagent workbooks, supporting multiple workflows during setup.

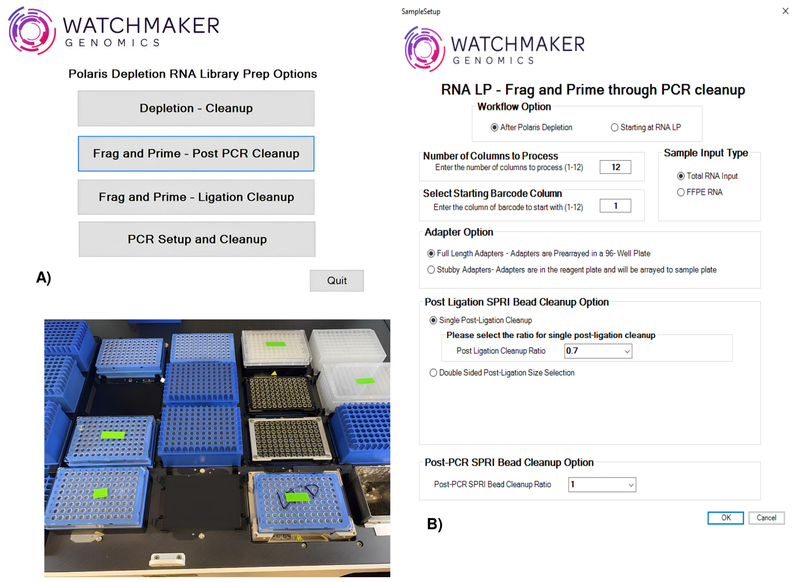

Figure 2: A) Intuitive user interface designed to enable flexibility for all safe start/stop points. B) Customizable runtime options provide adaptability across diverse workflows. Users can adjust parameters such as adapter types to meet sequencing requirements, bead cleanup ratios to optimize sample quality, and fragmentation conditions to achieve precise RNA sizing. This versatility ensures seamless compatibility with various library preparation needs, all within a single automated method. C) A clear and easy-to-follow deck layout guides users at the start of the run, ensuring smooth operation.

5 Method Performance & Results

The performance of the automated method was thoroughly evaluated through two low-throughput runs (24 samples each) and one high-throughput run (96 samples), with results compared to sixteen manually prepared samples. The 96-sample run incorporated both positive and negative controls in a checkerboard pattern to assess potential cross-contamination and residual plate effects. For all runs, 50 ng of Universal Human Reference (UHR) RNA (ThermoFisher® QS0639) was used as input per sample. The results demonstrated that the method successfully generated high-quality libraries with consistent yields and fragment sizes. Notably, all no-template controls (NTCs) showed no signs of cross-contamination, confirming the reliability and precision of the automated process. Fragmentation was performed at 85°C for 5 minutes. Revvity full length NEXTFLEX™ Unique Dual Index Barcodes (UDI) were used at a concentration of 1 µM. Post-ligation SPRI cleanup included a 0.7X cleanup followed by a 1X cleanup. PCR was conducted for 12 cycles, followed by a final 1X SPRI cleanup.

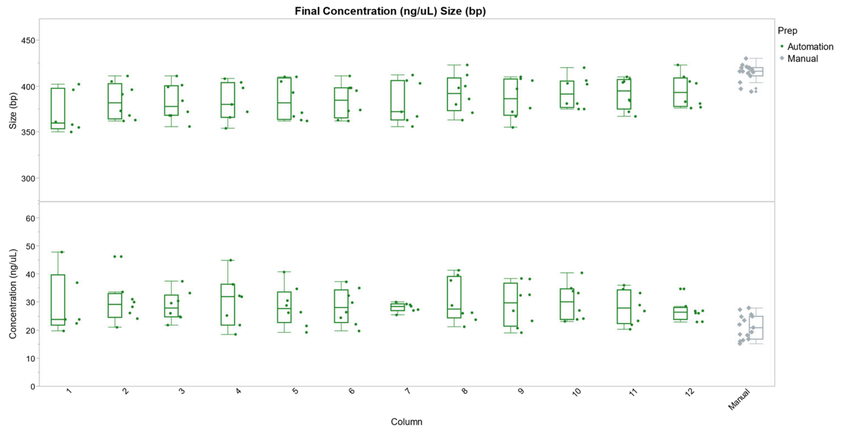

Figure 3: The automated and manual sample preparation methods yielded comparable results overall, with automation demonstrating notable efficiency and reliability. While manually prepared samples exhibited slightly larger insert sizes, the automated process provided consistent and scalable outcomes, making it particularly well-suited for high-throughput applications.

6 Sequencing Results

Three libraries prepared via automation underwent shallow sequencing (~1M reads) to check high level RNA-Seq QC metrics. Samples were sequenced on an Illumina® NextSeq™ 2000 sequencer using a P3 Flow Cell and 2 x 150 bp reads.

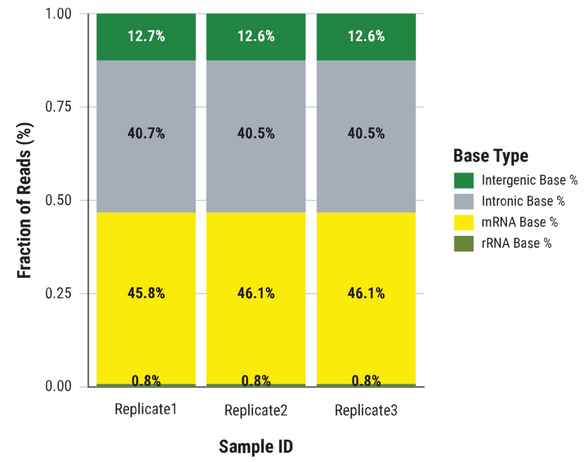

Figure 4. A comparison of the base type distribution in RNA-seq libraries prepared using the same automated protocol shows consistent distribution of bases across intergenic, intronic, mRNA, and rRNA regions. This uniformity across the three libraries suggests that the automated preparation method provides reliable and reproducible results, with similar proportions of bases mapped to these distinct genomic regions.

Replicate	PF Aligned Reads	Mean Insert Size (bp)	Insert Size SD	Median Insert Size (bp)	Unique Genes Detected	Strandedness
Replicate1	846,489	203	94	180	11,041	98.64%
Replicate2	899,359	211	96.9	188	11,242	98.70%
Replicate3	858,273	209	97	186	11,087	98.66%

Table 1. Comprehensive summary showcasing sequencing alignment metrics, insert size distribution and statistics, library complexity assessments, and the percentage of strandedness in the sequencing data. This table provides detailed insights into the quality and characteristics of the sequencing output.

7 Summary

The automated workflow demonstrated exceptional performance across multiple runs. Two 24-sample runs, and a 96-sample high-throughput run was conducted, with results compared to manually prepared libraries. Highlighted data shows:

- **High Quality Libraries Suitable for Sequencing:** All runs yielded high-quality libraries with consistent fragment sizes and reproducible yields.
- **No Cross-Contamination:** No-template controls (NTCs) confirmed the absence of cross-contamination across all runs, showing the precision of the automation.
- **Consistent Library Complexity:** Libraries underwent shallow sequencing (~1M reads) on an Illumina® NextSeq™ 2000 sequencer. High-level RNA-Seq QC metrics confirmed robust library performance, demonstrating suitability for downstream applications.

This automated workflow offers a flexible solution capable of producing high quality results, making it ideal for research and clinical WTS workflows.

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