High-Throughput Automation of mRNA Library Preparation for Reliable RNA-seq Workflows

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Introduction

Gene expression studies require precise, reproducible mRNA library preparation to accurately quantify expression across large sample sets. As studies scale, streamlined chemistries and reliable automation are essential for maintaining throughput and data quality. The Watchmaker mRNA Library Prep Kit supports high-performance gene expression analysis with an efficient workflow incorporating on-bead washes, shortened enzymatic reactions, and minimal cleanup steps—features that make it well-suited for automation (Figure 1). Here, we describe an automated implementation of this workflow on the Agilent Bravo NGS Workstation to enable robust, high-throughput mRNA-seq library preparation.

The automated method includes user-selectable runtime options (e.g., adapter type, bead-loading mode, SPRI cleanup ratios, and optional start/stop points) and requires minimal hands-on setup prior to run initiation. Total workflow turnaround time for a full 96-sample plate is ~7 hours, compared with 4.5 hours for the manual workflow for 8 samples, while substantially reducing hands-on time and operator intervention.

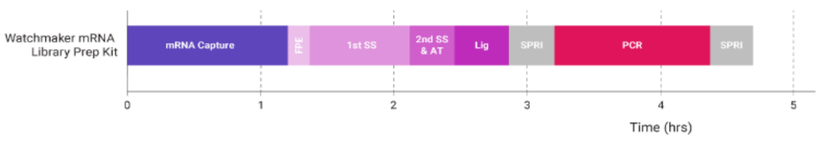

Figure 1: Watchmaker mRNA Library Prep Kit Workflow

Materials and Methods

Samples were prepared by normalizing a stock of Universal Human Reference (UHR) RNA sample to 1.0 ng/µL using the Qubit™ RNA High Sensitivity (HS) assay. The normalized stock was aliquoted into all test wells for automated processing and into tubes for the manual workflow. Manual and automated runs were performed concurrently looking for concordant results. During optimization, adjustments were made to mixing parameters and liquid-class settings—including aspiration and dispense speeds, heights, tip touches, and linearity—to refine liquid-handling performance.

Once the automated 16-sample run (two columns of eight wells in a 96-well plate) aligned with manual results, a high-throughput run was conducted using 48 samples arranged in a checkerboard pattern across the plate. Data was analyzed using both the Qubit™ RNA HS assay and High Sensitivity DNA ScreenTape on an Agilent TapeStation system.

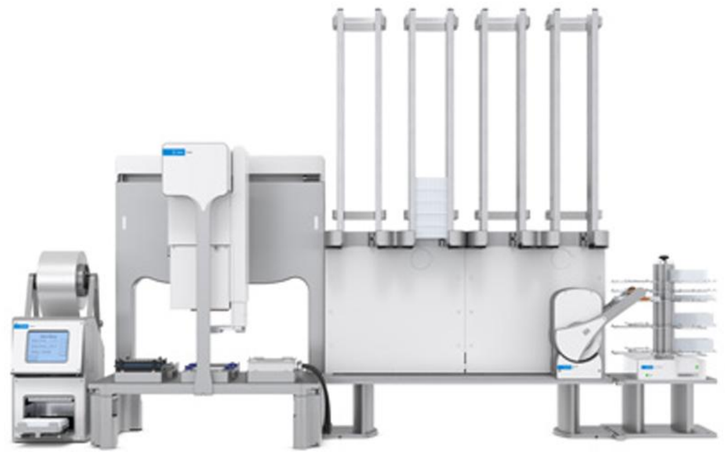

Figure 2: Agilent Bravo NGS Workstation

The automated workflow was designed in VWorks 13 software for the Agilent Bravo NGS Workstation (Figure 2). The workflow is made up of multiple runsets, each featuring a distinct deck layout, and a dynamic user interface (Figure 3) including selection of column number, adapter type, post-ligation cleanup steps, SPRI ratios, and final elution volume. Embedded tabbed worksheets enable advanced users to adjust labware or file paths without editing individual protocols and allow viewing of key global variables for troubleshooting. These variables were configured slightly differently from traditional Agilent Bravo NGS protocols to support both troubleshooting and standalone protocol execution while maintaining centralized control through the form.

Materials and Methods

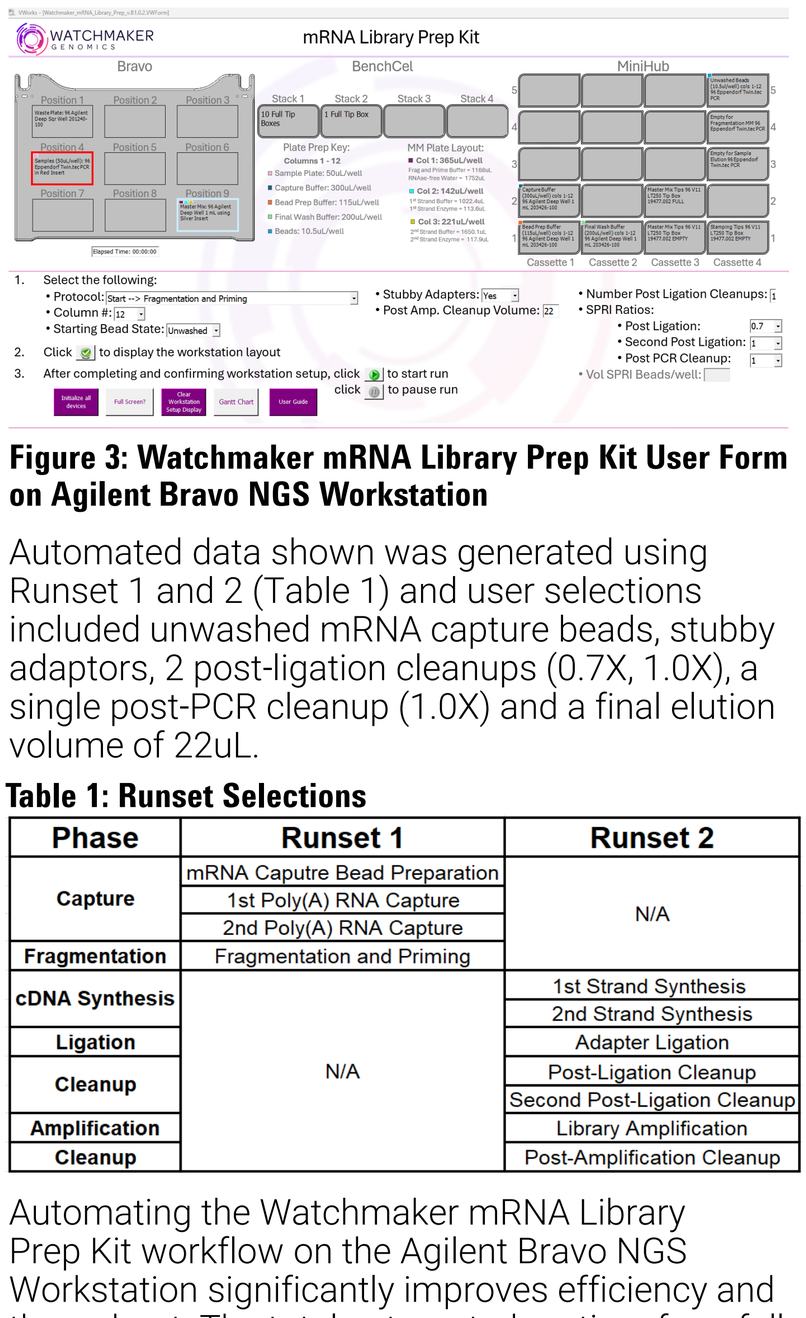

Figure 3: Watchmaker mRNA Library Prep Kit User Form on Agilent Bravo NGS Workstation

Automated data shown was generated using Runset 1 and 2 (Table 1) and user selections included unwashed mRNA capture beads, stubby adaptors, 2 post-ligation cleanups (0.7X, 1.0X), a single post-PCR cleanup (1.0X) and a final elution volume of 22uL.

Table 1: Runset Selections

Phase	Runset 1	Runset 2
Capture	mRNA Caputre Bead Preparation	N/A
	1st Poly(A) RNA Capture	
	2nd Poly(A) RNA Capture	
Fragmentation	Fragmentation and Priming	
cDNA Synthesis	N/A	1st Strand Synthesis
		2nd Strand Synthesis
Ligation		Adapter Ligation
Cleanup		Post-Ligation Cleanup
		Second Post-Ligation Cleanup
Amplification		Library Amplification
Cleanup		Post-Amplification Cleanup

Automating the Watchmaker mRNA Library Prep Kit workflow on the Agilent Bravo NGS Workstation significantly improves efficiency and throughput. The total automated runtime for a full 96-sample plate is approximately 7 hours, and notably, this runtime is nearly identical to that of an 8-sample automated run. By contrast, manual preparation requires approximately 4.5 hours for only 8 samples, underscoring the substantial time savings gained through automation. This increased throughput, combined with reduced hands-on time, enhances overall workflow efficiency and enables users to process larger studies with consistent performance. Table 2 and Figure 4 further illustrate the impact of automation on user time and intervention requirements.

Table 2: Protocol run times

Protocol	Hands-On Time	Walk-Away Time	Samples Processed
Start -> Fragmentation and Priming	45 minutes	2 hours	96
First Strand Synthesis -> Post Amplification Cleanup	15 minutes	4 hours	96

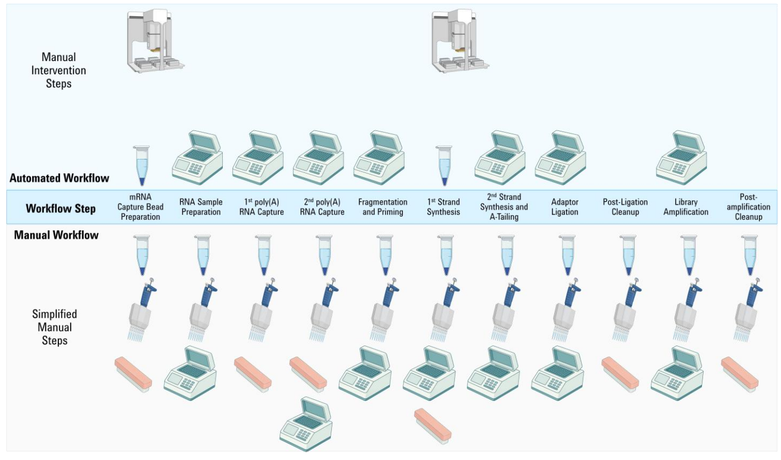

Figure 4: Manual intervention schematic

Results and Discussion

During automated workflow development, mixing and liquid-handling parameters were systematically optimized across multiple runs to achieve reproducible performance. To control for experimental variability, all test wells and manual reactions were prepared using material from a single normalized stock, thereby eliminating sample source as a potential contributor to performance differences.

Results and Discussion

A 16-sample low-throughput run demonstrated that automated library preparation produced concentrations and size profiles comparable to manual results, confirming method reliability before scaling. A high-throughput run consisting of a full 96-well plate containing 48 UHR samples and 48 NTCs arranged in a checkerboard layout demonstrated scalability via performance uniformity and no observed cross-contamination. QC Results showed the automated workflow generated libraries with an average final concentration of 22.4 ng/µL (9% CV), consistent with the manual workflow final library concentration of 19.6 ng/µL (8% CV) (Figure 5). Average library sizes were 388 bp for both methods, with tight reproducibility (2% CV automated; 3% CV manual) (Figure 6a and 6b). No plate-position effects were observed, and all NTC wells remained free of detectable library material (data not shown).

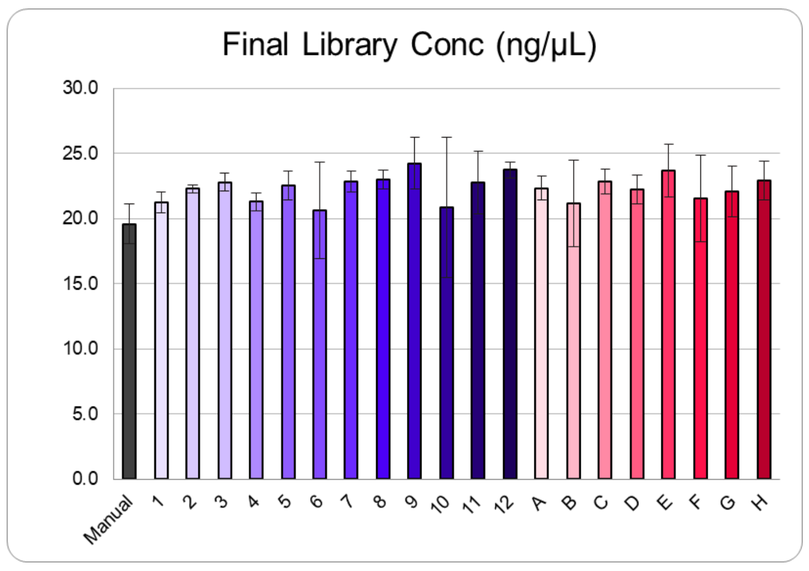

Figure 5: Manual v Automated Avg Final Library Conc

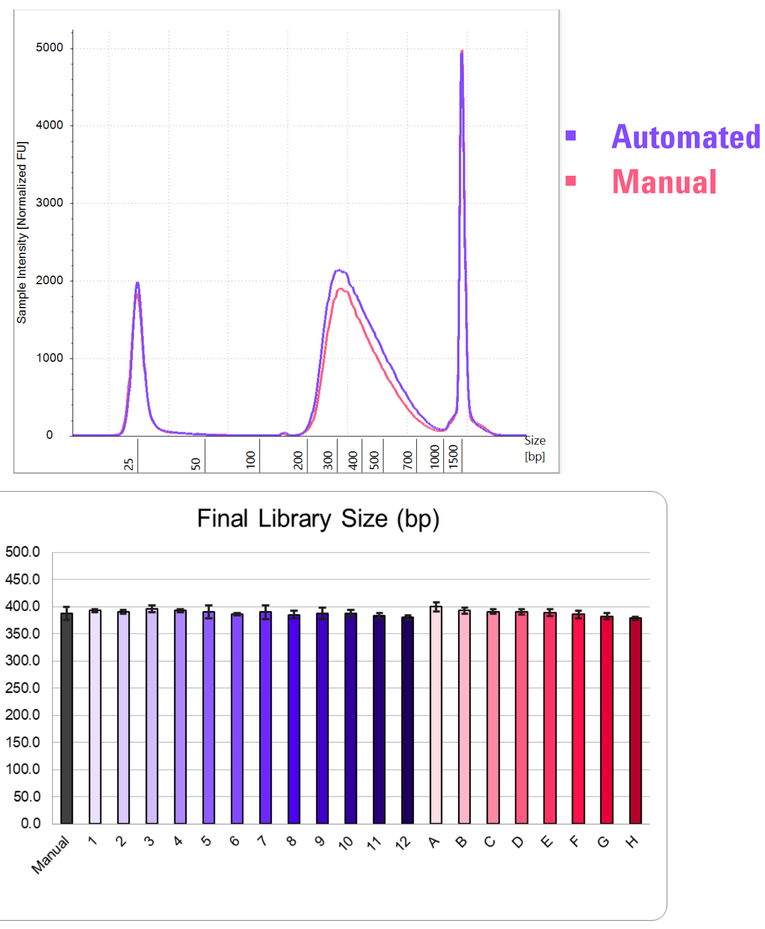

Figure 6: Manual v Automated Avg Final Library Sizes including electropherogram traces (A) and average library size across plate (B)

Conclusions

- Automating the Watchmaker mRNA Library Prep Kit on the Agilent Bravo NGS Workstation yields performance equivalent to manual workflows (Figures 5 and 6).
- The automation of the Watchmaker mRNA Library Prep Kit on the Agilent Bravo NGS Workstation is easy to implement and increases walkaway time for users (Table 2 and Figure 4).
- The combination of simplified chemistry, reduced hands-on time, and robust reproducibility provides a scalable solution for applications requiring consistent, high-quality mRNA-seq data.

References

1. Watchmaker mRNA Library Prep Kit User Guide v2.2.1224

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