Automating Watchmaker's RNA Library Prep Kit with Polaris™ Depletion on the Biomek i7 Hybrid NGS Workstation

Introduction

The Watchmaker RNA Library Prep Kit with Polaris™ Depletion was developed to address the highly specific needs of whole transcriptome sequencing (WTS) and the associated areas of variant calling, isoform/gene fusion identification, and gene expression analysis. To improve the detection of RNAs of interest, the Polaris™ Depletion Kit – rRNA/Globin (HMR) specifically targets and efficiently depletes:

- 28S, 18S, 5.8S, and 5S cytoplasmic rRNAs
- 16S and 12S mitochondrial rRNAs
- 45S ETS and ITS rRNAs (probes designed for human only)
- HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1, and HBZ globin RNAs (probes designed for human only)

The Watchmaker RNA Library Prep Kit with Polaris™ Depletion enables the highly streamlined preparation of stranded WTS libraries from 1 ng to 1 µg of total RNA with high library complexity and low coverage bias. The workflow is compatible with human, mouse, and rat species and both high- and low-quality samples, including FFPE material. The Watchmaker solution combines and shortens enzymatic steps and has fewer bead purifications in comparison to other commercially available kits, resulting in an easily automatable workflow with significantly reduced hands-on time and consumable use. The kit also includes generous overages and reasonable pipetting volumes making it more amenable to automated processes.

The automated workflow for the Biomek i7 Hybrid NGS Workstation has been designed to support multiple applications and workflow options at runtime, preparing up to 96 sequence-ready libraries in under 7 hours (including depletion) (Figure 4). Benefits of automating the Watchmaker RNA Library Prep Kit with Polaris™ Depletion on the Biomek i7 Hybrid NGS Workstation include:

- High-throughput scalable solution for RNASeq library preparation
- Run specific user-controlled method option flexibility
- Guided labware setup at runtime
- Reduced hands-on time and pipetting errors with no cross-contamination
- Optimized consumable use
- Knowledgeable technical support from experts at Watchmaker Genomics and Beckman Coulter Life Sciences

Figure 1. Biomek i7 Hybrid NGS Workstation

Automated Method

The automated Watchmaker RNA Library Prep Kit with Polaris™ Depletion method is constructed with modular sections to enable the use of safe stopping points throughout the workflow but can be run start to finish with full walk-away capability* (Figure 2). The method includes an intuitive user interface (Figure 3) presenting all workflow options available at start of run including:

1. Number of samples (1 – 96)
2. Optional up-front Polaris™ Depletion
3. On-deck vs. off-deck thermal cycling*
4. High quality (Protocol A) vs. FFPE samples (Protocol B)
5. Fragmentation parameters*
6. Dynamic bead cleanup ratios and elution volumes
7. Optional second post-ligation cleanup
8. Truncated vs. full-length adapters
9. Number of PCR cycles*

*On-Deck Thermal Cycler option required

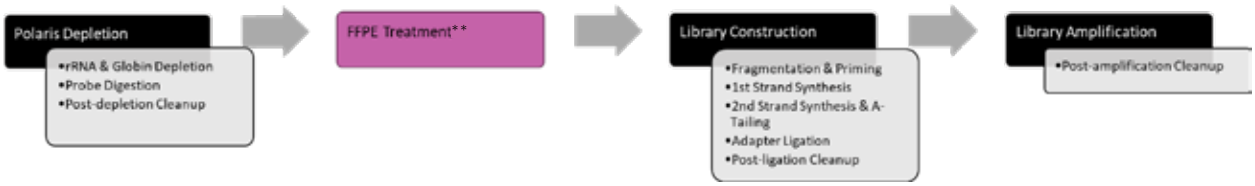

Figure 2. Automated workflow for Watchmaker RNA Library Prep including Polaris™ Depletion. **FFPE treatment is intended only for FFPE samples and will be omitted when processing high-quality or partially degraded samples.

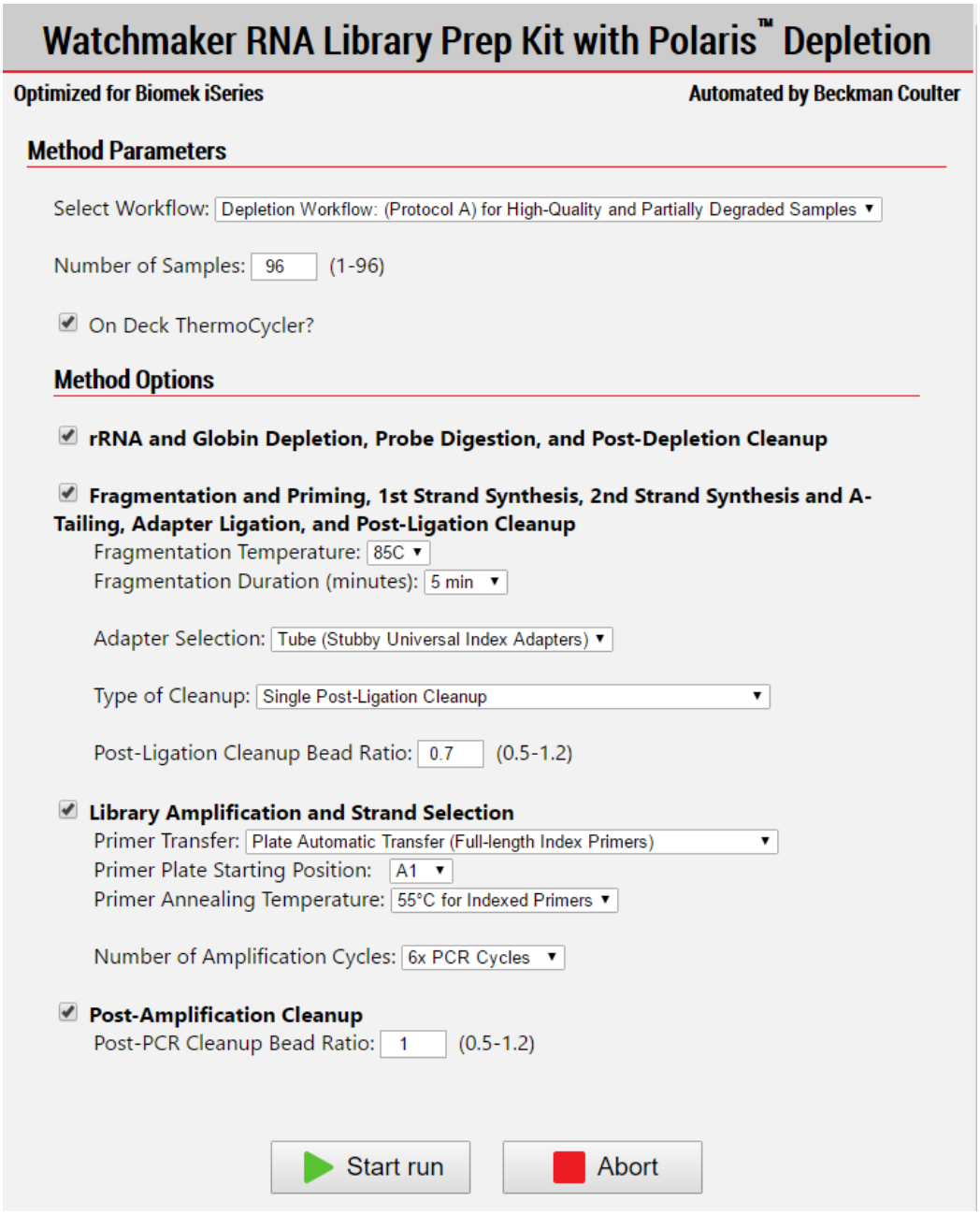

Figure 3. Intuitive Biomek i7 Hybrid NGS Workstation user interface for the Watchmaker RNA Library Prep Kit with Polaris™ Depletion presenting runtime options for workflow, sample number, fragmentation optimization, adapter transfer flexibility, and SPRI cleanup options.

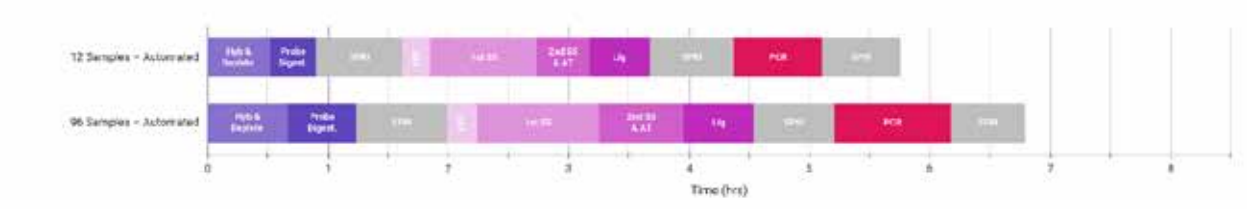

Figure 4: Calculated run times for 12-sample and 96-sample automation runs for the Watchmaker RNA Library Prep Kit with Polaris™ Depletion on the Biomek i7 Hybrid NGS Workstation. Method options included the Depletion Workflow (Protocol A), 5-minute fragmentation, single post-ligation cleanup, and 11 PCR cycles.

Experimental Design and Data

To initially test the method performance on the Biomek i7 Hybrid NGS Workstation, an automated run was performed using 12 technical replicates of Universal Human Reference (UHR) RNA diluted to a total of 50 ng per sample. Additionally, 4 samples were prepared in parallel manually with the same input material. An additional automated run was performed to test method capabilities for a 96-sample run using 48 technical replicates of UHR RNA diluted to a total of 50 ng per sample in a checkerboard pattern across a 96-well plate. The reagents used for the automated experimental run included:

- Watchmaker RNA Library Prep Kit (Watchmaker, 7K0078-096)
- Polaris™ Depletion Kit (Watchmaker, 7K0077-096)
- xGen™ Stubby Adapter, 96 rxn (IDT, 10005924)
- xGen™ UDI 10nt Primer Plates 1-4, 2nmol (IDT, 10008055)

Following library preparation, the libraries were analyzed using Agilent D1000 ScreenTape for library size and quality. The concentration of the libraries was measured using the Qubit™ 1X dsDNA High Sensitivity (HS) chemistry.

Results

The libraries from the first automated run had a mean concentration of 53.5 ng/µL with a standard deviation of 4.8 and a mean library size of 406 bp with a standard deviation of 5.6 (Figure 5, left). The libraries manually prepared had a mean concentration of 39.9 ng/µL with a standard deviation of 7.17 and a mean library size of 385 bp with a standard deviation of 10.14 (Figure 5, right).

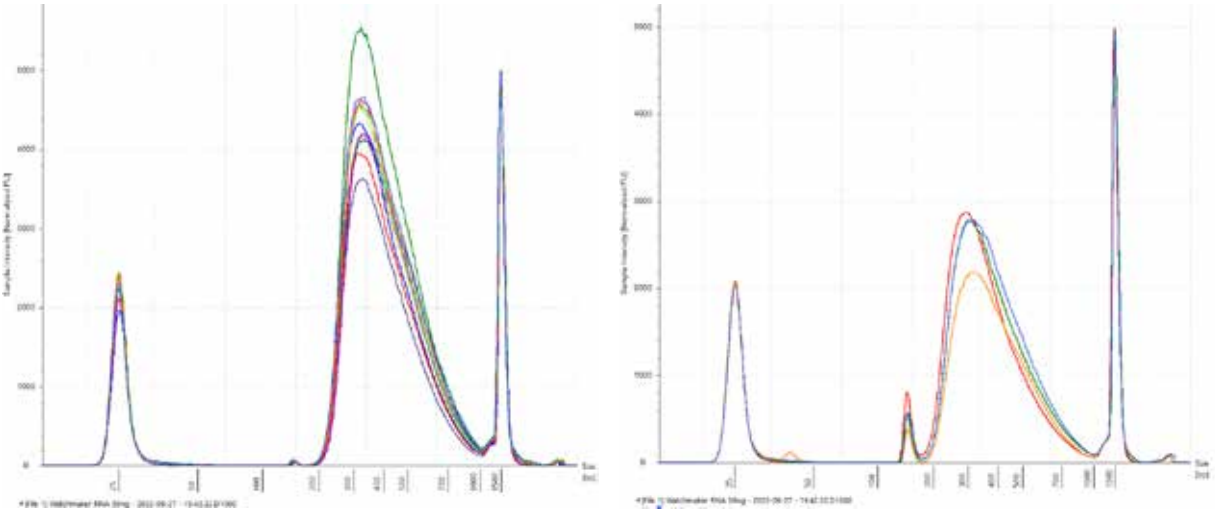

Figure 5: Comparison of the 12 libraries processed with the Biomek i7 Hybrid NGS Workstation (left) and 4 manual libraries (right) for the Watchmaker RNA Library Prep Kit with Polaris™ Depletion.

The libraries from the second run with a 96-well checkerboard plate had a mean concentration of 43.8 ng/µL (Figure 6) with a standard deviation of 4.32 and a mean library size of 380 bp with a standard deviation of 3.35 (Figure 7). There was no cross-contamination observed.

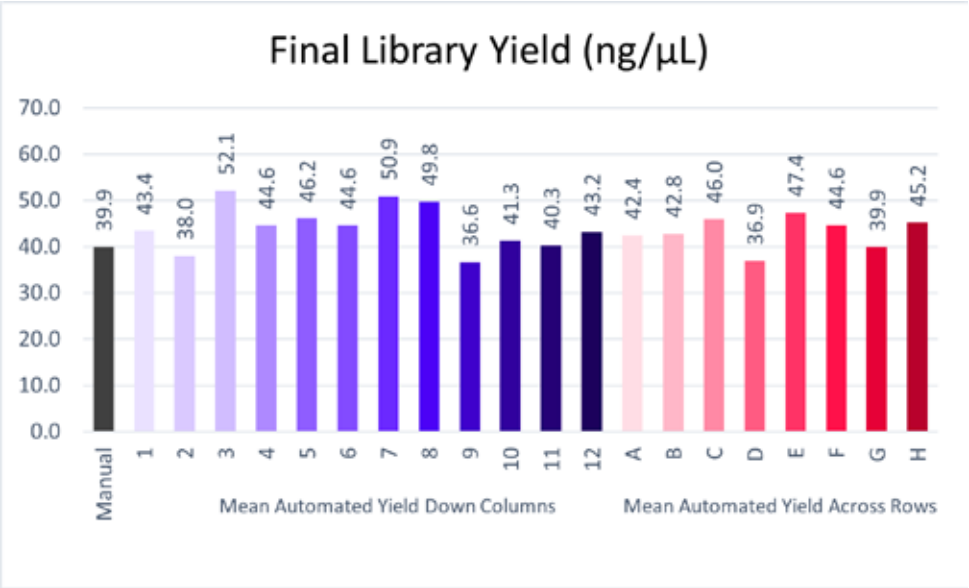

Figure 6: Final library yields were comparable to manual processing and consistent across a 96-well plate with no obvious plate effects.

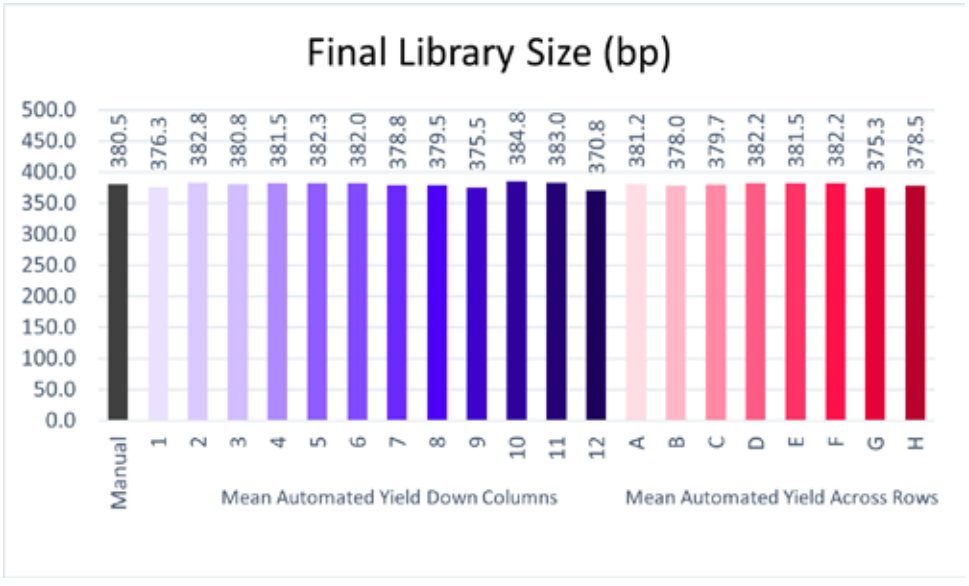

Figure 7: Final library sizes (bp) were comparable to manual processing and consistent across a 96-well plate with no obvious plate effects.

Summary

These results demonstrate automating Watchmaker's RNA Library Prep Kit with Polaris™ Depletion on the Biomek i7 Hybrid NGS Workstation provides an optimized, efficient, flexible workflow for high-throughput RNASeq library prep. This automated solution can generate 96 sequencing-ready libraries in under 7 hours with full walk-away capability. The libraries created with the Biomek i7 Hybrid NGS Workstation yield quality results for downstream workflows and can save valuable time and resources.

For further information on how to access this automated method or any other technical inquiries please contact us via:

Watchmaker Genomics Scientific Support Team: support@watchmakergenomics.com

Beckman Coulter Life Sciences Website: Beckman.com

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