Automating Watchmaker's mRNA Library Prep Kits on firefly® at a core genomics facility

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Abstract

At this genomics core facility, thousands of RNA samples are processed across multiple teams to identify and characterize therapeutic targets for cell rejuvenation and restoring cellular health on SPT Labtech's firefly® liquid handling platform. Watchmaker Genomics' mRNA Library Prep Kit streamlines sample processing, allowing for a dynamic input range and integrating optimized enzymes enabling an end-to-end workflow for up to 96 samples in less than 6.5 hours. This application note discusses the approach, benefits, and results of automating Watchmaker's mRNA Library Prep on firefly®.

Introduction

Multiple sequencing methods are used across three separate sites to build foundational datasets for the company's extensive computational ecosystem. To do this, the team leverages the cloud capabilities of firefly to seamlessly collaborate to automate and standardize their workflows.

Watchmaker Genomics is a leader in enzyme development, which has enabled simple, automatable, scalable and sensitive workflows for DNA and RNA library preparation. The Watchmaker mRNA Library Prep Kit is designed with automation in mind, provides generous overages, and leverages on-bead washing steps during poly(A) selection to reduce resuspension and wash times, as well as consumables use (Figure 1). In addition, the workflow enables users to process a broad range of RNA inputs (2.5 ng – 1 µg) without the need for secondary low-input solutions that fail to maintain strand origin information. These workflow improvements result in increased library complexity and gene detection sensitivity especially for more challenging and low-input sample types (Figure 2). Watchmaker's mRNA Library Prep Kit was developed to address the highly specific needs of mRNA sequencing in the associated areas of variant calling, isoform and gene fusion identification, and gene expression analysis.

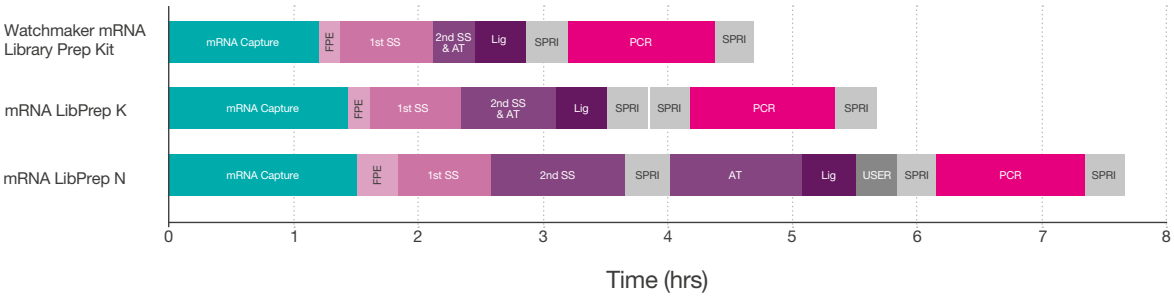

Figure 1. Workflow comparison of mRNA library prep kits. Watchmaker's mRNA Library Prep workflow features on-magnet washes for poly-A capture, combined and shortened enzymatic reactions, reduced number of SPRI cleanups and a novel engineered Reverse Transcriptase with improved RNA to cDNA conversion during first strand synthesis. Watchmaker's mRNA Library Prep on firefly can be executed in less than 6.5 hours for up to 96 samples and under 5 hours in a manual setting (8 samples).

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Unique Genes

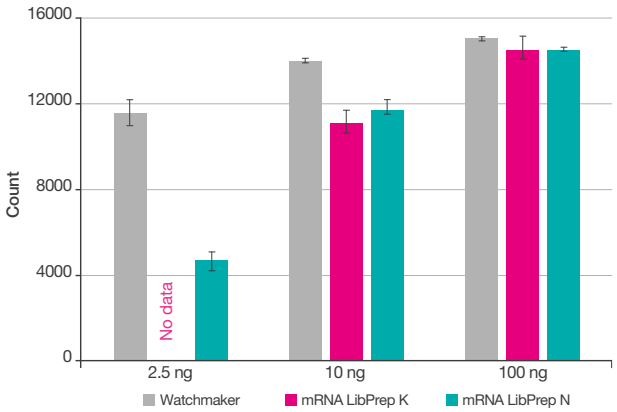

Figure 2. RNA extracted from breast tissue (RIN 7) was used to prepare libraries in quadruplicate from a range of RNA mass inputs, as indicated, using the Watchmaker mRNA Library Prep Kit and two other kits. Supplier recommendations were used for each workflow. mRNA LibPrep K failed to produce libraries at 2.5 ng with UHR, and thus 2.5 ng was not tested with tissue RNA. Libraries were downsampled to 5M reads.

Watchmaker's mRNA Library Preparation on firefly

firefly is used to automate multiple sequencing workflows, where methods can be shared seamlessly between instruments across all three sites without requirement for optimization. In addition to this ease-of-use, teams are able to reduce their plastic consumables consumption as firefly's non-contact dispense functionality reduces the need for multiple tip arrays (Figure 3). Dead volumes are also significantly reduced in comparison to other automation platforms, with three reservoir types available for the reservoir thermal module that enable users to batch multiple runs from a single kit without depleting reagents, further reducing costs and plastic consumption (Figure 4).

Standard Automation vs firefly Watchmaker mRNA Workflow Plastic (PP/HDPE) Consumption Comparison†§‡

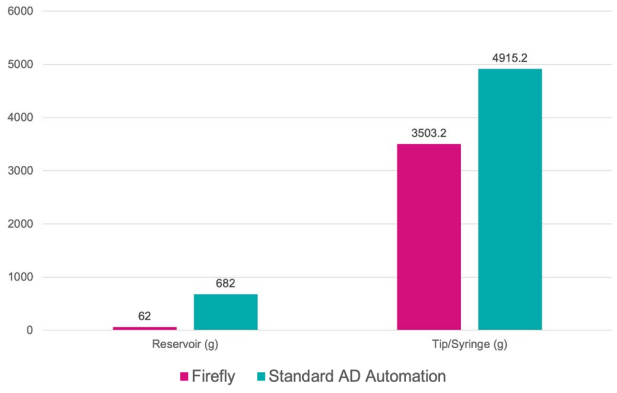

Figure 3. firefly's dispense head capabilities significantly reduce plastic consumption and cost compared to standard automation platforms. *LP SBS 96-well diamond bottom reservoir weight based on Axygen® RES-SW96-LP, other SBS reservoir types may differ. §Single tip weight (1.6 g) based non-filter 125 µL apricot® tips, other tip weights may differ. ‡Tip box not included in weight calculation.

Automated mRNA Workflow Reagent Dead Volume Comparison

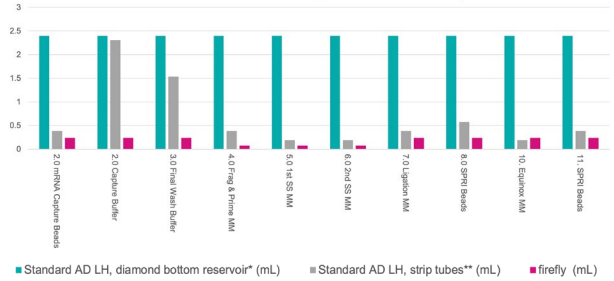

Figure 4. firefly's low dead volumes enable automation of batched samples <48 without depleting kit reagents for additional runs. Reservoir and tip type comparison. *LP 96-well diamond bottom reservoir dead volume based on Axygen (PO#) spec, other reservoir types may differ. **Strip tip dead volume calculation based on 2 µL per transfer from 12-tube strip, #12-tube strips required for volume for 96 samples, other automated transfer schemes may differ.

Watchmaker mRNA Library Preparation on firefly

Initial liquid class optimization and method development was performed with 15 high-quality control RNA samples. All samples included had a minimum RIN score of 7 and were diluted to 4 ng/µL (200 ng per sample) prior to running. For this initial experiment, the RNA libraries were prepared using xGen™ Stubby Adapter and UDI Primers and underwent 10 cycles of PCR, following recommendations provided in Watchmaker's mRNA Library Prep User Guide. Initial library QC metrics showed consistent library yield and size as seen in Figures 5 and 6.

Final Library Yields

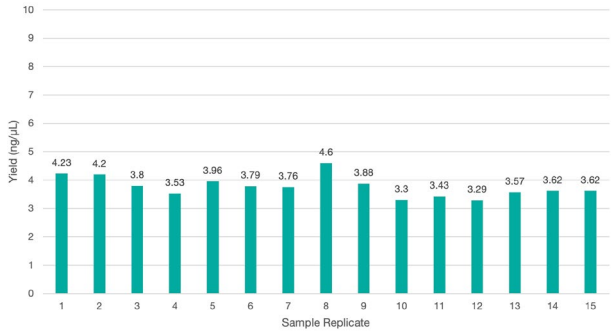

Figure 5. Automated final library yields as measured on the Thermo Qubit Flex Fluorometer displaying consistent and tight final library yields.

Final Library Sizes

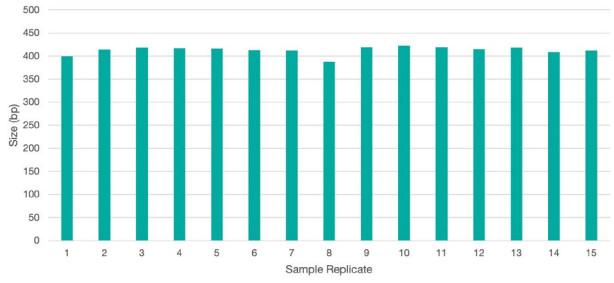

Figure 6. Automated final library size as measured on the Agilent 4200 TapeStation System displaying consistent final library size for all samples included.

After this initial performance assessment, additional testing was performed using samples at varying inputs. For these experiments, libraries were prepared at the following inputs in duplicate: 20 ng, 50 ng, and 200 ng. The 20 ng and 50 ng RNA samples were prepared using 12 cycles of PCR, and the 200 ng RNA samples were prepared using 10 cycles of PCR. Library QC and sequencing metrics are summarized in Table 1 demonstrating concordance between replicates. Final library traces (Figure 7) show consistent library sizes free of adapter dimer across all three input amounts.

	Sample Name	PCR Cycle Number	Total Yield (ng)	Total Sequences Reads (M)	% Duplication	% Unique Aligned	Expressed Gene Number
mRNA on firefly	20ng_r1	12	204.9	35.8	38.10%	93.20%	22034
	20ng_r2	12	213.3	32.4	36.30%	93.20%	21869
	50ng_r1	12	474.0	37	36.20%	93.30%	22155
	50ng_2	12	483.0	38.3	35.60%	93.30%	22155
	200ng_r1	10	138.0	35.1	40.50%	93.00%	21747
	200ng_r2	10	99.0	37.9	42.60%	93.10%	21758

Table 1. Summary QC and sequencing metrics from libraries prepared using Watchmaker's mRNA Library Prep on firefly across 20 ng, 50 ng and 200 ng inputs.

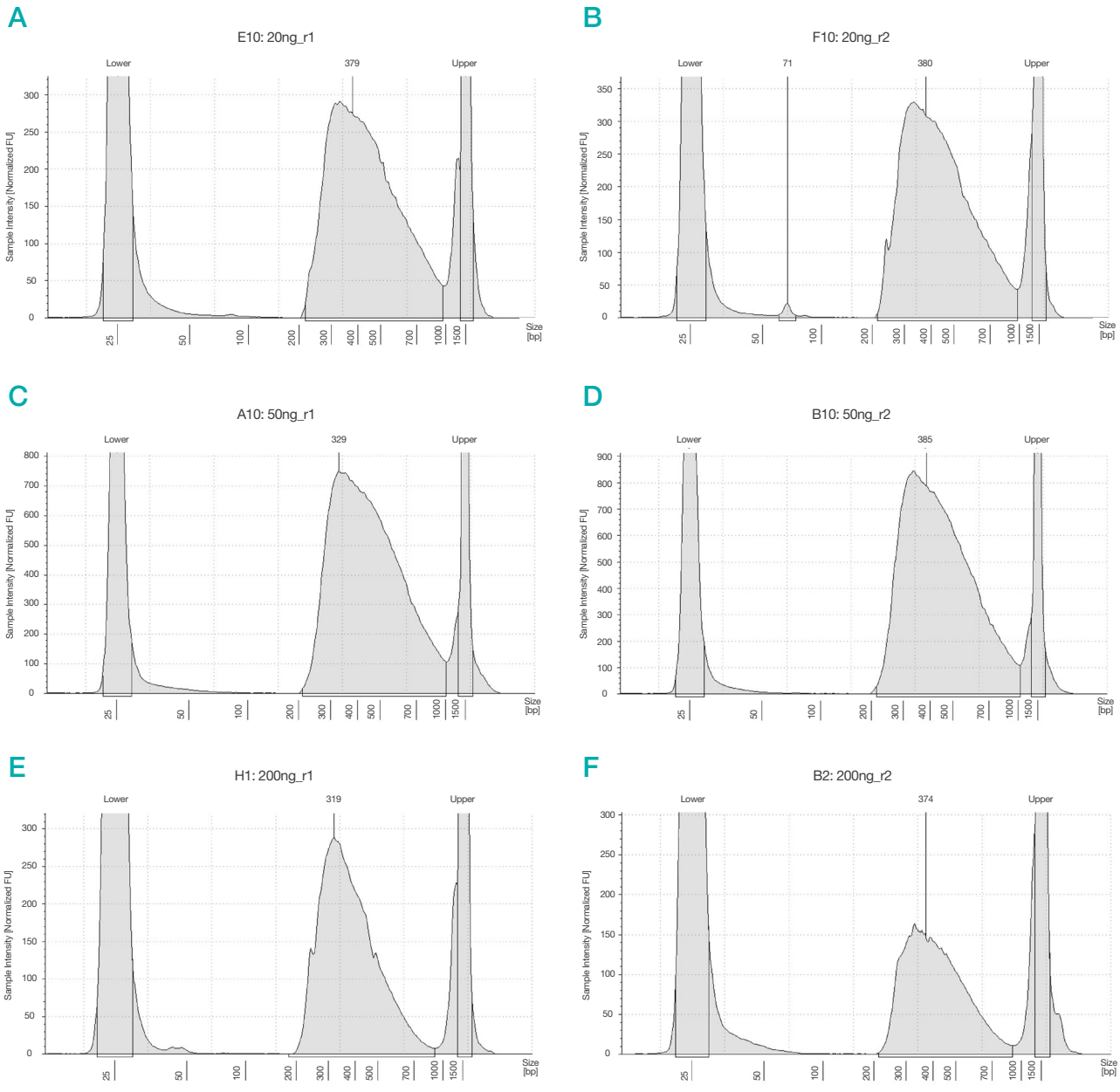

Figure 7. Final library traces for the 50 ng (a,b), 20 ng (c,d) and 200 ng (e, f) inputs show consistent size and absence of dimer (adapter or primer).

Conclusion

The automated workflow of the Watchmaker mRNA Library Prep Kit combined with the SPT Labtech firefly liquid handler delivers a high-throughput easy-to-use solution for users to generate high-quality sequenceable libraries while reducing hands-on time and the potential for human error during library preparation.

For more information, please contact:

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