Automating Watchmaker's High-Performance Next Generation Sequencing Chemistries on the Biomek i7 Liquid Handler for Rapid, High-Throughput Sample to Sequence Output

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Introduction

Expansion of Next Generation Sequencing (NGS) has revolutionized genomics research by enabling exploration of new applications and sample types where previously it may have been cost prohibitive. The utilization of laboratory automation coupled with streamlined library preparation chemistries has resulted in high-throughput cost-effective sample preparation with high-quality results. Here we showcase a novel automated solution for Watchmaker Genomics DNA Library Prep Kit with Fragmentation on the Biomek i7 Hybrid NGS Workstation.

Streamlined and Scalable DNA Library Prep

The Watchmaker DNA Library Prep with Fragmentation workflow harnesses the process benefits of enzymatic fragmentation, such as ease of automation, increased scalability, and preservation of low input samples, while mitigating the formation of associated library preparation artifacts, including false chimeric reads and hairpin artifacts, that can convolute variant calling. Further, use of the Equinox polymerase delivers ultra-high-fidelity, low-bias library amplification.

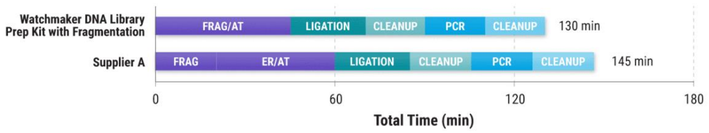
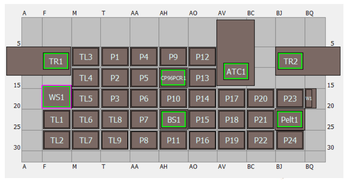
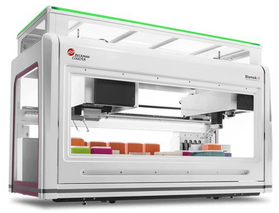


Figure 1. Simplified and easily automated workflow. The Watchmaker DNA Library Prep Kit with Fragmentation simplifies library construction using combined enzymatic steps (FRAG/AT) and a ready-to-use ligation master mix to reduce hands-on time compared to a widely used enzymatic fragmentation kit (Supplier A). The Watchmaker kit was designed with automation in mind, with generous overages to ensure sufficient fill volumes for automated library preparation.

Flexible High-Throughput Automated Methods

The Biomek i7 Hybrid NGS Workstation has a 96-channel head and a Span-8 with disposable tips. The Watchmaker DNA Library Prep with Fragmentation automated method offers a flexible workflow compatible with both high- and low-quality samples ranging from 1 to 500 ng, capable of creating up to 96 high-quality sequencing ready DNA libraries in under 4 hours. The method incorporates the use of tip washing to minimize consumable use and delivers high-yielding, high-quality libraries. The automated method includes a dynamic easy-to-use interface that supports multiple workflow options at runtime.





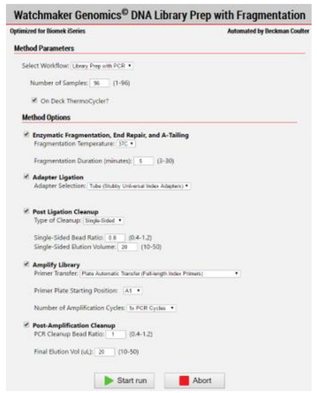


Figure 2. Dynamic easy-to-use automation. The Biomek i7 Hybrid NGS Workstation offers flexibility and efficiency with both multi-channel and Span-8 pipetting on board, in addition to standard on-deck process control elements such as shakers, temperature-controlled positions and optional thermocyclers. The Watchmaker DNA Library Prep with Fragmentation method includes an intuitive user interface presenting runtime options including number of samples, PCR vs. PCR-free workflows, adapter transfer flexibility and SPRI cleanup options that support multiple workflows within a single automated method.

Assessing Method Performance

To test the capabilities of the automated method, two different automated runs were performed. Initially, 48 replicates of human gDNA (GIAB, NA12878) were diluted to a total concentration of 10 ng per sample in a checkerboard pattern and run alongside 48 No Template Controls (NTCs) to check for cross-contamination. Manual libraries were prepared in parallel for comparison. The initial run generated libraries with consistent yield and sizes, both down and across the plate, with all NTCs being free of cross-contamination.

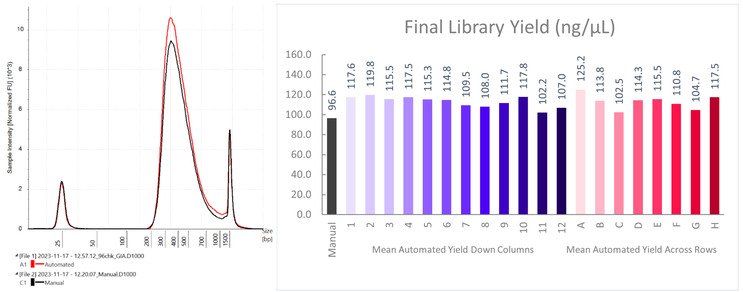


Figure 3. Consistent Yields and Sizes Comparable to Manual Processing. Final library yields and sizes were comparable to manual processing and consistent across a 96-well plate with no obvious plate effects.

The second automated run was set up to test the Watchmaker chemistry across multiple input levels and diverse genomes, again preparing manual libraries from the same inputs in parallel. The variety of DNA inputs included human (Coriell, NA12878), metagenomic (ATCC, MSA-1002), and plasmid (NEB, pUC19 Vector) at total concentrations of 1 ng and 10 ng. All final libraries, automated and manual, were sequenced on an Illumina NextSeq 2000 using a P1 Flow Cell and 2 x 150 bp reads.

Table 1. Experimental Conditions Tested Across Variable Sample Types and Inputs

Sample Label	Sample	Input (ng)	Frag Condition	Adapter Stock Concentration (µM)	PCR Cycle Number	Post-Ligation Cleanup	Post-Amplifcation Cleanup
H1	Human	1	37°C, 10 min	3	11	0.8X	1X
H10	Human	10	37°C, 10 min	3	11	0.8X	1X
M1	Metagenomic	1	37°C, 10 min	3	11	0.8X	1X
M10	Metagenomic	10	37°C, 10 min	3	11	0.8X	1X
P1	Plasmid	1	37°C, 10 min	3	11	0.8X	1X
P10	Plasmid	10	37°C, 10 min	3	11	0.8X	1X

Results

The results show remarkably consistent insert sizes for both automated and manual sequencing workflows, regardless of the sample type and input amount (Figure 4 and Table 2).

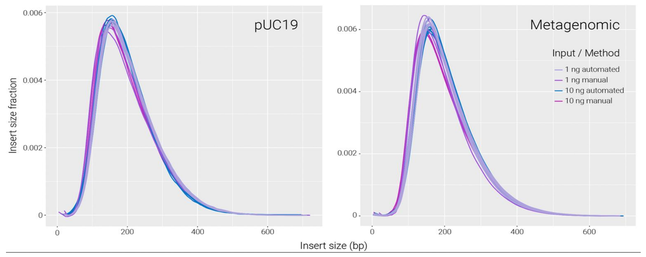


Figure 4. Consistent Insert Sizes Across Sample Type and Inputs. Sequencing data shows highly consistent and comparable library insert sizes across sample types, inputs and between automated and manual processes.

Results Continued

Table 2. Sequencing Alignment and Insert Size Statistics

Sample	Input (ng)	N	Method	PF Aligned reads (%)	Mean insert size	Insert size SD	Median insert size
NA12878	1	2	Manual	99.83%	192.6	78.1	177.0
		8	Automated	99.81%	202.5	79.8	187.0
	10	2	Manual	99.85%	194.7	83.2	178.0
		8	Automated	99.83%	201.1	81.0	185.9
MSA-1002	1	2	Manual	89.68%	192.6	85.8	178.5
		8	Automated	89.64%	200.9	86.5	187.3
	10	2	Manual	89.82%	193.7	88.8	178.5
		8	Automated	89.70%	200.9	86.5	188.0
pUC19	1	2	Manual	99.85%	198.8	85.2	182.0
		8	Automated	99.83%	206.8	83.0	191.6
	10	2	Manual	99.85%	193.5	82.4	177.5
		8	Automated	99.86%	200.1	80.2	186.3

These data highlight the reliability of the automated workflow, which doesn't exhibit any plate effects or cross-contamination (Figure 3). Additionally, there is uniform GC coverage for various microbial genomic DNA samples, as observed in both automated and manual preparations (Figure 5). The automated process maintains uniform coverage across a range of sample inputs, ensuring reliable results and optimal sequencing economy.

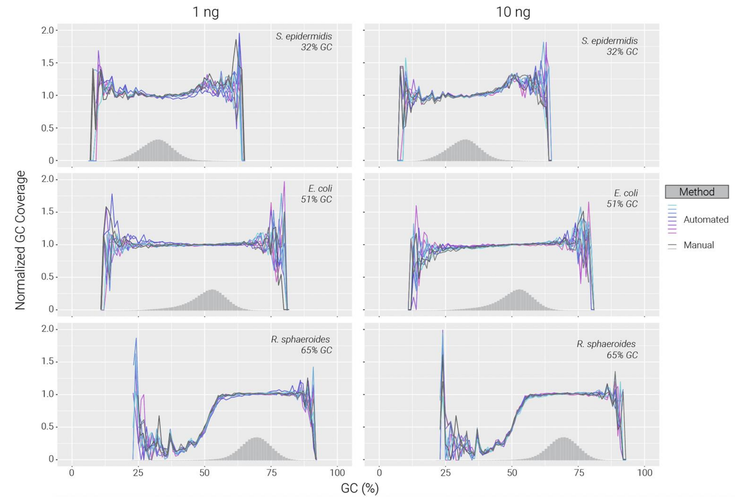


Figure 5. Uniform GC Coverage for a Variety of Microbial Genomic DNA. Normalized coverage plots are comparable across automated and manual libraries, and are consistent, indicating uniform coverage at varying sample inputs.

Conclusion

The Watchmaker DNA Library Prep Kit with Fragmentation on the Biomek i7 Hybrid NGS Workstation delivers:

- An all-in-one scalable DNA Library Prep solution supporting a wide range of workflow options, with a dynamic user-friendly interface
- Consistent high-quality libraries with equivalent insert sizes and uniform GC coverage across a variety of sample types and inputs, providing high sequencing economy
- Reduced risk of human error with walkaway capability and minimized hands-on time

Our collaboration continues to expand to additional Watchmaker workflows including the Watchmaker RNA Library Prep Kit with Polaris™ Depletion, now also available on the Biomek i7 automated workstation.