WATCHMAKER GENOMICS | BECKMAN COULTER Life Sciences

Automating Watchmaker DNA Library Prep Kit with Fragmentation on Biomek i7 Hybrid NGS Workstation

Introduction

The Watchmaker DNA Library Prep Kit with Fragmentation is designed for the highly efficient conversion of DNA, from both high- and low-quality samples, into sequencing ready libraries. The Watchmaker kit and streamlined protocol simplifies library construction using combined enzymatic steps (FRAG/AT) and a ready-to-use ligation master mix reducing hands-on time. The ligation module adds full-length or truncated adapters (not included) that have a 3′ overhanging T to DNA fragments with industry-leading efficiency. The kit supports PCR-free workflows for input DNA of sufficient mass and quality. For workflows where library amplification is desirable or required, the kit includes Equinox Amplification Master Mix (2X).

The Watchmaker DNA Library Prep Kit with Fragmentation chemistry and streamlined protocol has been optimized to produce libraries from 1 ng – 500 ng DNA while minimizing bias and artifacts. This library preparation kit is ideally suited for:

- High-quality genomic DNA
- Genomic DNA extracted from FFPE tissue, plasmid DNA and long PCR products
- High- and low-complexity genomes including genomes with extreme (15 – 85%) GC content

The Watchmaker kit was specifically designed with automation in mind featuring generous overages, fewer purifications, reagent additions, and reduced consumable usage. Here we highlight the Biomek i7 Hybrid NGS Workstation automated method for Watchmaker DNA Library Prep Kit with Fragmentation which can prepare up to 96 sequence-ready libraries in under 4 hours **(Figure 1)**.

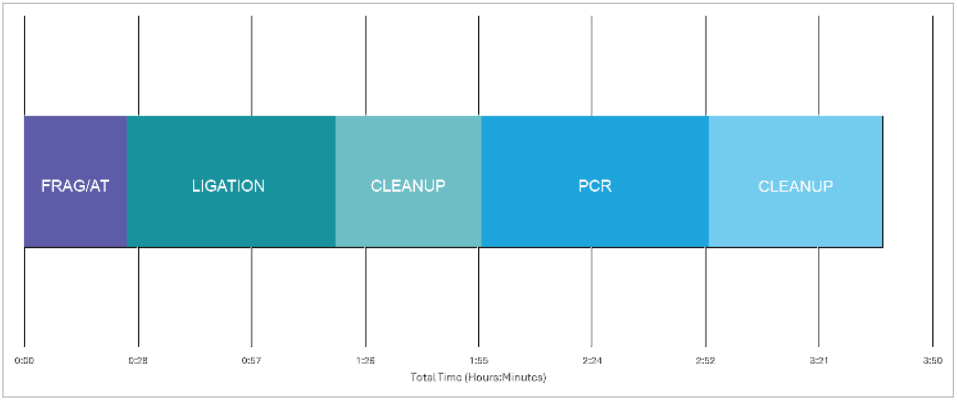

Figure 1. The Watchmaker DNA Library Prep Kit with Fragmentation automated on the Biomek i7 Hybrid NGS Workstation can deliver sequence-ready libraries in under 4 hours.

Benefits of automating the Watchmaker DNA Library Prep Kit with Fragmentation on the Biomek i7 Hybrid NGS Workstation include:

- High-throughput scalable solutions for DNA-Seq library preparation
- Run specific user-controlled method options
- 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

Spotlight

The features of the Biomek i7 Hybrid NGS Workstation include:

- 1200 µL multichannel head with 1-1000 µL pipetting capability
- Span-8 pod with fixed or disposable tips
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with up to 45 positions
- Shaking, heating/cooling, and tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck devices (e.g. thermocyclers)

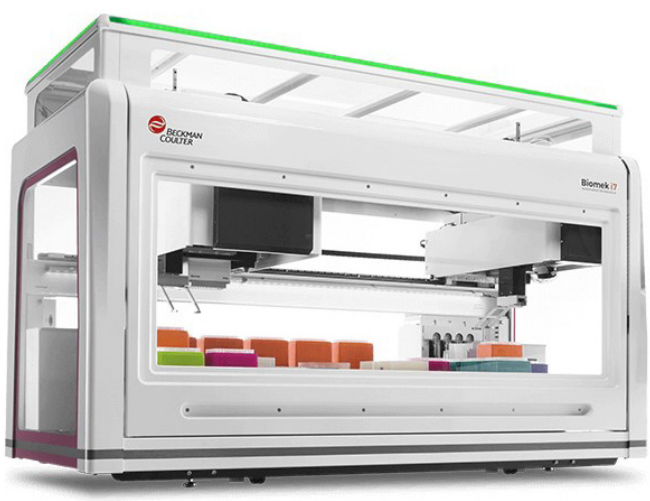

Figure 2. Biomek i7 Hybrid NGS Workstation.

Automated Method

Automated methods provide flexibility to users in scheduling their workflow and allowing method customizations for workflow selection, sample processing, and throughput at runtime. The automated Watchmaker DNA Library Prep Kit with Fragmentation method on the Biomek i7 Hybrid NGS Workstation is constructed with modular sections to enable the use of safe stopping points throughout the workflow. Alternatively, users can choose to run the entire workflow start to finish with full walk-away capability when the on-deck thermocycling is utilized **(Figure 3)**.

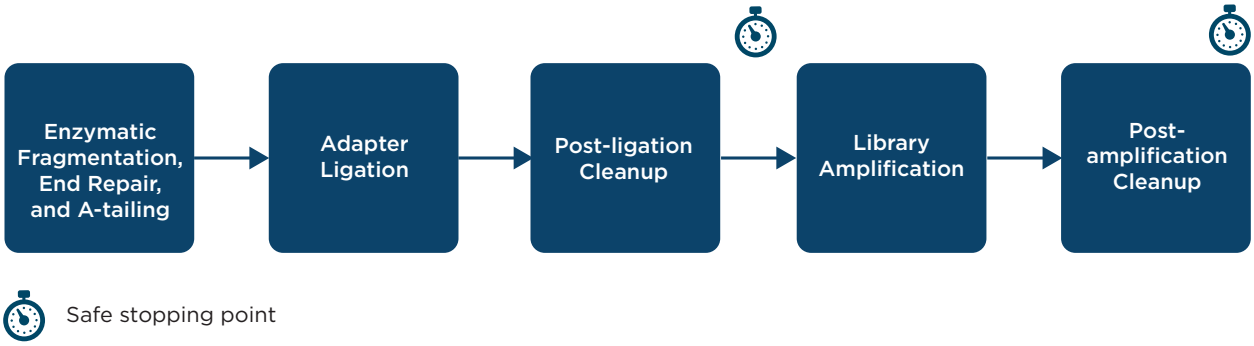

Figure 3. Automated workflow for the Watchmaker DNA Library Prep Kit with Fragmentation on the Biomek i7 Hybrid NGS Workstation.

Method Option Selector (MOS)

The automated method provides an intuitive user interface, the MOS, **(Figure 4)** presenting all workflow options available at the start of the method run including:

- Number of samples (1-96)
- Optional PCR-free workflow
- On-deck vs. off-deck thermocycling*
- Fragmentation parameters*
- Dynamic bead cleanup ratios and elution volumes
- Optional second post-ligation cleanup
- Truncated vs. full-length adapters
- Number of PCR cycles*

*On-deck thermocycler option required

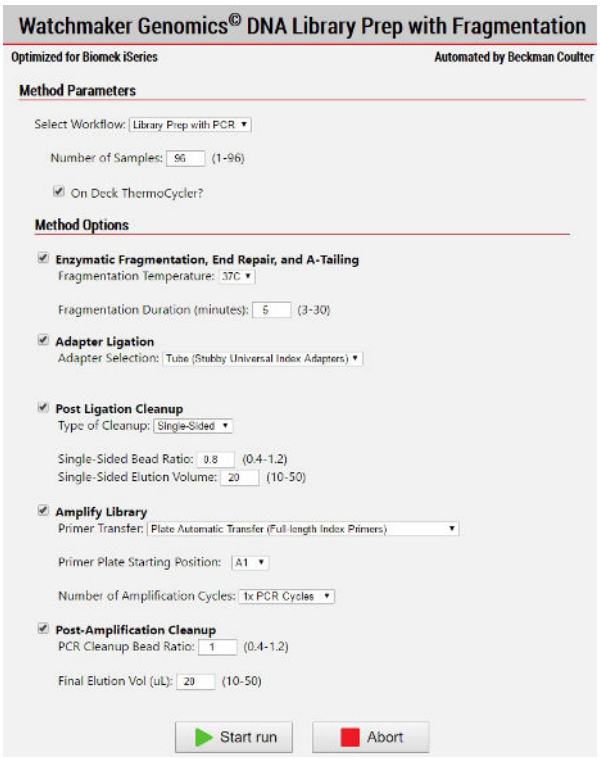

Figure 4. The automated Biomek method for Watchmaker DNA Library Prep Kit with Fragmentation provides an intuitive user interface, enabling users to select all workflow options available at the start of the automated run.

Guided Labware Setup (GLS)

The Watchmaker DNA Library Prep Kit with Fragmentation method utilizes the GLS **(Figure 5)**, a graphical interface that will walk the user through the deck setup of reagents and labware. The GLS is generated utilizing inputs from the MOS and will dynamically adjust the setup based on the sample number and workflow options needed for each run.

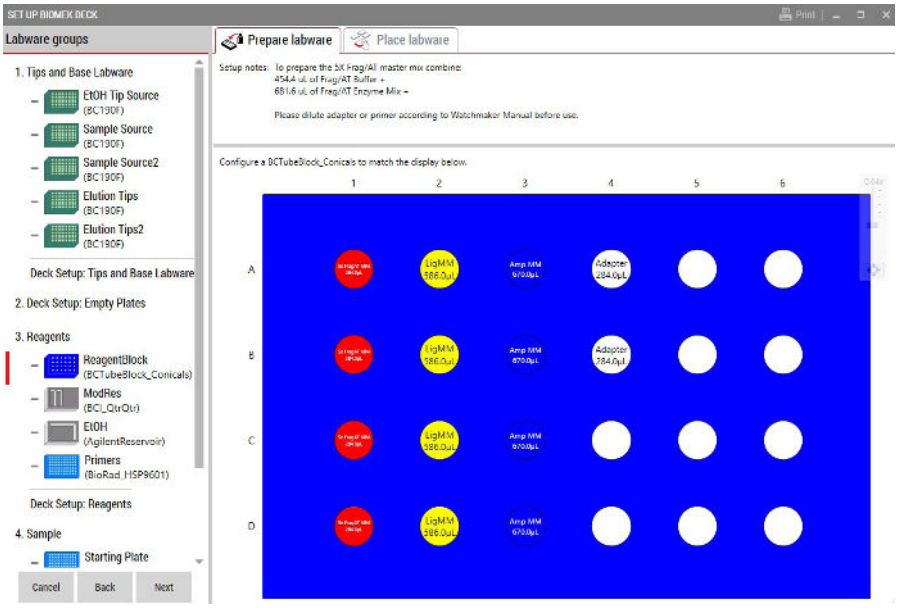

Figure 5. Guided Labware Setup (GLS) provides reagent volumes calculated by sample number, preparation notes, and a pictorial guide for user deck setup.

DeckOptix Final Check (DFC) software

At the end of the GLS the DFC is performed to analyze the Biomek deck setup utilizing the on-deck cameras **(Figure 6)**. The DFC reduces setup errors through the identification of missing, misplaced or incorrect labware or tips.

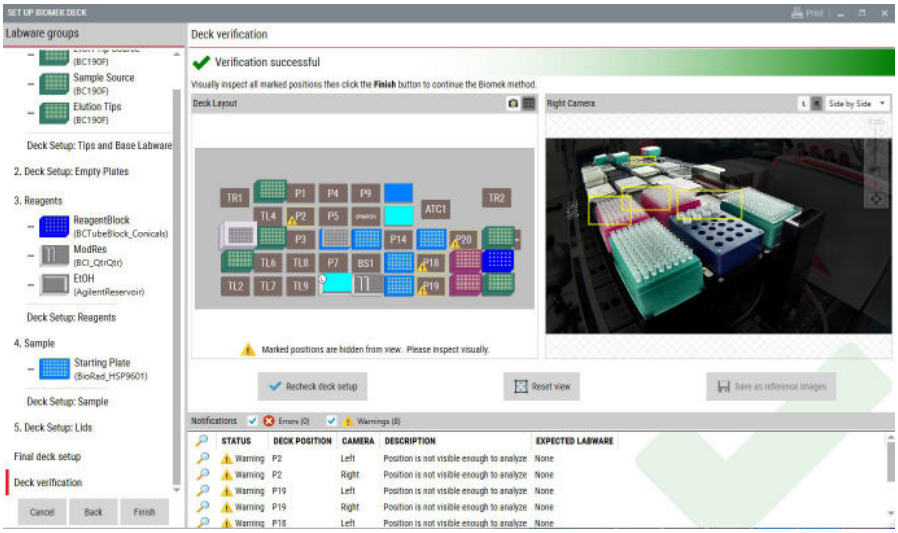

Figure 6. DeckOptix Final Check software uses on-board cameras to confirm the correct deck setup for the Watchmaker DNA Library Prep Kit with Fragmentation automated method for the Biomek i7 Hybrid NGS Workstation.

Experimental Design

To test the capabilities of the automated method, two different automated runs were performed. The first automated run consisted of 48 replicates of human gDNA (Coriell, NA12878) diluted to a total concentration of 10 ng per sample in a checkerboard pattern and run alongside 48 No Template Controls (NTCs) to assess cross-contamination. Manual libraries were prepared in parallel for comparison.

The second automated run was designed to test Watchmaker's chemistry across multiple inputs and DNA templates of varying complexity and GC content. Again, libraries were prepared manually in parallel from the same DNA inputs. The variety of DNA templates included human gDNA (Coriell, NA12878), metagenomic (ATCC, MSA-1002), and plasmid DNA (NEB, pUC19 Vector) at input masses of 1 ng and 10 ng. All libraries were prepared with Watchmaker's DNA Library Prep Kit with Fragmentation (Watchmaker, 7K0019-096), xGen Stubby Adapters, and xGen UDI 10nt primers (IDT, 10008055), according to Watchmaker's User Guide (see **Table 1** for additional experimental details). All libraries, both automated and manual, were sequenced on an Illumina NextSeq 2000 using a P1 Flow Cell and 2 x 150 bp reads.

Following library preparation, the libraries were analyzed using D1000 ScreenTape (Agilent, #5067-5582 and #5067-5583) on the Agilent TapeStation instrument for library size and quality. The Qubit dsDNA BR (Broad-Range) Assay was used for determination of final library concentration.

Sample Label	Sample	Input (ng)	Frag Condition	Adapter Stock Concentration (µM)	PCR Cycle Number	Post-Ligation Cleanup	Post-Amplification 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

Table 1. Experimental conditions tested across variable sample types and inputs.

Results

The initial run with 48 replicates of human gDNA (Coriell, NA12878) generated libraries with consistent yield and sizes, both down and across the plate, with all NTCs being free of cross-contamination **(Figure 7 and Figure 8)**.

The results from the multi-sample run show remarkably consistent insert sizes for both automated and manual sequencing workflows, highlighting the reliability of the automated workflow **(Figure 9 and Table 2)**. Additionally, uniform coverage was observed for the various microbial genomic DNA samples in both the automated and manual library preparations **(Figure 10)**. The automated process maintains uniform coverage across a range of sample inputs, ensuring reliable results and optimal sequencing economy.

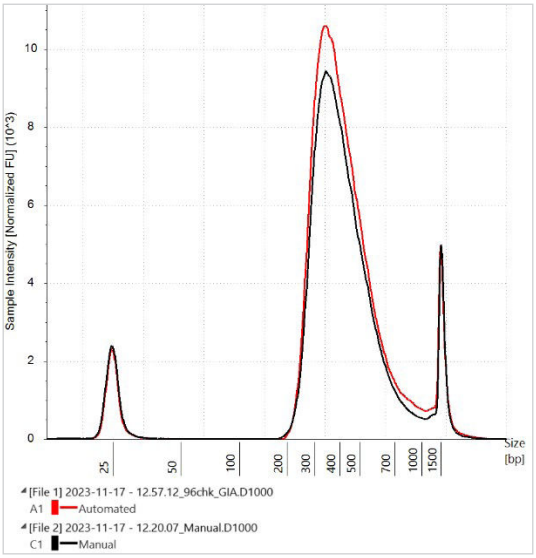

Figure 7. Automation and manual comparison libraries run on the D1000 ScreenTape showed consistent sizing.

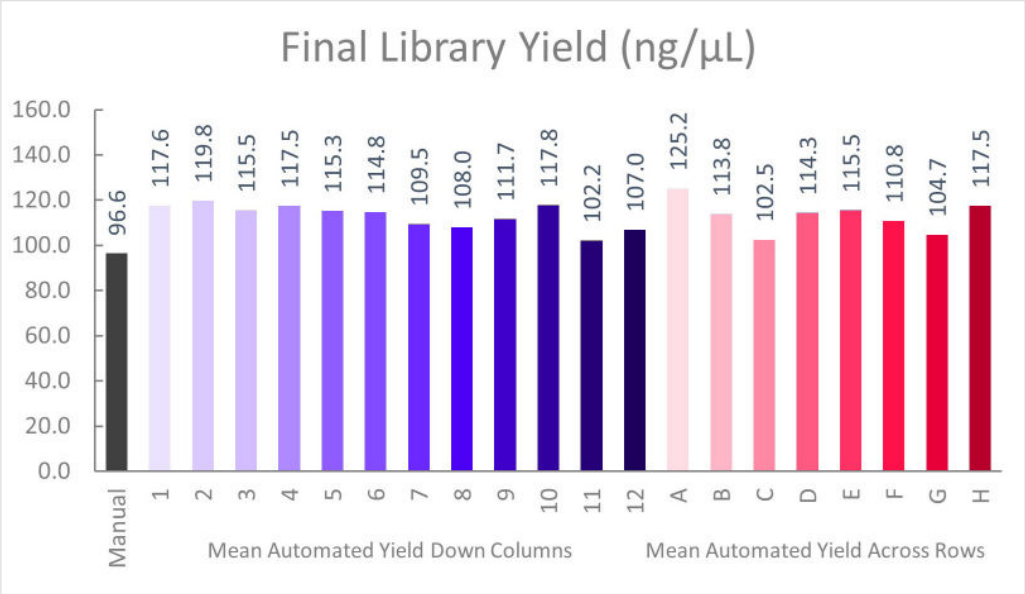

Figure 8. Final library yields assessed by Qubit were comparable between automated and manual processing and consistent across a 96-well plate with no obvious plate effects.

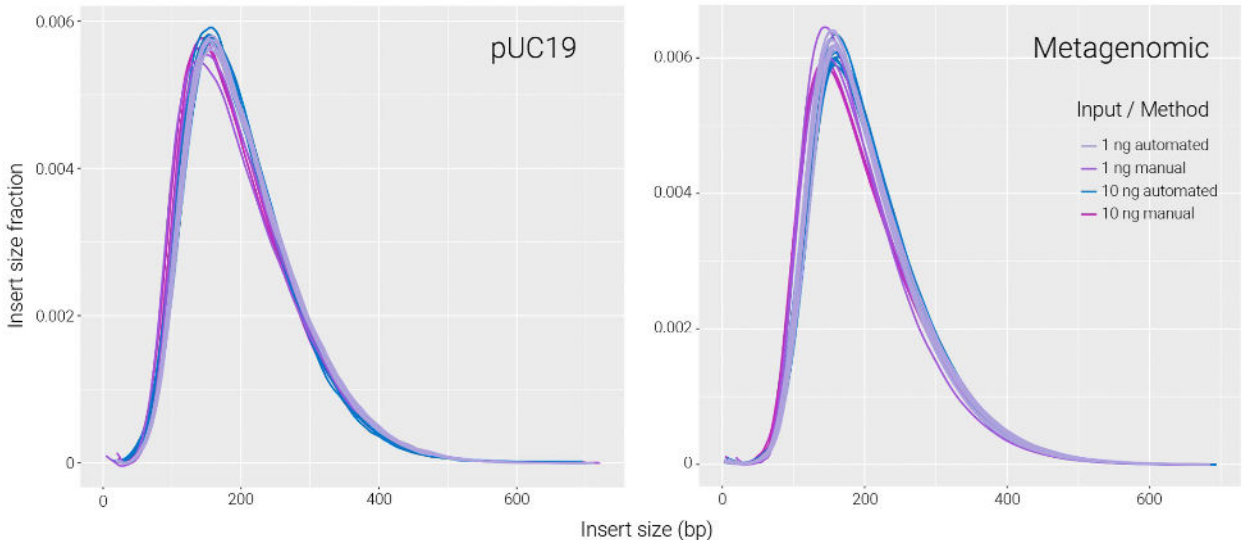

Figure 9. Sequencing data shows highly consistent and comparable library insert sizes across sample types, inputs and between automated and manual processes.

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	187.5
	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

Table 2. Sequencing alignment and insert size statistics.

* Insert size SD refers to the standard deviation within a library.

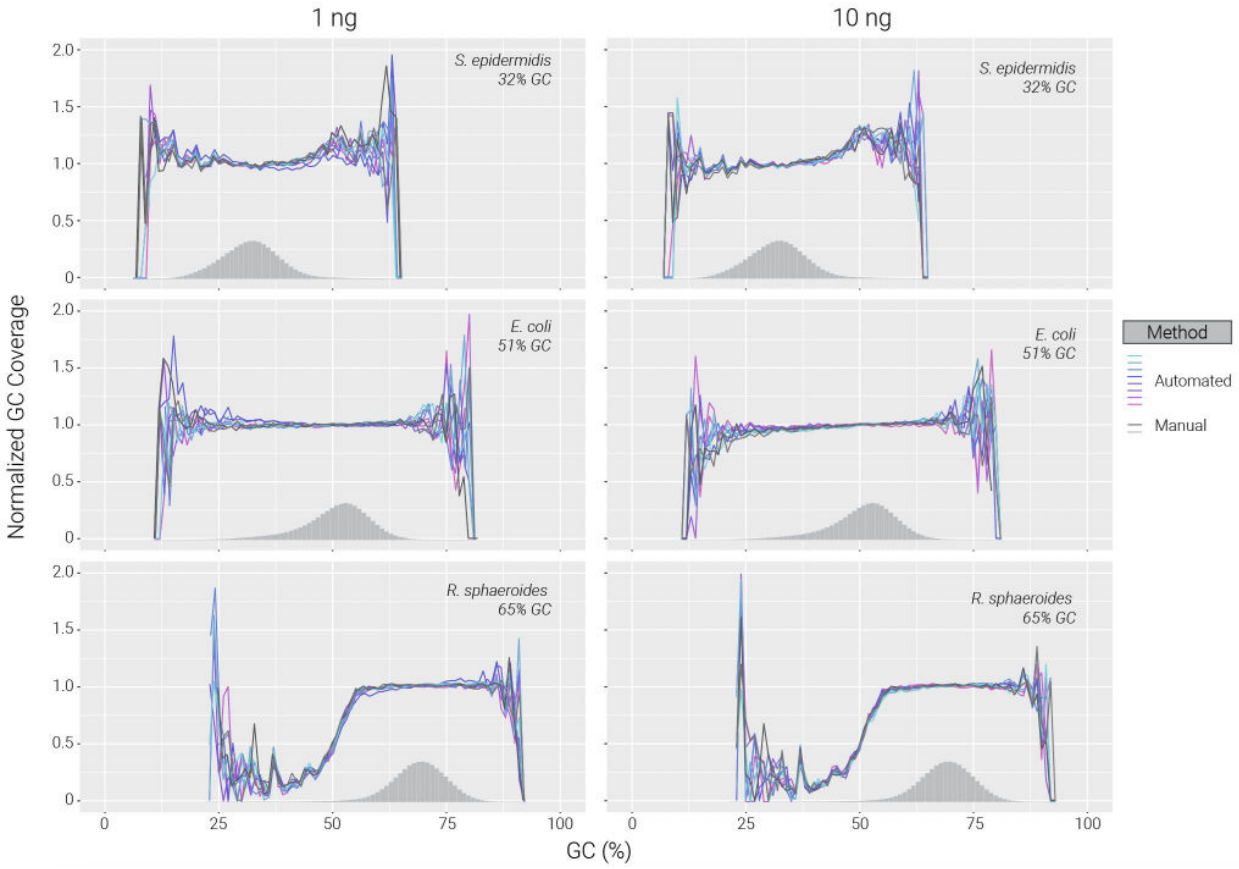

Figure 10. Uniform GC Coverage for a variety of metagenomic DNA. Normalized coverage plots are comparable across automated and manual libraries, and are consistent, indicating uniform coverage at varying sample inputs.

Summary

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
- High-quality libraries with consistent insert size distributions and uniform GC coverage across a variety of sample types and inputs to provide improved sequencing economy
- Reduced risk of human error with walkaway capability and minimized hands-on time

Acknowledgments

We would like to thank the scientists at Watchmaker Genomics for their collaborative work on the workflow implementation, experiemental design and data analysis.

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 Website: **Beckman.com**

2024-GBL-EN-105705-v1