Modernizing DNA library preparation: an automated workflow for translational research and clinical genomics

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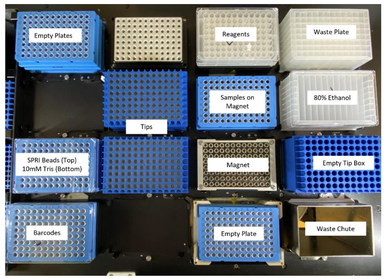
Introduction

The field of translational research and clinical genomics has seen significant advances, with NGS technology leading to increased throughput and lower sequencing costs. In light of this, traditional sonication methods are being re-evaluated to meet the need for more accurate and scalable workflows. Our goal was to evaluate a high-throughput DNA library preparation workflow that reduces the risk of human error, minimizes hands-on time, and is versatile across sample types and input masses. We present an automated workflow on the PerkinElmer Sciclone® G3 NGSx workstation, capable of processing up to 96 samples in a single run using the Watchmaker DNA Library Prep with Fragmentation Kit. The automated workflow was assessed using three different sample types (human gDNA, metagenomic DNA, and plasmids) at varied inputs highlighting the versatility and robustness of this high-throughput DNA library prep solution.

A



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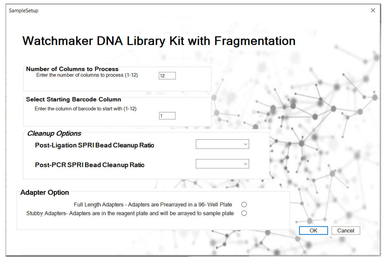


Figure 1. Watchmaker DNA Library Prep Kit with Fragmentation on the PerkinElmer Sciclone G3 NGSx. (A) Deck layout at the start of the run. **(B)** User interface and included options at the start of the run.

Scalable Library Preparation Workflow

Frag/AT ■ **Ligation** ■ **Cleanup** ■ **Amplification** ■ **Cleanup** ➡

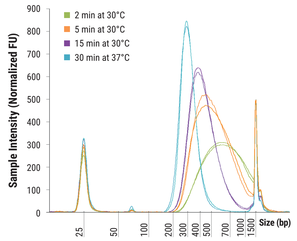
Library prep time: 1 hr 30 min / Hands-on time: 45 min

PCR time: 45 min / Hands-on time: 30 min

Figure 2. Simplified and automation-friendly workflow. The Watchmaker DNA Library Prep Kit with Fragmentation streamlines library construction by using combined enzymatic steps for Frag/AT and a Ligation Master Mix that were designed with automation in mind. Total processing time for 96 samples is between 3.5 – 4.5 hours, including reagent and deck setup, depending on the Frag/AT and PCR parameters.

Highly Tunable and Consistent Fragmentation

A



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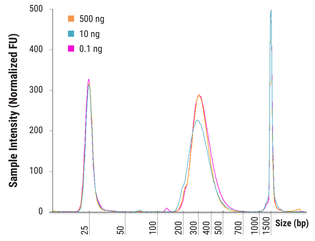

Figure 3. Consistent yet flexible fragmentation. (A) Libraries were constructed using different fragmentation times with 50 ng human genomic DNA. **(B)** Libraries were prepared in duplicate from 500, 10, and 0.1 ng of human genomic DNA fragmented for 20 minutes at 30°C.

Assessment of Automated Workflow Performance

A

Standardized fragmentation and clean protocol

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	9	0.8X	1X
H10	Human	10	37°C, 10 min	3	4	0.8X	1X
H100	Human	100	37°C, 10 min	15	4	0.8X	1X
M1	Metagenomic	1	37°C, 10 min	3	9	0.8X	1X
M10	Metagenomic	10	37°C, 10 min	3	4	0.8X	1X
P1	Plasmid	1	37°C, 10 min	3	9	0.8X	1X
P10	Plasmid	10	37°C, 10 min	3	4	0.8X	1X

B

Manual Sample Layout

H1	H10
M1	H100
P1	M10
H1	P10
M1	H10
P1	H100
NTC	M10
NTC	P10

C

Automated Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	H1	NTC	P1	NTC	M1	NTC	H10	NTC	H10	NTC	H10	NTC
B	NTC	M1	NTC	H1	NTC	P1	NTC	H10	NTC	H10	NTC	H10
C	M1	NTC	H1	NTC	P1	NTC	H100	NTC	H100	NTC	H100	NTC
D	NTC	P1	NTC	M1	NTC	H1	NTC	H100	NTC	H100	NTC	H100
E	P1	NTC	M1	NTC	H1	NTC	NTC	NTC	M10	NTC	NTC	NTC
F	NTC	H1	NTC	P1	NTC	M1	NTC	M10	NTC	M10	NTC	M10
G	H1	NTC	P1	NTC	M1	NTC	P10	NTC	P10	NTC	P10	NTC
H	NTC	M1	NTC	H1	NTC	P1	NTC	P10	NTC	P10	NTC	P10

Figure 4. Comparison of manual and automated workflows. (A) Libraries were prepared from Genome in a Bottle (NA12878), a mock metagenomic community (MSA-1002) and plasmid (pUC19) samples using the Watchmaker DNA Library Prep Kit with Fragmentation either **(B)** manually or **(C)** automated on the PerkinElmer Sciclone G3 NGSx. A total of 50 No Template Controls (NTCs) were included in a checkerboard pattern on the automated sample plate layout to assess cross-contamination. All samples underwent enzymatic fragmentation at 37°C for 10 min and were sequenced on the Illumina NovaSeq S4 flow cell with a 2 x 150 read length.

Manual and Automated Workflows Produce Comparable Results

Table 1. Alignment and insert size statistics

Sample	Input (ng)	N	Method	Reads Aligned (%)	Adapter (%)	Mean Read Length (bp)	Read Length (SD)
Human	1	2	Manual	99.59	0.0017	141.0	20.1
		8	Automated	99.68	0.0150	135.9	24.0
	10	2	Manual	99.72	0.0005	142.5	18.2
		6	Automated	99.76%	0.0015	137.5	23.0
	100	2	Manual	99.76	0.0003	142.6	18.0
		6	Automated	99.81	0.0004	138.7	21.6
Metagenomic	1	2	Manual	93.98	0.0047	141.6	19.3
		8	Automated	94.13	0.0074	137.2	23.1
	10	2	Manual	93.94	0.0004	142.3	18.2
		4	Automated	94.24	0.0020	137.7	22.3
Plasmid	1	2	Manual	99.56	0.0022	141.3	20.4
		8	Automated	99.65	0.0097	136.7	24.7
	10	2	Manual	99.66	0.0012	142.1	19.6
		6	Automated	99.65	0.0017	135.5	26.3

Scalable Library Preparation Workflow

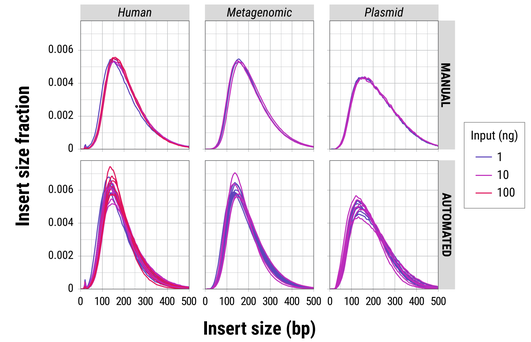

Figure 5. Highly consistent library sizes across inputs and workflows. Library insert sizes were comparable between manual and automated libraries, with no cross-contamination in NTCs (not shown). The automated workflow had no obvious plate effects and highly reproducible size distribution profiles across a 100-fold input range.

Uniform GC coverage

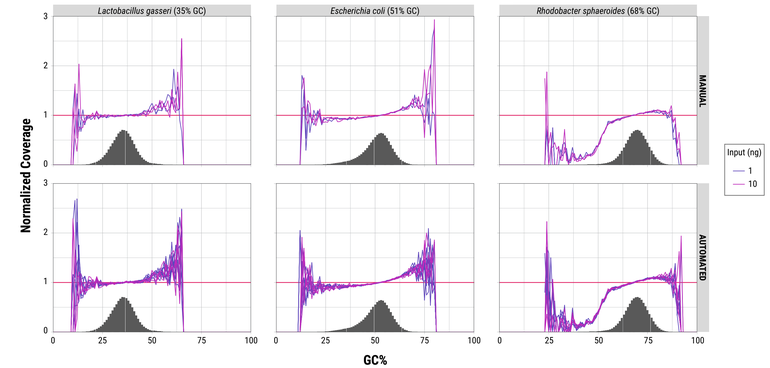

Figure 6. Uniform GC coverage for microbial genomic DNA. Normalized coverage plots for microbial genomes with low, medium, and high GC content were consistent, indicating uniform coverage over a broad range of GC content and varying input amounts. Ideal normalized coverage of 1.0 is indicated by the horizontal (red) line.

Conclusions

The Watchmaker DNA Library Prep Kit with Fragmentation automated solution for the PerkinElmer Sciclone G3 NGSx delivers:

- A high-throughput DNA library prep solution that reduces the risk of human error, minimizes hands-on time, and is easy to use
- Consistent fragmentation with a unified and streamlined workflow even across inputs and sample types
- High sequencing uniformity across a range of GC content that highlights the workflow's utility across a range of translational and research applications

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