

Introduction

DNA methylation is a critical epigenetic modification involved in gene regulation, disease progression, and therapeutic response. As epigenetic profiling becomes increasingly important in translational and clinical research, there is a growing need for methylation sequencing workflows that are accurate, scalable, and compatible with automation. Conventional bisulfite-based methylation sequencing methods introduce significant limitations, including DNA degradation, reduced sequence complexity, and workflow variability, which restrict throughput and reproducibility—particularly for low-input or clinical samples. TET-assisted pyridine borane sequencing (TAPS) offers a bisulfite-free alternative that preserves DNA integrity while enabling accurate detection of methylated cytosines. In this study, we evaluate the performance, reproducibility, and scalability of the Watchmaker DNA Library Prep Kit with TAPS+ chemistry when automated on the Hamilton NGS STAR MOA configuration.

Quick and robust methylation library prep

Watchmaker's TAPS+ chemistry offers several key advantages that make it particularly well-suited for automation and scalable methylation sequencing (Figure 1).

- Direct 5mC readout enables simultaneous detection of genetic variants (SNV/Indels and CNVs) and epigenetic modifications from the same library
- Greater than 98% 5mC conversion delivers high true positive and low false positive rates
- Non-damaging workflow delivers robust performance with degraded FFPE and low-input samples (down to 1 ng), including cfDNA
- Streamlined, automation-friendly workflow generates libraries in 6 hours – no columns required

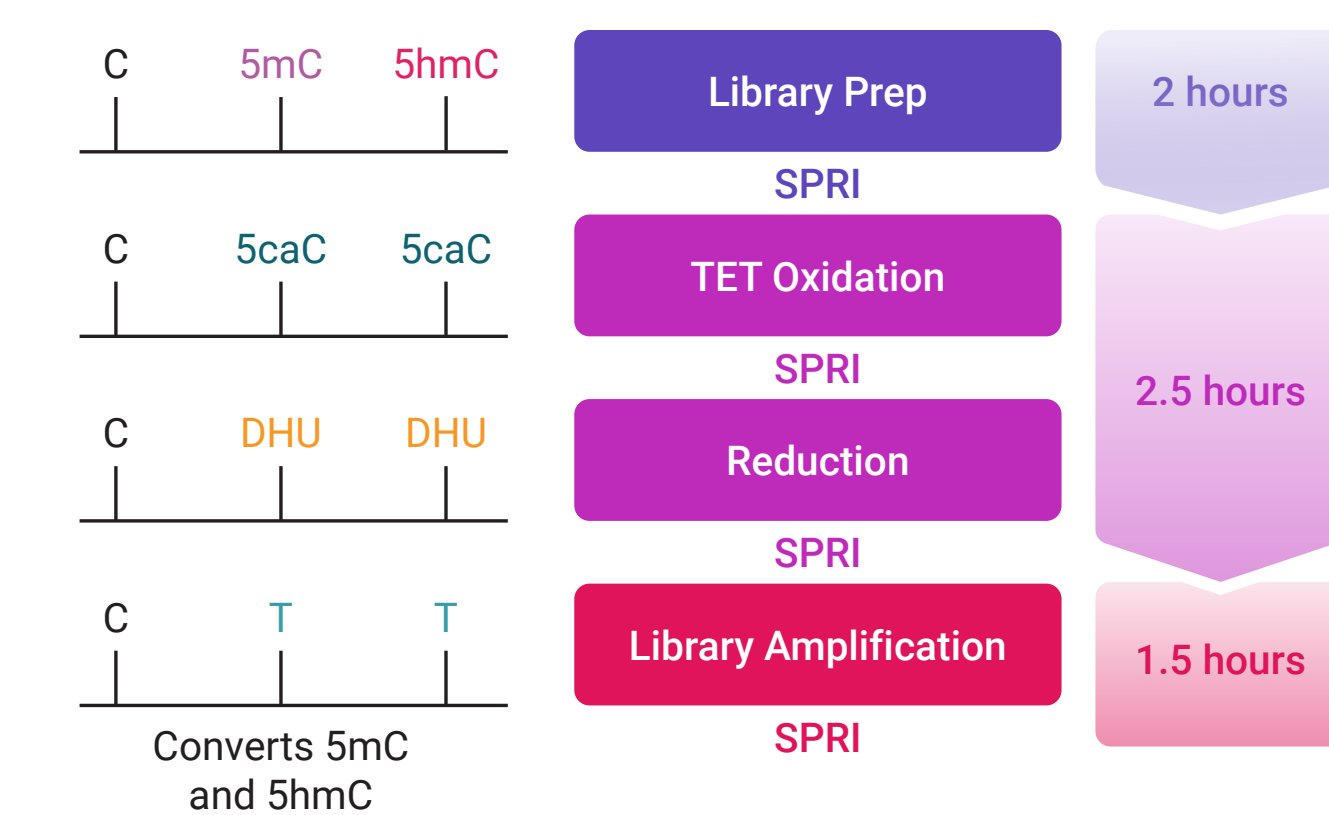


Figure 1. Reduce turnaround time with an automatable workflow. The Watchmaker DNA Library Prep Kit with TAPS+ delivers libraries in under 6 hours with a workflow that smoothly translates to automated liquid handlers for high-throughput processing. The method does not require specialty consumables (like conversion columns often used in bisulfite workflows).

Methylation sequencing at scale

An automated, dynamically configurable method was developed on the Hamilton NGS STAR MOA for the Watchmaker DNA Library Prep Kit with TAPS+, supporting methylation sequencing pipelines. The method enables users to process anywhere from 1 – 96 samples in a single run and can generate up to 96 high-quality sequencing ready libraries in under 9 hours. The method consists of running the Watchmaker DNA Library Prep Kit with TAPS+ with a user intervention between library preparation and TAPS+ conversion. The method uses dynamic run-time options supporting multiple workflows.

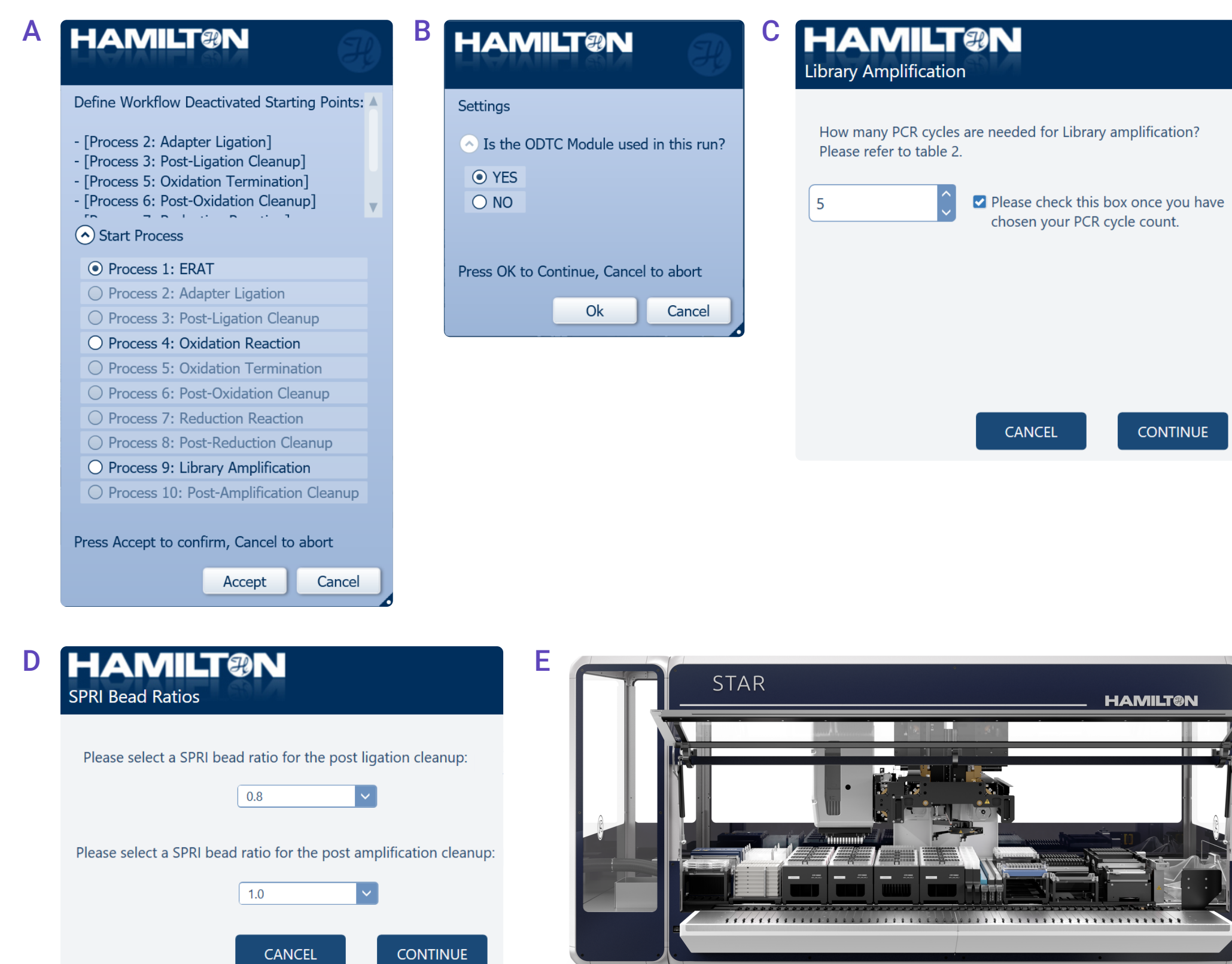


Figure 2. Dynamic method options. The Watchmaker DNA Library Prep Kit with TAPS+ script includes (A) dynamic runtime user options for safe start/stop points, (B) run conditions, (C) PCR cycles, (D) SPRI bead cleanup ratios, and more – supporting multiple workflows within a single automated method. The (E) Hamilton Microlab® NGS STAR MOA enables flexibility and speed utilizing both its MPH and 8-independent channels.

Evaluating method performance

The performance of the automated method was assessed using two separate runs: a low-throughput and a high-throughput run. Both preps underwent the following experimental conditions:

- Sample type: **1 ng high-quality DNA control (95% NA12878, 5% Fully Methylated Lambda DNA)**
- Adapter type: **Watchmaker Stubby Adapter with custom indexing primers**
- Adapter concentration: **15 μ M**
- PCR cycles: **10**
- Post-ligation SPRI cleanup ratio: **0.8X**
- Post-amplification SPRI cleanup ratio: **1.0X**

Automated library preparation was directly compared to manual workflows using standard library quality control and sequencing performance metrics. Initial assessment focused on library concentration and fragment size to evaluate yield and consistency.

For the low-throughput evaluation, 32 replicate libraries were generated from a high-quality DNA control alongside 8 manually prepared libraries. Automated libraries showed high reproducibility and were comparable to manual libraries across all QC and sequencing metrics.

Table 1. Final library QC

	Prep	Average	Std. Dev.	CV (%)
Concentration (ng/ μ L)	Low-throughput	8.0	0.7	8.5
	Manual	8.6	0.6	7.2
Size (bp)	Low-throughput	444	5.4	1.2
	Manual	457	4.5	1.0

Final library concentration was measured on the Qubit 4 Fluorometer using the 1X dsDNA High Sensitivity Assay, and final library size was measured by the Agilent TapeStation 4200 with a D1000 Screen Tape.

Following successful low-throughput testing, a high-throughput run was performed. A total of 88 libraries were generated along with 8 no-template controls (NTCs) to assess reproducibility, plate effects, and potential cross contamination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
B	DNA	NTC	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
C	DNA	DNA	DNA	NTC	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
D	DNA	DNA	DNA	DNA	NTC	DNA	DNA	DNA	DNA	DNA	DNA	DNA
E	DNA	DNA	DNA	DNA	DNA	NTC	DNA	DNA	DNA	DNA	DNA	DNA
F	DNA	DNA	DNA	DNA	DNA	DNA	DNA	NTC	DNA	DNA	DNA	DNA
G	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	NTC	DNA	DNA	DNA
H	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	NTC	DNA	DNA

Figure 3. High-throughput plate layout. Plate layout for the high-throughput automated run containing 88 samples and 8 NTCs.

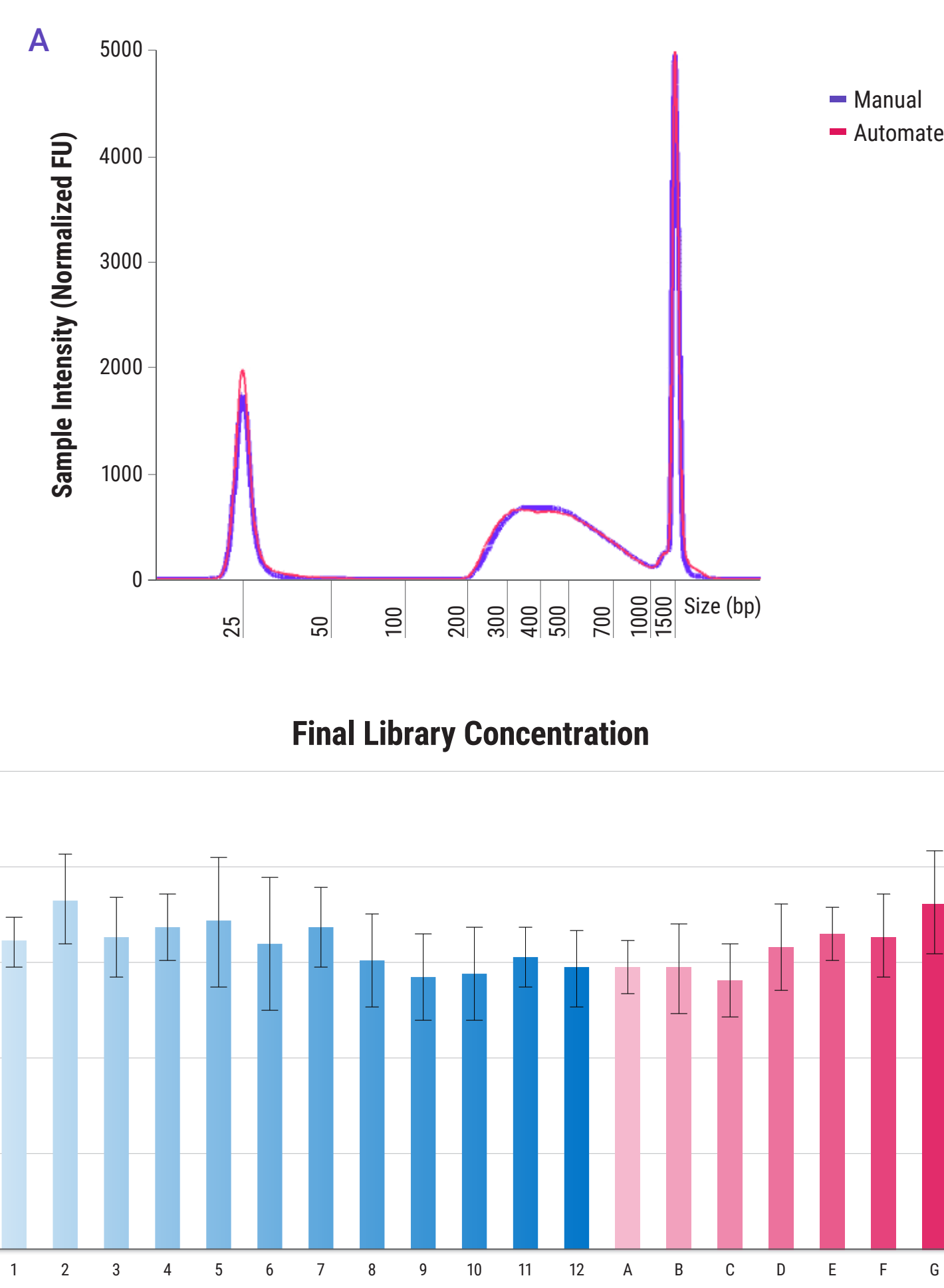


Figure 4. Final Library QC. (A) Final library size was measured using the TapeStation 4200 on a D1000 ScreenTape. (B) Final library concentration was measured on the Qubit 4 Fluorometer using the 1X dsDNA High Sensitivity Assay. Blue bars indicate average concentrations for each column of the plate prepared using automation; red bars indicate average concentrations for rows of the same plate.

Sequencing data summary

Following the high-throughput automated run and generation of manual libraries, all 88 automated libraries and 8 manually prepared libraries were sequenced on an Illumina NextSeq 2000 using a P2 flow cell with 2×150 bp paired-end reads. Sequencing data were downsampled to 1 million reads and were evaluated using methylation conversion efficiency, false positive rates, and M-bias profiles across read positions (Figures 5 and 6). These analyses were used to assess whether automated library preparation introduced bias or variability relative to established manual benchmarks.

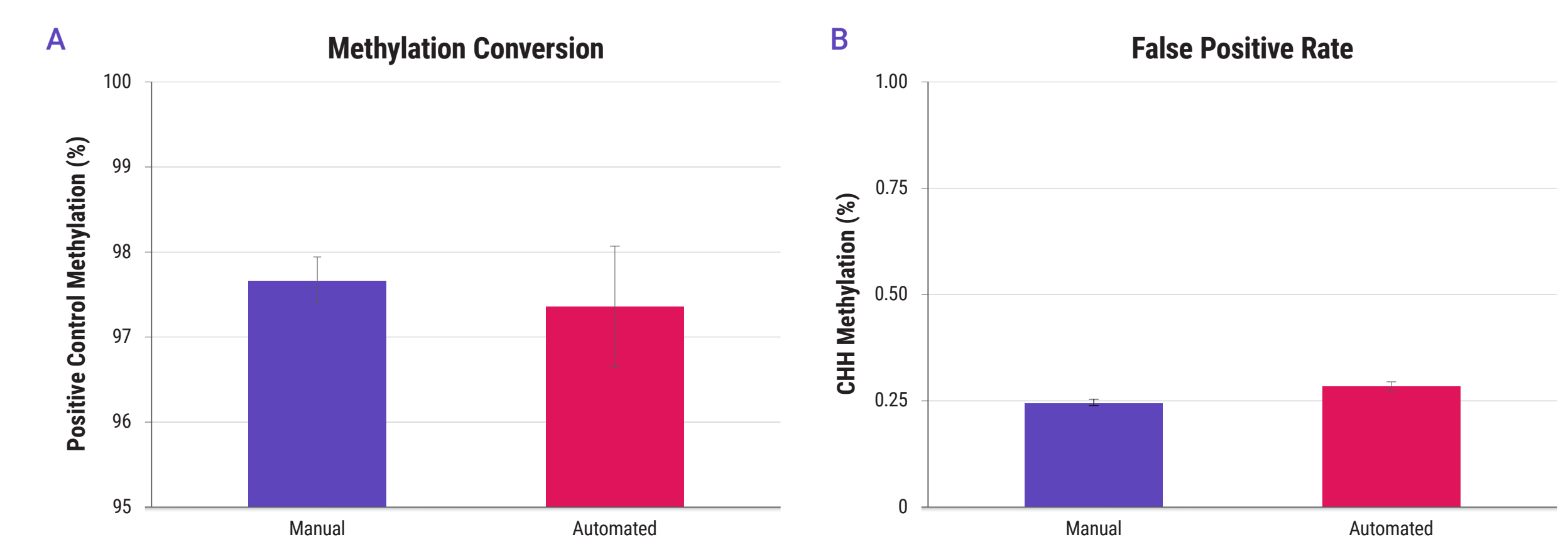


Figure 5. Assessment of methylation assay accuracy. (A) Methylation conversion efficiency, shown as the proportion of unmethylated cytosines successfully converted, demonstrating comparable conversion performance between automated and manual library preparation. (B) False positive rate, reflecting erroneous methylation calls, with similar low rates observed across workflows, indicating that automation does not introduce additional technical errors.

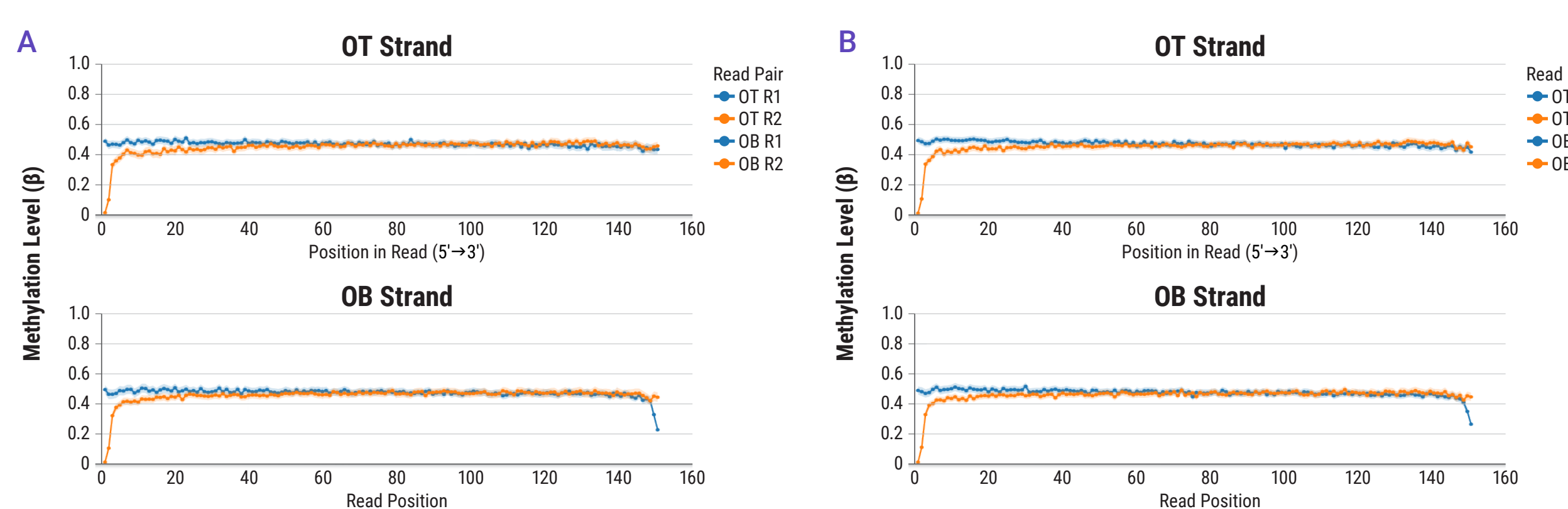


Figure 6. M-bias profiles comparing automated and manual library preparation. Methylation bias across read positions for representative (A) automated and (B) manual libraries is shown to assess position-specific artifacts. Similar bias patterns and signal stability across read length indicate that automated library preparation does not introduce additional positional bias relative to the manual workflow.

Library concentration and size distribution were evaluated to confirm comparable library yield and fragment integrity between automated and manual workflows, ensuring equivalent sequencing performance. Methylation conversion efficiency and false positive rates were used to assess assay accuracy, demonstrating that automation preserves true biological signal without introducing technical errors. M-bias profiles across read positions were examined to identify position-specific artifacts, with similar patterns observed across workflows, indicating no systematic bias introduced by automation. Collectively, these quality metrics show that automated library preparation produces data comparable to manual methods, supporting its use for reproducible, scalable, and high-throughput methylation sequencing applications.

Conclusions

The Watchmaker DNA Library Prep Kit with TAPS+ on the Hamilton Microlab NGS STAR MOA demonstrates the following:

- High reproducibility and performance equivalent to manual library preparation, even at low DNA input (1 ng) (Table 1 and Figure 4)
- Methylation conversion and false positive rates comparable to established manual benchmarks (Figure 5)
- Flat M-bias profiles indicating no position-specific artifacts introduced by automation (Figure 6)
- Significantly reduced hands-on time and operator variability while increasing throughput (Figure 1)