Rapid high quality next generation sequencing library preparation with Swift 2S™ Turbo DNA Library Kits on the Opentrons OT-2

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ABSTRACT

The Swift 2S™ Turbo DNA Library Kit was automated with the Opentrons OT-2 for fast and robust preparation of high quality libraries for next generation sequencing.

- Libraries prepared with 100 and 1 ng input were prepared from a mixture of bacteria with varied GC content including: *Staphylococcus aureus, Escherichia coli*, and *Streptomyces avermitilis*.
- Comparable performance was observed for libraries prepared manually and with the Opentrons OT-2 in terms of size and yield. Similar sequencing metrics such as complexity, coverage, and relative representation were also observed.

INTRODUCTION

Next-generation sequencing (NGS) library preparation requires multiple steps for fragmentation, polishing, adenylation, ligation, and PCR, requiring two to five master-mix additions, incubations, and SPRI clean-ups. Laboratory automation can reduce technical hands-on-time, while increasing the reliability of experiments due to human error and contamination. The Opentrons OT-2 is an affordable liquid handling platform, starting at $5,000, that enables the automation of complex library preparation. The implementation of automation and robust fluidics with the Swift 2S™ Turbo DNA Library Kits (1) ensures rapid and consistent performance for next generation sequencing. The two to three hour Turbo protocol allows varied input amounts of DNA (1 to 250 ng), volumes, and sample types. Demonstrated herein with a mock bacterial community, the Swift 2S™ Turbo DNA Library Kit with Opentrons OT-2 is a rapid and ideal library preparation solution for applications such as whole genome sequencing, targeted exome, genotyping, and metagenomics.

METHODS AND MATERIALS

Libraries were prepared with genomic DNA from three bacterial species with varied GC content, *Staphylococcus aureus (ATCC 25923D-5), Escherichia coli (ATCC 700926)* and *Streptomyces avermitilis (ATCC 31267)*, mixed at equal molar concentrations of DNA. Libraries were prepared manually by an experienced Swift developer with six or eight technical replicates of DNA with 100 ng and 1 ng input into each library prep. Eight technical replicates of both inputs were also prepared on the Opentrons OT-2. The fragmentation master mix used with 100 ng input samples prepared on the OT-2 included Regent DE to slow the fragmentation reaction (2). In detail, 5 ul of Reagent DE was added per reaction, Reagent K2 was not included, and the fragmentation time was increased to 15 min. Libraries were prepared for 350bp aligned insert size. PCR was used to incorporate indexes with 5 and 11 cycles for 100 ng and 1 ng, respectively. Eight libraries were randomly selected for sequencing including libraries prepared from both inputs on the OT-2 and manually. All sequencing was performed on an Illumina MiSeq Standard V2 PEx150. The Opentrons Temperature and Magnetic Modules were used for active cooling of enzymes and magnetic bead clean-up protocols on the OT-2. For comparable analysis, reads were normalized to 1.9 million reads per sample.

FIGURE 1

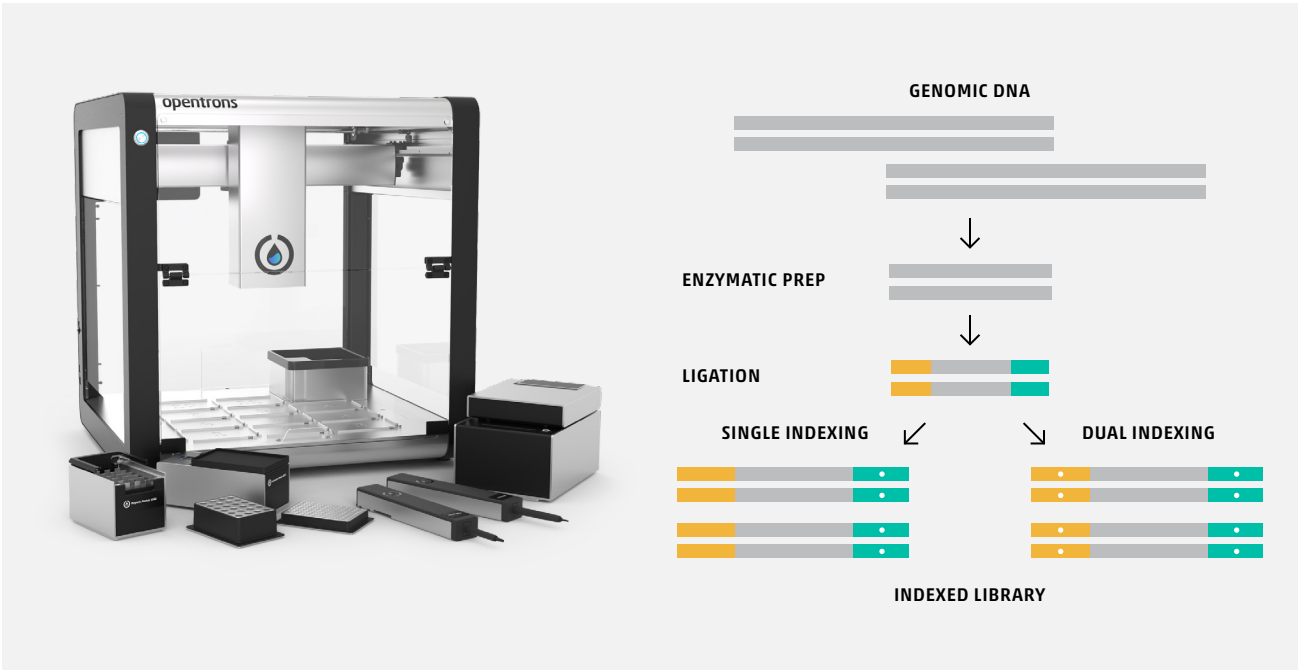

Figure 1. Opentrons OT-2 pipetting robot with single channel and multichannel pipettes required for library preparation using Swift 2S™ Turbo DNA Library Kit (left panel). The Opentrons Temperature Module, required for active cooling of the enzymes, and the Magnetic Module, required for bead-based clean-up, were used in all of these experiments. The Thermocycler module can be added onto the OT-2 deck for full automation. Swift 2S™ Turbo protocol includes enzymatic shearing of the input DNA, adapter ligation, and indexing or optional PCR. A SPRI clean-up was used after ligation and PCR steps.

RESULTS

Along with the time savings of automation, Swift 2S™ Turbo libraries prepared on the OT-2 were robust and comparable to libraries produced manually. To illustrate the unbiased performance of Swift 2S™ Turbo with the OT-2, libraries were prepared with inputs of 100 ng and 1 ng using a bacterial mixture of three strains with varied GC content (33 -71%). Libraries were prepared by both an experienced Swift 2S™ Turbo developer and on the Opentrons OT-2. Reagent DE, used to slow enzymatic fragmentation during extended durations at room temperature during automated liquid handling (2), was added to the fragmentation master mix. As a result, comparable library size and 85 % yield was obtained on the OT-2, compared to libraries prepared manually (Figure 2). For the eight technical replicates prepared on the OT-2, a CV of 14% in library yield was observed.

Sequencing metrics were also comparable for libraries prepared manually and on the OT-2. For example, ninety-three percent of reads mapped to reference genomes within the bacterial mix demonstrating libraries prepared on the OT-2 had no contamination or reduction in fidelity (Figure 3). The comparable percentage of duplicates observed in both manual and automated preparations demonstrates that DNA is retained throughout SPRI clean-ups performed on the OT-2 (Figure 3). Consistent coverage and relative abundance among automated and manual library preps further demonstrated comparable performance and sequence representation (Figure 4).

FIGURE 2

OPENTRONS	YIELD NG/UL	MANUAL	YIELD NG/UL
WellA	6.83	Tube1	9.52
WellB	6.12	Tube2	8.52
WellC	8.25	Tube3	9.00
WellD	6.69	Tube4	8.48
WellE	6.18	Tube5	7.68
WellF	7.92	Tube6	8.68
WellG	7.88		
WellH	9.01		

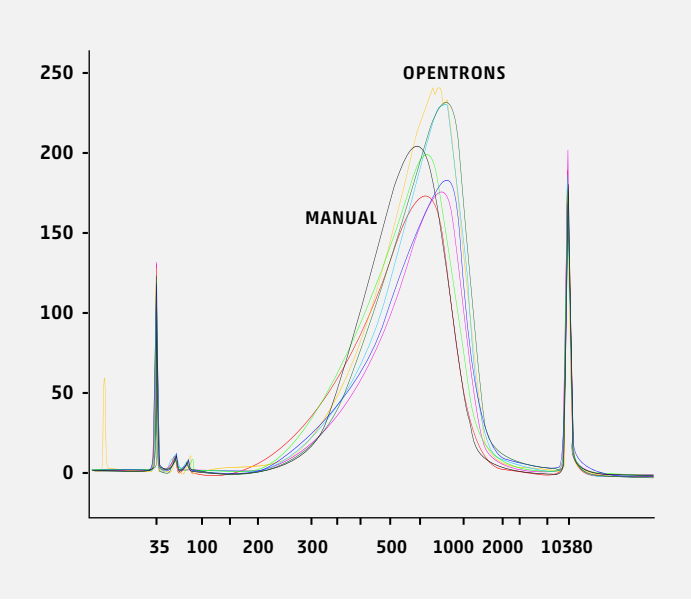

Figure 2. Comparable size and yield of all libraries prepared manually and on the Opentrons OT-2, illustrates robust library preparation. Electrophoretic rendering of 100 ng inputs for manual and automated library preparation.

FIGURE 3

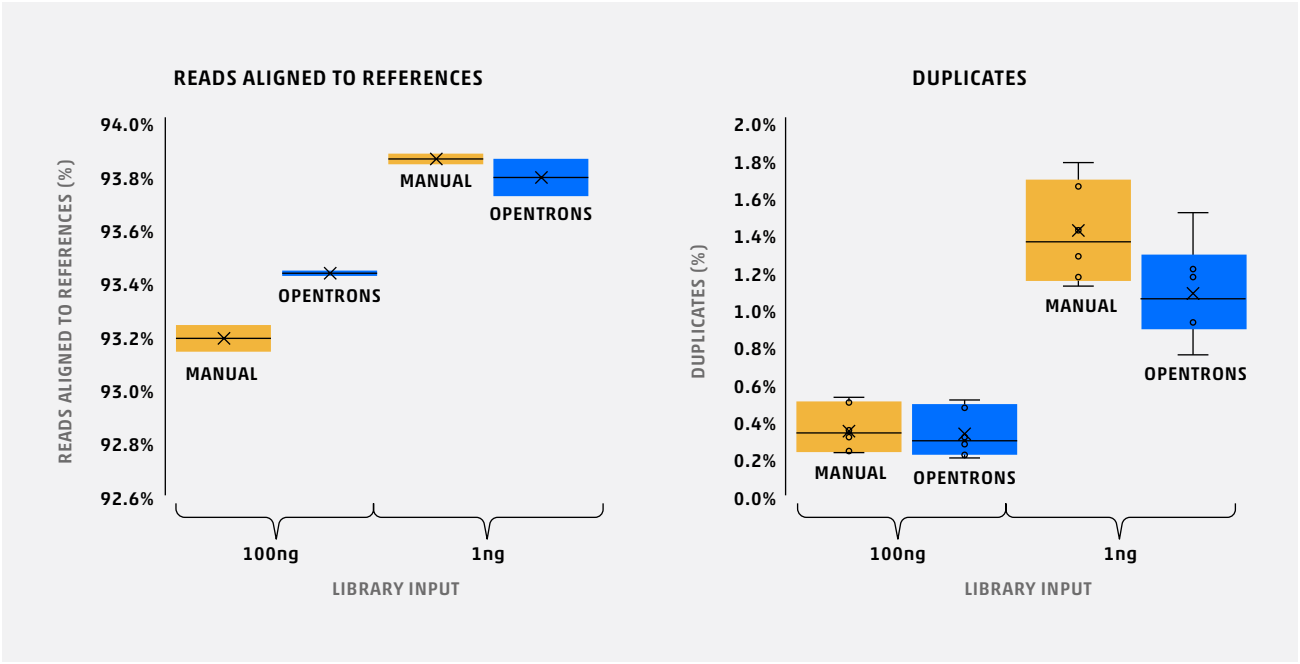

Figure 3. Comparable sequencing metrics libraries prepared manually and on the Opentrons. Manually prepared libraries are shown in yellow and libraries prepared on the Opentrons OT-2 are shown in blue. The left and right panels show comparable reads aligned to reference genomes and percent duplicates. These metrics indicate no contamination or loss of input DNA that would influence library complexity.

FIGURE 4

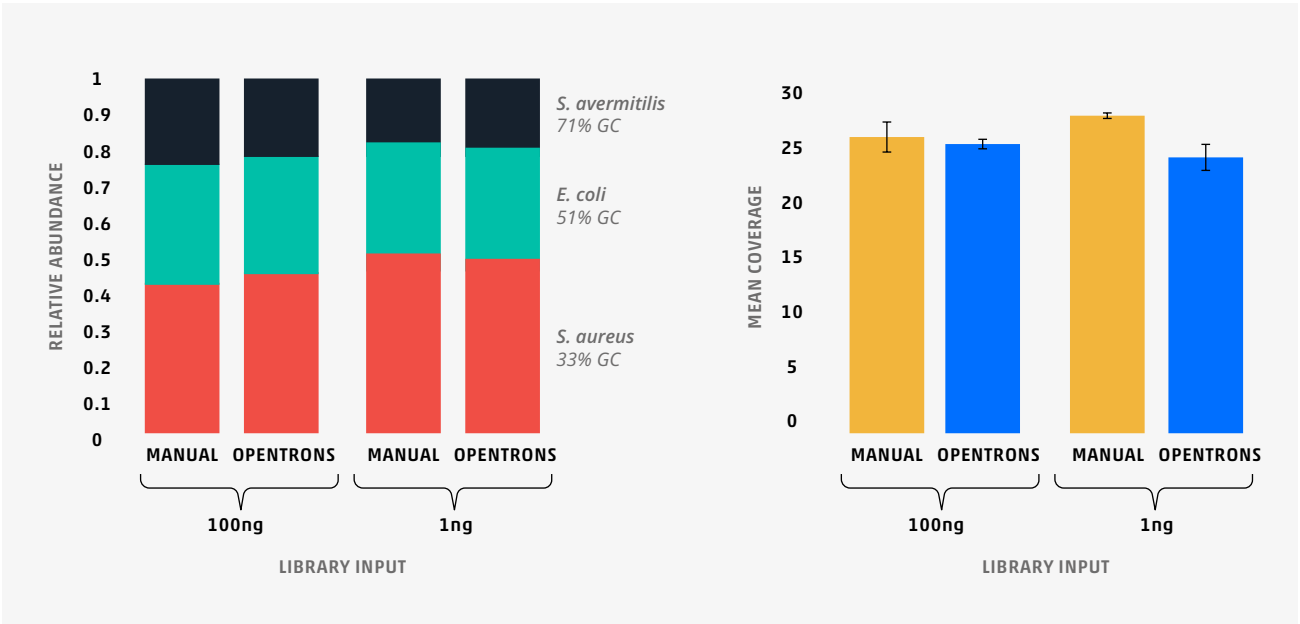

Figure 4. Comparable relative abundance and coverage for libraries prepared manually and on the Opentrons OT-2. Libraries were normalized to have the same number of sequencing reads (1.9 million reads) for comparative analysis.

CONCLUSIONS

Automated library preparation reduces the potential for human error, reduces hands-on-time and reagents, without sacrificing the quality of library preparation. The Swift 2S™ Turbo protocol automated on the OT-2 platform enabled robust preparation of eight or more libraries in two to three hours, with minimal variation in yield between technical replicates.

- Reagent DE (2) was used to slow fragmentation for automated setup at room temperature.
- Comparable and reproducible (CV=14%) library yields and sizes were observed for libraries prepared using the Opentrons OT-2 versus manual prep.
- Similar sequencing metrics were observed for libraries prepared manually and libraries prepared on the OT-2, with comparable complexity, bias, and coverage.

REFERENCES

1. **Masser, Dustin, Robert Stedtfeld, Justin Lenhart, Vladimir Makarov, and Laurie Kurihara. "Turbolase: A radically streamlined high throughput sample prep to sequencing workflow." (2019): 3542-3542.**
2. **https://swiftbiosci.com/wp-content/uploads/2019/12/PRT-022-Swift-Deceleration-Module-Rev-1.pdf**