Benchtop Automation for High-Throughput NGS with the Opentrons Flex® and Flex Stacker Module

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Abstract

Automated liquid handling has become a necessity for reliably scaling high-throughput Next-Generation Sequencing (NGS) library preparation. The new Opentrons Flex High-Throughput NGS Workstation includes all required components for fully automated NGS library preparation, including the Opentrons Flex Stacker, which automates replenishment of pipette tips and labware onto the robot deck. Here, we present high yielding and uniform libraries with coefficient of variation (CV) as low as 9%.

Introduction

The Opentrons Flex High-Throughput NGS Workstation supports NGS library preparation workflows at 96-sample throughput for common DNA library prep chemistries, as well as medium to high throughput for other common chemistries, including RNA, methylation and single-cell sequencing sample prep workflows. Key features of the Workstation include the **Flex 96-Channel Pipette**, which supports pipetting from 5 to 1000 µL and the **Flex Stacker Module**, which automates replenishment of pipette tips and labware onto the robot deck, as well as the Flex Gripper, to move tips and labware around the deck, and on-deck Thermocycler, Temperature and Magnetic Modules to support fragmentation, ligation, amplification and clean-up steps.

To demonstrate the effectiveness of the Opentrons Flex High-Throughput NGS Workstation, we successfully automated two popular DNA library prep chemistries, that use mechanical shearing and enzymatic fragmentation respectively, at 96-sample throughput.

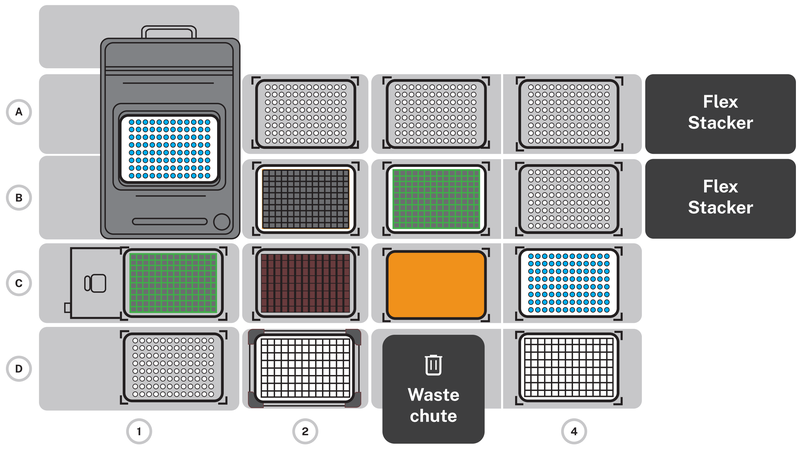


Figure 1. Deck layout for automating library preparation for 96 samples using IDT xGEN MC[1] and Twist EF 2.0[2] kits on the Opentrons Flex High-Throughput NGS Workstation

Row A: (A1) On Deck Thermocycler Module with Sample Plate, (A2-A3) Tip Racks. (A4) Flex Stacker Module with Tip Racks

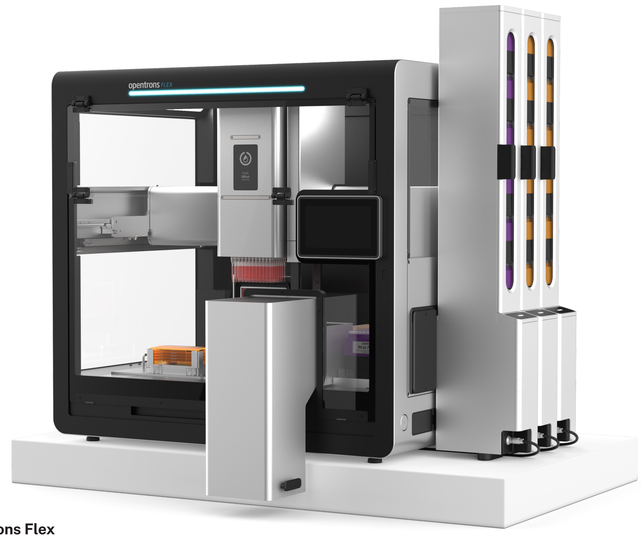
Row B: (B2) Waste Reservoir, (B3) Reagent Plate 2, (B4) Flex Stacker Module with Tip Racks

Row C: (C1) Temperature Module with Reagent Plate 1, (C2) Reagent Reservoir, (C3) Plate Lids, (C4) Deck Extension with Final Sample Plate

Row D: (D1) Tip Rack, (D2) Cleanup Plate 1, (D3) Waste Chute Extension, (D4) Deck Extension with Cleanup Plate 2

Methods

IDT xGen MC.[1] For starting input, samples of 100 ng of Lambda DNA were prepared and mechanically fragmented using a QSonica sonicator, ensuring uniform DNA shearing. The experiment was designed to process 12 samples within a 96-well plate, utilizing only a single row (row H) to minimize reagent costs while maintaining the ability to monitor the process across columns. Four samples were prepared manually for comparison.



Opentrons Flex
High-throughput NGS workstation with Flex Stacker

Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0.[2] Library preparation was conducted using 50 ng of 12 human genomic DNA samples processed alongside 12 negative water controls. The samples and controls were distributed across rows G and H of a 96-well plate in a checkerboard pattern to check for cross contamination. The enzymatic shearing incubation was 30 minutes and library amplification was 6 cycles. Human genomic DNA samples were enzymatically fragmented using Twist EF 2.0 reagents, following standard protocols for NGS preparation.

Library QC and Sequencing. Following the processing of the samples in both experiments, the resulting library yields (ng/µL) were quantified using a Qubit fluorometer and the average fragment sizes of the libraries were assessed using an Agilent TapeStation. Sequencing was performed on an Illumina MiSeq platform using a 2 × 150 paired-end configuration, and the Fastq were analyzed on Galaxy and aligned to either Lambda or Human Genome hg38.

Discussion

IDT xGen MC. The libraries prepared via automation on Opentrons Flex had an average yield of 5.6 ng/µL and a coefficient of variation (CV) of <10% (Fig 2). Average peak fragment sizes were as expected, also with low CV. The manual workflow generated slightly higher yields of 8.6 ng/µL, with a higher CV of 18.8%, indicating that the automated workflow gave more consistent results.

Twist EF Kit 2.0. Automated library preparation resulted in high-yielding, consistent libraries (Fig. 3). Negative control wells were near baseline values with no detectable peaks on the gel.

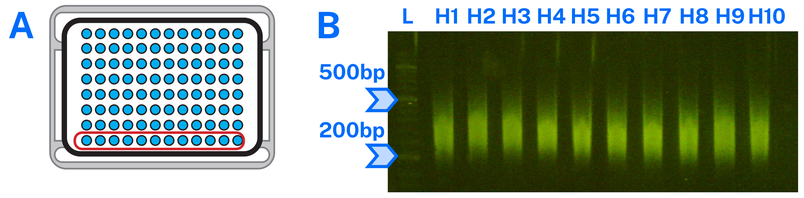
Sequencing. The libraries produced high quality data, as determined by their %PF, Q30, and %Mapped scores (Table 1).

Workflow. Total workflow time for IDT xGen MC after the manual fragmentation was approx. 2 h 30 mins, and for Twist EF 2.0 was approx. 2 h 50 mins. Opentrons Flex High-Throughput NGS Workstation supports walkaway automation of both workflows. Using the 96-channel pipette reduces time compared to processing a 96 sample plate with multi channels (about 5 Hours 40 Minutes).

Importantly, the libraries generated by the Flex platform performed comparably to manually prepared libraries in terms of quality metrics, demonstrating that automation with the Flex delivers both speed and reliability without compromising data integrity.

Key Finding

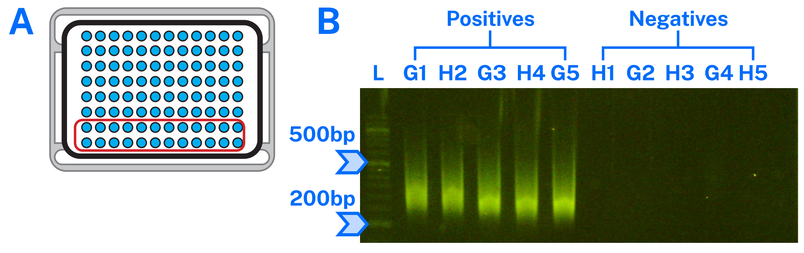
The Opentrons Flex High-Throughput NGS Workstation supports walkaway automation of popular DNA library preparation kits, resulting in high-yielding, consistent librairies.



C

	12 Samples Lambda Flex	4 Samples Lambda Manual
Yield AVE:	5.6 ng/µl	8.6 ng/µl
CV:	9.6%	18.8%
Peak Fragment Size AVE:	285 bp	303 bp
CV:	2.7%	6.3%

Figure 2. IDT xGen MC Library Preparation (A) Sample Plate Layout, (B) Library Fragment Sizes, 2% E-Gel, E-Gel Sizing DNA Ladder, (C) Library Yield, Flex and Manual Comparison.



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	12 Samples Human Genomic Flex
Yield AVE:	51.4 ng/µl
CV:	12.4%
Peak Fragment Size AVE:	365 bp
CV:	4.2%

Figure 3. Twist Library Preparation Enzymatic Fragmentation Kit 2.0 (A) Sample Plate Layout, Row G and H Tested in a Checkerboard pattern, (B) Library Fragment Sizes, Library Fragment Sizes 2% E-Gel, E-Gel Sizing DNA Ladder, representative Positive and Negative Samples, (C) Library Yield, Positive and Negative Control Comparison.

Table 1. Sequencing Results depicting the GC Content, Percent Passing Filter, Quality >30 and Percent Mapped for Lambda Samples prepared with IDT xGen MC and Human Genomic Samples prepared with Twist EF 2.0, on a 2 x 150 MiSeq Run.

	IDT xGen MC		Twist EF 2.0	
	Lambda	CV	Human Genomic	CV
GC content	49.21%	2.27%	41.85%	0.12%
% PF	98.55%	0.47%	99.40%	0.00%
Q30	94.44%	0.56%	94.74%	0.12%
% Mapped	98.73%	1.02%	100.00%	0.00%

References

1. Twist Bioscience. Library Preparation Enzymatic Fragmentation (EF) Kit 2.0 Product Sheet. Available at: https://www.twistbioscience.com/resources/product-sheet/library-preparation-enzymatic-fragmentation-ef-kit-20.

2. Integrated DNA Technologies (IDT). xGen NGS Library Prep MC Kit. Available at: https://www.idtdna.com/pages/products/next-generation-sequencing/workflow/xgen-ngs-library-preparation/dna-library-preparation/dna-library-prep-kit#dna-library-prep-mc.