Ultra-efficient automated whole genome sequencing: A library prep workflow using QIAseq® FX DNA Library Kit and QIAseq® Normalizer Kit on the Opentrons Flex™

Written by
Matthew Akana, MS,[1] Frances Slater, PhD,[1] Shu Boles, PhD,[2] Kinnari Watson, PhD,[1]
[1]Opentrons Labworks, Inc., [2]QIAGEN, LLC

ABSTRACT

As the ambition of sequencing projects and capacities of sequencing platforms grows, labs are increasingly looking for ways to streamline laborious and time-consuming preparation of libraries. One way of achieving this is through automating the library prep workflow. Additionally, library prep workflows are becoming more efficient as a result of advancements in library prep chemistry, with features that support automation, shorter workflows and/or fewer pipetting steps. Here we describe the automation of QIAGEN's QIAseq FX and Normalizer chemistries on the Opentrons Flex for efficient preparation of *E. coli*, human genomic and mixed microbial DNA libraries.

Key Features

- Automating QIAGEN's QIAseq FX and Normalizer chemistries on the Opentrons Flex results in a 96-sample workflow that can be completed in 4-5 hours, with <1 hour of hands-on time.
- This demonstrated workflow generated highly uniform, normalized, sequencing-ready libraries at 4 nM, which is well suited for high-throughput whole genome sequencing (WGS) application.

INTRODUCTION

Automation of library prep workflows is becoming increasingly common to satisfy the demands of increasing demands for sequencing. However, automation is a significant investment for most labs, and there are a number of additional considerations, including the compatibility with chosen chemistries, throughput, and total workflow and hands-on time.

Here we describe an automated QIAseq workflow for preparation of *E. coli*, human genomic and mixed microbial DNA libraries, with integrated library normalization on a benchtop liquid handler with up to 96-sample throughput, the Opentrons Flex. The QIAseq FX DNA Library Kit streamlines the NGS library preparation step by integrating enzymatic fragmentation and unique dual indexes for sequencing. The QIAseq Library Normalization Kit further improves the workflow by normalizing the libraries to 4 nM without requiring qPCR-based library quantification. The Opentrons Flex supports preparation of libraries up to 96 samples with integrated pipettes and labware gripper, and modular hardware, including on-deck thermocycler, temperature and heater-shaker modules and a magnetic block.

METHODS

Samples

DNA from three different sample types was used for whole-genome sequencing library prep: (1) *E. coli* (2) mixed microbial community and (3) human (see **Table 1** for further details).

Library preparation and normalization

Whole genome shotgun libraries were prepared using QIAseq FX DNA Library Kits automated on the Opentrons Flex (see **Tables 1 & 2** for further details). Normalization was carried out using the QIAseq Library Normalizer Kit using workflows A & B.

Sequencing

The normalized, pooled libraries were sequenced on Illumina MiSeq® (2 x 150 bp) and NextSeq® instruments (2 x 75 bp).

QC

Prior to sequencing, the finished samples were assessed for quality on Agilent Tapestation High Sensitivity D1000 Screentape for fragment size and concentration and quantified with KAPA Library Quantification kit for qPCR. The samples were then denatured and diluted for sequencing.

Data analysis

Sequencing data was demultiplexed and uploaded into Galaxy. Paired-end reads were analyzed with fastp and aligned to their respective genome with BWA-mem and their alignments analyzed with Samtools.

Table 1. Equipment, reagents and samples used.

Equipment, Reagents and Samples	Company / Cat. No. or SKU
Opentrons Flex NGS Workstation (2 x 8-Channel Pipette configuration)	Opentrons / 991-00116
QIAseq FX DNA Library UDI Kits (96)	QIAGEN / 180479–180482
QIAseq Library Normalizer Kit (96)	QIAGEN / 180605
E. coli Non-Methylated Genomic DNA	Zymo Research / D5016
Human Male Genomic DNA	Promega / G1471
20 Strain Staggered Mix Genomic Material	ATCC / MSA-1003™

Table 2. Input DNA and key library preparation and normalization parameters.

Input DNA	Key Workflow Parameters
100 ng *E. coli*	Fragmentation time: 15 mins Number PCR cycles: 6 Normalization workflow: B
1 ng mixed microbial	Fragmentation time: 14 mins (with 2.5 µL FX Enhancer) Number PCR cycles: 12 Normalization workflow: A
100 ng human	Fragmentation time: 15 mins Number PCR cycles: 6 Normalization workflow: B

RESULTS

Automating the QIAseq FX DNA Library Kit and QIAseq Normalizer workflows can be achieved using the Opentrons Flex NGS Workstation, with two pipette configurations possible, depending on desired sample throughput: Either two Flex 8-Channel Pipettes can be used (recommended for up to 48 sample throughput; see **Figure 1**) or a single Flex 96-Channel Pipette can be used (recommended for up to 96 sample throughput). For 96 samples, the timing of the manual and automated workflows was calculated, with considerable savings of both total and hands-on time **(Figure 2)**.

In order to test the performance of the automated workflow, we created whole genome shotgun libraries for different sample types, including *E. coli*, human genomic and mixed microbial DNA. *E. coli* DNA generated high-yielding and highly uniform libraries **(Figure 3)**.

DNA libraries from a mixed microbial sample were similarly high-yielding and balanced, with a profile close to the expected community profile across species with variable GC content **(Figure 4**). Similarly, for human genomic DNA, libraries were high yielding and uniform **(Table 3).**

The QIAseq Normalizer Kit streamlined library preparation by replacing time-consuming library quantification methods, and produced uniform libraries at appropriate concentrations for sequencing **(Figure 5)**.

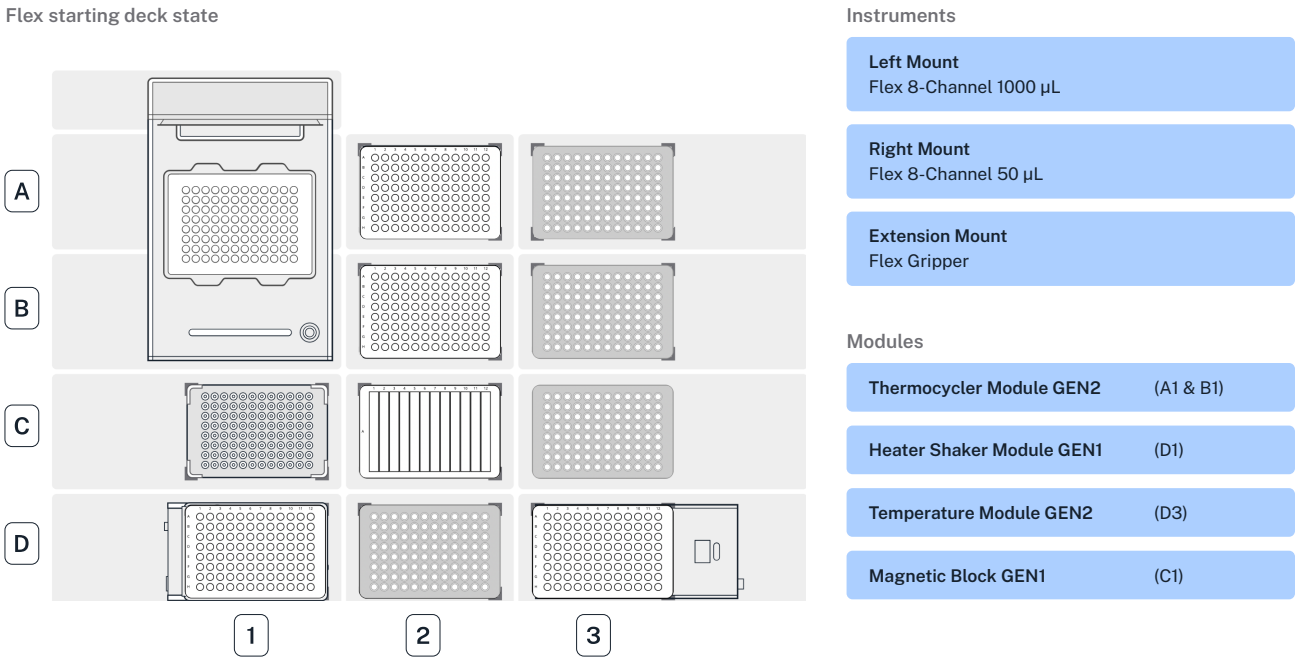


Figure 1. Starting deck layout for a QIAseq workflow on the Opentrons Flex NGS Workstation configured with two Flex 8-Channel Pipettes, Flex gripper and on-deck modules.

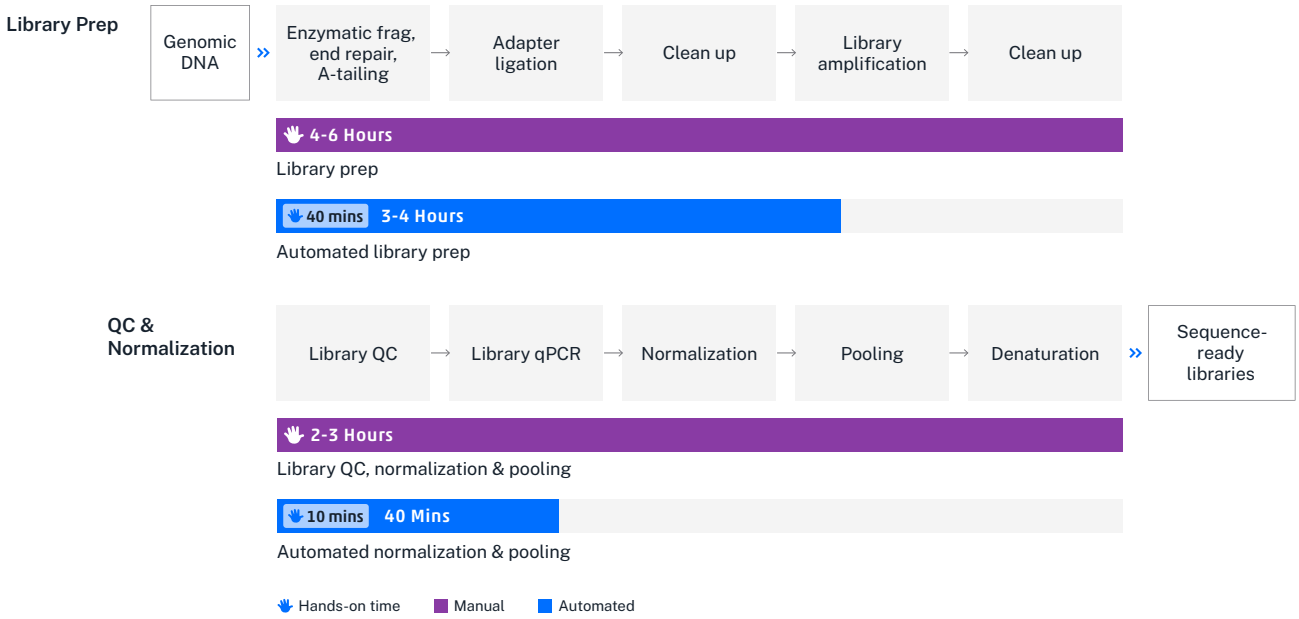
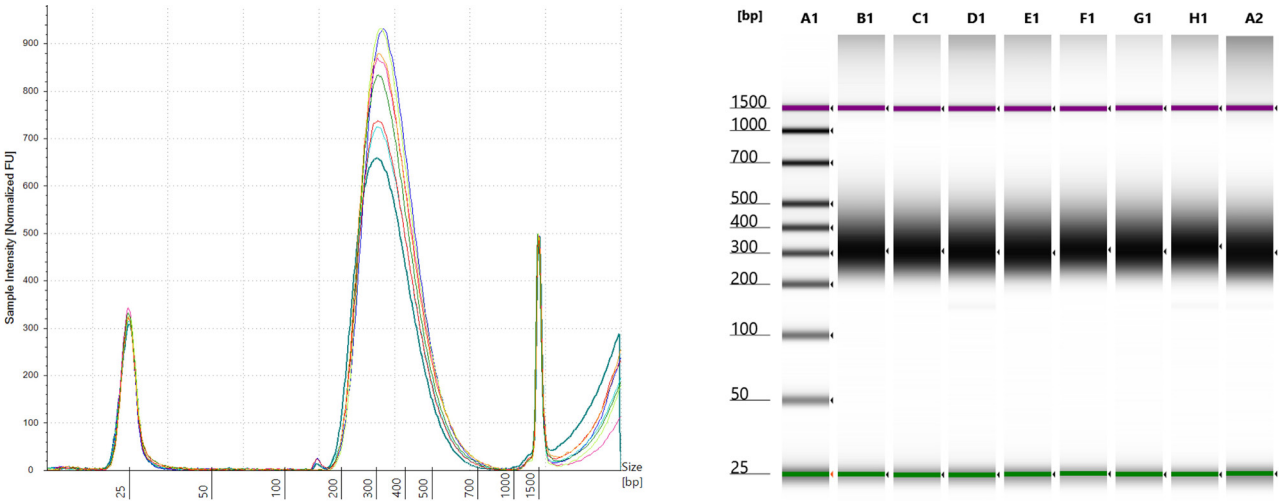
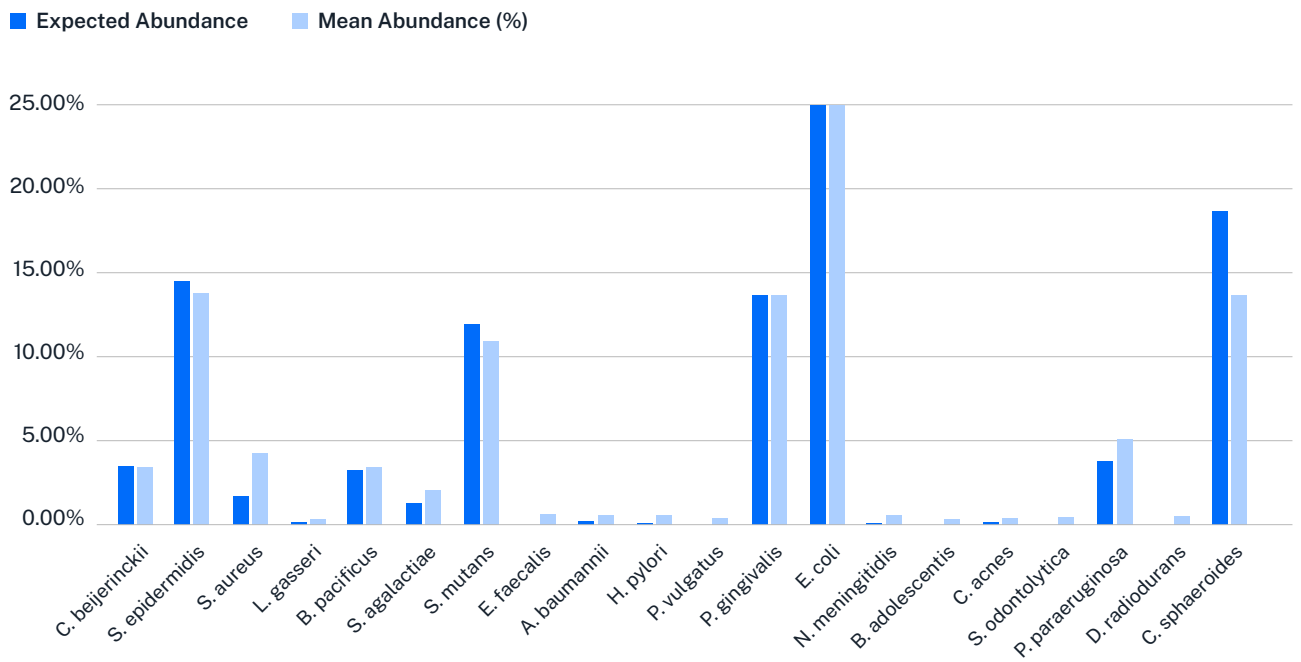


Figure 2. Automating the QIAseq FX and QIAseq Normalizer workflows on the Opentrons Flex reduces total workflow time by several hours and reduces hands-on time to less than one hour. Timings are approximate for 96-sample throughput.



	Yield (ng/µL)	Size (bp)	Mapped (%)	Index CV	Duplicate (%)	Error Rate (%)
Average	11.3	264	99.04	0.6	0.06	0.10
CV (%)	8.74	2.77	0.05	10.58	-	-

Figure 3. High-yielding and uniform libraries generated from *E. coli* genomic DNA. The data shown is from libraries generated from 8 samples of *E. coli* DNA using 100 ng input, with 15 min fragmentation and 6 cycles of amplification.

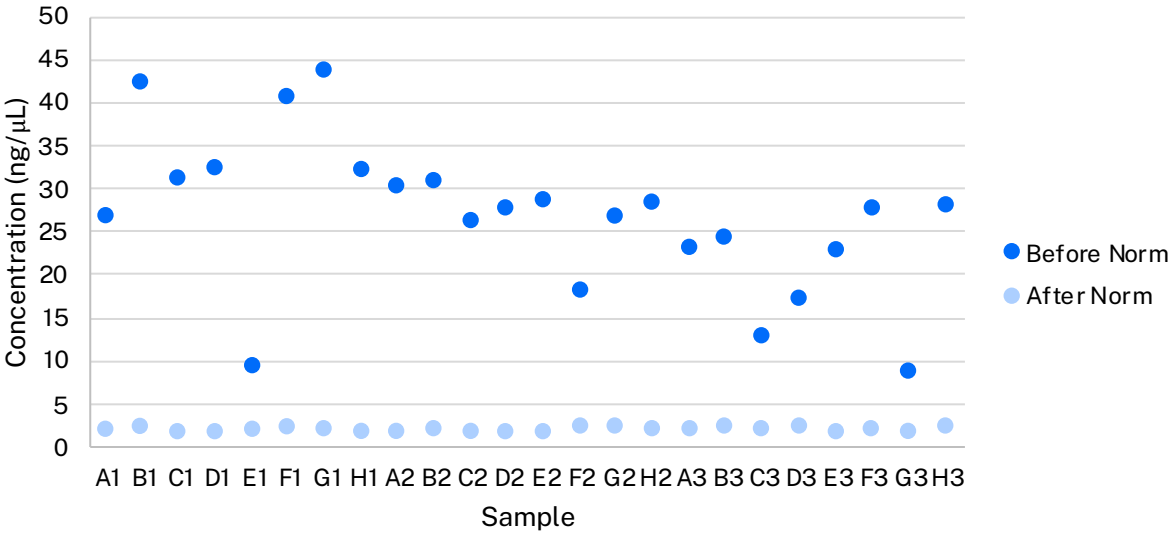


	Yield (ng/µL)	Size (bp)	Q30 Bases	Total Reads	Reads Passed Filters (%)
Average	46.8	258.4	83.0	33.4 M	90.7
CV (%)	15.27	11.42	1.16	18.41	2.04

Figure 4. Comparable community profiles were seen across species with variable GC content in mixed microbial samples. The data shown is from 8 samples of ATCC MSA-1003 generated using 1 ng input, with 14 mins fragmentation + enhancer, 12 cycles amplification, and 1:10 adapter dilution.

Table 3. High-yielding and uniform libraries generated from human genomic DNA. The data were generated from 8 samples of Promega Male Human Genomic DNA using 100 ng input, with 15 mins fragmentation and 6 cycles of amplification.

	Yield (ng/µL)	Size (bp)	Q30 Bases	Total Reads	Reads Passing Filter (%)
Average	13.3	386.3	92.4	40.1 M	96.8
CV (%)	16.71	1.05	0.74	19.48	0.32



	Input (ng/µl)	Library Before Normalization (ng/µl)	Library After Normalization (ng/µl)
Average	1.6	26.5	1.8
CV (%)	64.67%	33.85%	13.53%

Figure 5. QIAseq Normalizer produces reproducible, normalized DNA libraries from a sample batch with varied input DNA concentrations. To demonstrate this, we created a batch of 24 samples of E.coli DNA ranging from 10 to 100 ng input (CV = 64.67%). We processed the samples using QIAseq FX plus QIAseq Normalizer (Workflow B), and after the library prep, we removed aliquots both before and after normalization. Normalizer produced libraries with appropriate concentrations for sequencing (average of 1.8 ng/µl) and reduced variability in library concentration (CV decreased from 33.85% to 13.53%).

DISCUSSION

In this application note, we demonstrated an automated, ultra-efficient and streamlined WGS library preparation and normalization workflow. By coupling automation with enzymatic library prep and normalization chemistry, the total and hands-on time for the workflow is dramatically reduced, while delivering balanced libraries with high yields that generated high quality sequencing data.