Automating QIAseq® miRNA Library Kit on Opentrons Flex® NGS Workstation

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ABSTRACT

Preparing microRNA (miRNA) libraries for next-generation sequencing (NGS) requires several enzymatic and size selection steps. Automation of complex NGS library construction steps introduces standardization and minimizes potential errors associated with manual library construction. Here we present performance data showing that automation of the QIAseq® miRNA library kit on the Opentrons Flex® NGS workstation results in highly reproducible libraries with a significant reduction in hands-on time and removes potential batch effects associated with multiple manual operators.

Key features:

- Automating QIAseq miRNA Library Kit by QIAGEN on the Opentrons Flex generates high-quality miRNA libraries comparable to manual preparation.
- Automation reduces the number of touchpoints from six to one and saves approximately 2 hours of hands-on time.
- Automated library normalization with QIAseq Normalizer Kit reduces library QC time by an additional 1 to 2 hours and hands-on time by 1 hour 20 mins relative to qPCR-based normalization methods.

INTRODUCTION

MicroRNAs (miRNAs) are small noncoding RNAs that average 22 nucleotides in length. They regulate gene expression, typically by interacting with the 3' UTRs of target mRNAs to repress gene expression. Improper expression of miRNAs is associated with changes in development, cellular differentiation, signal transduction, and more. miRNAs have been shown to act as oncogenes and tumor suppressor genes while miRNAs from serum, plasma, and urine have been described as biomarkers for various processes.

Methods such as Northern blotting, RT-PCR, and microarray have been used to study miRNAs. In recent years, NGS technology has been increasingly adopted due to its single base pair resolution, capability of quantifying reads, and high data output. Thus, NGS has proven to be the method of choice of miRnome profiling.

To that end, QIAGEN has developed the QIAseq miRNA Library Kit for library preparation from isolated total RNA with the miRNeasy kits. First, adapters are ligated to mature miRNA molecules through a 3' hydroxyl group and a 5' phosphate group. Second, reverse transcription takes place while integrating unique molecular indices (UMIs), which can be used to remove PCR duplicates and PCR and sequencing errors. Next, the libraries are completed with 2nd strand cDNA synthesis and index adapter assignment. The workflow includes several optimized size selection steps which helps enrich for RNAs that are 16–40 bases in size and includes a method to limit the incorporation of Y4 RNA, an abundant small RNA species. The library kit has several indexing options with the possibility to combine up to 768 samples in a single sequencing run. Manual library construction takes 6.5 hours, with an additional 2 to 4 hours for library quantification, quality control, and pooling.

In order to make this miRNA library prep workflow more efficient, it has been fully automated on the Opentrons Flex to reduce hands-on time and human error. Here, we describe the automated workflow on the Opentrons Flex with 16 samples.

METHODS

Sample: 100 ng of Human XpressRef Universal Total RNA (QIAGEN Cat. No. 338112) was used to prepare miRNA sequencing libraries.

miRNA sequencing library preparation: miRNA sequencing libraries were prepared manually and by an automated method on the Opentrons Flex using the QIAGEN QIAseq miRNA Library Kit (QIAGEN Cat. No. 331505). Manual library preparation was done following the manufacturer's protocol. Automation on the Opentrons Flex was done using an end-to-end 1-day workflow (https://library.opentrons.com/p/QIAseq-miRNA-48x), capable of processing up to 8 to 48 samples (Figure 1). Libraries were normalized to 4 nM using the QIAGEN QIAseq Normalizer Kit (QIAGEN Cat. No. 180615), Workflow A (https://library.opentrons.com/p/QIAseq-Normalizer-Workflow-A-48x) (Figure 2).

Quality Control: miRNA libraries were assessed for quality and fragment size distributions using the Agilent Tapestation® High Sensitivity D1000 Screentape.

Sequencing: Libraries were sequenced on the Illumina MiSeq® with 2x75 reads.

Data analysis: FASTQ files were analyzed using the QIAGEN GeneGlobe pipeline for miRNA (https://geneglobe.qiagen.com/us/analyze).

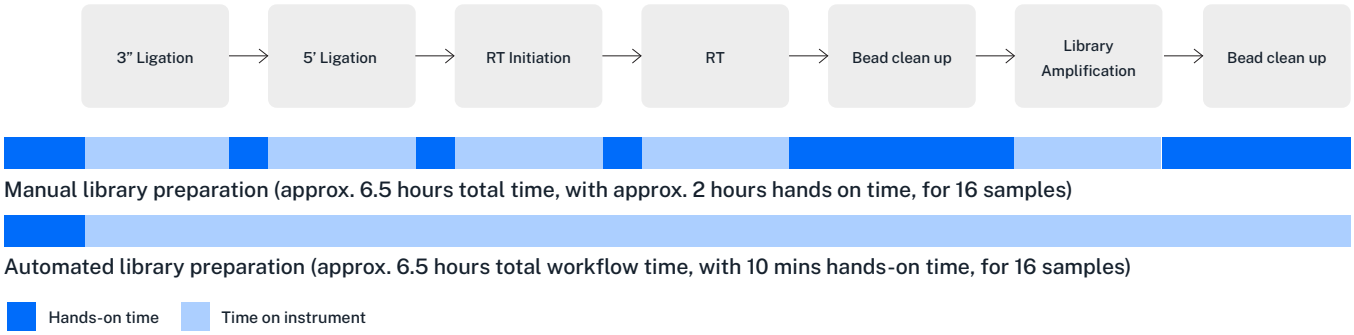

Figure 1. Fully automating the QIAGEN QIAseq miRNA library kit workflow on the Opentrons Flex reduces hands-on time by almost 2 hours. Timings are approximate for 16 sample throughput. Dark blue: hands-on time. Light blue: time on instrument.

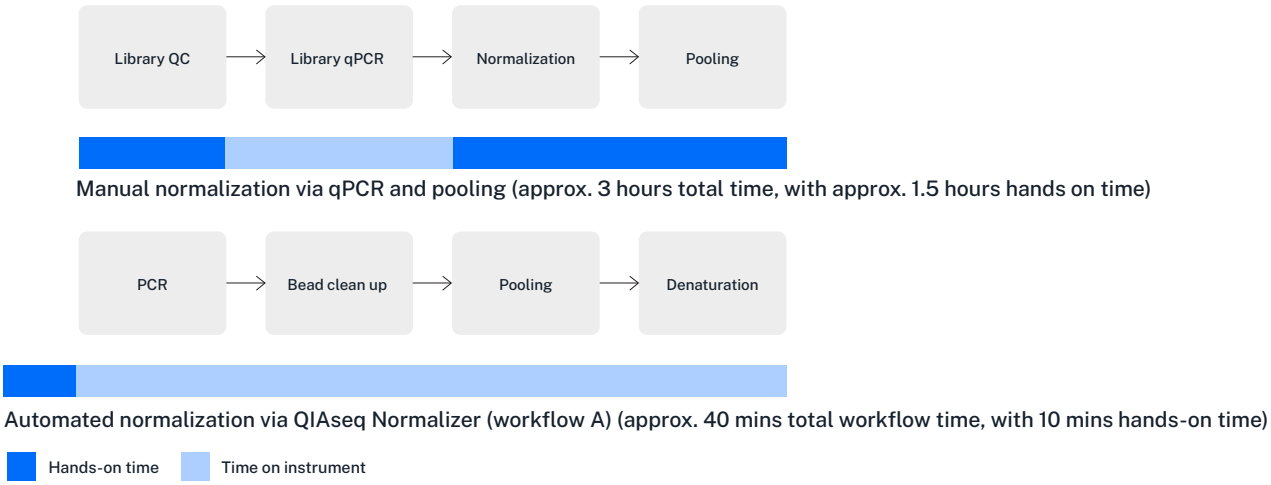

Figure 2. Automating normalization using QIAseq Normalizer reduces total time by approximately 2 hours and hands-on time by 1 hr and 20 minutes relative to normalization via qPCR-based methods. Timings are approximate for 8 sample throughput. Dark blue: hands-on time. Light blue: time in instrument.

RESULTS

Libraries prepared using the QIAGEN QIAseq miRNA Library Kit on the Opentrons Flex were analyzed on the TapeStation HS D1000 to confirm library fragment sizes and quality (Figure 3). The peak sample intensity was ~200 bp and matched the expected library size.

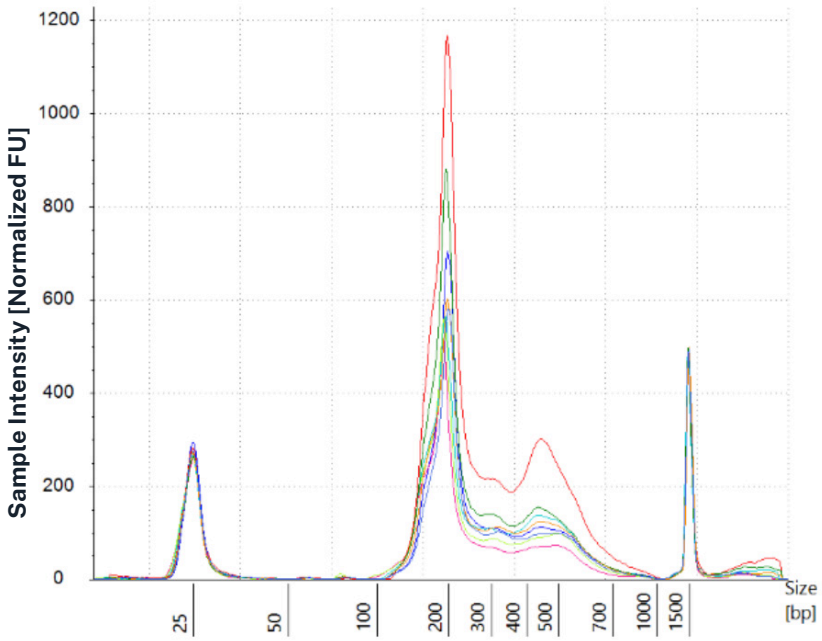

Figure 3. Fragment size distribution of miRNA libraries before normalization. Prepared libraries had the expected library size of ~200 bp with no evidence of adapter dimers.

After sequencing the normalized library, we examined quality control metrics including the total number of UMI reads, the percent of UMI reads annotated, and the Q score of the UMI reads (Table 1) for 16 samples prepared using Flex. On average, samples prepared using Flex had ~5 million UMI reads with 79% of UMI reads mapping to miRBase, a repository for miRNA sequences. The average Q score for UMI reads was 43, indicating over 99.99% base call accuracy. We found that libraries from samples prepared on the Flex had comparable percent of UMI reads annotated to reference data (provided by QIAGEN) and to manual runs performed in the same lab. These results demonstrate that the libraries generated via an automated workflow are of high sequencing quality.

Table 1. Quality control summary of prepared miRNA libraries.

Sample name	UMI reads	UMI reads annotated with miRBase (%)	Avg Q score, UMI reads
QMR_D6NOV22_S1	7,522,623	78.3	44.2
QMR_G7NOV22_S1	5,293,074	79.7	43.4
QMR_G5NOV22_S1	3,797,234	80.8	42.8
QMR_C6NOV22_S1	4,761,398	80.2	42.8
QMR_G6NOV22_S1	3,377,955	80.4	42.3
QMR_C5NOV22_S1	6,437,633	80.7	44.1
QMR_B6NOV22_S1	2,268,332	81.3	41.9
QMR_H5NOV22_S1	5,201,340	76.8	43.4
QMR_H7NOV22_S1	5,471,410	77.2	43.7
QMR_F6NOV22_S1	5,664,287	80.0	44.4
QMR_B5NOV22_S1	5,484,206	80.9	44.1
QMR_E6NOV22_S1	4,184,891	80.1	42.8
QMR_D5NOV22_S1	6,080,570	79.4	43.5
QMR_E5NOV22_S1	6,453,359	79.3	43.1
QMR_F5NOV22_S1	3,511,117	79.5	39.3
QMR_H6NOV22_S1	6,787,429	76.7	43.9

DISCUSSION

Here, we demonstrate an automated method for library prep using the QIAseq miRNA Library Kit on the Opentrons Flex NGS workstation. Libraries by automation produced high-quality results that matched the performance of manual methods, with a significant reduction in hands-on time and touchpoints. Furthermore, when adding the automated library normalization module by QIAseq Normalizer, the total time for library prep and normalization was shortened by approximately 2 hours with more than 3 hours of hands-on time savings. This allows our NGS technicians to complete library preparation in one 8-hour day with only 20 minutes of hands-on time. Libraries that are recovered from the workflow are 4 nM double stranded DNA and are ready to be pooled and sequenced or immediately stored.

While the protocol demonstrated here was designed for up to 48 samples, the Opentrons Flex Workstation can handle a standard 96 well plate format allowing for automation of up to 96 samples per run. This increased throughput is essential for researchers working on high volume sample numbers often seen in biomarker discovery projects.

ORDERING INFORMATION

Equipment, Reagents and Samples	Company	Cat. No. or SKU
Opentrons Flex NGS Workstation (2 x 8-Channel Pipette configuration)	Opentrons	991-00354
QIAseq miRNA Library Kit	QIAGEN	331505
QIAseq Library Normalizer Kit	QIAGEN	180615
Human XpressRef Universal Total RNA	QIAGEN	338112