Application Note

Automated library preparation using the QIAseq® FX DNA Library Kit for Illumina® on the Hamilton® NGS STAR™

Berthold Lechtenberg, Karim Benyaa, Markus Storbeck, Cornelia Mechlen, Alexander Vial and Peter Hahn
QIAGEN GmbH, Hilden, Germany

Introduction

Advanced sequencing platforms such as Illumina's NovaSeq® sequencer have increased sequencing capacities, with their higher speed and improved throughput. Using these platforms, sequencing projects such as large whole genome sequencing can now be completed more economically and are easier than ever before.

While the throughput of sample sequencing has improved markedly, the actual library preparation remains a labor-intensive and time-consuming step in the workflow. The QIAseq FX DNA Library Kit streamlines the NGS library preparation step by integrating enzymatic fragmentation of high-molecular-weight genomic DNA and fragment end-polishing. However, manual processing of multiple samples remains a bottleneck and is prone to variation.

To address this issue, we describe below the automation of the library preparation workflow on the Hamilton NGS STAR platform.

Materials and methods

DNA isolation and purification

E. coli gDNA was isolated from 1 x 10^{11} bacteria using the QIAGEN Genomic-tip 500/G and the Genomic DNA Buffer Set.

Library preparation

For manual library preparation, 25 ng *E. coli* gDNA per sample was processed according to the QIAseq FX library preparation protocol with a fragmentation time of 12 min. For automated library preparation, 25 ng *E. coli* gDNA was processed on the Hamilton NGS STAR using experimental parameters identical to the manual protocol.

Estimation of library yield, fragment size and quality

The QIAseq FX libraries were analyzed on the QIAxcel® Advanced system using the QIAxcel DNA High Resolution Kit. Library quantification was done using the QIAseq Library Quant Assay Kit for Illumina libraries.

Sequencing and sequence analysis

Library pools were loaded onto an Illumina MiSeq® instrument at 10 pM concentration and sequenced using the MiSeq V3 chemistry with 2 x 75 bp paired read length. From each pool, reads were demultiplexed according to all 96 dual sample indices, so that unintended (contaminant) reads from neighboring libraries could also be identified. From these data, the fractions of unintended versus intended reads were calculated for both library pools.

Results

Checking for cross-contamination and reproducibility using an off-deck cycling protocol

In a full 96-plex run, 25 ng *E. coli* gDNA per well was used for automated library preparation using 96-plex dual indexed adapters. Resulting libraries were split in a checkered pattern for sequencing in 2 MiSeq runs. All libraries (wells) marked in purple were pooled for the first run (run 1) and the ones marked in blue were pooled for a second run (run 2) as shown in Figure 1. After sequencing, the resulting reads of both runs were analyzed with particular emphasis on potential cross-contamination with barcodes not present in the respective library pool. Figures 2A and 2B show the sequence analyses for run 1 and run 2, respectively. The overall representation of each of the 48 samples per pool was as expected at around 2%, as shown in the respective box plots (Figure 2). The portion of unintended barcode reads (containing barcodes from wells that were not used in the pool) was very low with the highest level of 0.03% for both runs, revealing virtually no cross-contamination between the individual wells.

Manual versus automated library prep

To compare the automated library preparation protocol with the manual workflow, 48 x 25 ng *E. coli* gDNA were processed using the Hamilton NGS STAR with an integrated on deck cycler (ODTC) and analyzed in parallel with 48 similar samples processed manually.

Comparison of library yield and size

After cleanup, all resulting libraries were analyzed for median library size and yield. The median yield of all 48 libraries prepared using the automated liquid-handling system was 112 nM and identical to the median yield of 127 nM obtained using the manual library preparation (Figure 3A). The two groups showed very similar average fragment sizes – 391 bp for the manual protocol and 413 bp for the automated protocol (Figure 3B).

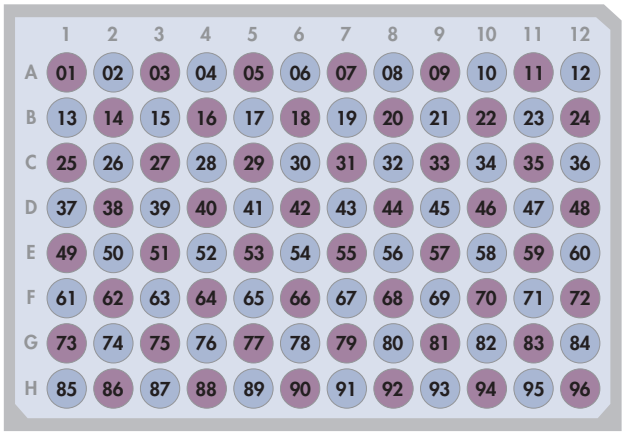

Figure 1. Sequencing setup to detect potential on deck cross-contamination. The 96 libraries were sequenced in two consecutive MiSeq runs. In each of the two sequencing runs, 48 libraries were pooled in a checkered pattern (purple-marked ones for the run 1 and blue-marked ones for the run 2).

Quality of libraries processed on the Hamilton NGS STAR

The capillary gel electrophoretic analysis of liquid-handler prepared libraries is shown for all 48 libraries (see Figure 4). The high uniformity of libraries prepared on the Hamilton NGS STAR, with regards to size as well as final yield, underlines the robustness and reliability of the automation protocol, also for larger sample sizes. The size distribution of all gDNA libraries was very similar (see Figure 4) with only one library showing detectable but still acceptable levels of adapter dimers after final bead purification (see Figure 4, Column 1). Comparing these libraries with those prepared manually as a reference further underscores the equal efficiency of the automated sample processing.

Figure 2. Sequence data analysis measuring potential cross-contamination between neighboring wells on the 96-well reaction plate. A) Box plot representing libraries in the first pool showing an even distribution of the 48 intended libraries and only negligible read numbers for unintended libraries. B) Similar results for libraries in the second pool with most of the reads assigned to intended libraries and only a few reads coming from unintended libraries.

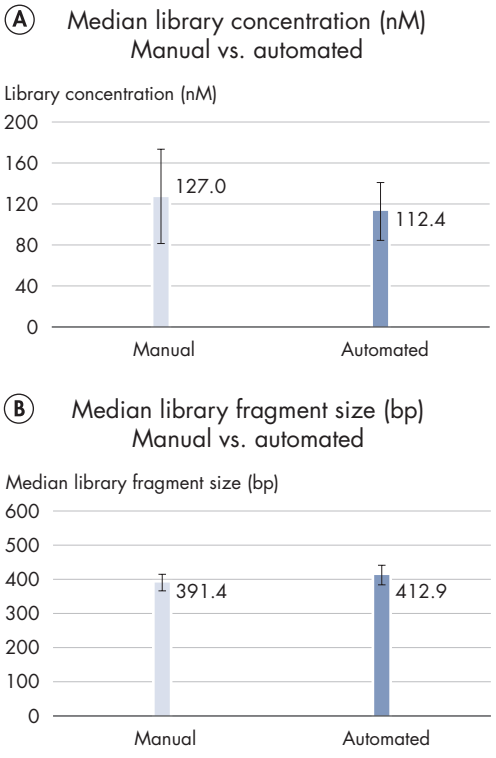

Figure 3. A comparison of library concentration and fragment sizes from 25 ng *E. coli* gDNA – manual vs. automated library prep. A) Median final library yield determined by qPCR shows equal library yields for manual and automated protocols. B) Median library sizes of all libraries were comparable between manual and automated library preps.

Discussion

We demonstrated the successful and reliable automation of the QIAseq FX DNA Library Kit for Illumina on the Hamilton NGS STAR liquid handler. Both configurations – with and without the ODTC module – are capable of generating up to 96 high-quality NGS libraries in parallel. A side-by-side comparison with manually prepared libraries shows the high efficiency of this automated workflow, even when large sample numbers are processed on the same run. Highly sensitive sequencing analysis demonstrated the virtually negligible level of on deck cross-talk between samples during a full 96-plex run.

Taken together, automated library construction using the QIAseq FX DNA Library Kit on the Hamilton NGS STAR enables you to effortlessly prepare up to 96 libraries in parallel, minimizing the risk of variation, inconsistency and potential handling errors arising due to high-throughput manual procedures, thereby streamlining and accelerating sample processing.

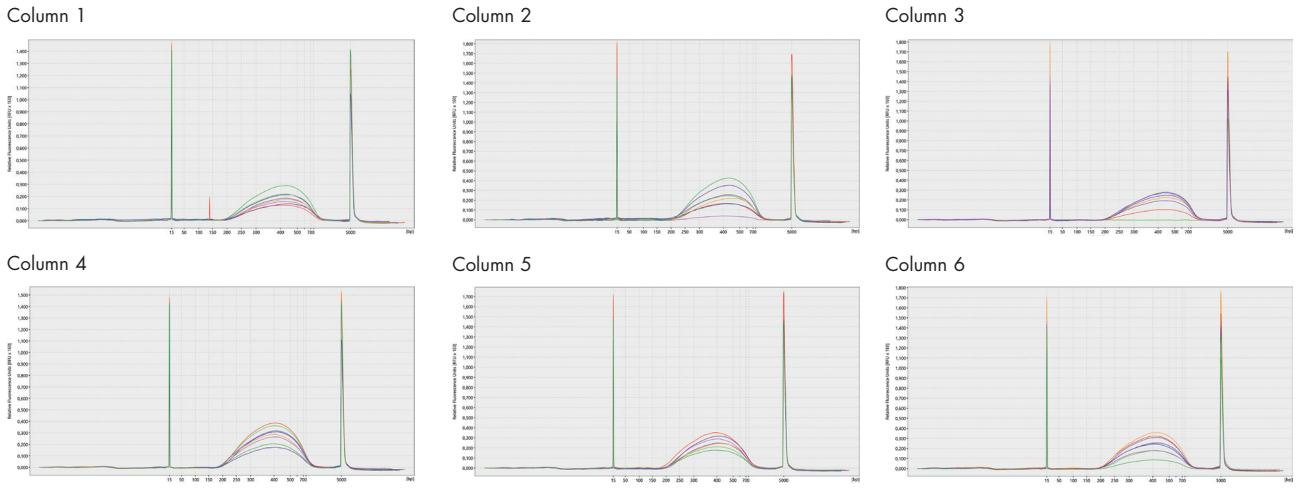

Figure 4. Library quality as shown by capillary gel analysis of liquid-handler prepared libraries. QIAxcel library QC from Hamilton NGS STAR prepared *E. coli* gDNA libraries. Shown here is an overlay of 8 libraries from each of the 6 columns used in the experiment.

Ordering Information

Product	Contents	Cat. no.
QIAseq FX DNA Library Kit (96)	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal, allowing usage of defined parts of plate)	180475
QIAGEN Genomic-tip 500/G	For isolation of up to 500 µg high-molecular-weight DNA from a wide range of samples; 10 columns	10262
Genomic DNA Buffer Set	Buffers, including specific lysis buffers for yeast, bacteria, cells, blood, and tissue: Y1, B1, B2, C1, G2, QBT, QC, QF; for 75 mini, 25 midi, or 10 maxi preps	19060
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, buffers, mineral oil, QX Intensity Calibration Marker, 12-tube strips	929002
QIAseq Library Quant Assay Kit	qPCR assays for quantifying Illumina libraries	QSTF-ILZ

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.