APPLICATION NOTE

Automation of the QIAGEN **QIAseq FX DNA Library Kit** on the Hamilton **NGS STARlet** Generates High-Quality Libraries for Whole-Genome Sequencing

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Introduction

Library preparation is a key requirement for Next-Generation Sequencing (NGS) applications and is among the most expensive segments of the sequencing workflow. It is not only a time-consuming step but can also result in sample loss or lower quality of the output library DNA due to handling errors. To reduce these issues, the streamlined QIAseq FX DNA Library protocol is optimized to perform adapter ligation directly after enzymatic fragmentation without the need for an intermediary clean-up step. Furthermore, the straightforward protocol (consisting of only three steps) ensures the smooth library preparation automation of high-priority samples and low-throughput sample numbers on the Hamilton NGS STARlet (Fig. 1).

- Standardized and reliable sample preparation for small sample throughput and high-priority samples
- Complete walk-away solution with no user interaction
- Streamlined workflow to reduce sample loss and process errors

Figure 1: The Hamilton NGS STARlet Assay Ready Workstation.

Method Description

The QIAGEN QIAseq FX DNA method automates the QIAseq FX DNA Library Kit protocol (HB-2015-004, Version 01/2021) on the NGS STARlet. This method allows for whole genome library preparation for sequencing on Illumina sequencers. The workflow facilitates conversion of 10 - 1000 ng of input DNA into high-quality NGS libraries (Fig. 2).

Visual Workflow

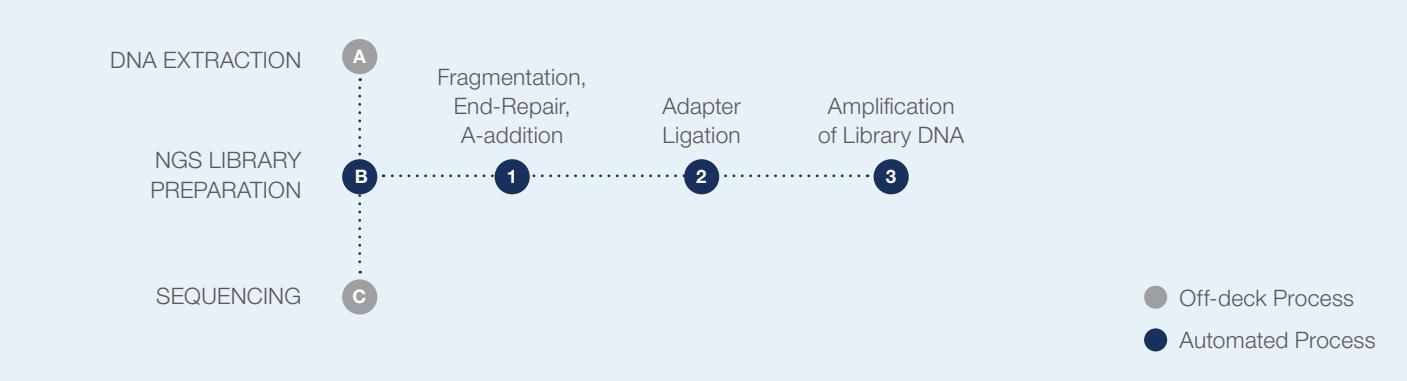
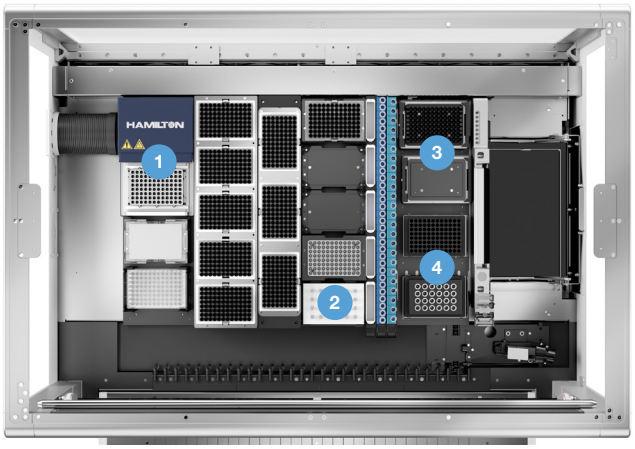


Figure 2: Graphical Overview of the QIAGEN QIAseq FX DNA Workflow.

System Description

The NGS STARlet is based on the Microlab STARlet platform and is equipped with 8 independent 1000 µL pipetting channels. The workspace (Fig. 3) is optimally tuned to generate high-quality DNA libraries for high-priority samples or low-throughput sample numbers. An On-Deck Thermal Cycler, two SBS cooling positions (CPACs), two Heater Shaker Modules (HHSs) and a magnet, together with carriers for tips, reagents and samples create the optimal deck for DNA library preparation with the NGS STARlet.

The NGS STARlet enables fully-automated processing of up to 24 samples, depending on the kit used. This reduces the amount of manual work to a minimum. The correct placement of samples, reagents, plates, and tips is guaranteed using automated barcode verification. In addition, the user can define in-process controls and a worklist with the combination of indexes and samples. The automated error handling and the easy-to-use framework ensure a smooth setup of the workflow, which can also be started and stopped at specific steps within the process.



1. On-Deck Thermal Cycler (ODTC)
2. Magnetic Stand
3. Heater Shaker Modules (HHSs)
4. High-Performance Cooling Modules (CPACs)

Figure 3: Deck Layout of the NGS STARlet.

Qualification Setup and Results

The performance of the QIAGEN QIAseq FX DNA method on the Hamilton NGS STARlet was evaluated by preparing NGS libraries, using the QIAseq FX DNA Library UDI-A Kit (96) (QIAGEN, #180479). Eight samples (including 1 negative control) as well as a maximum number of 24 samples (including eight negative controls) per run with 100 ng Human Genomic DNA (Roche, #11691112001) as input DNA were processed. Runs were conducted using a fragmentation time of eight minutes, as well as six PCR cycles for Library Amplification. The elution volume of the final libraries was 23 µL.

DNA concentration of the libraries obtained from the eight- and 24-sample biological verification runs were determined using the Thermo Fisher Scientific Qubit 4 Fluorometer with the Quant-iT 1x dsDNA HS Assay Kit (Thermo Fisher Scientific, #Q33232). The average sample concentration was 53.7 ng/µL (± 7.1 ng/µL) for the eight-sample run and 37.7 ng/µL (± 3.8 ng/µL) for the 24-sample run.

Subsequently, library size distribution of library DNA generated from both biological verification runs was measured with the Agilent TapeStation 4150 using the High Sensitivity D1000 ScreenTape (Agilent, #5067- 5584) and High Sensitivity D1000 Reagents (Agilent, #5067 5585) (Fig. 4). The average library size was 430 bp (± 11 bp) for the eight-sample run and 454 bp (± 15 bp) for the 24-sample run. TapeStation data from four randomly selected samples from the 24-sample run are depicted exemplarily in Figure 4.

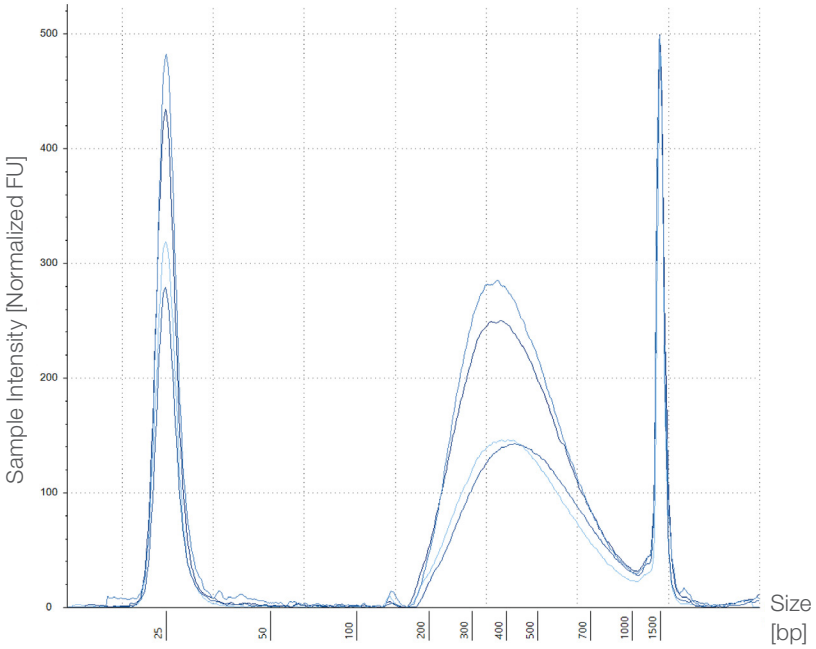


Figure 4: Size distribution of the library DNA generated with the QIAGEN QIAseq FX DNA method. Library size distribution was determined using the TapeStation 4150 with the High Sensitivity D1000 ScreenTape and High Sensitivity D1000 Reagents. Exemplary TapeStation curves from four samples randomly selected from of the 24-sample biological verification run are depicted.

To determine the sequencing metrics, the four libraries randomly selected out of the 24-sample biological verification run were sequenced at the Functional Genomics Center Zürich (FGCZ) on an Illumina NovaSeq 6000 sequencer (2x250 bp, SP Flowcell). Sequencing data was analyzed with the Sushi data analysis framework (Hatakeyama et al., BMC Bioinformatics: 17, 2016), using Bowtie 2 (v2.4.2) to align the sequencing reads to the human reference genome (GRCh38.p13) and estimate mapping rates (Figure 5).

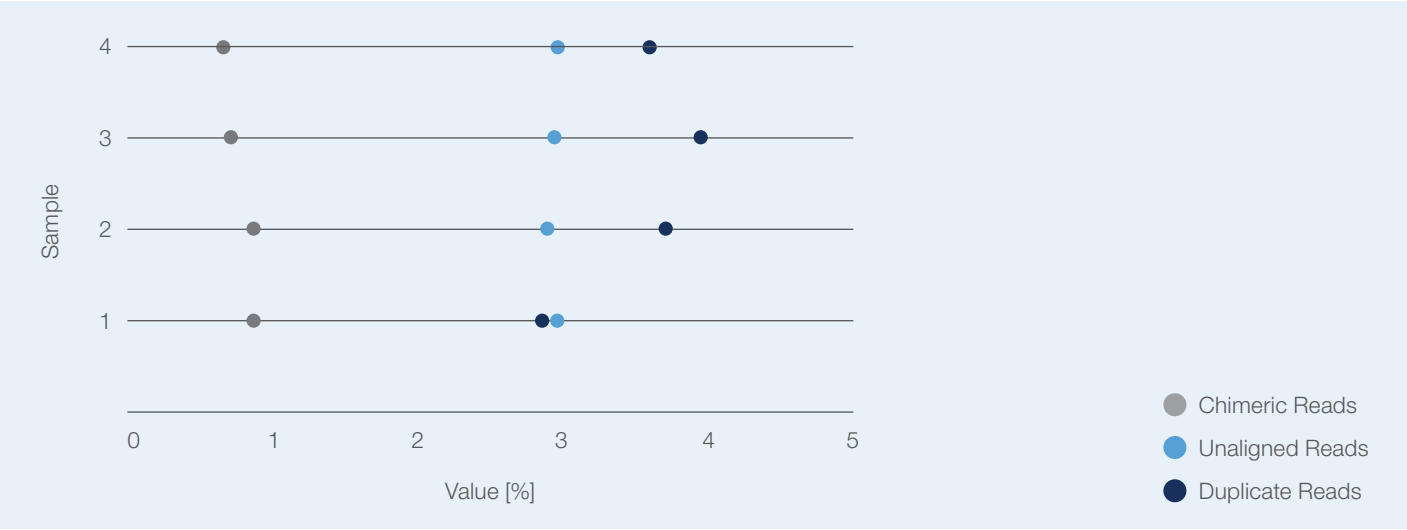


Figure 5: Mapping quality metrics of the library DNA generated with the QIAGEN FX DNA method. Library DNA generated from four samples out of the 24-sample biological verification run was sequenced with the Illumina NovaSeq 6000 sequencer at the Functional Genomics Center Zürich (FGCZ).

All four libraries displayed a high mapping rate of 97.07% (± 0.03%). Furthermore, they contained a low percentage of chimeric reads of 0.77% (± 0.09%), an average duplication rate of 3.53% (± 0.40%), and a low proportion of unaligned reads of 2.93% (± 0.07%).

Others

System Requirements	Provider	Part Number
NGS STARlet Base + Deck Components	Hamilton Bonaduz AG	806610
Adapter MIDI Plate	Hamilton Bonaduz AG	10087668

Labware Requirements	Part Number	Provider
50 µL CO-RE Filter Tips	Hamilton Bonaduz AG	235948
300 µL CO-RE Filter Tips	Hamilton Bonaduz AG	235903
1000 µL CO-RE Filter Tips	Hamilton Bonaduz AG	235905
PCR ComfortLid	Hamilton Bonaduz AG	814300
PCR FramePlate 96-well	Hamilton Bonaduz AG	814302
20 mL Reagent Reservoirs	Hamilton Bonaduz AG	96424-02
60 mL PP Reagent Trough with Lid	Hamilton Bonaduz AG	56694-01
Abgene 96-Well 0.8 mL Polypropylene Deep-Well Storage Plate	Thermo Fisher Scientific	AB0859
0.5 mL Screw Cap Micro Tubes	Sarstedt	72.730.006
2 mL Screw Cap Micro Tubes	Sarstedt	72.694.406
5 mL Screw Cap Micro Tubes	Sarstedt	62.611

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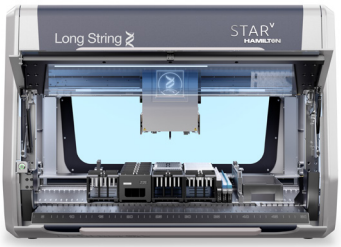
- More than 15 biologically-verified nucleid acid extraction protocols from leading kit vendors
- NOW available: Circulomics HMW DNA extraction application
- Configurable loading area for 96 samples

NGS STAR

- Tried and true performance for many years
- Proven design, including qualified methods from many kit providers
- Upgradable to on-deck thermal cycling and higher throughput needs



Long String STAR V

- Automated walk-away UHMW DNA extraction
- Optimal magnetic disk handling via Hamilton MagRod technology
- Scalable preparation of UHMW DNA for Bionano OGM for
  increased number of samples

NGS STAR V

- Temperature control devices meet reagent and sample needs
- Maximum process reliability via intelligent safety lights
- High-throughput library preparation with respect to usability, reproducibility, traceability, and safety needs

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