Application Note

Automation of the **QIAseq FX DNA Library Method** on the HAMILTON **NGS STAR V**

Authors: Kristina Ueffing[1], Anna Wachnowicz[1], Alexandra Avci[1], Cornelia Mechlen[2], Berthold Lechtenberg[2], Alexander Vial[2], Peter Hahn[2], Christian Vogler[1], Birgit Ottenwälder[1], Dominik Laubscher[1]

1 Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland
2 QIAGEN GmbH, Qiagen Strasse 1, 40724 Hilden, Germany

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 intermediate clean-up step. Furthermore, the straightforward protocol (consisting of only three steps) ensures the smooth library preparation automation of high-throughput sample numbers on the Hamilton NGS STAR V (Fig. 1).

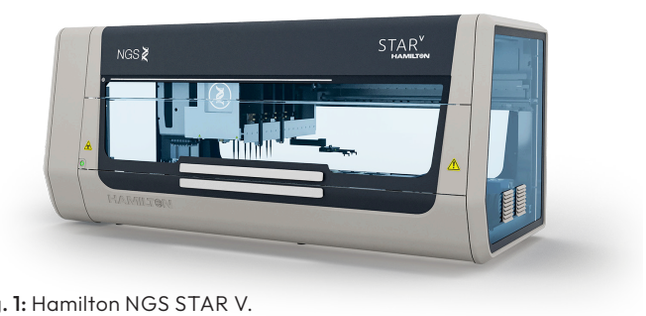


Fig. 1: Hamilton NGS STAR V.

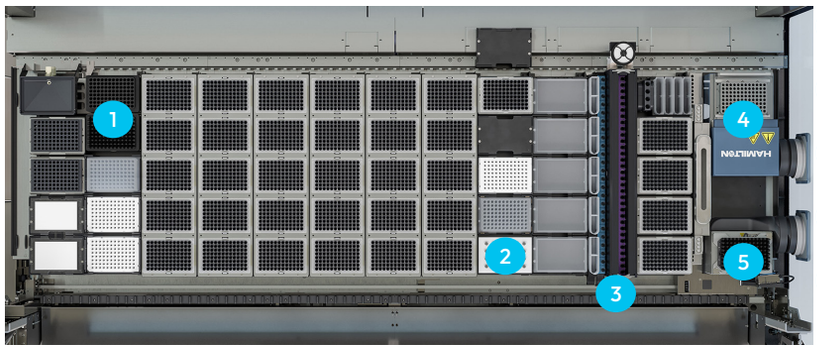
- Increased throughput – process up to 96 samples in parallel
- Enhanced reproducibility – minimized manual variability and human error
- Time efficiency – reduced hands-on time and more walk-away time
- Scalability – meet the growing sequencing demands without additional labor
- Automated barcode verification ensures error-free setup and traceability

System Description

The NGS STAR V (Fig. 2) is optimally equipped to generate high-quality NGS libraries even for the most technically demanding protocols. The Cooling Carrier Module, the optional On-Deck Thermal Cycler (ODTC), and two SBS cooling positions (CPACs) as well as a Heater Shaker module, ensure optimal temperature-controlled sample handling. A Magnetic Stand and Tip Carriers, together with carriers for samples and reagents complete the ideal deck for NGS library preparation on the NGS STAR V.

Technology

The method uses the 96 Multi-Probe Head to simultaneously perform up to 96 clean-ups, significantly reducing the time required for this procedure and reducing any column-based effects. Additionally, the method utilizes the capacitive Liquid Level Detection (cLLD) technology of Hamilton CO-RE Tips. Any qualified method for NGS library preparation can be leveled-up by integrating on-deck fluorescence-based nucleic acid quantification and normalization. Several integration concepts are available on the Hamilton NGS STAR V.



1 High Performance Cooling Modules (CPACs)
2 Magnetic Stand
3 Linear Cooling Carrier
4 On-Deck Thermal Cycler (ODTC)
5 Heating-Shaking Module

Fig. 2: Deck Layout of the NGS STAR V.

Method Description

The "QIAGEN QIAseq FX DNA Library Kit" method automates the QIAseq FX DNA Library Kit protocol (HB-2015-005, version 10/2023) on the NGS STAR V. 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. 3).

Visual Workflow

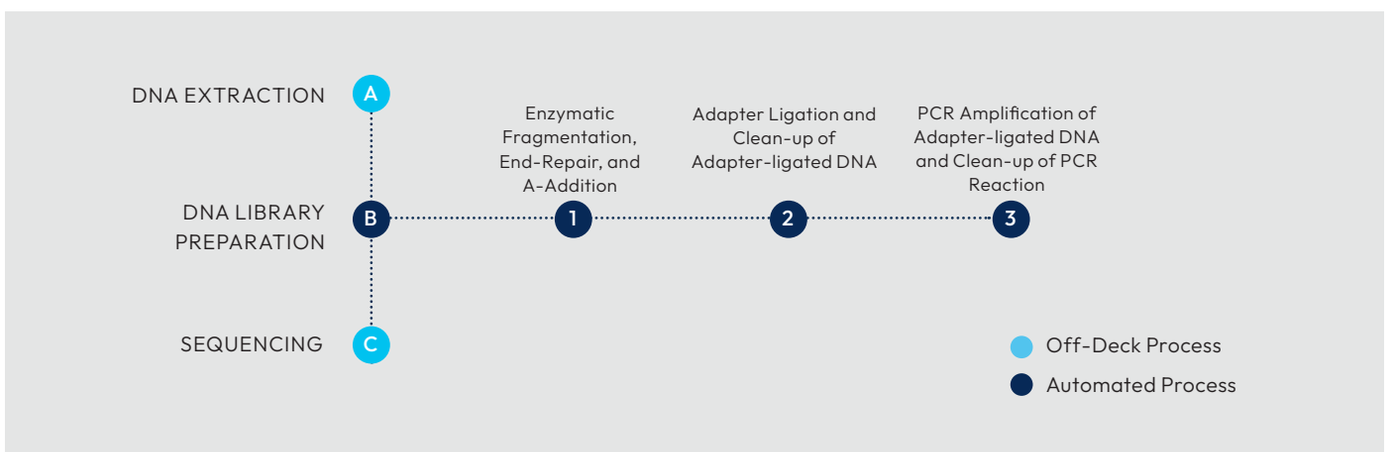


Figure 3: Graphical Overview of the QIAseq FX DNA Library Method Workflow.

Enhancement Option

Add the Hamilton FLUOREYE (Fig. 4) for even more walk-away time.

Figure 4: The Hamilton FLUOREYE.

Biological Qualification Results

The performance of the QIAGEN QIAseq FX DNA method on the Hamilton NGS STAR V was evaluated by preparing NGS libraries, using the QIAseq FX DNA Library Kit with this is repetitive and outlined below for the different samples Buffer EB. Three biological runs were conducted; two with eight samples using *E. coli* gDNA (DH10b) and either QIAseq Beads or Agencourt AMPure XP Beads (including one negative control), and one run with 96 samples using microbial community composition ATCC MSA-1003 (20 strains), ATTC MSA-1001 (10 strains) and *E. coli* gDNA (DH10b) and QIAseq Beads (including 12 negative controls). A fragmentation time of 10 min and seven PCR cycles was applied during the runs.

For biological qualification of the library preparation, final library concentration was determined with the QuantIT 1X dsDNA HS Assay Kit. Subsequently, library size distribution of all libraries of the eight-sample run,

as well as 15 samples of the 96-sample biological verification runs were analyzed with the Agilent Tape-Station 4150, using the D5000 ScreenTape with D5000 Reagents. Results are depicted in Table 1.

Table 1: Summary of Library Size and Yields.

Sample Number	8	8	96
Bead Type	AMPure XP	QIAseq	QIAseq
Input DNA Type	*E. coli*	*E. coli*	MSA-1003, MSA-1001, *E. coli*
Input DNA Amount [ng]	55	55	55
Insert Size [bp]	300	300	300
Library Yield [ng ± SD]	1239 ± 196	1135 ± 102	1197 ± 167
Library Size [bp ± SD]	422 ± 12	426 ± 13	458 ± 12

Out of all the libraries generated in the biological verification runs, 15 libraries were selected to represent three distinctive DNA types used to prepare the libraries. Sequencing of the libraries was performed at QIAGEN GmbH (Hilden, Germany) on an Illumina MiSeq, V2 cartridge (Paired-End 2x149 bp). Sequencing data was analyzed using proprietary QIAGEN software. On average, over 2.9 ± 0.7 million reads were generated per sample. 91.10% of the clusters passed filter, and 94.54% of these with quality score Q30 or higher. The mean paired distance was 235 bp (± 5 bp).

Furthermore, sequencing metrics of all 15 libraries were determined. The mapping rates and quality metrics are listed in Table 2 and depicted in Figure 5.
All 15 library DNA samples displayed high mapping rates, indicating high uniformity within the DNA type, and independent of plate position and bead type.

Table 2: Summary of Mapped Reads.

DNA Type	Mapped Reads [%± SD]
MSA-1003	92.2 ± 0.1
MSA-1001	87.2 ± 0.9
E. coli	99.8 ± 0.1

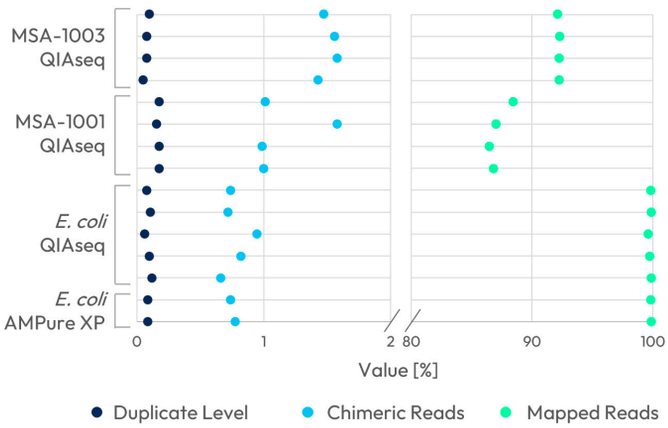


Figure 5: Mapping quality metrics of the library DNA generated with the QIAseq FX DNA Library Kit method.

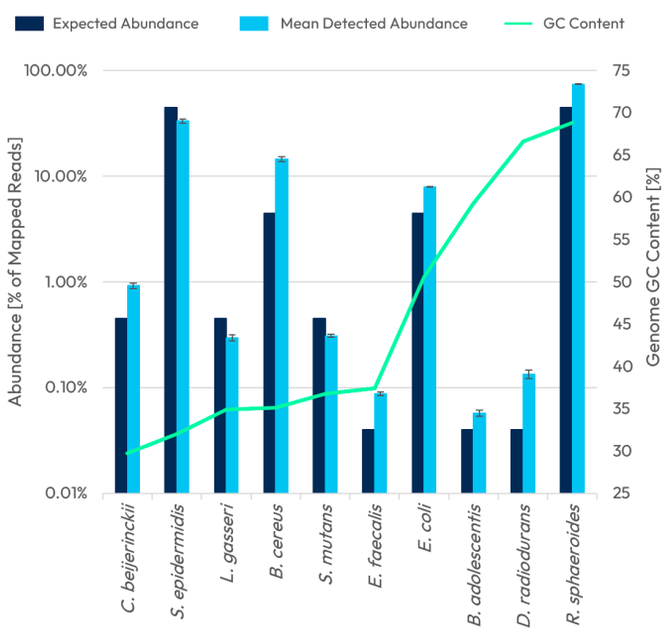


Figure 6: Microbial Community Composition ATCC MSA-1001 (10 Strains).

In addition to analyzing the quality metrics of the generated DNA libraries, the microbial community composition DNA ATCC MSA-1001 (10 strains) was performed. Results reveal reliable strain detection for species abundance as low as 0.02%, independent of the species' genomic GC content (Fig. 6).

In conclusion, the automation of the QIAseq FX DNA Library Kit on the Hamilton NGS STAR V provides a highly efficient and reliable solution for high-throughput NGS library preparation. By minimizing hands-on time and human error, the streamlined workflow ensures consistent library quality while meeting the demands of diverse sequencing applications. With its robust performance demonstrated across various sample types and library sizes, the integration of this automated system is a significant step forward in optimizing NGS workflows for laboratories of all scales.

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Requirements		
System Requirements	**Provider**	**Part Number**
NGS STAR V Product Line + ODTC Option	Hamilton Bonaduz AG	870021
2nd PCR Plate Adapter	Hamilton Bonaduz AG	10087667
Huber Mini Chiller Olé 280 for Cooling Carrier	Huber	3006.0105.98
Huber Tubing Adapter NW8	Huber	6086
Labware Requirements		
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 Container, natural color, no lid	Hamilton Bonaduz AG	10161052
60 mL PP Reagent Trough with Lid	Hamilton Bonaduz AG	56694-01
2 mL Screw Cap Micro Tubes	Sarstedt	72.694.006
Abgene 96-Well 0.8 mL Polypropylene DeepWell Storage Plate	Thermo Fisher Scientific	AB0859
Method Requirements		
QIAseq FX DNA Library Kit with UDI Y-Adapter Kit A	QIAGEN	180488
QIAseq FX DNA Library Kit with UDI Y-Adapter Kit B	QIAGEN	180489
QIAseq FX DNA Library Kit with UDI Y-Adapter Kit C	QIAGEN	180490
QIAseq FX DNA Library Kit with UDI Y-Adapter Kit D	QIAGEN	180491
QIAseq Beads	QIAGEN	333927
Buffer EB	QIAGEN	19086
AMPure XP Reagent	Beckman Coulter	A63880
Qubi 1X dsDNA High Sensitivity Assay Kit	Thermo Fisher Scientific	Q33266
D5000 ScreenTape	Agilent	5067- 5588
D5000 Reagents	Agilent	5067-5589

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