Application Note

Automation of the **QIAGEN QIAseq Targeted DNA Pro Panels** on the Hamilton **NGS STAR MOA** Generates High-Quality Libraries for **Targeted Next-Generation Sequencing**

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Introduction

Library preparation is essential for Next-Generation Sequencing (NGS) and one of the costliest steps in the workflow. It is time-consuming and prone to sample loss or reduced DNA quality due to handling errors. Furthermore, efficient genetic testing using targeted NGS applications demand precise detection of genetic variations, such as somatic mutations, single nucleotide polymorphisms, copy number variation, and small insertions/deletions, in defined genomic regions. The QIAseq Targeted DNA Pro Panels provide a powerful and flexible solution for targeted DNA sequencing. With their optimized workflow, the QIAseq Targeted DNA Pro Panels ensure smooth automation of library preparation with high-sample throughput on the Hamilton NGS STAR MOA (Fig. 1).

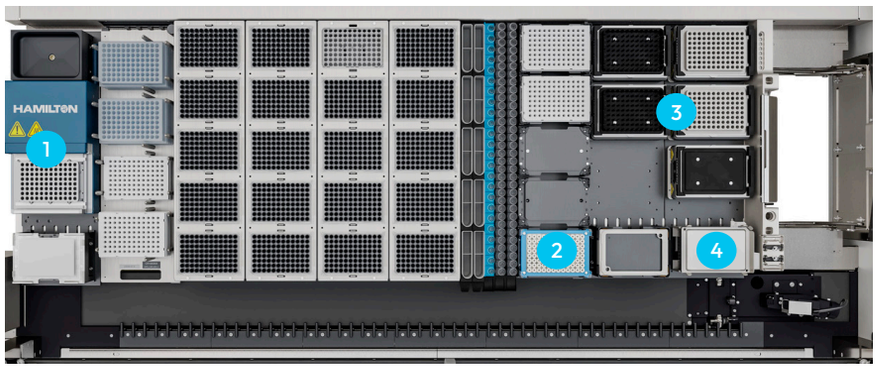
- Increased throughput – process up to 96 samples in parallel
- Enhanced reproducibility – minimized manual variability and human error
- Time efficiency – reduced hands-on time and more walk-away time
- Streamlined workflow – reduced sample loss and fewer process errors
- Automated barcode verification ensures error-free setup and traceability

Fig. 1: Hamilton NGS STAR MOA.

System Description

The NGS STAR MOA (Fig. 2) is designed for high-quality NGS library preparation in high-throughput workflows. The On-Deck Thermal Cycler (ODTC), one ANSI/SLAS cooling position (CPAC), and the Heater Shaker modules (HHSs) ensure optimal sample handling. A magnetic stand and tip carriers, together with carriers for samples and reagents, complete the ideal deck of the NGS STAR for NGS library preparation.

The NGS STAR MOA enables the fully-automated processing of up to 96 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 by using automated barcode verification. In addition, the user can define a worklist with the combination of indexes and samples. Automated error handling and the easy-to-use software 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 (CPAC)

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

Method Description

The QIAseq Targeted DNA Pro Panels - MOA v1.0 (further referred to as QIAseq Targeted DNA Pro Panels) method automates the QIAseq Targeted DNA Pro protocol (document 12/2022 HB-2979-002) on the NGS STAR MOA. The method covers the library preparation and the enrichment processes. It facilitates targeted NGS library preparation for sequencing on Illumina sequencers and allows for the processing of a maximum of 96 samples per run, with an input amount of 10 - 80 ng gDNA or cfDNA or 100 - 250 ng FFPE DNA (Fig. 3).

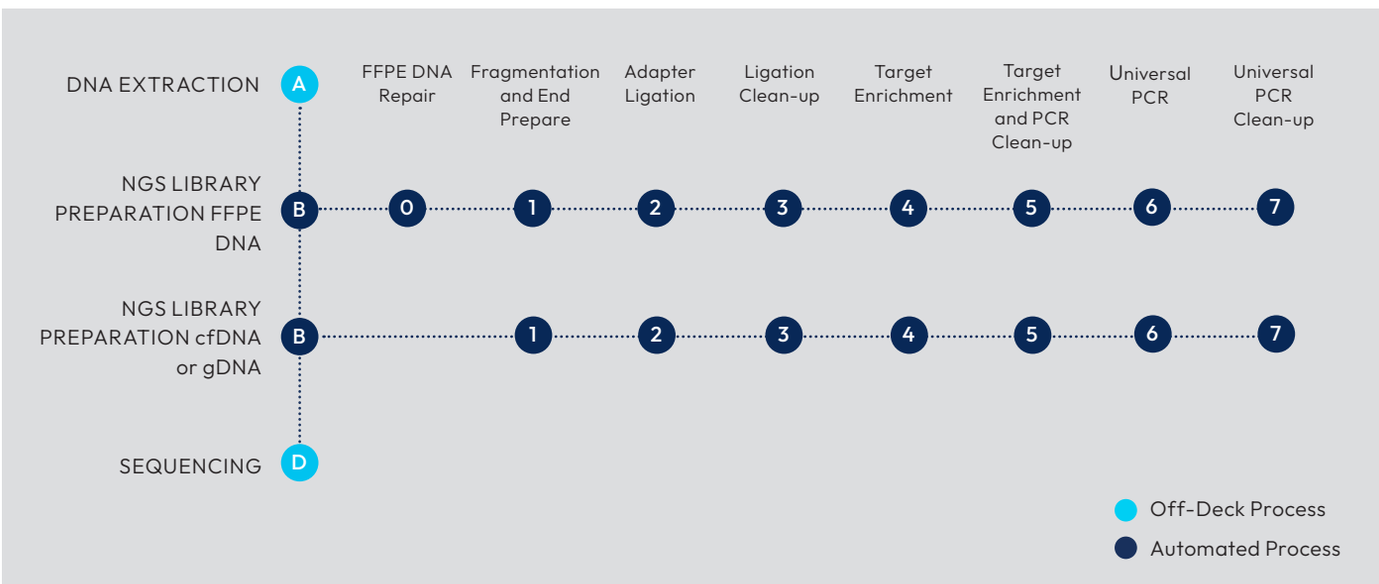


Fig. 3: Graphical Overview of the QIAseq Targeted DNA Pro Panel Method Workflow.

Enhancement Option

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

Fig. 4: The Hamilton FLUOREYE

Biological Qualification Results

To assess the biological performance of the QIAseq Targeted DNA Pro Panels method on the NGS STAR MOA, library preparation of 96 human genomic DNA samples was executed using a custom panel with 4110 primers. As input DNA, five distinct genotypes with two different input amounts each (10 ng and 40 ng) were used (see Method Requirements at the end of the Application Note). The run was conducted using 8 PCR cycles for Target Enrichment and 25 PCR cycles for Universal PCR. DNA concentration and total yield of the libraries obtained from the 96-sample biological verification run were determined using the Thermo Fisher Scientific Qubit 4 Fluorometer with the Quant-iT 1x dsDNA HS Assay Kit. Size distribution of library DNA was measured with the Agilent TapeStation 4150 using the High-Sensitivity D5000 ScreenTape with High-Sensitivity D5000 Reagents (Table 1).

Out of the generated libraries from the 96-sample biological verification run, three libraries per DNA type and input amount were selected for sequencing. They were sequenced using the NovaSeq6000 S1 Reagent Kit v1.5 on an Illumina NovaSeq 6000 at QIAGEN GmbH (Hilden, Germany). Sequencing data was analyzed by a QIAGEN specialist using CLC Genomics Workbench 24.0 and smCounter2. On average, each sample generated over 59 million (±7 million) reads, with 95% of clusters passing the filter.

Tab. 1: Summary of Library Size and Yields and Sequencing Results.

DNA Type	HD734		HD752		HD827		HD829		NA12878	
Input DNA Amount [ng]	10	40	10	40	10	40	10	40	10	40
Insert Size [bp]	200 - 1000									
Sample Number	6	6	6	6	6	6	6	6	24	24
Library Yield [ng ± SD]	106 ± 18	174 ± 52	82 ± 41	189 ± 46	107 ± 49	226 ± 50	76 ± 7	139 ± 78	87 ± 27	250 ± 148
Library Size [bp ± SD]	472 ± 16	466 ± 20	467 ± 30	469 ± 18	477 ± 39	464 ± 14	465 ± 27	449 ± 30	479 ± 23	479 ± 37
Total Number of Reads [Mio ± SD]	57 ± 4	59 ± 8	60 ± 4	62 ± 10	51 ± 6	66 ± 9	49 ± 9	63 ± 7	51 ± 16	71 ± 9
Mean Read Fragments per UMI [± SD]	8.6 ± 0.5	5.8 ± 2.3	11.1 ± 2.4	6.3 ± 1.3	9.1 ± 0.6	4.8 ± 0.9	13.3 ± 1.4	6.2 ± 1.1	12.3 ± 4.3	4.5 ± 0.6
Specificity [% ± SD]	79.8 ± 0.6	81.3 ± 3.7	77.7 ± 0.7	81.0 ± 1.9	79.9 ± 1.7	82.6 ± 1.5	77.0 ± 2.2	81.3 ± 1.0	77.5 ± 3.2	84.0 ± 0.4

The accuracy of Variant Allelic Frequency (VAF) estimation for HD827, HD829, and HD734 gDNA libraries depicts a strong match between expected and detected frequencies (Fig. 5 and Fig. 6). Libraries made from 10 ng and 40 ng input DNA performed consistently well. This accuracy highlights the method's ability to detect important variants, even with low DNA input, proving the workflow's reliability in various experiments.

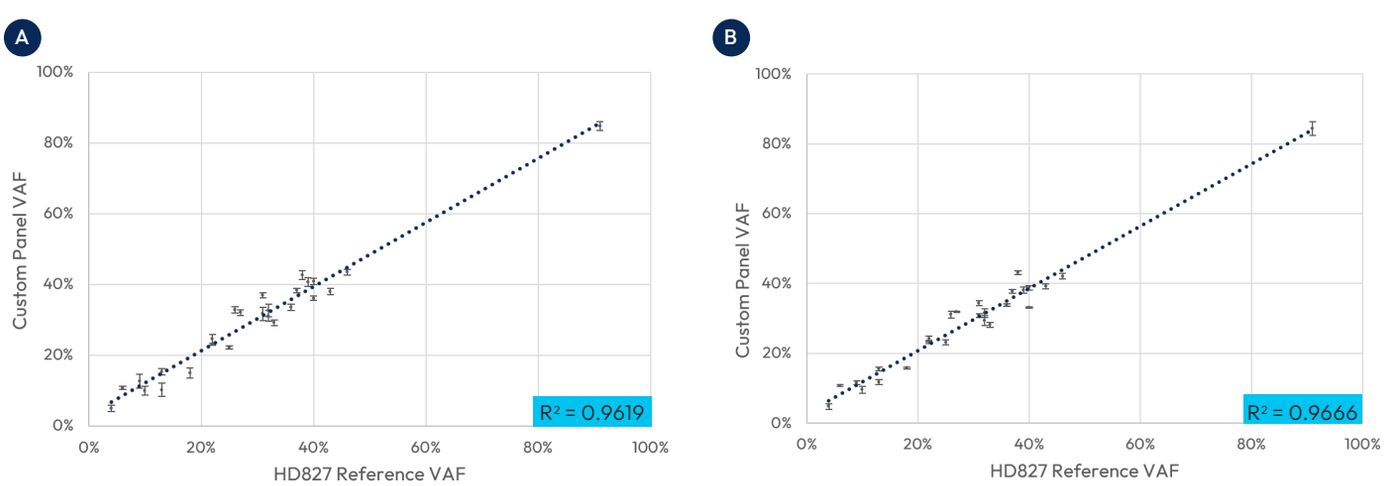


Fig. 5: Accuracy of Variant Allelic Frequency (VAF) estimation for (A) 10 ng and (B) 40 ng HD827 gDNA NGS libraries.

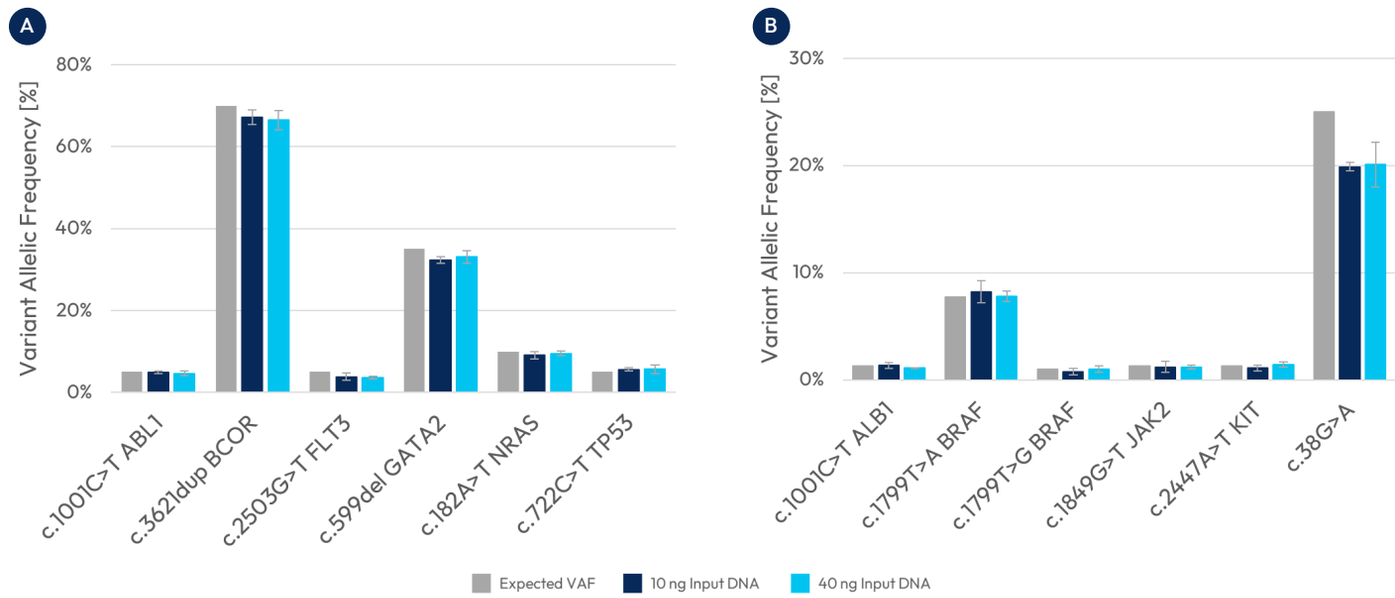


Fig. 6: Accuracy of Variant Allelic Frequency (VAF) estimation of selected mutations of the for (A) HD829 and (B) HD734 gDNA NGS libraries.

In conclusion, automating the QIAseq Targeted DNA Pro Panels method on the Hamilton NGS STAR MOA offers an efficient and reliable solution for high-throughput NGS library preparation. It reduces hands-on time and errors, ensuring consistent library quality for various sequencing applications. This system's robust performance across different samples and library sizes optimizes NGS workflows for all lab scales.

All Hamilton Consumables can be ordered from our Web Shop.

HAMILTON'S CONSUMABLES
WEBSHOP
Web: eshop.hamilton.com

Requirements		
System Requirements	**Provider**	**Part Number**
NGS STAR MOA / NGS STAR MOA UV incl. Deck Components	Hamilton Bonaduz AG	806800
ODTC 96	Hamilton Bonaduz AG	10147734
iSWAP	Hamilton Bonaduz AG	190220
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
0.5 mL Screw Cap Micro Tubes	Sarstedt	72.730.006
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		
CEPH1463	Coriell Institute	NA12878
Myeloid DNA Reference Standard	Horizon Discovery	HD829
OncoSpan gDNA	Horizon Discovery	HD827
Tru-Q 7 (1.3% Tier) Reference Standard	Horizon Discovery	HD734
Tru-Q 0 (100% Wildtype) Reference Standard	Horizon Discovery	HD752
QIAseq Targeted DNA Pro (96)	QIAGEN	333655
QIAseq Targeted DNA Pro HC (96)	QIAGEN	333665
QIAseq Targeted DNA Pro UDI Set A	QIAGEN	333455
QIAseq Targeted DNA Pro UDI Set B	QIAGEN	333465
QIAseq Targeted DNA Pro UDI Set C	QIAGEN	333475
QIAseq Targeted DNA Pro UDI Set D	QIAGEN	333485
High Sensitivity D5000 Reagents	Agilent	5067-5593
High Sensitivity D5000 ScreenTape	Agilent	5067-5592
NovaSeq6000 S1 Reagent Kit v1.5	Illumina	20028317
QuantIT 1X dsDNA HS Assay Kit	Thermo Fisher Scientific	Q33232

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