Application Note

HAMILTON

Automation of the **QIAseq Targeted DNA Pro Panel** Method on the HAMILTON **NGS STAR V**

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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. Furthermore, 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 Panel Kit provides a powerful and flexible solution for targeted NGS of DNA. With its optimized workflow, the QIAseq Targeted DNA Pro Panel Kit ensures smooth automation of library preparation with high sample throughput 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 growing sequencing demands without additional labor
- Automated barcode verification ensures error-free setup and traceability

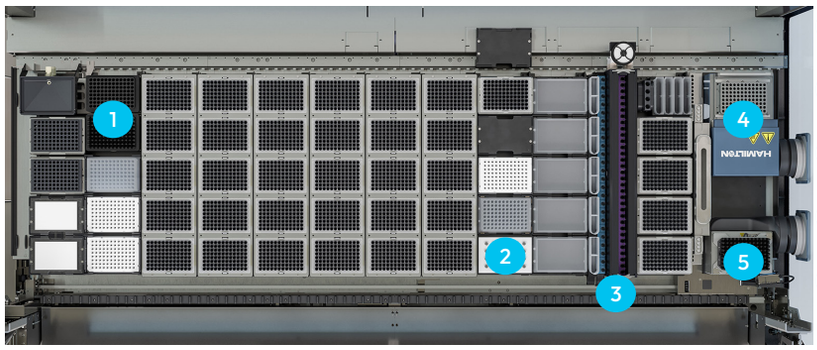
System Description

The NGS STAR V workspace (Fig. 2) is optimally equipped to generate high-quality NGS libraries, also for highly demanding protocols. The Cooling Carrier Module, the optional On-Deck Thermal Cycler (ODTC), and two SBS cooling positions (CPACs) as well as a heating-shaking 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 of 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.

QIAGEN



1 High Performance Cooling Module (CPAC)
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 QIAseq Targeted DNA Pro 2.0 method, including library preparation and enrichment workflow, automates the QIAGEN QIAseq Targeted DNA Pro Kit for ultrasensitive targeted next-generation sequencing of DNA for Illumina NGS systems (HB-2979-002, version 12/2022) on the NGS STAR V (Fig. 3). This method allows for targeted NGS library preparation of 96 samples and up to 20 panels per run with an input amount of 10 – 80 ng genomic DNA or cfDNA or 250 ng FFPE DNA per sample.

Visual Workflow

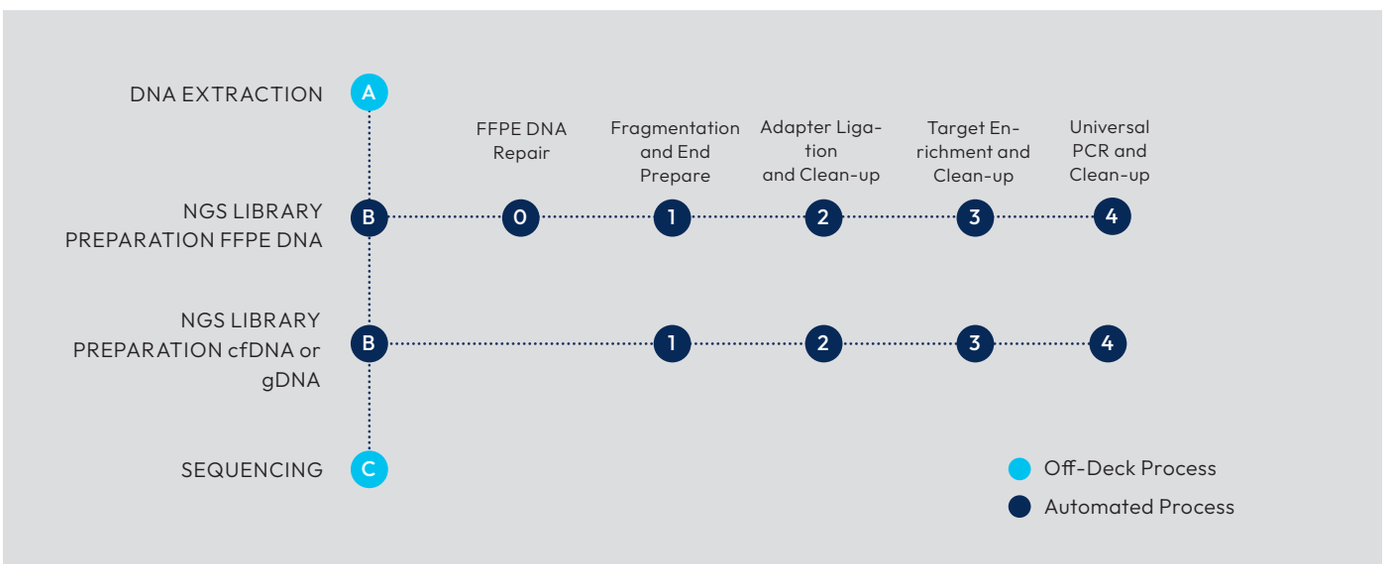


Figure 3: Graphical Overview of the QIAseq Targeted DNA Pro Panel Method Workflow.

Enhancement Option

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

Figure 4: The Hamilton FLUOREYE.

Biological Qualification Results

The performance of the QIAseq Targeted DNA Pro 2.0 method on the Hamilton NGS STAR V was evaluated by preparing NGS libraries using the QIAseq Targeted DNA Pro Kit. A biological verification run with 96 samples (including 12 negative controls) was conducted using 10 ng or 40 ng of human genomic DNA (Horizon Onco-Span HD827 or NA12878) as input DNA. Lung Cancer Research Panel (PHS-005Z) was used for library preparation of 10 ng and 40 ng input DNA samples, Breast Cancer Research Panel (PHS-001Z) was used for library preparation of 40 ng input DNA samples. No size selection was performed. For biological qualification of the library preparation, final library concentration was determined with the QuantIT 1X dsDNA HS Assay Kit.

Library size distribution of randomly selected samples of the 96-sample biological verification run was analyzed with the Agilent TapeStation 4150, using the D5000 ScreenTape with D5000 Reagents. Results are depicted in Table 1.

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

DNA Type	OncoSpan (HD827)	OncoSpan (HD827)	OncoSpan (HD827)	NA12878	NA12878	NA12878
Input DNA Amount [ng]	10	40	40	10	40	40
Panel	PHS-005Z Lung Cancer	PHS-005Z Lung Cancer	PHS-001Z Breast Cancer	PHS-005Z Lung Cancer	PHS-005Z Lung Cancer	PHS-001Z Breast Cancer
Insert Size [bp]	50 - 800	50 - 800	50 - 800	50 - 800	50 - 800	50 - 800
PCR Cycles	26	26	26	26	26	26
Sample Number for Library Yield Analysis	3	3	3	18	18	18
Library Yield [ng ± SD]	277 ± 32	552 ± 68	503 ± 21	191 ± 72	635 ± 84	589 ± 133
Sample Number for Library Size Analysis	3	3	3	5	6	5
Library Size [bp ± SD]	430 ± 9	426 ± 12	439 ± 9	469 ± 17	457 ± 33	467 ± 19
Sample Number for Sequencing	3	3	3	2	2	2
Total Number of Reads [Mio ± SD]	19.0 ± 4.9	23.8 ± 1.7	22.0 ± 5.1	15.4 ± 0.6	22.3 ± 4.2	27.2 ± 0.5
Read Fragments with Primer Found [Mio ± SD]	8.1 ± 2.9	13.5 ± 1.9	9.9 ± 2.3	7.7 ± 1.5	12.5 ± 3.2	12.9 ± 2.1
Mean Primer UMI Depth [± SD]	481 ± 280	1601 ± 181	1403 ± 191	236 ± 85	1991 ± 359	1952 ± 189
Mean Read Fragments per UMI [± SD]	7.1 ± 1.4	3.3 ± 0.3	2.8 ± 0.8	13.1 ± 2.1	2.5 ± 0.2	2.6 ± 0.1

Out of the generated libraries from the 96-sample biological verification run, 15 libraries were selected for sequencing. They were sequenced at QIAGEN GmbH (Hilden, Germany) on an Illumina NextSeq, HighOutput cartridge (Paired-End 2x149 bp). Sequencing data was analyzed by a QIAGEN specialist using QIAGEN CLC Genomics Workbench 24.0 software, and UMI-aware variant caller smCounter2 (Table 1). On average, over 21.6 million (± 4.6 million) reads were generated per sample and 93,7% of clusters passed filter. The uniformity of sequencing depth further supports the workflow's efficiency and reliability. Additionally, the smCounter2 UMI-aware variant caller effectively identified variants, highlighting the utility of this method for targeted sequencing applications.

Variant calling capacity results demonstrate a strong detection capability for well-defined and low-frequency variants, achieving ~90% sensitivity for 1% minor allele frequencies (MAFs) with 40 ng DNA inputs. This confirms the suitability of the workflow for high-sensitivity variant detection applications.

For 10 ng inputs, while variant detection was achievable, the increased read per unique molecular identifier (UMI) ratios (~4x higher than 40 ng inputs) suggest that lower inputs require further optimization. Despite this, the system successfully maintained variant calling accuracy, as reflected in the strong correlation between expected and observed variant frequencies ($R^2 > 0.97$, Fig. 6). These results highlight the method's robustness while indicating potential for further refinement to improve resolution at low input levels. (Fig. 5)

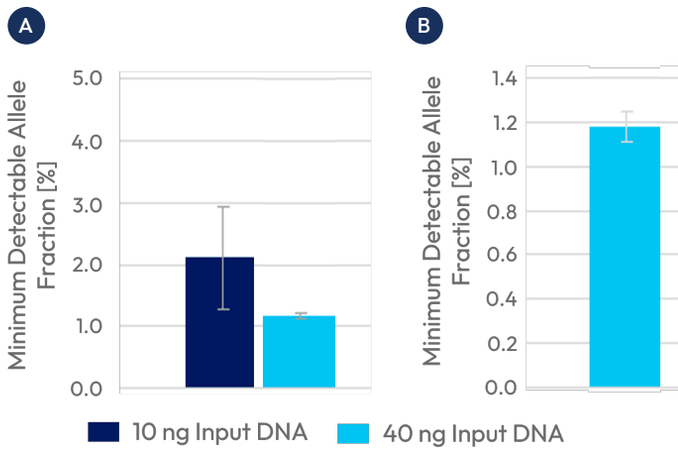


Figure 5: Variant calling capacity for (A) Lung Cancer Research Panel and (B) Breast Cancer Research panel for OncoSpan (HD827) gDNA NGS libraries. Expected variant calling capacity for 40 ng input DNA: 1% variant allele frequency based on 90% sensitivity on the entire panel region.

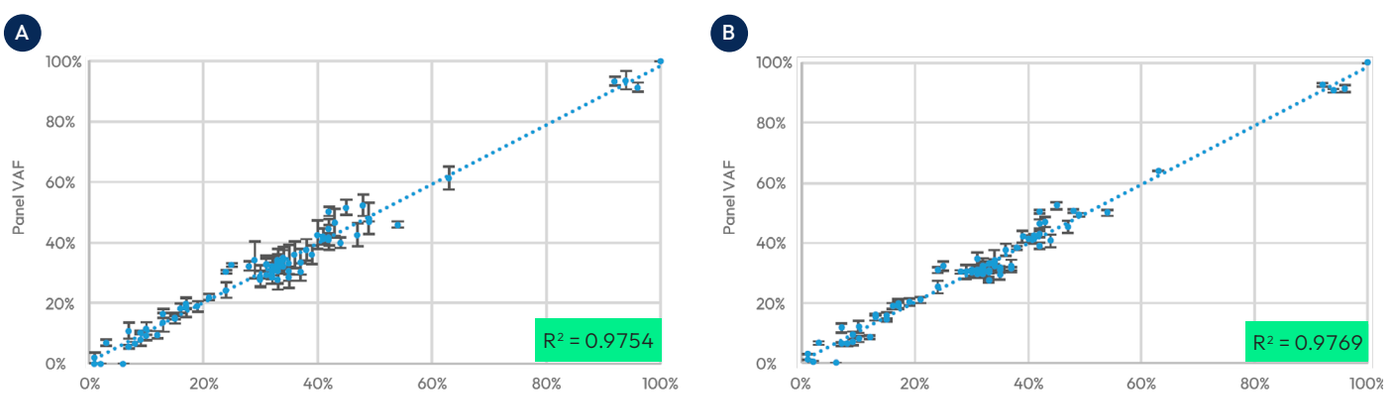


Figure 6: Accuracy of variant allelic frequency (VAF) estimation of the Lung Cancer Research Panel for (A) 10 ng and (B) 40 ng OncoSpan (HD827) gDNA NGS libraries.

Furthermore, variant allelic frequency (VAF) accuracy estimation of selected mutations of the Lung Cancer Research Panel for OncoSpan (HD827) gDNA NGS libraries exhibited a strong correlation between expected and detected frequencies (Fig. 7). For libraries generated from 40 ng input DNA, the performance was highly consistent, with low variability. For libraries generated from 10 ng input DNA, although variability was slightly higher due to the reduced input amount, the observed accuracy demonstrates the method's capacity for detecting clinically relevant variants, even in low-input workflows. These findings underscore the workflow's reliability across diverse experimental scenarios.

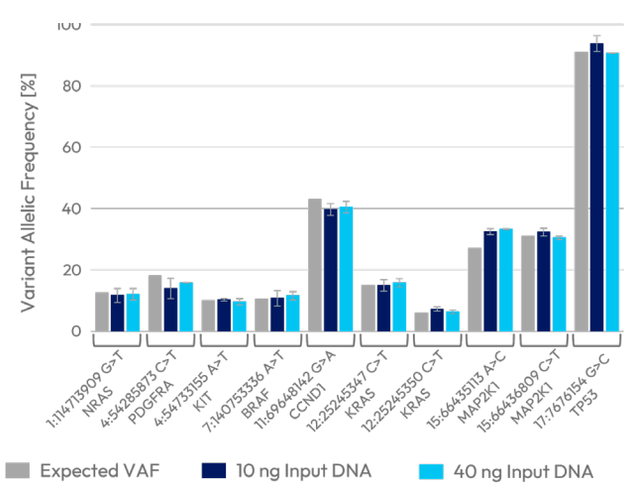


Figure 7: Accuracy of variant allelic frequency (VAF) estimation of selected mutations of the Lung Cancer Research Panel for OncoSpan (HD827) gDNA NGS libraries.

In conclusion, the automation of the QIAseq Targeted DNA Pro Panel method 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.

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 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
Breast Cancer Research Panel	QIAGEN	PHS-001Z
Lung Cancer Research Panel	QIAGEN	PHS-005Z
Mimix™ OncoSpan gDNA Reference Standard	Horizon	HD827
DNA from LCL	Coriell Institute	NA12878
QuantIT 1X dsDNA HS Assay Kit	Thermo Fisher Scientific	Q33266
D5000 ScreenTape	Agilent	5067- 5588
D5000 Reagents	Agilent	5067-5589

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