Technical Note

Pillar Biosciences oncoReveal® Nexus 21 Gene Panel Method

For NGS STAR MOA Assay Ready Workstation

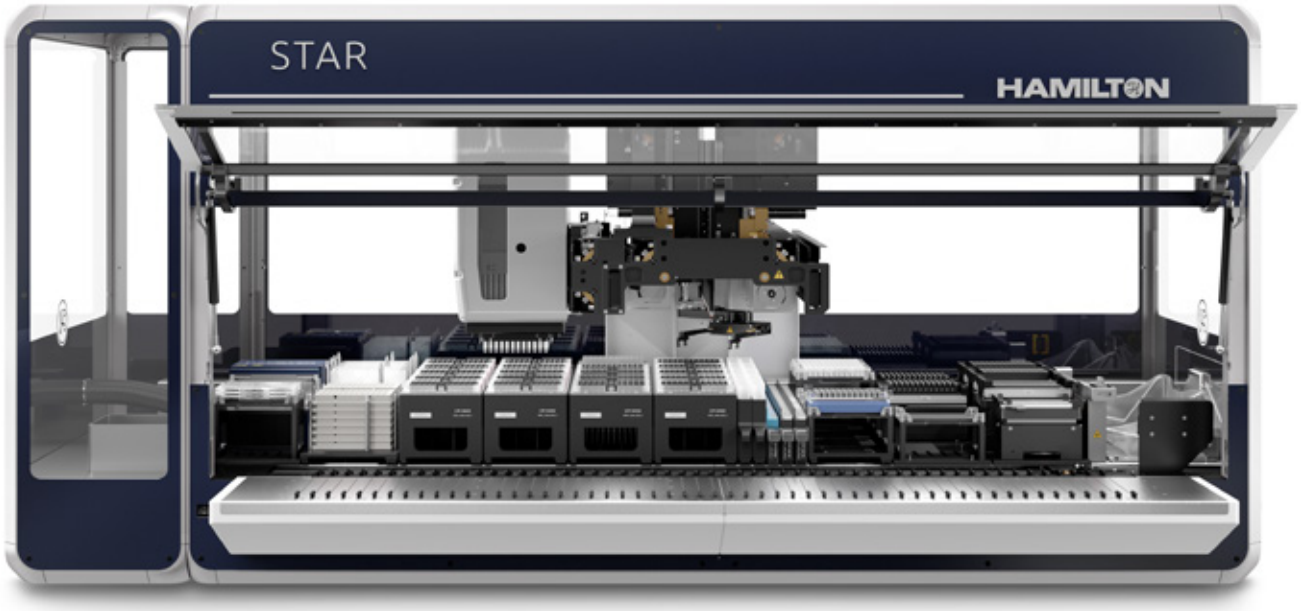


Figure 1: Hamilton Microlab NGS STAR MOA Assay Ready Workstation.

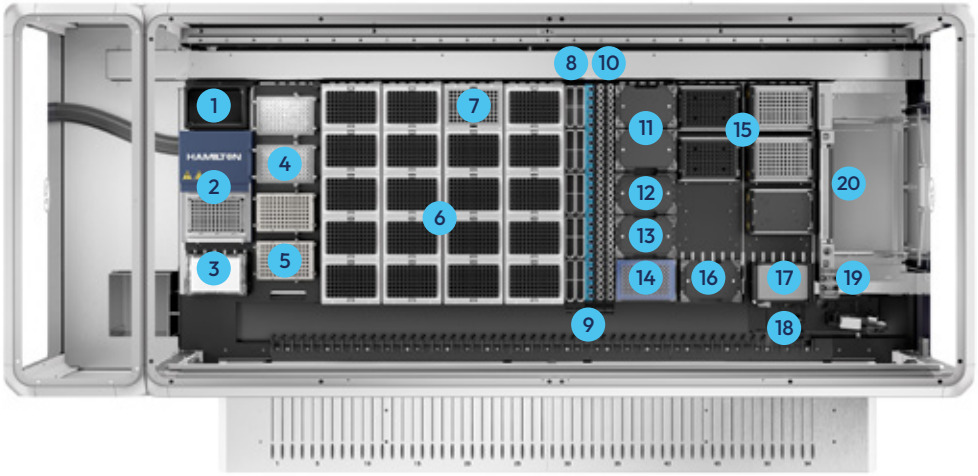
Introduction

Manual library preparation for Next-Generation Sequencing (NGS) can introduce significant variability in turnaround time, library yields, and overall sequencing quality due to run-to-run and sample-to-sample inconsistencies in pipetting technique, quality, and speed. High-throughput manual library preparation can amplify this variance via repetitive pipetting fatigue, heightening the risk of delayed results and costly reruns. These factors can impact workflow scalability at higher batch sizes. To address these limitations, Hamilton Robotics and Pillar Biosciences developed the oncoReveal® Nexus 21 Gene Panel method for the Hamilton Microlab® NGS STAR MOA Assay Ready Workstation (Figure 1), which standardizes library prep for the Nexus 21 Gene Panel with reduced hands-on time and improved consistency.

oncoReveal Nexus 21 Gene Panel

Pillar Biosciences' oncoReveal Nexus 21 Gene Panel is a robust NGS assay designed to analyze key genomic regions in both hematologic malignancies and solid tumors. It supports testing of blood cancer samples as well as DNA extracted from formalin-fixed, paraffin-embedded (FFPE) solid tumor tissue. The panel uses proprietary Stem-Loop Inhibition-Mediated amplification (SLIMamp®) technology, a tiled amplicon-based library prep chemistry for efficient single-tube target enrichment.

NGS STAR MOA Deck Layout



1 Gravity Waste	6 Conductive Tips	11 96-Well PCR Plate	16 DWP Module
2 On-Deck Thermal Cycler	7 Shifted CORE II Tip Adapter	12 96-Well MIDI Plate	17 Cold Plate Air Cooled (CPAC)
3 Plate Lid Park	8 60 mL Reagent Troughs	13 Deep-Well Plate	18 Autoload
4 Stacked 96-Well MIDI Plates	9 15-17 mm Tubes	14 Alpaqua Magnum FLX without springs	19 CORE Gripper
5 Stacked 96-Well PCR Plates	10 Microtubes	15 Heater Shakers	20 Solid/Liquid Waste

Figure 2: Deck Layout of The NGS STAR MOA.

NGS STAR MOA Assay Ready Workstation

The Hamilton Microlab NGS STAR MOA (Multi-probe head On-deck thermal cycler Add-on) workstation enables consistent high-throughput library preparation via its 96-channel pipetting head and On-Deck Thermal Cycler module (Figure 2, position 2). The system's eight independent 1-1000 µL pipetting channels allow for rapid reagent aliquoting and precise mixing, while the cold block module (Figure 2, position 17) can preserve temperature-sensitive reagents or samples over extended processing runs. The NGS STAR MOA Assay Ready Workstation is ideal for automating mid to high-throughput library preparation workflows with enhanced reproducibility and efficiency.

Method Description

The oncoReveal Nexus 21 Gene Panel method is designed to prepare up to 96 oncoReveal Nexus 21 Gene Panel libraries on the NGS STAR MOA system in a single contiguous run using an oncoReveal Nexus 21 Gene Panel kit (HDA-HS 1012-24) and Pillar Biosciences Indexing Plate A or B (IDX-PI-1009-96, IDX-PI-1010-96). Alternatively, indices can be manually paired and loaded using Pillar Custom Indexing Primers Kit A (IDX-PI-1001-96) or Pillar Custom Indexing Primers Kit D (IDX-PI-1004-96). The method encompasses the Gene-Specific PCR, Purify PCR Product, Indexing PCR, and Purify Libraries portions of the Nexus 21 Gene Panel workflow (Figure 3).

The method can prepare 8-96 libraries in ~5–6 hours, with ~0.75–1.25 hours upfront hands-on time and ~4.1–4.5 hours contiguous hands-off processing time. Hands-on time refers to the manual preparation of PCR reaction mixes, aliquoting of bulk reagents, setting up the sample plate, loading the instrument deck, and waiting for the initial on-deck index storage process to complete. Sample input volume is 4.75 µL, with a recommended input mass of 20-60 ng as specified in the Nexus 21 Gene Panel User Guide (UM-0081). After run completion, the purified libraries will be kept in the thermal cycler at 10 °C until an operator can collect them.

Key Features

- **High Throughput Processing:** Can prepare up to 96 libraries in ~5-6 hours.
- **Scalable Flexibility:** Supports processing of 8-96 libraries per-run, selectable in multiples of 8.
- **User-Friendly Interface:** Detailed dialogs guide the user through the loading, processing, and unloading steps.
- **Streamlined Workflow:** Minimal hands-on time for sample and reagent preparation, no mandatory mid-run tip reloads.
- **Efficient Reagent Usage:** Pipetting is optimized to reduce dead volume requirements.

Workflow

Figure 3: Sections of the Pillar Biosciences oncoReveal Nexus 21 Gene Panel Workflow Automated by oncoReveal Nexus 21 Gene Panel Method.

Method Qualification

Qualification Setup

To evaluate Nexus 21 Gene Panel automated library preparation sequencing performance across batch sizes and control types, a research-use-only method qualification was conducted on 26 replicates of Wild-Type Controls (Coriell Institute NA12878), 11 replicates of Horizon Myeloid (Horizon Discovery HD829), 14 replicates of Horizon Moderate (Horizon Discovery HD799), and 93 Negative Controls (Nuclease-Free Water) were processed across four oncoReveal Nexus 21 Gene Panel method runs (Table 1). Sample input mass for each run was 20 ng. After processing, libraries were quantified and sequenced on an Illumina® MiSeq™ i100 System. After sequencing, key sequencing metrics and variants, reported by PiVAT® (Pillar Variant Analysis Toolkit) software, were analyzed, including single-nucleotide variants (SNVs), insertions or deletions (INDELs), and internal tandem duplications (ITDs).

Qualification Results

Across the four runs, overall Q=30 was > 95% for all samples, with averages of 96.67, 96.1, and 96.55 for Wild-Type, Horizon Myeloid, and Horizon Moderate replicates, respectively (Table 2). The Effective On-Target Rate (% of total sequenced reads that map to the targets of interest) was > 95% for all sample replicates, with averages of 98.94, 98.82, and 97.49 for Wild-Type, Horizon Myeloid, and Horizon Moderate replicates, respectively (Table 2). 104/104 (100%) expected variants were called for Wild-Type replicates (Table 3). 99/99 (100%) expected variants were called for Horizon Myeloid replicates (Table 4). 98/98 (100%) expected variants were called for Horizon Moderate replicates (Table 4). No variants were called for Negative Control replicates. These results indicate that the automated workflow produces samples with high sequencing quality and target enrichment efficiency, with on-target metrics exceeding 95% across all sample replicates.

Table 1: Qualification Run Parameters

Parameter	Run 1	Run 2	Run 3	Run 4
Batch Size (n)	8	24	96	16
Input DNA Amount (Genomic DNA)	20 ng	20 ng	20 ng	20 ng
Indexing method	Pre-paired index plate	Pre-paired index plate	Pre-paired index plate	Manually paired indices
Wild-Type Control (n)	3	3	16	4
Horizon Myeloid (n)	2	3	4	2
Horizon Moderate (n)	1	3	8	2
Negative Control (n)	2	15	68	8
Sequencer	MiSeq i100	MiSeq i100	MiSeq i100	MiSeq i100

Table 2: Sequencing Metrics

Library Type	Overall: Q=30	Mapping Rate (%)	On Target Rate (%)	Effective On Target Rate (%)
Wild-Type Control	96.67 ±1.0	99.76 ±0.23	99.18 ±0.31	98.94 ±0.34
Horizon Myeloid	96.1 ±1.23	99.76 ±0.15	99.06 ±0.54	98.82 ±0.61
Horizon Moderate	96.55 ±1.09	99.39 ±0.32	98.09 ±1.11	97.49 ±1.18

Table 3: Wild-Type Control (NA12878) Expected Variant Calls

Library Type	Chromosome	Gene	Variant	Variant Type	Hit Rate
Wild-Type control (NA12878)	4	PDGFRA	chr4:55141055-55141055	SNV	26/26
	4	PDGFRA	chr4:55152040-55152040	SNV	26/26
	7	EGFR	chr7:55249063-55249063	SNV	26/26
	17	TP53	chr17:7579472-7579472	SNV	26/26

Table 4: Horizon Myeloid and Horizon Moderate Expected Variant Calls

Library Type	Chromosome	Gene	Variant	Variant Type	Hit Rate
Horizon Myeloid	13	FLT3	D835Y	SNV	11/11
	2	IDH1	R132C	SNV	11/11
	15	IDH2	R172K	SNV	11/11
	9	JAK2	F537-K539>L	deletion	11/11
	9	JAK2	V617F	SNV	11/11
	12	KRAS	G13D	SNV	11/11
	5	NPM1	W288Cfs*12	Insertion	11/11
	1	NRAS	Q61L	SNV	11/11
	17	TP53	S241F	SNV	11/11
Horizon Moderate	1	NRAS	Q61K	SNV	14/14
	7	BRAF	V600E	SNV	14/14
	7	EGFR	L858R	SNV	14/14
	7	EGFR	G719S	SNV	14/14
	4	KIT	D816V	SNV	14/14
	12	KRAS	G13D	SNV	14/14
	12	KRAS	G12D	SNV	14/14

Conclusion

The oncoReveal Nexus 21 Gene Panel method on the Hamilton Microlab NGS STAR MOA Assay Ready Workstation enables standardized, high-quality automated library preparation with minimal hands-on time. The qualification data demonstrates that this automated method maintains and improves analytical performance while providing significant operational benefits.

Qualification data confirms consistent performance across diverse sample types and batch sizes, ensuring readiness for research applications. With 100% variant detection accuracy and strong operational efficiency, this solution meets the demands of high-throughput genomic research while maintaining precision for scientific studies. Successful validation across multiple batch sizes, control types, and indexing methods demonstrates reliability and position as an ideal choice for improving the efficiency and robustness of myeloproliferative neoplasm research workflows.

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