Technical Note

HAMILTON

Pillar Biosciences oncoReveal® Essential MPN Method

For NGS STAR MOA Assay Ready Workstation

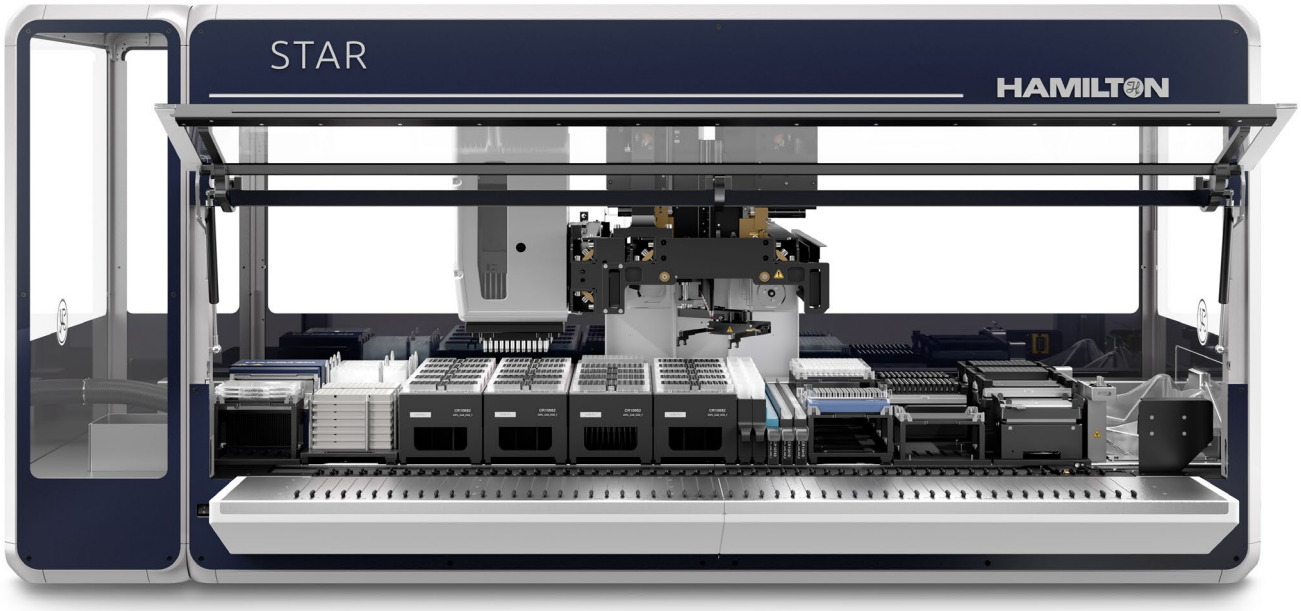


Figure 1: Hamilton Microlab NGS STAR MOA Assay Ready Workstation.

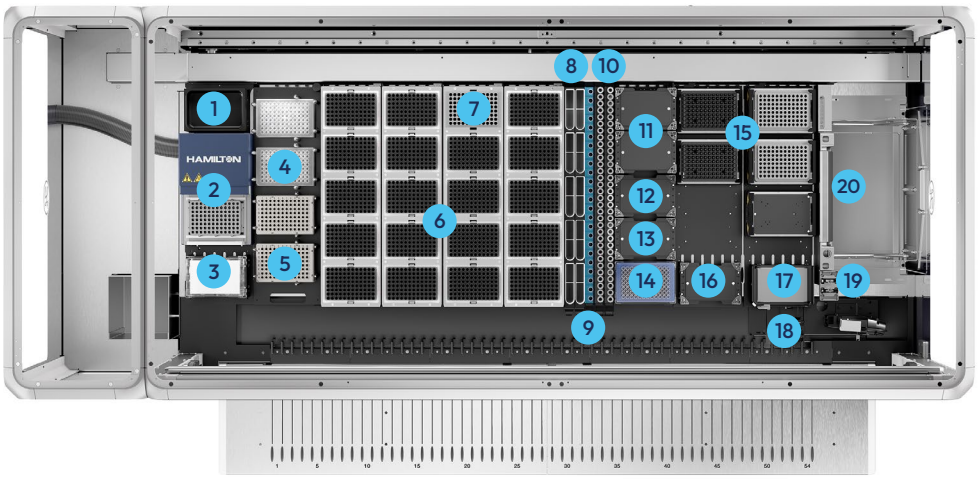
Introduction

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® Essential MPN method for the Hamilton Microlab® NGS STAR MOA Assay Ready Workstation (Figure 1), which standardizes library prep for the Essential MPN panel with reduced hands-on time and improved consistency.

oncoReveal Essential MPN panel

Pillar Biosciences oncoReveal Essential MPN panel is a NGS assay that targets numerous gene regions of interest for researchers looking to explore mutations in JAK2, CALR, and MPL genes from blood samples (WBCs and PBMCs) that are associated with myeloproliferative neoplasms. 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
2 On-Deck Thermal Cycler
3 Plate Lid Park
4 Stacked 96-Well MIDI Plates
5 Stacked 96-Well PCR Plates
6 Conductive Tips
7 Shifted CORE II Tip Adapter
8 60 mL Reagent Troughs
9 15-17 mm Tubes
10 Microtubes
11 96-Well PCR Plate
12 96-Well MIDI Plate
13 Deep-Well Plate
14 Alpaqua Magnum FLX without springs
15 Heater Shakers
16 DWP Module
17 Cold Plate Air Cooled (CPAC)
18 Autoload
19 CORE Gripper
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 through its 96-channel pipetting head and on-board thermal cycler module (Figure 2, position 2). The systems 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 Essential MPN method is designed to prepare up to 96 oncoReveal Essential MPN libraries on the NGS STAR MOA system in a single contiguous run using an oncoReveal Essential MPN Automation-Ready kit (HDA-MY-1004-96) and Pillar Biosciences Indexing Plate A or B (IDX-PI-1009-96, IDX-PI-1010-96). The method encompasses the Gene-Specific PCR, Purify PCR Product, Indexing PCR, and Purify Libraries portions of the Essential MPN workflow (Figure 3). The method can prepare 8-96 libraries in ~5–6 hours, with ~.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 10 µL, with a recommended input mass of 20-60 ng as specified in the Essential MPN User Guide (UM-0015). 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 Essential MPN workflow automated by oncoReveal Essential MPN method.

Method Qualification

Qualification Setup

To evaluate Essential MPN automated library preparation sequencing performance across batch sizes and control types, a research-use-only method qualification was conducted on 22 replicates of Wild-Type Controls (Coriell Institute NA12878), 10 replicates of Mutant Control High (SeraCare 0710-0408), 11 replicates of Mutant Control Low (1:1 dilution of Coriell Institute NA12878 and SeraCare 0710-0408), and 85 Negative Controls (Nuclease-Free Water) were processed across three oncoReveal Essential MPN method runs at batch sizes of 8, 16, and 24 replicates (Table 1). High and Low Mutant Controls were used to assess variant call accuracy, Wild-Type Controls were used to assess sequencing metric uniformity, and Negative Controls were used to screen for cross-contamination. Sample input mass for each run was 20 ng (Table 1). After processing, libraries were quantified and sequenced. Runs 1 and 2 were sequenced on a MiSeq i100, and run 3 was sequenced on a MiSeq RUO (Table 1). After sequencing, key sequencing metrics and variants reported by PiVAT® (Pillar Variant Analysis Toolkit) software were analyzed.

Qualification Results

Across the three runs, overall Q=30 was > 95% for all samples, with an average of 96.93, 97.73, and 96.98 for Wild-Type, Mutant Control High, and Mutant Control Low replicates respectively (Table 2). Effective On-Target Rate (% of total sequenced reads that map to the targets of interest) was > 95% for all sample replicates, with an average of 99.77, 99.75, and 99.80 for Wild-Type, Mutant Control High, and Mutant Control Low replicates respectively (Table 2). 87/88 (98.9%) expected variants were called for Mutant Control High and Mutant Control Low replicates (Table 3). A CALR L367fs*46 variant was not called for one Mutant Control Low replicate due to being below the 2% PiVAT VAF (Variant Allele Frequency) threshold (1.85%). No unexpected variants were called for Wild-Type and Negative Control replicates. These results indicate that the automated workflow prepares samples of high sequencing quality and target enrichment efficiency, with Q30 and on-target metrics exceeding 95% across all Mutant and Wild-type controls.

Parameter	Run 1	Run 2	Run 3
Batch Size (n)	8	24	96
Input DNA Amount (Genomic DNA)	20 ng	20 ng	20 ng
Wild-Type Control (n)	3	3	16
Mutant Control High (n)	3	3	4
Mutant Control Low (n)	0	3	8
Negative Control (n)	2	15	68
Sequencer	MiSeq i100	MISeq i100	MiSeq RUO

Table 1: Qualification Run Parameters.

Library Type	Overall: Q=30	Mapping Rate (%)	On Target Rate (%)	Effective On Target Rate (%)
Wild-Type Control	96.93 ±1.18	99.88 ±0.07	99.9 ±0.12	99.77 ±0.11
Mutant Control High	97.73 ±1.29	99.91 ±0.05	99.83 ±0.13	99.75 ±0.11
Mutant Control Low	96.98 ±1.48	99.89 ±0.06	99.9 ±0.11	99.8 ±0.1

Table 2: Sequencing Metrics.

Library Type	Chromosome	Variant	Hit Rate	Average VAF (%)
Mutant Control High	19	CALR L367fs*46	10/10	5.4 ±0.5
	9	JAK2 N542_E543del	10/10	8.51 ±0.67
	9	JAK2 V617F	10/10	6.52 ±0.29
	1	MPL W515L	10/10	5.28 ±0.42
Mutant Control Low	19	CALR L367fs*46	11/12*	2.38 ±0.31
	9	JAK2 N542_E543del	12/12	4.44 ±0.56
	9	JAK2 V617F	12/12	3.21 ±0.3
	1	MPL W515L	12/12	2.94 ±0.55

* One replicate VAF below 2% VAF threshold (1.85%).

Table 3: Expected Variant Calls.

Conclusion

The oncoReveal Essential MPN 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 shows 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 >98% variant detection accuracy, >95% Q30 sequencing quality, 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 sequencing platforms demonstrates its reliability and positions it as an ideal choice for improving the efficiency and robustness of myeloproliferative neoplasm research workflows.

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