Evaluation of oncoReveal Essential MPN Panel on Illumina Miseq i100 for Key Driver Mutation Testing in Myeloproliferative Neoplasms

Sadie Carbajal, MLS (ASCP), Paul Ollila, B.S, M.B.A., David Viswanatha, MD, Rong He, MD

Molecular Hematopathology Laboratory, Mayo Clinic Rochester

H069

INTRODUCTION

BCR::ABL1 negative myeloproliferative neoplasms (MPNs) are driven by mutations in *JAK2*, *CALR*, and *MPL*, converging on the Jak/STAT pathway. Mutations can be logically assessed by a reflex approach (*JAK2* >*CALR* > *MPL*). This involves complex workflows, extended turnaround times, and possibly variable analytical sensitivities (AS). In this study, we evaluated the performance of an amplicon-based targeted NGS panel, which utilizes stem-loop inhibition mediated amplification (SLIMamp®) to mitigate reamplification of overlapping regions and ensure uniform target coverage.

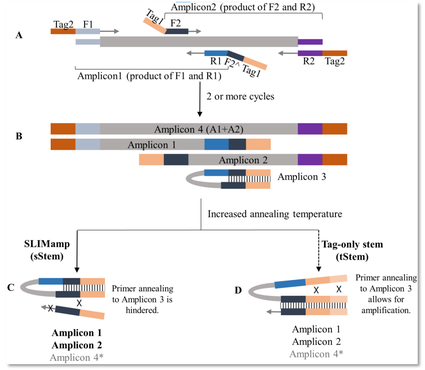
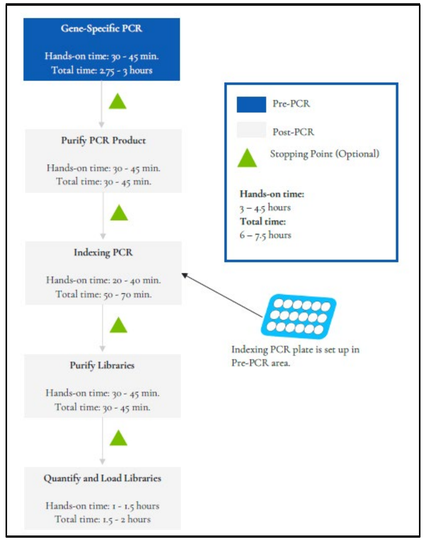


FIGURE 1-SLIMamp CHEMISTRY (LEFT):
Pillar SLIMamp enrichment chemistry facilitates overlapping multiplex PCR in a single reaction tube. The incorporation of stem-loop structures selectively inhibits undesired amplicon formation, allowing efficient and specific amplification of target regions. (Image from Pillar Biosciences Inc. 2025)

FIGURE 2-WORKFLOW (RIGHT):
Stepwise Pillar workflow illustrating estimated hands-on and total processing times, emphasizing the streamlined design that allows completion within a single day. Optional stopping points are included to support workflows performed in multiple sessions. (Image from oncoReveal Essential MPN Panel User Manual, 2025)



RESULTS

- 94 samples tested by oncoReveal
  - 82 positive results and 12 negative results for *MPN* mutations
  - VAF ranged from 0.1-91.8% *JAK2*, 2.9-52.9% *CALR*, and 2.1-51.0% *MPL*
- 32 cases had concurrent NGSHM with 91% concordance
  - Mean VAF difference of 2.2% (range -16.6 to 4.5%).
- 3/32 (9%) cases were positive by onocReveal with low VAF (<2%) and negative by NGSHM, attributable to the 2% AS cut-off of NGSHM.
- 50 cases had *JAK2 V*617F AS-PCR performed with 92% concordance.
  - 4/50 (8%) negative by oncoReveal had low level *JAK2*V617F (<0.2%) detected by AS-PCR.
  - 26/30 cases with a low level *JAK2* V617F by AS-PCR (<1%) were detected by oncoReveal.
- 3 cases with *JAK2* non-V617F were performed by SS and NGS with 100% concordance
- 32 cases with concurrent *CALR* FA (20 positive, 12 negative for indels) with100% concordance.
- 13 cases with the *MPL* Sanger Sequencing done (9 positive and 4 negative) had a concordance rate of 92%.
  - One case was positive by oncoReveal and negative by SS due to the differences in AS.

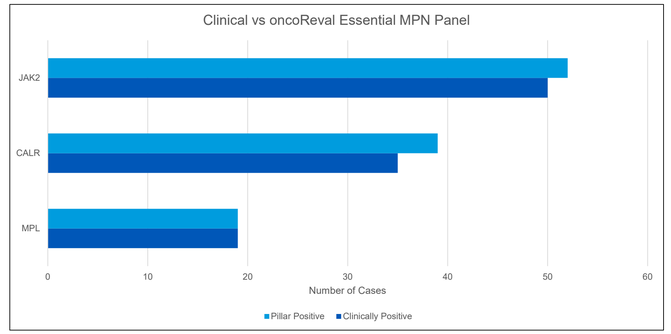
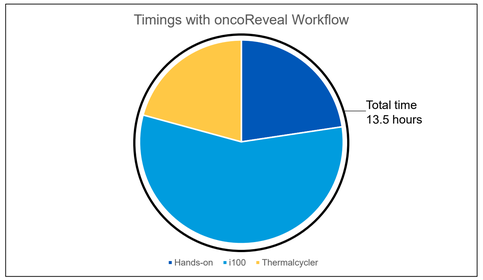
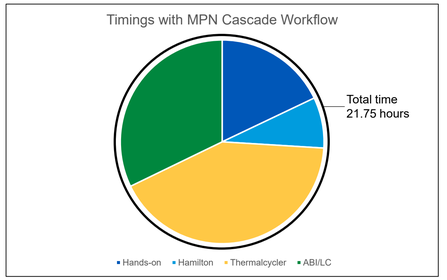


FIGURE 5:
Comparison of clinical results and oncoReval Essential MPN panel findings for three key mutations (*JAK2, CALR, MPL*). The chart shows strong concordance between clinically positive and panel-detected (Pillar Positive) cases, with slight variations in *CALR* and *JAK2* detection rates.

METHODS

- DNA was extracted from peripheral blood and/or bone marrow specimens using the Promega SimplyDNA kit.
- Samples were prepared for sequencing using the Pillar Biosciences oncoReveal™ Essential MPN Panel
  - Targets *JAK2*, *CALR*, and *MPL,* following manufacturer's instructions.
  - NGS was performed on Illumina MiSeq i100
  - Data analysis was conducted using the vendor's analysis toolkit (PiVAT).
- Results were compared to existing laboratory results, including
  - Capture-based myeloid NGS panel (NGSHM) run on Illumina NovaSeq 6000 (AS 2%)
  - *JAK2* V617F quantitative allele-specific PCR (AS-PCR) on the Roche LightCycler 480 (AS 0.06%)
  - *MPL* Sanger sequencing (SS, AS 10-20%) using Integrated DNA Technologies primers on ThermoFisher ABI platforms.
  - *CALR* fragment analysis (FA, AS 5%) using Integrated DNA Technologies primers on ThermoFisher ABI platforms.





FIGURES 3 AND 4:
Hands on, machine, and total time of the oncoReveal MPN Panel compared to clinical MPN cascade. Both timings do not include analysis and MPN Cascade does not include pulling of samples for the next set-up in the cascade if needed. The cascade having multiple tests significantly increased the turn around time compared to the oncoReveal Panel having one set-up.

DISCUSSION

Overall, the results demonstrated a high level of concordance, with most discrepancies arising from differences in the limits of detection (LOD) across the platforms. Samples that were positive for the *JAK2* V617F mutation by AS-PCR but negative by the oncoReveal Panel were low-level positives (<0.2%), which seemed to be below the oncoReveal LOD. Conversely, samples that were positive by oncoReveal but negative by NGSHM were present because of the NGSHM LOD limit of 2%. One NGSHM-discrepant case involved an *MPL* variant located outside of exon 10 and was not detected by oncoReveal. The final discrepant case was an *MPL* variant detected by the oncoReveal panel but not by Sanger sequencing, due to a VAF present below the typical sensitivity threshold for Sanger.

Positive CALR results show larger VAF have differences attributed to the use of capture based NGS in clinical testing versus amplicon based NGS from the oncoReveal panel.

The Pillar set up is efficient. In current practice, the MPN cascade can take 5-7 days to generate results after DNA/RNA extraction, and a standard NGS sample can require 10-14 days. The Pillar oncoReveal MPN panel on the Illumina i100 provides results within 24 hours after extraction—even with all processing performed manually. The workflow is also well-suited for an automated set up. Implementing an automated setup along with pre-made index plate could further reduce turnaround time, increasing efficiency and minimizing hands on time.

CONCLUSIONS

The oncoReveal™ Essential MPN Panel demonstrated strong concordance with existing clinical assays for detecting JAK2, CALR, and MPL mutations. This is observed by its robust performance on detection of low VAF mutations. Additionally, it streamlines workflow and compatibility with a compact sequencing platform, supports its utility as an efficient and consolidated test solution for MPN upon further validation.

© 2025 Mayo Foundation for Medical Education and Research