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JAK2 Exon 12-15, *CALR* and *MPL* Essential MPN NGS Panel

Li Cai, Danny Overman, Carrie Wilson, Binbin Huang, Dan Wang, Ying Wang, Jodie Williams, Melissa Calvert, Caron Glotzbach, Lauren Kam-Morgan, Anjen Chenn

Center for Molecular Biology and Pathology, Labcorp, Research Triangle Park, NC 27709

Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem-cell disorders involving granulocytic, erythroid, megakaryocytic, and mast cell lineages. *BCR/ABL* negative MPNs include primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET). They are characterized by stem cell-derived clonal proliferation, harbor Janus kinase 2 (*JAK2*), or calreticulin (*CALR*), or myeloproliferative leukemia virus oncogene (*MPL*) driver mutations and utilize an over activated JAK-signal transducer and activator of transcription (STAT) pathway. Single gene tests and large myeloid panels are available for MPNs. Mutational testing for *JAK2*, *CALR*, and *MPL* is well accepted as standard of care in the workup of myeloproliferative neoplasms and is included in clinical guidelines. In this study, we have evaluated the clinical and analytical performance features of an essential NGS panel only including *JAK2*, *CALR* and *MPL* for high throughput testing and quick turn-around time.

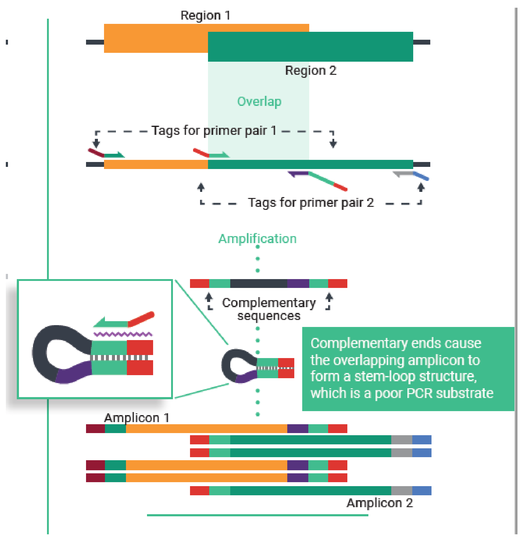
Methods

MPN Panel Coverage

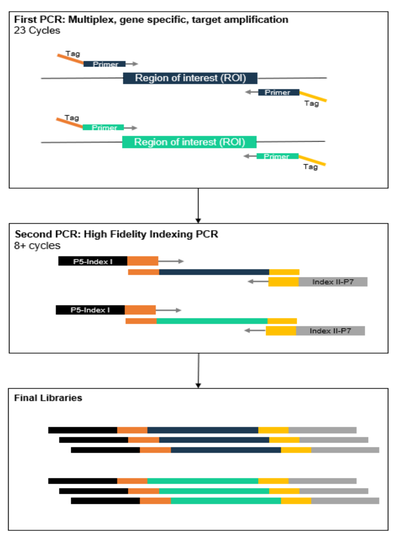
Target Name	Region	Segment Size	GC Content_(%)
CC004.MPL.X10_ROI	chr1:43814928-43815092	165	62.34
CC181.JAK2.X12_ROI	chr9:5069903-5070080	178	30.38
CC182.JAK2.X13_ROI	chr9:5072469-5072653	185	35.83
CC183.JAK2.X14_ROI	chr9:5073694-5073803	110	35.92
CC184.JAK2.X15_A01_ROI	chr9:5077380-5077542	163	23.61
CC185.JAK2.X15_A02_ROI	chr9:5077476-5077628	153	26.89
CC314N-CALR-NM_004343-X09^52bpDel_ROI	chr19:13054522-13054710	189	54.74

The oncoReveal™ Essential MPN Panel (Pillar Biosciences) utilizes proprietary SLIMamp™ (stem-loop inhibition mediated amplification) technology, allowing amplification of regions of interest in a single tube, multiplex reaction. Subsequent libraries are designed for sequencing on the Illumina MiSeq™ platform using a paired-end read length of 150bp. The oncoReveal™ Essential MPN Panel contains the key regions of interest in *JAK2*, *CALR*, and *MPL*. DNA from blood, bone marrow and cell pellet were used to evaluate the assay's accuracy, repeatability, reproducibility, and analytical sensitivity. Identified reportable mutations were confirmed by a secondary method.

Stem-Loop Inhibition-Mediated Amplification (SLIMamp)



Pillar Biosciences NGS Workflow

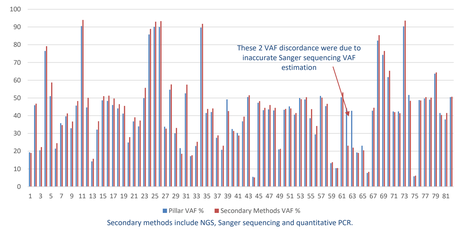


Results

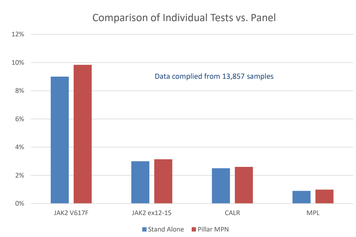
Of the 126 specimens tested during validation, variants identified were confirmed by a secondary method and showed 100% concordance. Repeatability (intra-assay precision) and reproducibility (Inter-assay revision) were 100% from 30 specimens. The sensitivity of this assay is 1% for *JAK2* p.V617F variant and 2.5% for all other variants detected with 40ng input DNA. EDTA or heparin blood or bone marrow specimens stored at 4°C could be used for testing up to 30 days. DNA stored at 2-8°C could be used for testing up to 36 days. DNA extraction and test set up were automated with Chemagen and Tecan robotic systems. 93 specimens could be processed simultaneously with no template, positive and negative controls.

This panel has been offered as a clinical test based on the successful performance features. In a set of 5000 clinical specimens, the percentage of mutations identified in *JAK2*, *CALR* and *MPL* is comparable with our cumulative standalone testing results. The average laboratory turn-around time was shortened by 4 days using this essential MPN panel compared to the previous cascading test orders, such as *JAK2* p.V617F with reflex to *JAK2* exon 12-15 sequencing and multiple other reflex ordering pathways as data were generated simultaneously and masked or unmasked according to the test order.

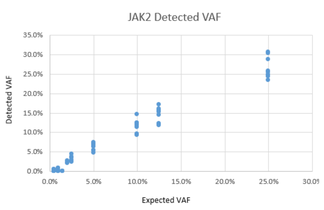
Concordance



Positive Rate Comparison

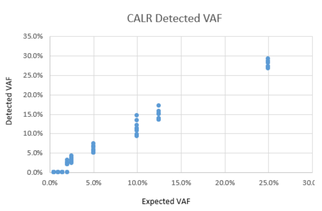


JAK2 Analytical Sensitivity



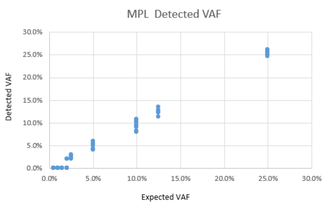
Valid to 2.5% for JAK2 except exon 14 p.V617F which is 1%

CALR Analytical Sensitivity



Valid to 2.5% for CALR

MPL Analytical Sensitivity



Valid to 2.5% for MPL

Conclusions

The essential MPN high throughput panel is a robust and reproducible assay using blood, bone marrow and cell pellet. The molecular alterations detected by this assay can assist in diagnosis, prognosis and making cancer treatment decisions involving targeted therapies in a clinically relevant turn-around time.

References

1.Pillar Biosciences. *oncoReveal Essential MPN Panel Reference Guide version 0.8*. October 2021