Specifications Sanquin Molecular diagnostics for MDS/MPN (X068)

Design MDS/MPN Panel

The MDS/MPN Panel (IAD160996_182) exists of 186 amplicons and is covering 100% of submitted areas (all coding regions (exons) and is able to analyze variants in 21 genes implicated in MDS/MPN. Indicated exons (Table 1) include flanking intronic regions based on 5 base exon padding. For some genes the 5 base exon padding is not achieved or only a hotspot location is covered. See Table 2 for detailed coverage information about these aberrant regions.

Table 1. Design MDS/MPN panel

Gene	Chromosome	NCBI Transcript	Exon	Coverage %
ASXL1	Chr20	NM_015338.5	13	100
BCOR	ChrX	NM_001123385.1	2-15 (full)	100
CALR	Chr19	NM_004343.3	8, 9	100
CBL	Chr11	NM_005188.3	8, 9	100
CSF3R	Chr1	NM_156039.3	12-17	100
ETNK1	Chr12	NM_018638.4	3	100
ETV6	Chr12	NM_001987.4	3-8 (full)	100
EZH2	Chr7	NM_004456.4	2-20 (full)	100
IDH1	Chr2	NM_005896.3	4	100
IDH2	Chr15	NM_002168.3	4	100
JAK2	Chr9	NM_004972.3	12, 14	100
KRAS	Chr12	NM_033360.3	2, 3	100
MPL	Chr1	NM_005373.2	4, 10, 12	100
NRAS	Chr1	NM_002524.4	2, 3	100
SETBP1	Chr18	NM_015559.2	4	100
SF3B1	Chr2	NM_012433.3	12-16	100
SRSF2	Chr17	NM_003016.4	1	100
STAG2	ChrX	NM_001042749.2	3-35 (full)	100
TP53	Chr17	NM_000546.5	2-11	100
TP53β	Chr17	NM_001126114.2	alt 10	100
TP53γ	Chr17	NM_001126113.2	Alt 10	100
U2AF1	Chr21	NM_006758.2	2, 6	100
ZRSR2	ChrX	NM_005089.3	1-11 (full)	100

Table 2. Aberrant covered regions MDS/MPN panel

Gene	Exon	Coding region
KRAS	3	c.112-1 - c.290+5
MPL	1	c.539 - c.690+5
MPL	12	c.1677 - c.1871
SETBP1	4	c.2302 - c.2753
SF3B1	12	c.1540-1 - c.1719+5
SF3B1	14	c.1807-3 - c.2077+5
SF3B1	15	c.2078-1 - c.2223+5
TP53	2	c.-5 - c.74+2
TP53	3	c.75-5 - c.96+2

Coverage of the NGS MDS/MPN Panel

Coverage is the number of times a base is sequenced. The deeper the coverage of each base the greater the reliability and sensitivity of the sequencing assay. The minimum depth of coverage required for detection of somatic variants with the MDS/MPN Panel is 500X. The percentage of Target Base coverage (%Base500x) is the percentage of target bases in a panel that is covered at least 500 times.

Coverage for the NGS MDS/MPN panel is in silico validated and is 100% for all amplicons representing 21 different genes. The percentage of target bases that is covered at least 500 times (%Base500x) is at least 99.04% at 2.000.000 Mapped Reads. With this acceptance criteria two amplicons failed to yield >500 times coverage, specific locations are listed in Table 3. One amplicon in BCOR results in a too low coverage of 197bp at the 5'side of exon 4. Besides BCOR, one amplicon in MPL has less than 500x coverage. This amplicon is covering the coding region of exon 10 and 5 base flanking intronic regions on both sides (total 106bp). There are several MDS/MPN related variants described in both regions.

Table 3. Information failed amplicons MDS/MPN panel

	GRCh37/hg19 coordinates					
Chr.	**Start**	**End**	**Gene**	**Bases failing coverage 500X**	**Exon**	**Missing COSMIC**
ChrX	39933684	39933486	BCOR	197	4	Several
Chr1	43814929	43815035	MPL	106	10	Several

Reporting: addition hematological malignancies variants

This test does not distinguish between somatic and germ line alterations in analyzed gene regions, particularly when variant allele frequencies (VAF) are near 50% or 100%. If nucleotide alterations in genes associated with germline mutation syndromes are present and there is also a strong clinical suspicion or family history of malignant disease predisposition, appropriate genetic counselling may be indicated.

Variants detected between 5% and 10%. Variant Allele Frequency may indicate sub clonal tumor populations. However, the clinical significance of these findings may not always be distinct. It is demonstrated that in blood DNA samples from individuals with advancing age and who do not have a hematologic neoplasm, a low incidence of gene variants that are associated with myeloid neoplasms can be detected. This phenomenon of clonal hematopoiesis of indeterminate potential (CHIP) may not be clearly distinguishable from tumor-associated mutations, especially if detected as a sole abnormality (DOI: 10.1182/blood-2015-03-631747).

Correlation with clinical, histopathologic and additional laboratory findings is required for final interpretation of the results. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician.

NGS data are interpreted with the current knowledge concerning variants in relation to disease or as explanation of a phenotype. For reporting variants, the following guidelines will be followed: 'Best Practice Guidelines for Reporting Molecular Genetics results' written by R.J.L. Treacy and D.O. Robinson. The authorization of the results is done by a recognized Clinical molecular geneticist. All variants are annotated and reported as designated by the Human Genome Variation Society (HGVS) nomenclature, as described at their website.