Specifications Sanquin Molecular diagnostics for AML (P042)

Design AML-MDS NGS Panel

The AML-MDS Panel (IAD245850_243), request code P042, exists of 348 amplicons and is in silico covering 100% of submitted areas (all coding regions (exons)) and is able to analyze variants in 31 genes related to AML. Indicated exons (Table 1) include flanking intronic regions based on 10 base exon padding. For some genes only a hotspot location is covered. See Table 1 and 2 for detailed coverage information about these aberrant regions.

Table 1. Design AML-MDS panel

Gene	Chromosome	Transcript	Exon
ASXL1	Chr20	NM_015338.6	1, 2, 4-13
BCOR	ChrX	NM_001123385.2	2-15 (full)
CALR	Chr19	NM_004343.4	9
CBL	Chr11	NM_005188.3	8-9
CEBPA	Chr19	NM_004364.4	1
CSF3R	Chr1	NM_000760.4	12-17
DDX41	Chr5	NM_016222.4	1-17 (full)
DHX34	Chr19	NM_014681.6	2-17 (full)
DNMT3A	Chr2	NM_022552.4	2-23 (full)
ETNK1	Chr12	NM_018638.4	3
ETV6	Chr12	NM_001987.5	1-8 (full)
EZH2	Chr7	NM_004456.5	2-20 (full)
FLT3	Chr13	NM_004119.3	13,14,15,16,20,21
GATA2	Chr3	NM_032638.5	2-6 (full)
IDH1	Chr2	NM_005896.3	4
IDH2	Chr15	NM_002168.3	4
JAK2	Chr9	NM_004972.4	12 and 14
KIT	Chr4	NM_000222.3	8-19
KRAS	Chr12	NM_033360.3	2-5 (full)
KRAS	Chr12	NM_004985.5	alt 5
MPL	Chr1	NM_005373.3	3,4,6,10,12
NPM1	Chr5	NM_002520.6	11
NRAS	Chr1	NM_002524.5	2-3
RUNX1	Chr21	NM_001754.4	2-9 (full)
SETBP1	Chr18	NM_015559.2	4 (hotspot)
SF3B1	Chr2	NM_012433.4	12-24
SRSF2	Chr17	NM_003016.4	1
STAG2	ChrX	NM_001042749.2	3-35 (full)
TET2	Chr4	NM_001127208.2	3-11 (full)
TP53	Chr17	NM_000546.5	2-11 (full)
TP53β	Chr17	NM_001126114.2	alt 10
TP53γ	Chr17	NM_001126113.2	alt 10
U2AF1	Chr21	NM_006758.2	2, 6
ZRSR2	ChrX	NM_005089.3	1-11 (full)

Table 2. Aberrant covered regions AML-MDS panel

Gene	Exon	Coding DNA region
SETBP1	4	c.2477-c.2760

Coverage of the NGS AML-MDS 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 AML-MDS AmpliSeq 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.

The percentage of target bases that is covered at least 500 times (%Base500x) is at least 99% at 2.200.000 Mapped Reads. With this acceptance criteria two regions failed to yield >500 times coverage, specific locations are listed in Table 3. One amplicon covering exon 1 in ASXL1 failed to reach >500 times coverage. A region in the middle of exon 1 of CEBPA also did not reach >500 times coverage. This does **not** concern the bZIP domain of CEBPA, which is used for the AML risk classification. The bZIP domain is covered >500 times. In the regions of ASXL1 and CEBPA that are covered <500 times, germline variants will be called, but for somatic variants the coverage might be too low.

Table 3. Information amplicons AML-MDS panel that fail to reach >500 times coverage

	GRCh37/hg19 coordinates				
Chr	**Start**	**End**	**Gene**	**Number of bases**	**Exon**
Chr20	30946569	30946645	ASXL1	77	1
Chr19	33792690	33793122	CEBPA	433	1

Design AML Fusion Transcript Panel

The AML-Fusion Transcript Panel (IAD84643) is able to detect fusion transcripts BCR::ABL1, CBFB::MYH11, PML::RARA and RUNX1::RUNX1T1 (AML1::ETO).
Fusion of genes may result in the usage of different donor and/or acceptor exons. Consequently, the corresponding fusion gene transcripts will have different exon compositions. In Table 4, a summation is listed with the number of fusion forms that are detected by the AML Fusion Transcript Panel.

Table 4. Overview fusion transcripts in AML Fusion Transcript Panel

Fusion Gene	Transcript Variants
BCR::ABL1	18
CBFB::MYH11	11
PML::RARA	5
RUNX1::RUNX1T1	5

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% may indicate subclonal 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.