See below for Additional Technical Information topics
**Myeloid Malignancies Mutation Panel by Next Generation Sequencing**
**Cytogenomic Microarray – Oncology**

# Myeloid Malignancies Mutation Panel by Next Generation Sequencing

## Indications for Ordering

Assess for single gene variants, including substitutions and insertions and deletions, that may have diagnostic, prognostic, and/or therapeutic significance in

- Acute myeloid leukemia
- Myelodysplastic syndromes (MDS)
- Myeloproliferative neoplasms (MPN)
- MDS/MPN overlap disorders such as chronic myelomonocytic leukemia

## Test Description

Myeloid Malignancies Mutation Panel by Next Generation Sequencing

- Next generation sequencing (NGS) library construction from genomic DNA
- Enrichment for regions of interest by hybridization
- Massively parallel sequencing

## Tests to Consider

**Primary tests**

- Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117
- Myeloid Malignancies Somatic Mutation and Copy Number Analysis Panel 2012182

**Related tests**

- *CEBPA* Mutation Detection 2004247
- *NPM1* Mutation Detection by RT-PCR, Quantitative 3000066
- *IDH1* and *IDH2* Mutation Analysis, exon 4 2006444
- *KIT* Mutations in AML by Fragment Analysis and Sequencing 2002437

## Disease Overview

**Diagnostic issues**

- Genetic targets contained in panels are relevant across the spectrum of myeloid malignancies
- Identification of one or more clonal genetic abnormalities may aid in establishing the diagnosis of a neoplasm
- Identification of certain variants or patterns of variants may aid in diagnostic subclassification

**Prognostic and treatment issues**

- Certain variants or patterns of variants may have prognostic significance
- Certain variants may allow for the use of targeted therapies

## Genetics

**Genes** – *ASXL1, ASXL2, BCOR, BCORL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT1, DNMT3A, EED, ELANE, ETNK1, ETV6, EZH2, FAM5C, FLT3, GATA1, GATA2, HNRNPK, IDH1, IDH2, JAK2, JAK3, KDM6A, KIT, KRAS, LUC7L2, MAP2K1, MLL, MPL, NOTCH1, NPM1, NRAS, NSD1, PHF6, PRPF40B, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, U2AF2, WT1, ZRSR2*

- See Myeloid Panel Coordinates table below for full list of targeted regions

## Test Interpretation

**Results**

- Positive – a somatic variant in one of the 57 tested genes was detected
  - Clinical relevance (diagnosis, prognosis, therapy) will be correlated, if known
- Negative result – no variants were detected in the sequenced genes

**Limitations**

- Variants may be present below the limit of detection (LOD) of 5% allele frequency
- Lower limit of detection for large variants (>30bp) has not been validated
- Not intended to detect minimal residual disease

## Analytical Sensitivity

| Variant Class | No. Variants Tested | PPA | 95% Tolerance Interval at 95% Reliability |
|---|---|---|---|
| SNV | 414 | 96% | 94-97% |
| Insertions and duplications (small,[a] medium,[b] large[c]) | 281 | 100% | 94-100% |
| Deletions (small[a] and medium[b]) | 132 | 100% | 97-100% |
| Deletions (large[d]) | 5 | 80% | 37-98% |

[a]Small variant (<30 bp)
[b]Medium variant (30-60 bp)
[c]Large variant, insertions/duplications (60-224 bp)
[d]Large variant, deletions (60-1675 bp)

## Myeloid Panel Coordinates

| Gene | Accession No. | Sequenced Exons |
|---|---|---|
| ASXL1 | NM_015338.5 | 12 |
| ASXL2 | NM_018263.4 | 10, 11, 12 |
| BCOR | NM_001123385.1 | All[a] |
| BCORL1 | NM_021946.4 | All |
| BRAF | NM_004333.4 | 15 |
| BRINP3/FAM5C | NM_199051.1 | All[a] |
| CALR | NM_004343.3 | 9 |
| CBL | NM_005188.3 | 8, 9 |
| CEBPA | NM_004364.4 | All |
| CSF3R | NM_156039.3 | 14, 17 |
| DNMT1 | NM_001130823.1 | 1-4, 6-41 |
| DNMT3A | NM_175629.2 | All[a] |
| EED | NM_003797.4 | All |
| ELANE | NM_001972.2 | All |
| ETNK1 | NM_018638.4 | 3 |
| ETV6 | NM_001987.4 | All |
| EZH2 | NM_004456.4 | All[a] |
| FLT3 | NM_004119.2 | 14,15, 16, 20 |
| GATA1 | NM_002049.3 | 2 |
| GATA2 | NM_001145661.1 | 4-7 |
| HNRNPK | NM_002140.3 | 3-17 |
| IDH1 | NM_005896.3 | 4 |
| IDH2 | NM_002168.3 | 4 |
| JAK2 | NM_004972.3 | 12-14 |
| JAK3 | NM_000215.3 | 2-4, 11, 15-22 |
| KDM6A | NM_001291415.1 | All |
| KIT | NM_000222.2 | 8, 9, 11, 13, 17, 18 |
| KMT2A/MLL | NM_001197104.1 | All |
| KRAS | NM_004985.4 | 2-4 |
| LUC7L2 | NM_016019.4 | All |
| MAP2K1 | NM_002755.3 | 2,3 |
| MPL | NM_005373.2 | 10 |
| NOTCH1 | NM_017617.3 | 26-28, 34 |
| NPM1 | NM_002520.6 | 10, 11 |
| NRAS | NM_002524.4 | 2-4 |
| NSD1 | NM_022455.4 | All[a] |
| PHF6 | NM_001015877.1 | All[a] |
| PRPF40B | NM_001031698.2 | All |
| PRPF8 | NM_006445.3 | All[a] |
| PTPN11 | NM_002834.3 | 3, 4, 12, 13 |
| RAD21 | NM_006265.2 | All[a] |
| RUNX1 | NM_001754.4 | All |
| SETBP1 | NM_015559.2 | 4(codons 799-965) |
| SF1 | NM_004630.3 | All |
| SF3A1 | NM_005877.5 | All |
| SF3B1 | NM_012433.3 | 6-8, 12-18 |
| SMC1A | NM_006306.3 | All |
| SMC3 | NM_005445.3 | All |
| SRSF2 | NM_003016.4 | All |
| STAG2 | NM_001042749.2 | All[a] |
| SUZ12 | NM_015355.2 | 10-16 |
| TET2 | NM_001127208.2 | All[a] |
| TP53 | NM_000546.5 | All[a] |
| U2AF1 | NM_006758.2 | 2, 6 |
| U2AF2 | NM_007279.2 | All |
| WT1 | NM_024426.4 | 3, 6-9 |
| ZRSR2 | NM_005089.3 | All |

[a]Excludes noncoding exons

# Cytogenomic Microarray – Oncology

## Indications for Ordering

- Preferred test at time of diagnosis for detecting prognostically important genomic abnormalities in leukemias/lymphomas and solid tumors involving
  - Loss/gain of DNA
  - Loss of heterozygosity (LOH)
- Monitor disease progression and response to therapy

## Test Description

**Cytogenomic Molecular Inversion Probe Array, FFPE Tissue – Oncology**

- Platform – Affymetrix OncoScan
- Contains 220,000 SNP probes across the entire genome
- Average functional resolution – 20 consecutive markers

**Cytogenomic SNP Microarray – Oncology**

- Platform – Affymetrix CytoScan HD
- Oligo copy number and single-nucleotide polymorphism (SNP) array
- Contains >2.6 million copy number markers
- Includes 750,000 SNP probes
- Detects copy number changes and LOH
- Average marker spacing
  - Intragenic – 880 base pairs (bp)
  - Intergenic (nongene backbone) – 1,700 bp
  - Overall (gene and nongene backbone) – 1,100 bp
- Average functional resolution
  - Deletion of 25 consecutive markers
  - Duplication of 50 consecutive markers

## Tests to Consider

**Primary tests**

- Offer whole genome coverage
- Detect copy number changes and LOH
- Differ in type of specimen and array platform

Cytogenomic Molecular Inversion Probe Array, FFPE Tissue – Oncology 2010229

- Formalin-fixed, paraffin-embedded (FFPE) tissue specimens

Cytogenomic SNP Microarray – Oncology 2006325

- Bone marrow or blood specimens

**Related tests**

- Fluorescence in situ hybridization (FISH) testing for specific balanced translocations may be considered, based on indication
- For a complete list of ARUP's oncology FISH tests, including probe targets and genes, see "Oncology FISH" on the ARUP Genetics website (www.aruplab.com/genetics/tests/fish)

Myeloid Malignancies Somatic Mutation and Copy Number Analysis Panel 2012182

- Panel for myeloid malignancies that combines cytogenomic microarray with a next generation sequencing panel targeting genes with diagnostic, prognostic, and/or therapeutic significance

## Disease Overview

**Diagnostic issues**

- Gains, losses, and LOH occur in malignancies – identification may be helpful for
  - Diagnosis
  - Prognosis and therapeutic decisions
  - Monitoring disease progression and response to therapy
- Conventional cytogenetic (CC) analysis for detection of genetic abnormalities in oncology is hampered by
  - Lack of tumor cell growth in cell culture
  - Subtle chromosomal abnormalities that are often missed
- FISH
  - Improved rate of detection of clonal abnormalities when compared to CC, but only for the targeted region
  - Detects balanced translocations
  - Limited because only a few loci examined at a time
- Neither conventional karyotyping nor FISH testing can detect copy-neutral events that are associated with hematological malignancies
  - Often due to mutations and subsequent selection of mutant tumor-suppressor genes and oncogenes
- SNP microarray detects many of the chromosomal variants involving gains or losses in chromosomes with complex karyotypes across the genome

## Test Interpretation

**Results**

- Abnormal microarray
  - Well-documented and clinically significant gain or loss or LOH detected
- Copy number change detected, clinical significance unknown
  - Copy number variation detected for which insufficient evidence is available to determine unequivocally the clinical significance
- Normal microarray
  - No clinically significant abnormalities detected based on current knowledge at time of reporting

**Limitations**

- Low-level mosaicism (<15-20%) may not be detected
- May not be appropriate for individuals with expected lower levels of malignant cells
- FFPE specimens must contain a region with ≥50% tumor
- Not recommended for minimal residual disease
- Does not detect
  - Balanced rearrangements
    - FISH should be used to evaluate specific balanced rearrangements according to indication
  - Base pair mutations and very small deletions/duplications
  - Imbalances of the mitochondrial genome
  - Positional information for chromosome rearrangements
  - Low-level clones