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AmpliSeq™ for Illumina Myeloid Panel

Targeted panel for investigating somatic variants associated with hematological malignancies.

Highlights

- **Relevant gene content**
  Target biomarkers across 69 genes relevant to hematological malignancies
- **Fast, streamlined workflow**
  Prepare sequencing-ready libraries in a single day from as little as 20 ng high-quality DNA or 10 ng high-quality RNA
- **Accurate data**
  Detect somatic mutations down to 5% frequency using local or cloud-based analysis
- **Excellent performance**
  Provides high coverage uniformity of GC-rich genes such as *CEBPA*

Introduction

The AmpliSeq for Illumina Myeloid Panel enables concurrent analysis of both DNA and RNA from blood and bone marrow samples in a single assay to study biomarkers associated with hematologic malignancies (Table 2). From as little as 20 ng input DNA or 10 ng input RNA, 40 DNA genes, 29 fusion driver genes, and 5 gene expression levels associated with several myeloid cancers can be interrogated from sample to report in 2-3 days.

Relevant gene content

The AmpliSeq for Illumina Myeloid Panel targets the most relevant genes in major myeloid disorders: acute myeloid leukemia (AML), myeloid dysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML) (Table 1). This ready-to-use panel saves researchers the time and effort of identifying targets, designing amplicons, and optimizing performance.

Table 2: AmpliSeq for Illumina Myeloid Panel at a glance

Parameter	Specification
No. of genes	40 key DNA target genes, 29 RNA fusion driver genes
Targets	Genes relevant to myeloid cancer
Variant types	SNVs, indels, gene fusions[a]
Amplicon size	DNA: 230 bp on average, RNA: 100 bp on average
No. of amplicons	DNA: 526, RNA: 700
Input DNA/RNA requirement	20 ng high-quality DNA, 10 ng high-quality RNA (10 ng recommended per pool)
No. of pools per panel	DNA panel: 2 pools, RNA panel: 1 pool
Compatible sample types	Blood, bone marrow, not FFPE-compatible
Percent targets covered at minimum 1000× at recommended throughput	> 95%
Coverage uniformity (percent of targets with >0.2× mean coverage)	≥ 90%
Percent on-target aligned reads	≥ 80%
Total assay time[b]	5-6 hours
Hands-on time	< 1.5 hours
DNA/RNA-to-data time	2.5 days

a. SNVs: single nucleotide variations; indels: insertions/deletions, FFPE, formalin-fixed, paraffin-embedded

b. Time represents library preparation only and does not include library quantification, normalization, or pooling.

Data on file at Illumina, Inc. 2017

Table 1: Gene list for the AmpliSeq for Illumina Myeloid Panel

Hotspot gene (23)									
ABL1	*BRAF*	*CBL*	*CSF3R*	*DNMT3A*	*FLT3*	*GATA2*	*HRAS*	*IDH1*	*IDH2*
JAK2	*KIT*	*KRAS*	*MPL*	*MYD88*	*NPM1*	*NRAS*	*PTPN11*	*SETBP1*	*SF3B1*
SRSF2	*U2AF1*	*WT1*							
Full genes (17)									
ASXL1	*BCOR*	*CALR*	*CEBPA*	*ETV6*	*EZH2*	*IKZF1*	*NF1*	*PHF6*	*PRPF8*
RB1	*RUNX1*	*SH2B3*	*STAG2*	*TET2*	*TP53*	*ZRSR2*			
Fusion driver genes (29)									
ABL1	*ALK*	*BCL2*	*BRAF*	*CCND1*	*CREBBP*	*EGFR*	*ETV6*	*FGFR1*	*FGFR2*
FUS	*HMGA2*	*JAK2*	*KMT2A (MLL)*	*MECOM*	*MET*	*MLLT10*	*MLLT3*	*MYBL1*	*MYH11*
NTRK3	*NUP214*	*PDGFRA*	*PDGFRB*	*RARA*	*RBM15*	*RUNX1*	*TCF3*	*TFE3*	
Expression genes (5)					**Expression control genes (5)**				
BAALC	*MECOM*	*MYC*	*SMC1A*	*WT1*	*EIF2B1*	*FBXW2*	*PSMB2*	*PUM1*	*TRIM27*

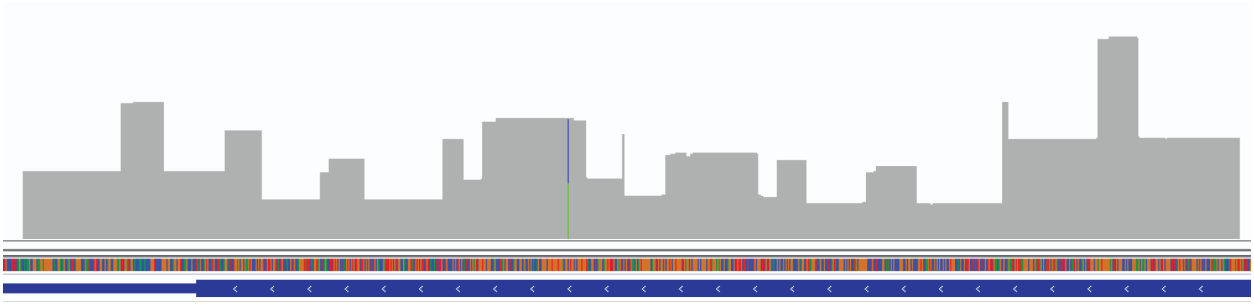

Figure 1: High coverage of CEBPA gene—DNA from Coriell sample NA12878 was evaluated using the AmpliSeq for Illumina Myeloid Panel and the MiSeq System. Analysis with the IGV app shows high read depth coverage across the entire transcript of the challenging, GC-rich *CEBPA* gene.

Simple, streamlined workflow

The AmpliSeq for Illumina Myeloid Panel is part of an integrated solution that offers streamlined content, easy-to-perform library preparation, push-button sequencing systems, and simplified data analysis.

Library preparation follows a straightforward, PCR-based protocol that can be completed in as little as 5-6 hours, with < 1.5 hours hands-on time. Resulting libraries can be normalized, pooled, and then loaded on to a flow cell for sequencing. Prepared libraries are sequenced using proven SBS chemistry on a compatible Illumina sequencing system (Table 3).

Resulting data can be analyzed locally with Local Run Manager or easily streamed into BaseSpace™ Sequence Hub. Local Run Manager and BaseSpace Sequence Hub can access the Integrative Genomics Viewer (IGV) app for sequence alignment and visualization. Resulting data files can be imported diretly into BaseSpace Variant Interpreter for rapid interpretation and reporting of variant data.

Table 3: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Myeloid Panel

Instrument	DNA Samples per Run	RNA Samples per Run	DNA/RNA pooling ratio[a]	Run Time
MiniSeq™ System (mid output)	4	32	8:1	17 hours
MiniSeq System (high output)	12	96	8:1	24 hours
MiSeq System (v2 chemistry)	7	60	8:1	24 hours
MiSeq System (v3 chemistry)	12	96	8:1	32 hours

a. Recommended DNA to RNA pooling ratio is based on the read coverage ratio.

Accurate data

To demonstrate assay capabilities and sensitivity, a Seraseq Myeloid Fusion RNA Mix sample was evaluated using the AmpliSeq for Illumina Myeloid Panel and the MiSeq™ System. Results show accurate detection of somatic variants and gene fusions (Table 4). In addition, the AmpliSeq for Illumina Myeloid Panel provides high coverage of the CCAAT/enhancer binding protein alpha (*CEBPA*) gene (Figure 1). Mutations in the *CEBPA* gene have known associations in AML, but sequencing has proven a challenge due to the high GC content in the gene. In addition, samples were evaluated for variant calling accuracy. Data showed high concordance between expected and detected variant frequency (Figure 2).

Learn more about Illumina sequencing systems

Learn more about AmpliSeq for Illumina informatics

Table 4: High call rates for gene fusions

Fusion	No. samples NOT detected	No. samples detected	Call rate
FIP1L1-PDGFRa	0	16	100%
TCF3-PBX1	0	16	100%
ETV6-ABL1	0	16	100%
DAT6A-CREBBP	0	16	100%
PCM1-JAK2	0	16	100%
BCR-ABL1	0	16	100%
ETV6-ABL1	0	16	100%
RUNX1-RUNX1T1	0	16	100%
PML-RARA	0	16	100%

a. Seraseq Fusion RNA Mix v2, a fusion-positive RNA sample, was used to generate RNA libraries with the AmpliSeq for Illumina Myeloid Panel and sequenced on the MiniSeq and MiSeq Systems.

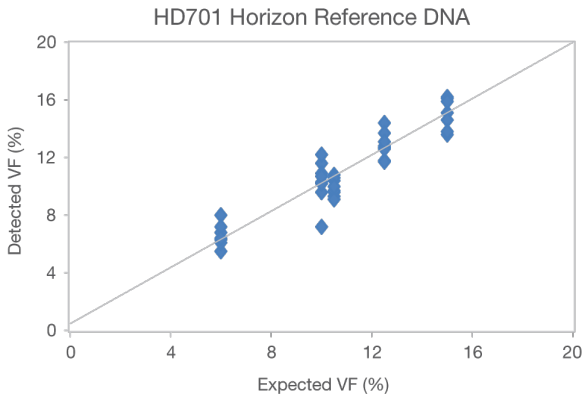

Figure 2: High concordance between expected and detected variant frequency— DNA from HD701 Horizon Reference DNA was evaluated using the AmpliSeq for Illumina Myeloid Panel and sequenced on the MiSeq System. Results show that 100% of expected variants were detected.

Ordering information

Order AmpliSeq for Illumina products online at www.illumina.com

Product	Catalog No.
AmpliSeq for Illumina Myeloid Panel (24 reactions)	20024478
AmpliSeq for Illumina Library PLUS (24 reactions)	20019101
AmpliSeq for Illumina Library PLUS (96 reactions)	20019102
AmpliSeq for Illumina Library PLUS (384 reactions)	20019103
AmpliSeq for Illumina CD Indexes Set A (96 indexes, 96 samples)	20019105
AmpliSeq for Illumina cDNA Synthesis (96 reactions)	20022654
AmpliSeq for Illumina Library Equalizer	20019171

Learn more

Learn more about the AmpliSeq for Illumina Myeloid Panel

Learn more about the AmpliSeq for Illumina targeted sequencing solution

References

1. Mannelli F, Ponzani V, Bencini S, et al. CEBPA-double-mutated acute myeloid leukemia displays a unique phenotypic profile: a reliable screening method and insight into biological features. *Haematologica*. 2017;102(3):529–540.

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