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TruSight™ Myeloid Sequencing Panel

Using expert-defined content and proven next-generation sequencing technology to efficiently and cost-effectively identify somatic mutations in hematological malignancies.

Highlights

- **Expert-Defined Content**
  Designed by a consortium of recognized experts to target 54 genes mutated frequently in myeloid malignancies
- **Streamlined, Comprehensive Method**
  Single workflow includes library preparation, sequencing, data analysis, and data annotation
- **Cost-Effective, Time-Efficient Solution**
  Assess multiple genes simultaneously for approximately the same cost as a single-gene assay
- **High-Accuracy, High-Sensitivity Analysis**
  Limit of detection down to 5% mutant allele frequency with 500× minimum coverage of each region

Introduction

Approximately one person in the United States is diagnosed with a blood cancer every four minutes[1]. Current methods for assessing these myeloid malignancies can be effective, but are time-consuming and expensive when looking at multiple variants, and may not determine the underlying genetic cause of the disease.

The TruSight Myeloid Sequencing Panel uses next-generation sequencing (NGS) technology to provide a comprehensive assessment of 54 genes (tumor suppressor genes and oncogenic hotspots) in one test (Table 1). The panel targets mutations with known involvement in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML). The result is a single assay for accurate, economical, and rapid profiling of liquid tumors for disease status and prognosis, in multiple samples.

Targeted Content Design Strategy

A consortium of recognized experts in blood cancer disorders designed the content for TruSight Myeloid. The sequencing panel specifically targets 54 genes known to be frequently mutated in hematological malignancies, focusing on leukemia and myeloproliferative disorders. Targeted genes include those with an associated clinical relevance, providing a comprehensive picture of the disease and its progression. Targeted genes include those involved in the MDS-AML continuum.

Superior Coverage

TruSight Myeloid features a highly optimized oligo pool specific for investigating genomic changes associated with hematological malignancies. The panel focuses on ~141 kb of genomic content, consisting of 568 amplicons of ~250 bp in length designed against the human NCBI37/hg19 reference genome. The oligo pool targets 15 full

Table 1: Gene Regions Assessed by the TruSight Myeloid Sequencing Panel

Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)
ABL1	4–6	*DNMT3A*	full	*KDM6A*	full	*RAD21*	full
ASXL1	12	*ETV6/TEL*	full	*KIT*	2, 8–11, 13 + 17	*RUNX1*	full
ATRX	8–10 and 17–31	*EZH2*	full	*KRAS*	2 + 3	*SETBP1*	4 (partial)
BCOR	full	*FBXW7*	9 + 10 + 11	*MLL*	5–8	*SF3B1*	13–16
BCORL1	full	*FLT3*	14 + 15 + 20	*MPL*	10	*SMC1A*	2, 11, 16 + 17
BRAF	15	*GATA1*	2	*MYD88*	3–5	*SMC3*	10, 13, 19, 23, 25 + 28
CALR	9	*GATA2*	2–6	*NOTCH1*	26–28 + 34	*SRSF2*	1
CBL	8 + 9	*GNAS*	8 + 9	*NPM1*	12	*STAG2*	full
CBLB	9, 10	*HRAS*	2 + 3	*NRAS*	2 + 3	*TET2*	3–11
CBLC	9, 10	*IDH1*	4	*PDGFRA*	12, 14, 18	*TP53*	2–11
CDKN2A	full	*IDH2*	4	*PHF6*	full	*U2AF1*	2 + 6
CEBPA	full	*IKZF1*	full	*PTEN*	5 + 7	*WT1*	7 + 9
CSF3R	14–17	*JAK2*	12 + 14	*PTPN11*	3 + 13	*ZRSR2*	full
CUX1	full	*JAK3*	13				

genes (exons only) plus exonic hotspots of an additional 39 genes, providing nearly 100% coverage of all targeted regions (Table 2).

This optimized oligo pool provides uniform coverage of the target regions, enabling > 500× coverage for > 95% of amplicons at > 5,000× mean coverage. This translates into 8 samples per run on a MiSeq® system with v3 reagents, providing the required sensitivity and accuracy to call rare variants with confidence.

Simple, Integrated Workflow

The TruSight Myeloid Sequencing Panel offers a fully integrated DNA-to-data solution, including a streamlined workflow and automated data analysis with specific variant calling (Figure 1). Starting with 50 ng DNA isolated from blood, bone marrow, or fine needle aspirates (FNA), libraries are generated with the highly multiplexed oligonucleotide probes. Sample-specific indexes are added to each library, enabling pooling of libraries before sequencing for higher throughput. Pooled libraries are loaded into the MiSeq system for automated sequencing and data analysis. Analyzed data can be imported into VariantStudio software for accurate variant annotation, classification, and reporting. The entire process is completed in just three days.

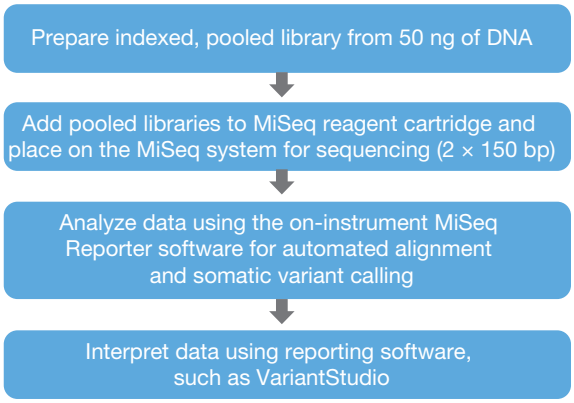
Optimized Assay Chemistry

The TruSight Myeloid assay begins with hybridizing a highly multiplexed pool of oligo pairs, one upstream and one downstream of each region of interest (Figure 2). Each oligo contains unique, target-specific sequence as well as a universal adapter sequence that is used in a subsequent amplification reaction. A proprietary extension–ligation reaction extends across the region of interest, followed by ligation to unite the two probes and yield a library of new templates with common ends. The resulting extension-ligation templates are PCR-amplified, incorporating two unique, library-specific indexes. Final reaction products are converted to a single-stranded, adapter-ligated normalized library using a bead-based protocol. The sequence-ready library can be loaded into the MiSeq reagent cartridge ready for sequencing on the MiSeq system without additional processing.

Table 2: Coverage Details

Cumulative target region size	~141 kb
Number of target genes	54
Amplicon size	~250 bp
Number of amplicons	568
Recommended mean coverage	5,000×
Target minimum coverage	500×
Percent exons covered at 500×	95

Figure 1: Simple, Integrated Workflow



TruSight Myeloid offers integrated library preparation and sequencing, as well as automated data analysis, creating a streamlined workflow that goes from DNA to data in 3 days.

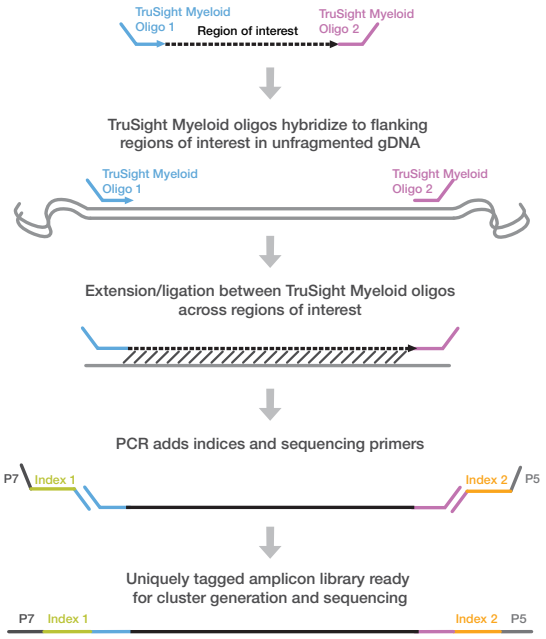
Data Analysis

Sequence data generated from TruSight Myeloid libraries are analyzed using the on-instrument MiSeq Reporter software. After demultiplexing and FASTQ file generation, the software uses a custom banded Smith-Waterman aligner to align the reads against the human hg19 reference genome to create BAM files. The Somatic Variant Caller then performs variant analysis for the specified regions. The outputs are VCF or gVCR files, which are text files that contain SNPs and small indels.

Data Interpretation

VCF and gVCR files generated by the MiSeq Reporter software can be imported directly into the Illumina VariantStudio software application. This powerful tool enables clinical researchers to identify and classify disease-relevant variants quickly, and then communicate significant findings in concise, actionable reports. The intuitive framework offers flexible filtering options, streamlined variant classification, rapid and rich annotation, and customizable reporting options.

Figure 2: Optimized Assay Chemistry



The TruSight Myeloid assay enables simple, streamlined hybridization and amplification of targeted regions.

Summary

The TruSight Myeloid Sequencing Panel enables clinical research laboratories to access expert-defined content for investigating genomic features associated with hematological malignancies. The optimized probe set provides comprehensive coverage of the regions known to be frequently mutated in myeloid cancers and myeloproliferative disorders. Using this panel, 54 genes can be analyzed in a single assay, saving time, money, and resources over current single-gene assays.

Ordering Information

Product	Catalog No.
TruSight Myeloid Sequencing Panel (96 samples)	FC-130-1010

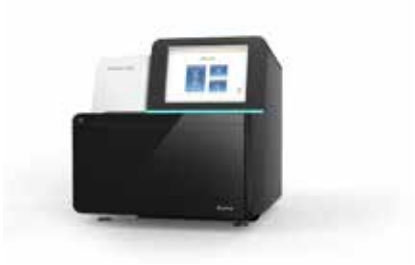
Learn More

To learn more about the TruSight Myeloid Sequencing Panel and Illumina NGS technology, visit www.illumina.com/trusightmyeloid.

References

1. Facts & Statistics. The Leukemia & Lymphoma Society. (www.lls.org/#/diseaseinformation/getinformationsupport/factsstatistics/)

The TruSight Myeloid Sequencing Panel can also be used with the high-throughput, easy-to-use NextSeq™ 500 Desktop Sequencer. Learn more at **www.illumina.com/nextseq**.



Note regarding biomarker patents and other patents unique to specific uses of products.

Some genomic variants, including some nucleic acid sequences, and their use in specific applications may be protected by patents. Customers are advised to determine whether they are required to obtain licenses from the party that owns or controls such patents in order to use the product in customer's specific application.

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