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TruSight™ Myeloid Sequencing Panel

Using expert-defined content and proven next-generation sequencing (NGS) technology to identify somatic mutations in hematologic 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 and analytical sensitivity**
  Limit of detection down to 5% mutant allele frequency with 500× minimum coverage of each region

Introduction

Blood cancers affect more than one million people in the United States alone.[1] Current methods for studying 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 NGS technology to provide a comprehensive assessment of 54 genes (tumor suppressor genes and oncogenic hotspots) in one assay (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).

Targeted content design strategy

A consortium of recognized experts in blood cancer disorders designed the content for the TruSight Myeloid Sequencing Panel . The sequencing panel specifically targets 54 genes known in the peer-reviewed literature to be frequently mutated in hematologic malignancies, focusing on leukemia and myeloproliferative disorders. Targeted genes include those involved in the MDS-AML continuum as cited by professional organizations including the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO),[2-4] providing researchers with a comprehensive picture of the disease and its progression.

Advantages of NGS and targeted resequencing

A key advantage that NGS holds over single-gene assays is the ability to assess up to thousands of genes in a single experiment. NGS provides the efficiency of library preparation in a single tube with a large number of targets. Potentially eliminating sequential testing by covering all possible targets in the first round may be a valuable feature for researchers trying to conserve limited samples. Furthermore, by providing sequence information at the single nucleotide level of resolution, NGS also enables discovery of previously unidentified variants.

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	8–10,17–31	*EZH2*	full	*KRAS*	2,3	*SETBP1*	4 (partial)
BCOR	full	*FBXW7*	9–11	*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				

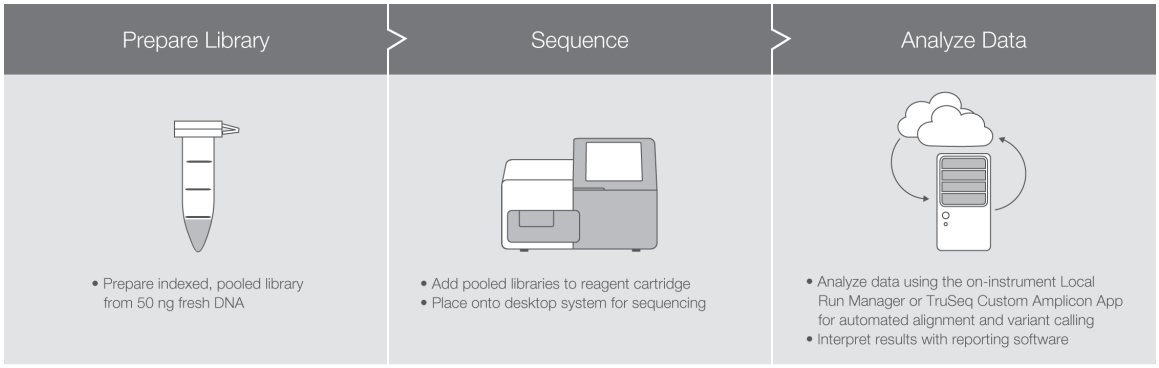

Figure 1: Simple, integrated workflow—TruSight Myeloid offers integrated library preparation, sequencing, and automated data analysis, creating a streamlined workflow that can go from DNA to data in three days.

Targeted resequencing focuses the power of NGS on a subset of genes or target regions (a sequencing panel) that are enriched before the sequencing step. This offers several advantages, such as improved analytical sensitivity and throughput capabilities. Distributing millions of sequencing reads over a limited number of targets results in a greater number of reads per target (higher depth of coverage), enabling higher confidence for calling low-frequency variants in heterogenous neoplasms. By focusing on a limited number of relevant genes, targeted resequencing also offers higher multiplexing capacity and lower data analysis requirements compared to whole genome sequencing. The result is a single assay for accurate, economical, and rapid profiling of liquid tumors.

Simple, integrated workflow

The TruSight Myeloid Sequencing Panel offers an integrated DNA-to-data solution, including a streamlined workflow and automated data analysis with specific variant calling (Figure 1). Starting with 50 ng of DNA isolated from blood, bone marrow, or fine needle aspirates (FNA), libraries are generated with highly multiplexed oligonucleotide (oligo) probes. Sample-specific indexes are added to each library to enable pooling for higher throughput. Pooled libraries are loaded into an Illumina benchtop sequencing system for automated sequencing and data analysis. These benchtop sequencers include the MiniSeq™, MiSeq™, and MiSeqDx (run in research mode). 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 (Figure 2). Each oligo contains a unique, target-specific sequence, and 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 reagent cartridge ready for sequencing on the benchtop system without additional processing.

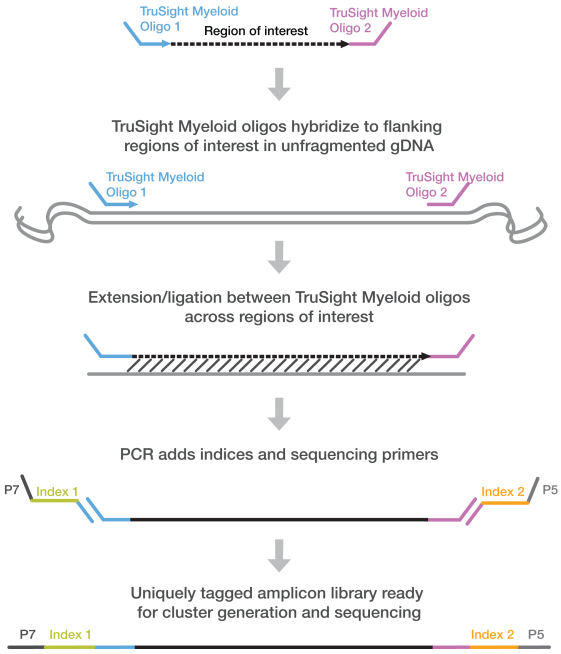

Figure 2: Optimized assay chemistry—The TruSight Myeloid assay enables simple, streamlined hybridization and amplification of targeted regions.

Data analysis

Sequence data generated from TruSight Myeloid libraries are analyzed using on-instrument software or the TruSeq™ Amplicon app in BaseSpace™ Sequence Hub. 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 gVCF files, which are text files that contain single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels).

Table 3: Variant detection in titrated Horizon DNA

Gene and Variant	100% Horizon DNA		50% Horizon DNA		25% Horizon DNA	
	Expected	Observed	Expected	Observed	Expected	Observed
BRAF V600E	10.5%	9.7%	5.3%	6.2%	2.6%	3.2%
KRAS G13D	15.0%	16.7%	7.5%	6.7%	3.8%	2.8%
KRAS G12D	6.0%	6.9%	3.0%	2.3%	1.5%	0.0%
NRAS Q61K	12.5%	11.7%	6.3%	5.2%	3.1%	3.0%

Data interpretation

VCF and gVCF files generated by the on-instrument software or the TruSeq Amplicon app 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 reports. The intuitive framework offers flexible filtering options, streamlined variant classification, rapid and rich annotation, and customizable reporting options.

Deep coverage of targeted genes

The TruSight Myeloid Sequencing Panel features a highly optimized oligo pool specific for researching genomic changes associated with hematologic 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 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, providing the required analytical sensitivity and accuracy to call rare variants with confidence.

Table 2: Coverage details

Parameter	Specification
Cumulative target region size	~141 kb
Number of target genes	54
Amplicon size	~250 bp
Number of amplicons	568
Recommended mean coverage	5000×
Target minimum coverage	500×
Percent exons covered at ≥ 500×	95
Sample throughput - MiniSeq High Throughput Kit	8 samples/run
Sample throughput - MiSeq v3 chemistry	8 samples/run

Analytical performance

To demonstrate analytical performance, HDx Quantitative Multiplex Reference Standard DNA[5] (Horizon Diagnostics) samples were prepared with the TruSight Myeloid assay and sequenced on the MiSeq System using MiSeq v3 reagents. The reference DNA was serially diluted with control DNA at 50:50 and 25:75 proportions to generate different variant allele frequencies (Table 3). Limit of detection testing showed that variants can be called reliably down to 5% allele frequency. Variants of lower frequency were also detected. Laboratories should establish their own minimum detection threshold for calling low-frequency variants with confidence.

Summary

The TruSight Myeloid Sequencing Panel enables clinical research laboratories to access expert-defined content for investigating genomic features associated with hematologic 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, conserving time, money, and resources over single-gene assays.

Ordering information

Product	Catalog No.
TruSight Myeloid Sequencing Panel (96 samples)	FC-130-1010
TruSeq Custom Amplicon Index Kit	FC-130-1003
TruSeq Index Plate Fixture and Collar Kit	FC-130-1007
TruSeq Custom Amplicon Filter Plate	FC-130-1000

Learn more

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

References

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5. Quantitative Multiplex Reference Standard. www.horizondiscovery.com/quantitative-multiplex-reference-standard-hd701. Accessed October 6, 2017.

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 to use the product in customer's specific application.

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