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# TruSight® Myeloid Sequencing Panel

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

Blood cancers affect more than 1 million people in the United States alone[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 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). The result is a single assay for accurate, economical, and rapid profiling of liquid tumors.

## 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 in the peer-reviewed literature to be frequently mutated in hematological malignancies, focusing on leukemia and myeloproliferative disorders. Targeted genes include those with cited 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 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×

**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 | | | | |

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AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
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mean coverage. This translates into 8 samples per run, providing the required sensitivity and accuracy to call rare variants with confidence.

**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 | 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 |

## 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 an Illumina desktop sequencing system for automated sequencing and data analysis. These desktop sequencers include the MiniSeq™, MiSeq®, and MiSeqDx (run in RUO mode). Analyzed data can be imported into VariantStudio software for accurate variant annotation, classification, and reporting. The entire process is completed in just 3 days.

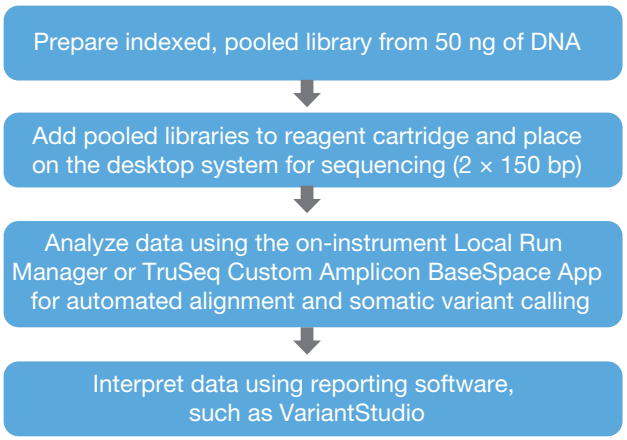


**Figure 1: Simple, Integrated Workflow**—TruSight Myeloid offers integrated library preparation, sequencing, and automated data analysis, creating a streamlined workflow that goes from DNA to data in 3 days.

## 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, 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 2 probes and yield a library of new templates with common ends. The resulting extension-ligation templates are PCR-amplified, incorporating 2 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.

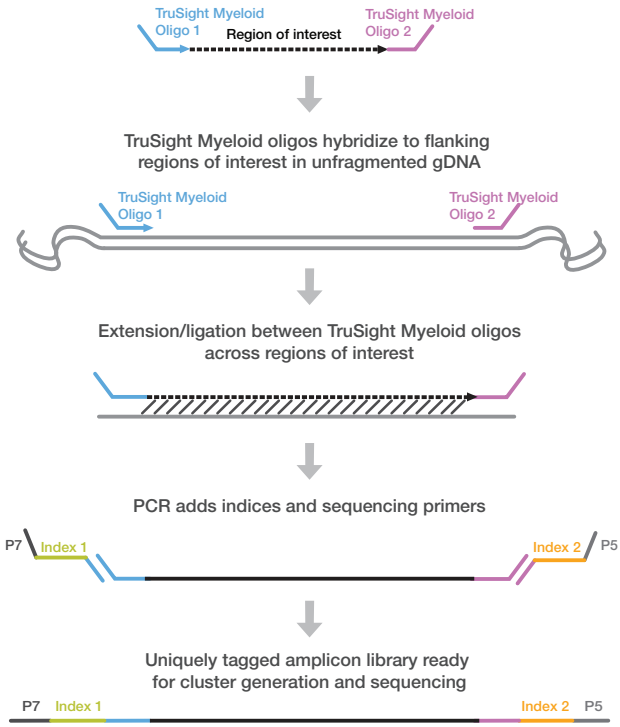


**Figure 2: Optimized Assay Chemistry**—The TruSight Myeloid assay enables simple, streamlined hybridization and amplification or targeted regions.

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Table 2: Next-Generation Sequencing Simplified

| 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 Analysis

Sequence data generated from TruSight Myeloid libraries are analyzed using on-instrument software or the TruSeq® Amplicon BaseSpace® App. 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 SNPs and small indels.

## Data Interpretation

VCF and gVCF files generated by the on-instrument software or TruSeq Amplicon BaseSpace 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.

## Analytical Performance

To demonstrate analytical performance, HDx Quantitative Multiplex Reference Standard DNA[2] (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 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 |
| 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


1. Facts and Statistics | The Leukemia & Lymphoma Society (www.lls.org/diseaseinformation/getinformationsupport/factsstatistics) Accessed 02 September 2014.
2. Horizon Diagnostics (www.horizondx.com/what-we-offer/q-seq-NGS-reference-standards/multi-gene-multiplex) Accessed 02 September 2014.


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AAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAA
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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.**

**Illumina** • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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