NGS Disease Research Panels

ClearSeq AML^{HS}

Access Targets for Acute Myeloid Leukemia (AML) Research

Now with Molecular Barcodes!

Benefits

Expert-Defined Content

- Designed in collaboration with Dr. Robert Ohgami and Dr. Daniel Arber at Stanford University
- Targets 20 key genes frequently mutated in AML

Premium Performance You Can Trust

- Provides 99.9% design coverage of targeted exons
- Confidently discern variants of very low frequency, down to 0.5%
- Library-prep free target enrichment in six hours

Comprehensive Workflow Solution

- Get from raw data to mutation report in 3 simple steps using SureCall analysis software
- Obtain all reagents for sample preparation, QC and automation tools from one trusted partner

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Overview

Acute myeloid leukemia (AML) is the most common myeloid neoplasm affecting adults and the role of chromosomal structural variations in its molecular pathogenesis have been well-established.[1] In recent years, next generation sequencing has led to a revolution in the study of hematological malignancies and shown that mutations and indels play an essential part in the pathogenesis of AML.

The ClearSeq AML was designed in collaboration with Dr. Robert Ohgami and Dr. Daniel Arber at Stanford University. It targets 48 selected exons in 20 genes found to be commonly mutated in AML and to be associated with myelodysplastic syndromes and myeloproliferative neoplasms.

The identification of rare variants, such as those in heterogeneous tumor samples, can be practically limited by the error rate associated with the sequencing process itself.[2] The ClearSeq AML^{HS} is an enhanced, high-sensitivity version of ClearSeq AML that addresses this limitation. Molecular barcodes have been incorporated to increase the accuracy of variant calls by allowing users to identify and remove false positive calls due to PCR and sequencing artifacts. It also has greater uniformity of coverage due to the addition of more overlapping probes in target genes, increasing the coverage of targeted regions to 99.4% at 20x read depth (Figure 1). Finally, the product supports DNA input of 50 ng compared with 200 ng input DNA required by the original kit.

More Accurate and Sensitive Variant Calling

The ClearSeq AML^{HS} incorporates HaloPlex^{HS} technology, which is an amplicon-based, NGS sample preparation method that employs molecular barcodes to enable detection of variant allele frequencies at below 1%.[3] Molecular barcodes

Gene List (targeted exons)							
ASXL1	12	**EZH2**	8, 17, 18	**MPL**	10	**SF3B1**	13–15, 17
CSF3R	14, 17	**FLT3**	14, 20	**NPM1**	11	**SRSF2**	1
CBL	8, 9	**IDH1**	4	**NRAS**	2, 3	**TET2**	3, 9, 10, 11
CEBPA	1	**IDH2**	4	**RUNX1**	3, 4, 8	**TP53**	5–8
DNMT3A	4, 8, 13, 15, 16, 18 19, 20, 22, 23	**JAK2**	12, 14	**SETBP1**	3	**U2AF1**	2, 6

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are short oligonucleotides that are added to targeted sample molecules to serve as unique identifiers.[4] After alignment, reads containing the same molecular barcode are analyzed and a consensus sequence is selected. Sequence differences observed in the population minority are considered artifacts of amplification or sequencing and are created. While typical amplicon-based technologies detect allele frequencies as low as 3-5%, the addition of molecular barcodes increases the ability to confidently detect low allelic fraction mutations associated with cancer.

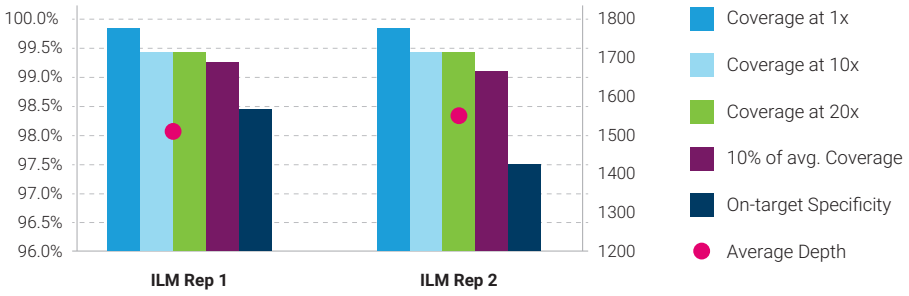

Figure 1. ClearSeq AML^{HS} coverage and specificity at varying sequencing depths on Illumina MiSeq instrumentation. Samples were sequenced to 4 Gb with over 99.0% of bases covered at 20x, with an on-target specificity exceeding 97.0% in all cases and reaching above 98.0% in most replicates. Over 95% of bases are covered at ≥ 10% of average coverage with normalized 2000x sequencing depth. The improved coverage and specificity means important mutations are not missed. ILM: MiSeq (Illumina).

Comprehensive Sample-to-Analysis Solution

SureCall software is critical to enable the high-sensitivity analysis of ClearSeq AML^{HS} data. The SureCall software analyzes the molecular barcodes to remove duplicate reads and corrects sequencing or PCR amplification errors. Using pre-configured cancer analysis workflows in the SureCall software, go from raw data to a mutation report containing categorized known variants of interest within minutes, greatly reducing time-to-results. Together with robust sample QC and throughput scalability by automation, ClearSeq AML^{HS} accelerates the confident detection of disease-associated variants.

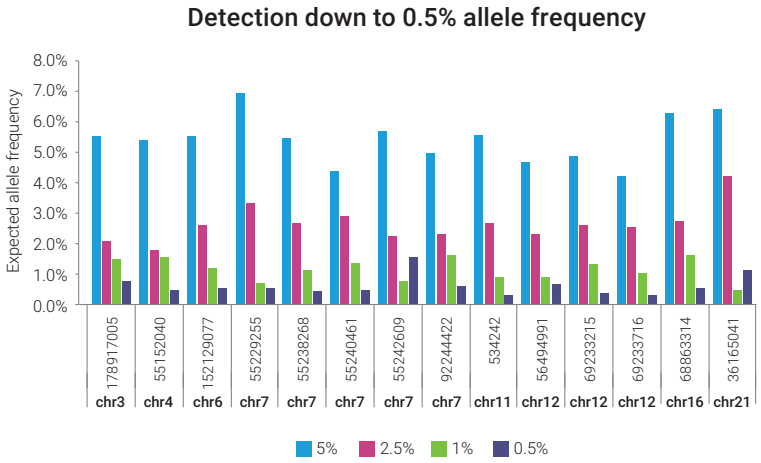

Figure 2. HapMap cell lines, NA18507 and NA10831, were mixed to generate allelic fractions ranging from 0.5% – 5%. The close agreement between expected and observed frequency at various chromosomal positions demonstrates the high sensitivity of HaloPlex^{HS} for low frequency variant detection. Data shown is representative of replicates (sequencing depth= 2000x – 4000x).

References

1. Betz BL, Hess JL. (2010) Arch Pathol Lab Med. 134(10): 1427-1433. Doi: 10.1043/2010-0245-RA.1.
2. Gundry, M and Vijg, J. (2012) Mutation Research. 729 (1-2): 1-15. Doi: 10.1016/mrfmmm.2011.10.001. Epub 2011 Oct 12.
3. M.A. Quail, Yong, Gu *et al*. (2012) BMC Genomics. 13: 341-354. Doi:10.1186/1471-2164-13-341.
4. L. Mamanova, D.J. Turner *et al*. (2010) Nature Methods. 7(2): 111-118. Doi: 10.1038/nmeth.1419.

Request more information or buy online:
www.agilent.com/genomics/AML

Product Description	Part Number
ClearSeq, AML, HS, ILM, 16 samples	G9963A
ClearSeq, AML, HS, ILM, 96 samples	G9963B

Kits to process 96 samples are also available.

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