MEDGENOME

Sy No 94/1C and 94/2, Tower-1, Veerasandra Village, Attibele Hobli,
Electronic City, Phase-1, Bangalore, Karnataka, 560100.

Fluorescent In Situ Hybridization (FISH) REPORT: FISH for Chronic Lymphocytic Leukemia (CLL) Panel- 6 markers (del6q, del17p, del13q, ATM del/Trisomy 11, Trisomy 12, IGH rearrangement)

Patient Name	I. Kathriarachchi	**Requesting Clinician**	Dr. Mahendra Perera
Gender	Female	**Hospital Information**	Aegle Omics Private Limited
Age/Date of Birth	65 Years	**Sample Source**	Peripheral Blood in Sodium Heparin.
Sample ID	9045266	**Samples Collected(Date & Time)**	25-03-2025 5:48 pm
Order ID(s)	1244031	**Samples Received(Date & Time)**	27-03-2025 11:40 am
Clinical Indication	Case of CLL.	**Report Date**	04-04-2025 5:02 pm
Collection Center/ Partner Lab	0		

RESULT SUMMARY

Sl no	Probe Name	FISH Result	ISCN 2020
1	IGH Gene Rearrangement	Positive	nuc ish(5'IGHx1,3'IGHx2)(5'IGH con 3'IGHx1)[140/200]
2	CEP12/D13S319 (13q14.2) (Tri12 and Del 13q)	Positive	nuc ish(D12Z3x3,D13S319x0~1)[120/200]
3	CEP6/MYB	Negative	nuc ish(CEP6,MYB)x2[198/200]
4	RB1/ATM	Negative	nuc ish(D11Z1,ATM)x2[198/200]
5	TP53/CEP17 (Del17p)	Negative	nuc ish(TP53,CEP17)x2[196/200]

Interpretation:

The FISH test is positive for Trisomy 12, Deletion 13q14.2 and IGH rearrangement and negative for Deletion 6q, Deletion 11q and Deletion 17p.

There is presence of IGH rearrangement with loss of spectrum green signal indicating deletion of IGHV segments within the green probe target as a result of normal somatic V-D-J recombination.Kindly correlate with clinical, hematological and cytogenetic findings.

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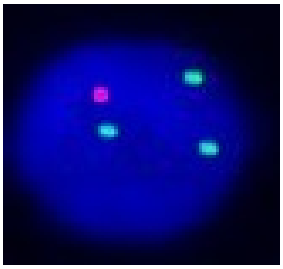
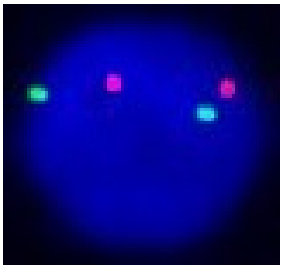
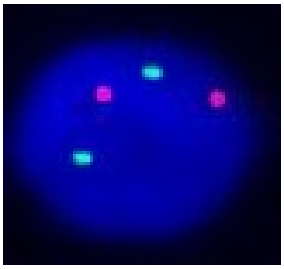
DETAILED REPORT



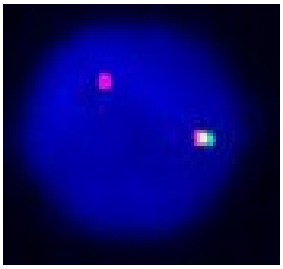
CEP12/D13S319 (13q14.2) (Tri12 and Del 13q)



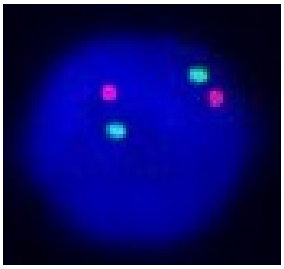
CEP6/MYB



TP53/CEP17 (Del17p)



IGH Gene Rearrangement



RB1/ATM

1) CEP12/D13S319 (13q14.2) (Tri12 and Del 13q) :

Spectrum Green (G)	**CEP 12**	**Spectrum Orange (O)**	**D13S319 (13q14.2)**

Loci Analyzed	Signal Pattern	Normal Cut Off (%)	Percentage of Cells showing signal pattern	Result
CEP12/D13S319 (13q14.2) (Tri12 and Del 13q)	**3G/1O**	**> 2 %**	**60 %**	**Positive**

2) CEP6/MYB :

Spectrum Green (G)	**CEP6**	**Spectrum Orange (O)**	**MYB(6q23)**

Loci Analyzed	Signal Pattern	Normal Cut Off (%)	Percentage of Cells showing signal pattern	Result
CEP6/MYB	**2G/2O**	**>= 98 %**	**99 %**	**Negative**

3) TP53/CEP17 (Del17p) :

Spectrum Green (G)	**CEP17**	**Spectrum Orange (O)**	**TP53**

Fluorescent In Situ Hybridization (FISH) REPORT: FISH for Chronic Lymphocytic Leukemia (CLL) Panel- 6 markers (del6q, del17p, del13q, ATM del/Trisomy 11, Trisomy 12, IGH rearrangement)

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Loci Analyzed	Signal Pattern	Normal Cut Off (%)	Percentage of Cells showing signal pattern	Result
TP53/CEP17 (Del17p)	2G2O	>= 98 %	98 %	Negative

4) IGH(14q32) :

Spectrum Green (G)	5'IGH	Spectrum Orange (O)	3'IGH

Loci Analyzed	Signal Pattern	Normal Cut Off (%)	Percentage of Cells showing signal pattern	Result
IGH(14q32)	2F/0G/0O	>= 98 %	30 %	Positive

5) RB1/ATM :

Spectrum Green (G)	RB1	Spectrum Orange (O)	ATM

Loci Analyzed	Signal Pattern	Normal Cut Off (%)	Percentage of Cells showing signal pattern	Result
RB1/ATM	2G/2O	> 98 %	99 %	Negative

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Testing methodology: FISH is a molecular cytogenetic technique used to detect the presence or absence and location of specific gene sequences. FISH involves co-denaturation and hybridization of fluorescent labelled specific DNA probes to target DNA sequence in the interphase cells. The excess unbound probe is removed during post hybridization washes. The sample is stained with a DAPI (4',6-Diamidino-2-phenylindole) counter-stain to demarcate the nuclei. Each fluorescent labelled probe that hybridizes to region of interest in interphase cells is visualized as signal using suitable optical filters under Epi fluorescent microscope. 200 interphase cells are counted for each probe manually by two readers. Interpretation of results are done based on the signal patterns observed. Based on these interpretations, the results of the test are reported. Metasystems probes are used for the CLL panel. Trisomy 12 is a tricolor probe, it can detect trisomy 12 and deletion 13q.Appropriate controls are run along with patient samples.
Chronic lymphocytic leukemia is a neoplasm composed of monomorphic small mature B-Cells. About 80-905 of cytogenetic abnormalities are detceted by FISH or copy number arrays. As per WHO, the most common abnormalities seen in CLL are Del13q14.3, trisomy 12, Del11q22-23, Del17p13 and Del6q. NCCN guidelines recommends testing for t(11;14), Del 13q, Del 11q, +12,Del 17p by FISH. **If Del 17p/TP53 mutation is present, ibrutinib is given as first line treatment.**

(i)NCCN adopted Prognostic information for CLL based on Interphase FISH results:

Prognosis	Cytogenetic markers
Unfavorable	Del (17p) , del (11q)
Neutral	Normal, Trisomy 12
Favorable	Del (13q) as sole abnormality

References:

1. National comprehensive cancer care network. NCCN guidelines for Chronic lymphocytic leukemia or Small lymphocytic leukemia (CLL/SLL).Version 5.2018-March 26,2018.
2. WHO Classification of tumors of hematopoietic and lymphoid tissues, Revised edition 2017, 215-220.

Disclaimers:

1. This test was developed, and its performance characteristics determined by MedGenome. It has not been cleared or approved by the US Food and Drug Administration.
2. The finding of this test must be correlated with other clinical, haematopathological and cytogenetic findings for complete analysis.
3. Genetic changes other than those assayed cannot be ruled out on the basis of this testing.

Prepared by:
Sruthi M

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Lab Director

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Molecular Pathologist
KMC Reg No. - 71468

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Gender	Female	**Hospital Information**	Aegle Omics Private Limited
Age/Date of Birth	65 Years	**Sample Source**	Peripheral Blood in Sodium Heparin.
Sample ID	9045266	**Samples Collected(Date & Time)**	25-03-2025 5:48 pm
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Collection Center/ Partner Lab	0		

End of Report

I. Kathriarachchi

AGE: 65 years | **Gender:** Female | **Coupon/UID:** NA **/** AOMG94

MEDGENOME

MGM1342 : IGHV gene Mutation Analysis

Report Details		Specimen Information		Ordering Clinician	
Sample ID / Order ID:	9045262 / 1244031	**Specimen Site:**	NA	**Clinician:**	Dr. Mahendra Perera
Collection Date:	25th March 2025	**Specimen Received:**	Peripheral Blood in EDTA	**Affiliation:**	Aegle Omics Private Limited
Date Received:	27th March 2025			**Serviced By:**	18718
Report Date & Time:	12th Apr 2025 10:04 AM	**Specimen Tested:**	Peripheral Blood in EDTA	**Report Status:**	Final
		Tumor Content (%):	NA		

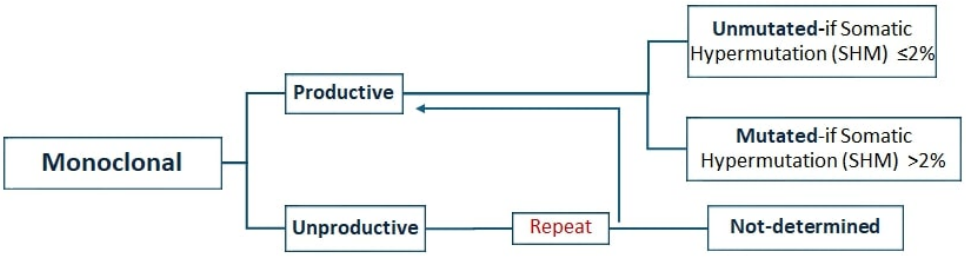
Clinical Summary:

Chronic Lymphocytic Leukemia (CLL) [as per the clinical details provided along with the Test Requisition Form]. The FISH test is positive for Trisomy 12, Deletion 13q14.2 and IGH rearrangement and negative for Deletion 6q, Deletion 11q and Deletion 17p. There is presence of IGH rearrangement with loss of spectrum green signal indicating deletion of IGHV segments within the green probe target as a result of normal somatic V-D-J recombination [as per the FISH for CLL report dated; 04-04-2025].

TEST RESULT SUMMARY

This is a **Monoclonal** sample, and the IGHV gene status of the subject under investigation is **MUTATED**

Result Summary	Clone Information
IGHV gene Status	MUTATED
V-Gene and Allele	IGHV3-74*01
J-Gene and allele	IGHJ4*02
Functionality	Productive
% SHM	11.10
Subset	Unassigned



ERIC Guidelines- 35614318, NCCN Guidelines Version 3.2024, [PMID: 10477713, 10477712, 21455216, 28439111].

INTERPRETATION AND CLINICAL SIGNIFICANCE

The mutation status of *IGHV* gene represents a predictive biomarker for identifying patients that may benefit the most from chemoimmunotherapy with Fludarabine, Cyclophosphamide and Rituximab.

In the CLL8 study the prognosis in FC versus FCR as first-line therapy was evaluated and unmutated *IGHV* was the strongest predictor of shorter progression-free survival and overall survival [PMID: 26486789]. In the long-term follow-up from the CALGB 9712 study where outcome in concurrent versus sequential Fludarabine and Rituximab as first-line therapy was evaluated, it was reported that the unmutated *IGHV* was a significant independent predictor for shorter survival [PMID: 21321292].

As per the latest NCCN Guidelines Version 3.2024, in the first-line setting, CLL patients greater than 65 years of age without del(17p)/*TP53* mutation, are suggested Ibrutinib or Acalabrutinib ± Obinutuzumab or Venetoclax ± Obinutuzumab.

The above recommendation holds good even in patients <65 years of age without significant co-morbidities. Nevertheless, in absence of del(17p)/ *TP53* mutation, and in presence of *IGHV* mutation, FCR is a preferred regimen.

The choice of FCR regimen is based on the *IGHV* mutation status. Those patients who are *IGHV* mutated are known to respond to FCR regimen.

TEST DESCRIPTION

The International Prognostic Index for CLL (CLL-IPI) stratifies patients into four risk groups (low, intermediate, high, and very high) based on *TP53* and IGHV mutation status, serum beta-2 microglobulin concentration, clinical stage, and age [PMID: 29540348]. Clonal *IGHV* gene hypermutation status provides important prognostic information for patients with CLL and small lymphocytic lymphoma (SLL). Additionally, the knowledge of stereotypy subset for instance unmutated IGHV or the VH3-21 gene usage was shown to be an independent predictor of shorter treatment-free interval and/or survival outcomes, even when high-risk genetic abnormalities were included in the multivariable regression models [PMID: 35614318, 32509784, NCCN Guidelines Version 3.2024]

TEST METHODOLOGY

Sample Type: Peripheral blood or BMA in EDTA.

Extraction and Amplification:DNA is extracted from the sample and amplified using multiplex primers sets flanking the V-J region. The test employs two different master mixes: Hypermutation Mix 1 v2.0 (M1) and Hypermutation Mix 2 v2.0. The Hypermutation Mix 1 v2.0 targets sequences between the leader and joining regions, while The Hypermutation Mix 2 v2.0 (M2) targets sequences between the framework 1 (FR1) and joining (J) regions. The QC of the amplified products are verified on capillary electrophoresis. The gaussian distribution obtained represents the heterogenous population of V-D-J rearrangements. The gel extracted M1 and M2 amplicons are then sequenced by Sanger sequencing (ABI platform).

Data analysis and Reporting: GeneMapper software is used for detection of clonal immunoglobulins heavy chains gene rearrangements. The FASTQ files are interpreted using the NCBI analysis tool IgBlast [https://www.ncbi.nlm.nih.gov/projects/igblast/] and the ARResT/AssignSubsets online tool as per the manufacturer's recommendation [PMID: 26249808].

DISCLAIMER

- **IGHV SOMATIC HYPERMUTATION ANALYSIS IS FOR INVESTIGATIONAL PURPOSE ONLY. TREATMENT DECISIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.**
- The prognostic value of somatic IGHV mutation status is applicable currently only to B-CLL and the test is not intended for use in other B-cell neoplasms or hematopoietic tumors.
- This test requires a minimum of 5% of monoclonal CLL B-cell percentage (as determined by flow cytometric immunophenotyping) to amplify the clonal IGH gene rearrangement. A CLL population below 5% will not have a reliable or reproducible clonal gene rearrangement.
- The IGH gene mutation status reporting is limited to IGHV and IGHJ allele due to the perceived difficulties in the identification of IGHD.
- The prognostic significance of SHM status is only known when a single productive IGH rearrangement is identified with in-frame junctional coding region and no premature stop codons.
- If greater than one productive rearrangement is identified, the clinical significance may vary depending on the mutation status of each productive rearrangement.
- Only 12-13% of IGHV segments could be classified into one of the 19 major subsets.

Dr. Ritika Chauhan, Ph.D
Lead Genome analyst

Dr. Shruthi. P. S., M.D. Pathology
Senior Hematopathologist
KMC Reg No.-78159

END OF REPORT

I. Kathriarachchi

AGE: 65 years | **Gender:** Female | **Coupon/UID:** NA **/** AOMG94

MEDGENOME

MGM499: Leukemia Panel (SNVs, small INDELs and CNVs) by NGS

Report Details		Specimen Information		Ordering Clinician	
Sample ID / Order ID:	9045262 / 1244031	**Specimen Site:**	NA	**Clinician:**	Dr. Mahendra Perera
Collection Date:	25th March 2025	**Specimen Received:**	Peripheral Blood in EDTA	**Affiliation:**	Aegle Omics Private Limited
Date Received:	27th March 2025	**Specimen Tested:**	Peripheral Blood in EDTA	**Serviced By:**	18718
Report Date & Time:	11th Apr 2025 15:14 PM	**Tumor Content (%):**	NA	**Report Status:**	Final

CLINICAL BACKGROUND

Chronic Lymphocytic Leukemia (CLL) [as per the clinical details provided along with the Test Requisition Form]. The FISH test is positive for Trisomy 12, Deletion 13q14.2 and IGH rearrangement and negative for Deletion 6q, Deletion 11q and Deletion 17p. There is presence of IGH rearrangement with loss of spectrum green signal indicating deletion of IGHV segments within the green probe target as a result of normal somatic V-D-J recombination [as per the FISH for CLL report dated; 04-04-2025].

Test Result Summary

Result - NEGATIVE

NO CLINICALLY RELEVANT VARIANT/S DETECTED

Gene/AMP Classification ^	Clinical relevance	Therapeutic relevance $	Interpretation
No significant variants detected			

No clinically significant SNVs, Small INDELs and CNVs have been detected in this sample

^ Refer to Glossary section for the classification criteria details.

$ Drug Approvals are based on US-FDA Guidelines. Kindly refer to local guidelines if required.

ADDITIONAL BIOMARKERS DETECTED

This section provides information about variants that do not have any therapeutic value. However, these variants may or may not have a likely oncogenic effect.

GLOSSARY

AMP Classification Criteria: Displays the classification of a biomarker according to the recommendations of the Association for Molecular Pathology (AMP) [PMID: 27993330].

Tier	Criteria
Tier IA	Variants of strong clinical significance. FDA-approved therapy or biomarkers included in professional guidelines.
Tier IB	Variants of strong clinical significance. Well-powered studies with consensus from experts in the field.
Tier IIC	Variants of potential clinical significance. FDA-approved therapies for different cancer types or investigational therapies. Multiple small published studies with some consensus.
Tier IID	Variants of potential clinical significance. Preclinical trials or a few case reports without consensus.
Tier III	Variants of unknown clinical significance.
Tier IV	Benign or likely benign variants.

Drug approval:

The development stage of the treatment for the patient's indication as per US-FDA guidelines.

Stage	Definition
Approved	This drug is launched for the primary or a secondary patient disease
Off-Label	This drug is launched for a disease other than the primary or secondary patient diseases
Investigational	This drug is currently under clinical development in the patient disease.
Other	None of the other stages are applicable. The drug or drug class is, for example, suspended, discontinued, or withdrawn.

DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MedGenome later for any change.
- Intronic variants are not assessed using this method.
- Rearrangements cannot be assessed using this method.
- Certain genes may not be covered completely, and few mutations could be missed.
- This NGS test used does not allow definitive differentiation between germline and somatic variants.
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and small indels, cannot be ruled out.

TEST DESCRIPTION

The whole genome sequencing of different subtypes of leukemia revealed new recurrent genetic and chromosomal abnormalities that could add value to the existing prognostic scoring index in different subtypes of leukemia. Several studies have been reported wherein clinical outcome was measured to correlate the significance of the mutational findings from whole genome sequencing. The scope of this Leukemia Panel (SNVs, small INDELs and CNVs) by NGS testing includes a panel of genes, wherein prognostic significance of these genes and their mutations has been well studied and documented in medical literature. The panel is designed on targeted sequencing of multiple genes for the coding regions through NGS.

TEST METHODOLOGY

Sample type: Peripheral blood or bone marrow in EDTA tube.

Extraction and Library Preparation: Nucleic acid extracted from blood or bone marrow was used to perform targeted gene capture by custom capture kit.

Sequencing: The QC passed libraries were sequenced on validated Illumina sequencing platform.

Data Analysis: The sequences obtained were aligned to human reference genome (GRCh38.p13/) using BWA program [PMID: 20080505, 23155063] The validated UMI-based analysis pipeline was designed to accurately detect all classes of genomic alterations (SNVs, InDels and CNVs). Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation.

Limit of Detection (LOD): The LOD for SNVs and InDels is 5% Variant allele Frequency (VAF)

Variant : The mutations were annotated using our in-house annotation pipeline (VariMAT). Gene annotation of the variants was performed using VeP program [PMID: 27268795] against the Ensembl release 99 human gene Model Clinically relevant mutations were annotated using peer-reviewed publications, public clinical databases (ClinVar, HGMD, CiViC, OncoKb), medical guidelines (NCCN, ASCO, AMP). The common variants are filtered out based on the minor allele frequency (MAF) in various population databases (1000G, ExAC, gnomAD, GAsP, dbSNP, OncoCrDb (in-house curated database)) and only variants with MAF <0.01% are considered for reporting [PMID: 29155950,27535533, 26292667,31526404]

Reporting : Clinically relevant mutations were prioritized, and reports were prepared based on AMP-ASCO-CAP, WHO, ASH guidelines [PMID: 27993330,WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues; Revised 4th Edition, Volume 2] and also based on annotation metrics from OncoMD [PMID: 26928227],MedGenome's curated somatic database which includes somatic mutations from TCGA.

#The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 99 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on in-complete and nonsense mediated decay transcripts will not be reported. This test has been developed and its performance characteristics are verified at MedGenome labs.

COVERAGE OF ANALYZED GENES

Gene	Coverage (%)	Gene	Coverage (%)	Gene	Coverage (%)
ABL1	100	*ARAF*	100	*ARID1A*	98.24

Gene	Coverage (%)	Gene	Coverage (%)	Gene	Coverage (%)
ASXL1	100	*ASXL2*	100	*ATM*	100
ATRX	100	*B2M*	100	*BCL2*	100
BCL6	100	*BCOR*	100	*BCORL1*	100
BIRC3	100	*BRAF*	100	*CALR*	100
CARD11	100	*CBL*	100	*CBLB*	100
CBLC	100	*CCND1*	100	*CCND3*	100
CCR4	100	*CCR7*	100	*CD28*	100
CD58	100	*CD79B*	100	*CDKN1A*	100
CDKN2A	100	*CDKN2B*	100	*CDKN2C*	100
CEBPA	78.58	*CREBBP*	100	*CSF3R*	100
CTNNA1	100	*CUX1*	98.02	*CXCR4*	100
CYLD	100	*DIS3*	100	*DNMT3A*	100
EP300	100	*EPHA7*	100	*ETNK1*	100
ETV6	100	*EZH2*	100	*FAS*	100
FBXW7	100	*FGFR3*	100	*FLT3*	100
FYN	100	*GATA1*	100	*GATA2*	100
GATA3	100	*GNA13*	100	*GNAS*	99.9
HNRNPA2B1	100	*HRAS*	100	*ID3*	100
IDH1	100	*IDH2*	100	*IKZF1*	100
INPP5D	100	*IRF4*	100	*ITPKB*	100
JAK1	100	*JAK2*	100	*JAK3*	100
KDM6A	100	*KIT*	100	*KLF2*	71.27
KLHL14	100	*KMT2A*	99.34	*KMT2D*	100
KRAS	100	*MAP2K1*	100	*MEF2B*	100
MFHAS1	100	*MPL*	100	*MYBBP1A*	100
MYD88	100	*NF1*	100	*NFE2*	100
NOTCH1	100	*NOTCH2*	100	*NPM1*	100
NRAS	100	*OSBPL10*	100	*PAX5*	100
PDCD1LG2	100	*PDGFRA*	100	*PDGFRB*	100
PHF6	100	*PIK3CA*	100	*PLCG1*	100
POT1	100	*PRDM1*	100	*PRKCB*	99.92
PRPF8	100	*PTEN*	100	*PTPN1*	100
PTPN11	100	*RAD21*	100	*RB1*	100
REL	100	*RHOA*	100	*RUNX1*	100
SETBP1	100	*SETD2*	100	*SF3B1*	100
SGK1	100	*SMARCA4*	100	*SMC1A*	100
SMC2	100	*SMC3*	100	*SOCS1*	100
SRSF2	100	*STAG2*	100	*STAT3*	100
STAT5B	100	*STAT6*	100	*SUSD2*	100
TCF3	100	*TENT5C*	100	*TET1*	100
TET2	100	*TNFAIP3*	100	*TNFRSF14*	100
TP53	100	*TRAF3*	100	*U2AF1*	100
VAV1	98.62	*WT1*	100	*XPO1*	100

Gene	Coverage (%)	Gene	Coverage (%)	Gene	Coverage (%)
ZRSR2	100				

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END OF REPORT