A Sample to Insight® NGS solution for myeloid neoplasms: Redefined amplicon sequencing for low-variant detection and interpretation

Introduction

Myeloid neoplasms are a group of diseases characterized by a wide range of mutations across many genes, including oncogenes and tumor suppressor genes. Genes commonly mutated in myeloid neoplasms include *CALR* and *CEBPA* in acute myeloid leukemia (AML) and *TP53* and *RB1* in chronic myeloid leukemia (CLL). These genes can acquire a variety of mutations, and each myeloid neoplasm can have mutations in multiple genes. These mutations are relevant for tumor classification and, therefore, require extensive investigation to understand disease development and progression (1).

A next-generation sequencing (NGS) run on a panel of key genes commonly mutated in myeloid neoplasms can rapidly capture these changes across many genes. However, NGS analysis is challenging for several reasons, including low allele frequency of variants, high GC content and low enrichment of target DNA (see Table 1).

Table 1. An overview of NGS challenges and the corresponding QIAseq® solutions

Challenge	QIAseq solution
Detection of low-allele-frequency variants	Incorporation of unique molecular indices (UMIs) to reduce false positives
Low enrichment and sequencing uniformity	Utilization of a proprietary approach for target enrichment
Incompatibility with GC-rich regions	Optimized chemistry that enriches GC-rich regions
Low complexity of amplicon-based libraries	Target enrichment approach to increase library complexity by defining targets with one (instead of two) target-specific primer
High DNA input requirement	As low as 10 ng DNA is enough
Mechanical shearing	Enzymatic fragmentation in a single reaction
Long turnaround time	DNA to library in just 6 hours
Low-throughput sample processing	Automation-friendly workflow for high-throughput applications
Multiple primer pools for enrichment	High primer multiplexing capabilities with up to 20,000 primers in a single pool
Limited sample multiplexing capabilities	Unique dual index (UDI) multiplexing approach; up to 384 sample indices for Illumina® platforms; up to 96 for Ion Torrent™ platforms
Hotspot coverage only	Flexibility in primer design for genome-wide coverage
Limited ability to increase panel content	Flexibility to easily increase the content of any panel
Inefficient customization of panels	Robust primer design algorithms
Time-consuming bead purifications	New enzymatic process for preventing adapter and primer carryover contamination, eliminating tedious bead cleanup steps

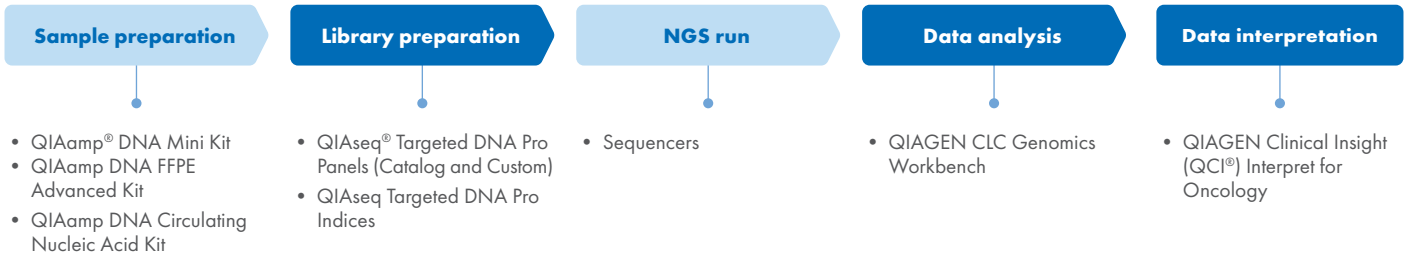


Figure 1. A Sample to Insight NGS solution for myeloid neoplasms.

The QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel is a targeted enrichment NGS panel that overcomes many of these challenges by enabling:

- High-sensitivity detection of <1% variant allele frequency (VAF) using UMIs
- Full *CEBPA* coverage using chemistry compatible with GC-rich regions
- Detection of *CALR* deletions enabled by robust bioinformatics pipelines
- Comprehensive coverage of genes driven by high primer multiplexing capabilities
- Automation-friendly, sample to sequencing-ready library in under 6 hours

Figure 1 shows the complete workflow from sample preparation to data interpretation.

Prepare NGS libraries with QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel

The QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel enriches genes and constructs libraries for NGS analysis of 164 genes commonly mutated in myeloid neoplasms. We specifically collected targeted actionable and interpretable genes and variants from:

- Clinical practice guidelines recommended by multiple organizations, including the American College of Medical Genetics (ACMG), the American Society of Hematology (ASH), the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), the College of American Pathologists (CAP), the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN)
- NIH NCBI ClinVar and Online Mendelian Inheritance in Man (OMIM) resources that compile variants with known associations to cancers cited by peer-reviewed publications
- Clinical trial documents from resources such as Cancer.gov, ClinicalTrials.gov, and the UMIN Clinical Trials Registry
- Requests and suggestions from QIAGEN® key opinion leaders and subject matter experts
- Other peer-reviewed publications

The panel covers all coding sequences with 20 bp intronic padding across all transcripts, giving researchers comprehensive insights into myeloid neoplasms. Tables 2–3 provide the list of genes covered by the panel. Coverage bed files should be consulted for complete coverage details.

Table 2. List of genes covered by QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel

ABL1	*BRCA2*	*CEBPA*	*ETV6*	*IL7R*	*NOTCH1*	*RAD21*	*SMC1A*	*XPO1*
ABL2	*BRIP1*	*CHEK1*	*EZH2*	*INO80*	*NOTCH2*	*RAD51B*	*SMC3*	*ZAP70*
ANKRD26	*BTK*	*CHEK2*	*FANCL*	*JAK1*	*NPM1*	*RAD51C*	*SOCS1*	*ZEB2*
ARHGAP26	*CALR*	*CREBBP*	*FBXW7*	*JAK2*	*NRAS*	*RAD51D*	*SRP72*	*ZRSR2*
ARID1A	*CARD11*	*CRLF2*	*FGFR3*	*JAK3*	*NUMA1*	*RAD54B*	*SRSF2*	
ARID1B	*CASP10*	*CSF3R*	*FLT3*	*KDM6A*	*NUP214*	*RAD54L*	*STAG2*	
ASXL1	*CBFB*	*CUX1*	*FOXO1*	*KIT*	*PALB2*	*RARA*	*STAT3*	
ATM	*CBL*	*CXCR4*	*GATA1*	*KLF2*	*PAX5*	*RB1*	*STAT5B*	
ATRX	*CBLB*	*DCK*	*GATA2*	*KMT2A*	*PDGFRA*	*RBBP6*	*STAT6*	
B2M	*CBLC*	*DDX41*	*GATA3*	*KMT2D*	*PDGFRB*	*RET*	*TERC*	
BARD1	*CCND1*	*DHX15*	*GNA13*	*KRAS*	*PHF6*	*RHOA*	*TERT*	
BCL10	*CCND2*	*DKC1*	*GNAS*	*LUC7L2*	*PICALM*	*RPS14*	*TET1*	
BCL2	*CCR4*	*DNMT3A*	*GNB1*	*MAP2K1*	*PIGA*	*RUNX1*	*TET2*	
BCL6	*CD38*	*DNMT3B*	*H1-4*	*MEF2B*	*PIK3CA*	*SAMD9*	*TNFAIP3*	
BCOR	*CD79A*	*EBF1*	*HRAS*	*MET*	*PIM1*	*SAMD9L*	*TNFRSF14*	
BCORL1	*CD79B*	*EGFR*	*IDH1*	*MPL*	*PLCG2*	*SETBP1*	*TP53*	
BCR	*CDC25C*	*ELANE*	*IDH2*	*MTOR*	*PPM1D*	*SETD2*	*TPSAB1*	
BIRC3	*CDKN1B*	*EP300*	*IKZF1*	*MYC*	*PRPF8*	*SF3B1*	*U2AF1*	
BRAF	*CDKN2A*	*ERBB2*	*IKZF2*	*MYD88*	*PTEN*	*SH2B3*	*U2AF2*	
BRCA1	*CDKN2B*	*ETNK1*	*IKZF3*	*NF1*	*PTPN11*	*SLC29A1*	*WT1*	

Table 3. Gene list by functional disease groupings

Leukemia	
Acute lymphoblastic leukemia	*ABL1, ABL2, BIRC3, BRAF, BTK, CDKN2A, CDKN2B, CREBBP, CRLF2, EBF1, EP300, ETV6, FBXW7, FLT3, GATA3, HRAS, IKZF1, IL7R, JAK1, JAK2, JAK3, KDM6A, KMT2A, KRAS, MYC, NOTCH1, NRAS, PAX5, PDGFRB, PLCG2, PTEN, PTPN11, RUNX1, SF3B1, SH2B3, TP53*
Acute myeloid leukemia	*ANKRD26, ASXL1, ATRX, BRCA1, BRCA2, CBFB, CBLB, CBLC, CCND2, CDC25C, CEBPA, CUX1, DCK, DDX41, DHX15, DNMT3A, ELANE, ETV6, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, IKZF2, IKZF3, JAK2, KDM6A, KIT, KMT2A, KRAS, LUC7L2, MTOR, NPM1, NRAS, NUP214, PDGFRA, PICALM, PRPF8, PTPN11, RB1, RBBP6, RPS14, RUNX1, SF3B1, SLC29A1, SMC1A, TERT, TET1, TET2, TP53, U2AF1, U2AF2, WT1, ZEB2*
Acute promyelocytic leukemia	*CEBPA, NPM1, NUMA1, RARA, RUNX1*
Atypical chronic myeloid leukemia	*ASXL1, CBL, CEBPA, EZH2, FLT3, JAK2, KRAS, NPM1, NRAS, RUNX1, SETBP1, SRSF2, TET2, U2AF1*
Chronic lymphocytic leukemia	*ATRX, BCL2, BIRC3, BRAF, CBLB, CCND2, CD38, CUX1, GNAS, GNB1, LUC7L2, MYD88, NOTCH1, NRAS, PTPN11, SF3B1, TP53, U2AF2, XPO1, ZAP70*
Chronic myeloid leukemia	*ABL1, ASXL1, BCOR, BCR, BRAF, CBL, CREBBP, DNMT3A, DNMT3B, EZH2, IDH1, IDH2, IKZF1, JAK2, KRAS, NPM1, NRAS, RUNX1, TET1, TET2, TP53, WT1*
Chronic myelomonocytic leukemia	*ASXL1, BCOR, BCORL1, CBL, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PHF6, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, ZRSR2*
Hairy cell leukemia	*BRAF, CDKN1B, MAP2K1, TP53*
Juvenile myelomonocytic leukemia	*ARHGAP26, CBL, KRAS, NRAS, PTPN11*
Lymphomas	
Acute lymphocytic lymphomas	*BIRC3, BTK, CDKN2A, CDKN2B, MYC, NOTCH1, PLCG2, SF3B1, TP53*
B-cell lymphomas	*BCL6, BRAF, CD79A, CHEK2, EZH2, MAP2K1, MYD88, STAT6, TNFRSF14, TP53*
Burkitt lymphomas	*CBLB, GNA13, H1-4, MTOR, MYC*

Table continued from previous page.

Lymphomas	
Diffuse large B-cell lymphomas	*B2M, BCL2, CBLB, GNA13, H1-4, KMT2D, MTOR, PIM1, PRPF8, SOCS1, TNFAIP3*
Follicular lymphomas	*ARID1A, BCL10, BCL2, CARD11, CREBBP, EP300, EZH2, FOXO1, KMT2D, MEF2B*
Mantle cell lymphomas	*ATM, CCND1*
Marginal zone lymphomas	*KLF2, MYD88, NOTCH2, TNFAIP3, TP53*
Mucosa-associated lymphoid tissue (malt) lymphomas	*BCL10, TNFAIP3*
Non-Hodgkin's lymphomas	*BRAF, CASP10, NRAS, PIK3CA, RAD54B, RAD54L, TP53*
Primary cutaneous lymphomas	*CD79B, MYD88*
Small lymphocytic lymphomas	*ATRX, BCL2, CBLB, CCND2, CUX1, GNAS, LUC7L2, U2AF2, XPO1*
T-cell lymphomas	*ARID1B, ATM, CBLB, CCR4, DNMT3A, IDH1, IDH2, INO80, JAK1, JAK3, RHOA, SETD2, STAT3, STAT5B, TET2*
Myelodysplastic syndromes and myeloproliferative neoplasms	
Myelodysplastic syndromes	*ANKRD26, ASXL1, ATRX, BCOR, BCORL1, CALR, CBL, CEBPA, CSF3R, CUX1, DDX41, DKC1, DNMT3A, ETNK1, ETV6, EZH2, FLT3, GATA2, GNAS, GNB1, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MPL, NF1, NPM1, NRAS, PHF6, PIGA, PPM1D, PTPN11, RAD21, RUNX1, SAMD9, SAMD9L, SETBP1, SF3B1, SMC3, SRP72, SRSF2, STAG2, STAT3, TERC, TERT, TET2, TP53, U2AF1, WT1, ZRSR2*
Myeloproliferative neoplasms	*ASXL1, BCOR, CALR, CBL, CSF3R, DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, MPL, NOTCH1, NRAS, PDGFRA, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, STAT3, STAT5B, TET2, TP53, U2AF1, ZRSR2*
Other myeloid neoplasms	
Essential thrombocythemia	*ASXL1, CBL, IDH2, JAK2, MPL, SF3B1, TET2, TP53*
Multiple myeloma	*ATM, BARD1, BRAF, BRCA1, BRCA2, BRIP1, CHEK1, CHEK2, CXCR4, EGFR, ERBB2, FANCL, FGFR3, IDH1, IDH2, KIT, KRAS, MET, NRAS, PALB2, PDGFRA, RAD51B, RAD51C, RAD51D, RET, TP53*
Plasma cell myeloma	*HRAS, MTOR, RB1, SLC29A1*
Polycythemia vera	*ASXL1, DNMT3A, EZH2, IDH1, JAK2, NRAS, TET2, TP53*
Primary myelofibrosis	*ASXL1, CBL, DNMT3A, EZH2, IDH1, IDH2, JAK2, MPL, NF1, NRAS, SETBP1, SF3B1, SRSF2, TET2, TP53*
Systemic mastocytosis	*ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH2, JAK2, KIT, KRAS, NPM1, NRAS, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TPSAB1, U2AF1*
Waldenstrom macroglobulinemia	*CXCR4, MYD88*

The panel can be customized to include additional genes or specific genes, exons, hotspots or genomic loci. Visit the QIAseq Targeted DNA custom panel builder at **www.qiagen.com/QIAseqDNAcustom**.

Obtain high sensitivity using unique molecular indices

The QIAseq panel incorporates UMIs to reduce false positive rates, increasing confidence in calling low-allele-frequency variants. Tagging unique DNA molecules with UMIs before amplification enables UMI-aware pipelines to condense reads back to the original DNA molecules, thereby overcoming the issue of PCR duplicates.

The QIAseq panel, the UMI-aware data analysis pipelines and a powerful knowledge base for interpretation together deliver a complete Sample to Insight solution for myeloid neoplasms analysis. The streamlined workflow enables routine detection of known and novel myeloid neoplasm mutations in any research laboratory with access to NGS platforms. Tables 4, 5 and 6 outline the performance specifications, coverage and sample multiplexing, respectively.

Table 4. Performance specifications

Attribute	Specification
DNA input	≥10 ng
Targeted region size (bp)	574 kb
Targeted regions	Exonic regions with 20 bp intronic padding
Types of variants called	SNVs, Indels, CNVs*
Variant allele frequency called	<1%
Specificity (on-target reads)	96.1%
Uniformity (0.2x mean coverage)	99.7%
Recommended mean coverage for 5% VAF	7200x
Sequencer compatibility	Illumina, Ion Torrent, Element AVITI™† and MGI†sequencers

* Depends on the secondary analysis pipeline; the Biomedical Genomics Analysis plugin to QIAGEN CLC Genomics Workbench enables detection of SNVs, Indels and CNVs.
† Element AVITI and MGI instruments would require a conversion kit.

Table 5. Coverage

Recommended coverage depends on the required VAF and DNA input			
VAF (%)	**DNA input (ng)**	**Read pairs/UMI**	**Mean read**
5	10	4	7200x
1	40	4	25,600x

Table 6. Sample multiplexing for Illumina sequencing instruments

Instrument	Version	Sequencing capacity (in million PE reads)	5% VAF 10 ng DNA	1% VAF 40 ng DNA
NextSeq® 550	Mid output	260	5	1
NextSeq 550	High output	800	16	5
NextSeq 1000/2000	P1 flow cell	200	4	1
NextSeq 1000/2000	P2 flow cell	800	16	5
NextSeq 2000	P3 flow cell	2400	49	14
NovaSeq® 6000	SP (flow cell)	1600	32	9
NovaSeq 6000	S1 (flow cell)	3200	65	18
NovaSeq 6000	S2 (flow cell)	8200	166	47
NovaSeq 6000	S4 (flow cell)	20,000	406	114

Get to sequencing-ready libraries in one day

The workflow of the QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel is straightforward and can be finished in one day with minimal hands-on time (Figure 2). The workflow can be easily automated on liquid handlers for high-throughput applications. Tables 7 and 8 show the performance data of the panel with reference DNA.

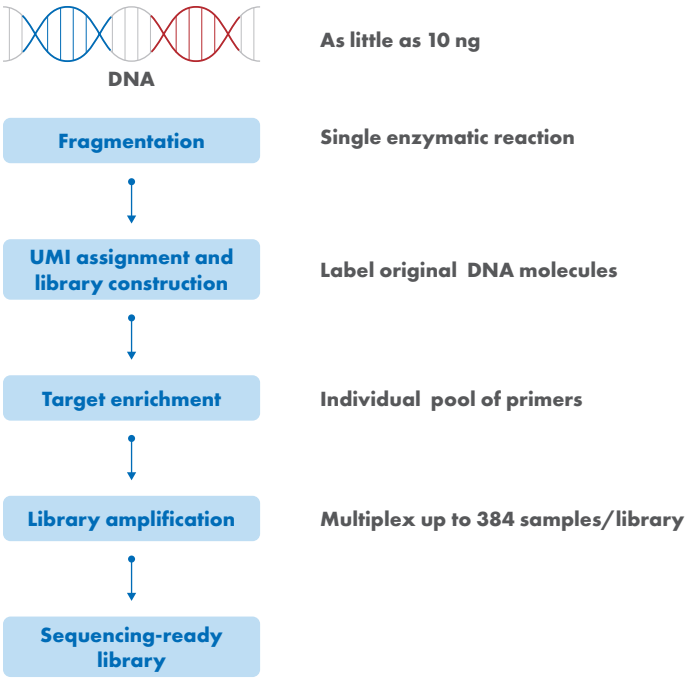


Figure 2. Workflow of the QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel.

Table 7. Variant detection in Seraseq® Myeloid Mutation DNA Mix (10 ng DNA input)

Gene ID	Variant type	COSMIC	HGVS	Expected VAF (%)	Detected VAF (%)
CEBPA	Insertion	COSV57195669	c.68_69insC	15	12.8
CEBPA	Insertion	COSV57195607	c.939_940insAAG	15	8.9
ABL1	SNV	COSV59323790	c.944C>T	10	7.6
ASXL1	Deletion	COSV60102280	c.1900_1922del23	10	9.6
ASXL1	Insertion	COSV60102155	c.1934_1935insG	10	9.3
BRAF	SNV	COSV56056643	c.1799T>A	10	17.5
CBL	SNV	COSV50629675	c.1259G>A	10	17
CBL	SNV	COSV50630049	c.1139T>C	10	18.9
FLT3	ITD		duplication of chr13:28,608,250-28,608,277 (hg19), insGCCCC between duplicated and native seq	10	26.3
FLT3	SNV	COSV54042116	c.2503G>T	10	12.2
JAK2	Deletion	COSV67575778	c.1624_1629delAATGAA	10	16.2
MYD88	SNV	COSV57169334	c.794T>C	10	14.2
U2AF1	SNV	COSV52341059	c.101C>T	10	N/A
CALR	Deletion	COSV57116546	c.1092_1143del52 (c.1099_1150del)	5	3.3
CSF3R	SNV	COSV58963463	c.1853C>T	5	10.3
FLT3	ITD	COSV54045898	c.1759_1800dup	5	4.5
IDH1	SNV	COSV61615256	c.394C>T	5	6.5
JAK2	SNV	COSV67569051	c.1849G>T	5	7.4
MPL	SNV	COSV65243776	c.1544G>T	5	8.4
NPM1	Insertion	COSV51542664	c.863_864insTCTG	5	8.9
SF3B1	SNV	COSV59205318	c.2098A>G	5	9.1
SF3B1	SNV	COSV59205799	c.1998G>T	5	2.5
SRSF2	Deletion	COSV57969801	c.284_307del24	5	5

Table 8. Variant detection in Horizon™ Quantitative Multiplex Reference Standard fcDNA (moderate; HD799; 40 ng DNA input)

Gene	Variant	Expected VAF (%)	Detected VAF (%)
EGFR	T790M	1	0.86
EGFR	ΔE746 - A750	2	1.06
EGFR	L858R	3	3.42
KRAS	G12D	6	5.92
cKIT	D816V	10	9.17
BRAF	V600E	10.5	12.9
NRAS	Q61K	12.5	11.77
KRAS	G13D	15	16.04

Analyze your data with QIAGEN CLC Genomics Workbench

The combined solution – QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel and the QIAseq Targeted Panel Analysis plugin within QIAGEN CLC Genomics Workbench – detects difficult variants, such as *SRSF2*, *CEBPA* and *CALR* mutations, and calls variants below 1% VAF (Figure 3).

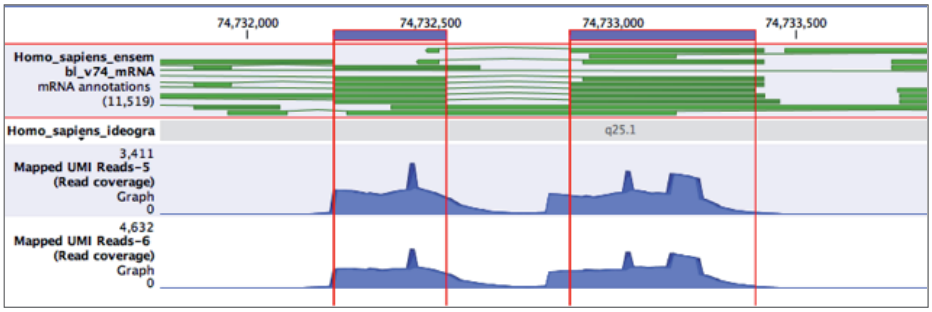


Figure 3. Coverage of *SRSF2*. Coverage plot showing the amount of coverage achieved using the QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel and analyzed using QIAGEN CLC Genomics Workbench (maximum coverage in the two samples are 3411x and 4632x); the coverage is summarized over a small window chosen by the user; light blue represents minimum value and dark blue maximum value. Coverage is sufficient to call variants below 1% VAF.

Full coverage of *CEBPA* gene

CEBPA, a putative tumor suppressor, is frequently mutated in patients with acute myeloid leukemia. It encodes a transcription factor called CCAAT enhancer-binding protein alpha, involved in granulocyte differentiation (2). *CEBPA* is a GC-rich gene (75% of the coding region), which makes NGS assays for *CEBPA* mutation testing challenging. Moreover, the presence of a trinucleotide repeat region in *CEBPA*, the complexity of the mutations, and the frequent occurrence of mutations in mononucleotide repeats add to the challenge.

The optimized chemistry of the QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel facilitates full coverage of *CEBPA* enabling accurate mutant calling within this GC-rich gene (Figure 4).

Figure 4. Analysis plot from the QIAseq Targeted Panel Analysis plugin within QIAGEN CLC Genomics Workbench. The plot shows the presence of a biologically relevant deletion in *CEBPA*.

Detection of *CALR* deletions

CALR encodes calreticulin, a calcium-binding protein with multiple cellular functions, including protein quality control and transcriptional regulation. *CALR* mutations occur in myeloproliferative neoplasms, a heterogeneous group of chronic myeloid neoplasms that can progress to acute leukemia (3). *CALR* sequencing is challenging due to the presence of low-complexity regions, making detecting insertions and deletions difficult.

The optimized chemistry of the QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel facilitates uniform and robust coverage of all *CALR* exons. Additionally, the powerful algorithms in the QIAseq Targeted Panel Analysis plugin of the QIAGEN CLC Genomics Workbench enable precise detection of *CALR* deletions (Figure 5).

Figure 5. The QIAseq Targeted Panel Analysis plugin of QIAGEN CLC Genomics Workbench accurately and confidently calls a 52 bp deletion in *CALR*.

Interpret your data with QCI Interpret for Oncology

QIAGEN CLC Genomics Workbench not only enables accurate variant detection but also makes it easy to explore variants down to the read level. Once variants have been detected using any QIAseq DNA Pro Panel and the QIAseq Targeted Panel Analysis plugin in the QIAGEN CLC Genomics Workbench, the user can easily export these variants via VCF file for further interpretation in QCI Interpret for Oncology (Figures 6–7).

QCI Interpret for Oncology is a variant interpretation and reporting software that transparently computes the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant classifications, enabling users to generate evidence-based reports with efficiency, confidence and reproducibility.

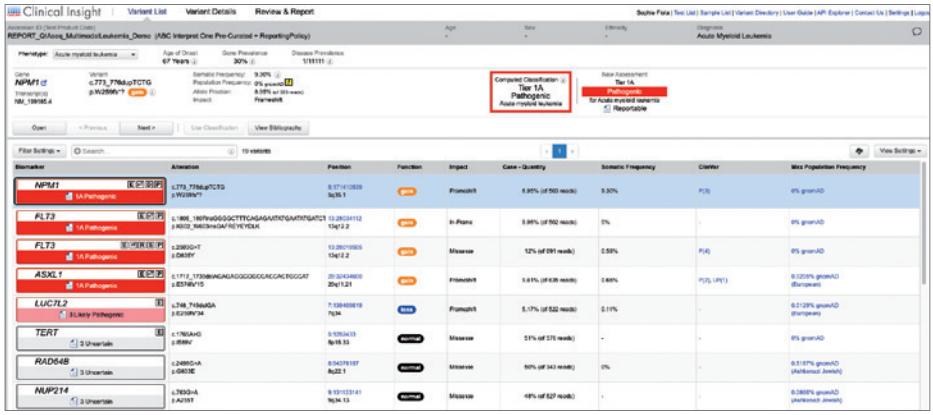


Figure 6. Variant pathogenicity classifications in QCI Interpret for Oncology. The software ranks detected variants based on the ACMG/AMP-defined 28 criteria for variant classifications. The software also identifies the alteration, position, molecular function, impact, somatic frequency and population frequency.

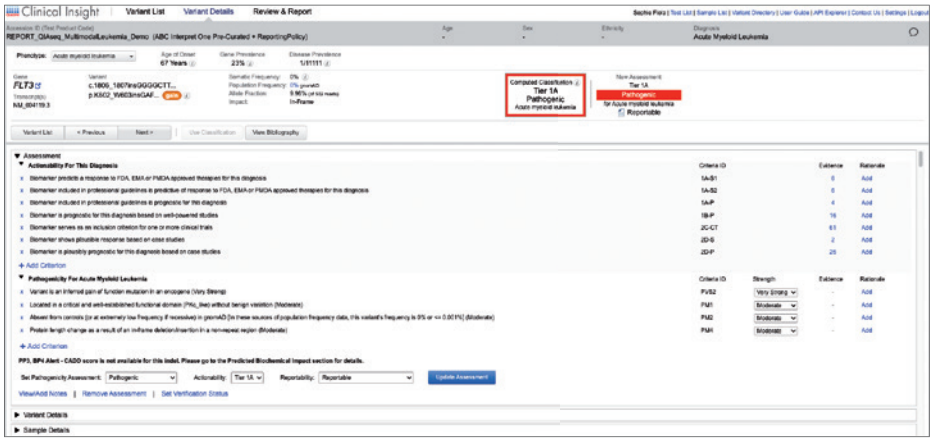


Figure 7. Actionability and pathogenicity criteria in QCI Interpret for Oncology. Actionability and pathogenicity classifications in QCI Interpret are accompanied by clear visibility into the evidence and criteria supporting the classifications. In addition, you can manually add a rationale for each of the rules triggered if desired and adjust the strength of the final assessment if additional data is available.

Connected to the exclusive QIAGEN Knowledge Base, the industry's most comprehensive, manually curated resource updated weekly, QCI Interpret for Oncology dynamically computes pathogenicity and actionability based on the AMP/ASCO/CAP Guidelines with full transparency. The amount of detail offered by the QIAGEN Knowledge Base triggers all levels of evidence of the AMP/ASCO/CAP Guidelines (Figure 7). For every variant in over 31,000 cancer types, QCI Interpret for Oncology automatically provides a computed AMP/ASCO/CAP classification, computed molecular function, the alteration's incidence in disease, an oncologist-reviewed interpretation summary, information on hereditary clinical cases, treatment information, including alteration-specific drug sensitivity and resistance, and a list of open and recruiting clinical trials specific to a geographic region.

In addition, QCI Interpret for Oncology identifies and lists co-occurring variants in each sample, providing evidence on the clinical effect with reference to relevant guidelines. The software allows you to filter variants according to genes in which actionable mutations are detected and visualize the co-mutations in the sample. If the mutation occurs in the same gene, the software provides a "protein view" that shows the presence of mutations, their position, and their effect on the protein. Users also receive an expert explanation of the clinical effect of the co-occurring mutations with supporting references.

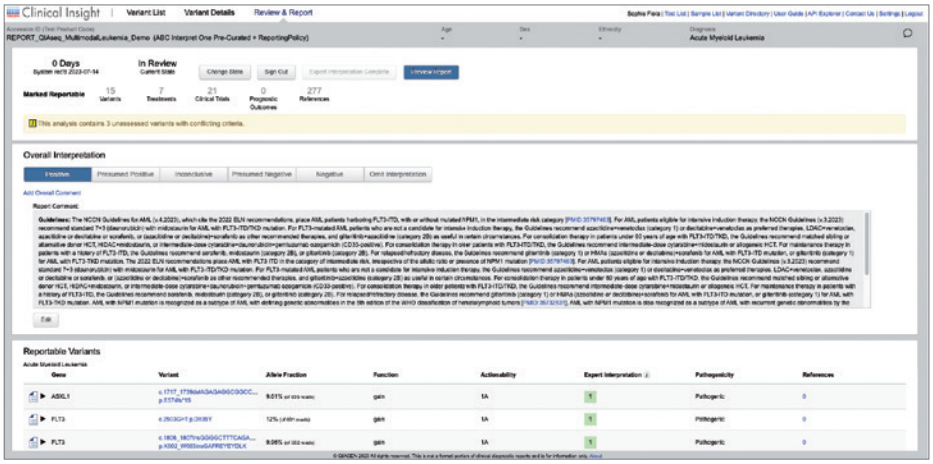
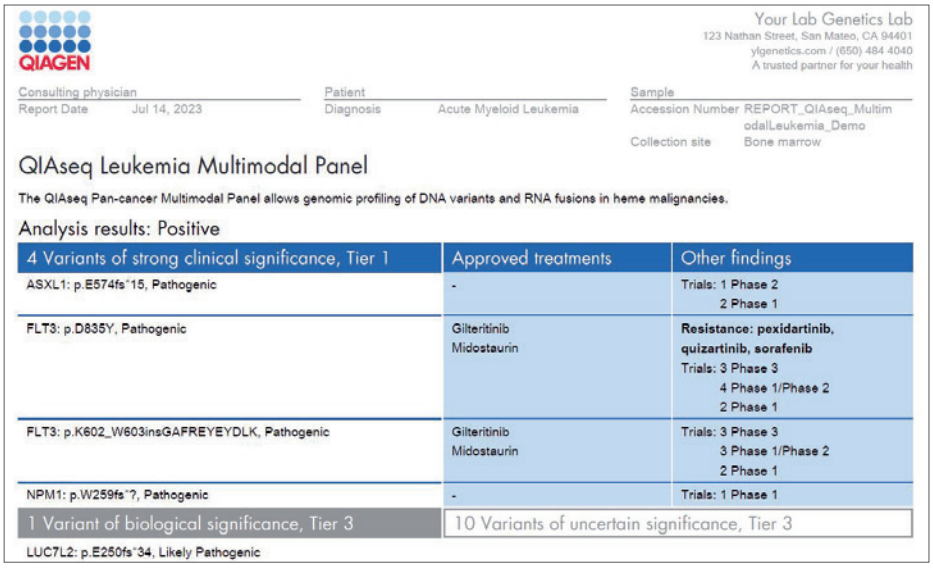


Figure 8. Pre-formulated, oncologist-reviewed interpretative comments in QCI Interpret for Oncology.

To simplify and accelerate interpretation, the software instantly delivers concise oncologist-reviewed evidence for each biomarker in the context of the cancer subtype, listing information on the mutation's molecular characteristics, roles in disease and therapeutic, prognostic and diagnostic implications (Figure 8). To date, the software provides access to over 490,000 decision-ready interpretive comments to help accelerate test turnaround time and increase caseload volume.

QCI Interpret for Oncology enables easy and rapid report building. Each clinical report contains the following information (Figure 9):

- Molecular function
- Therapeutic, prognostic and diagnostic relevance
- Variant interactions, such as the effect of co-occurring variants on therapies, drug resistance and sensitivities
- Clinical practice guideline recommendations
- Relevant local recruiting clinical trials
- FDA-approved drug therapies
- Primary literature references



QIAGEN

Your Lab Genetics Lab
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Consulting physician
Report Date Jul 14, 2023

Patient
Diagnosis Acute Myeloid Leukemia

Sample
Accession Number REPORT_QIAseq_MultimodalLeukemia_Demo
Collection site Bone marrow

QIAseq Leukemia Multimodal Panel

The QIAseq Pan-cancer Multimodal Panel allows genomic profiling of DNA variants and RNA fusions in heme malignancies.

Analysis results: Positive

4 Variants of strong clinical significance, Tier 1	Approved treatments	Other findings
ASXL1: p.E574fs*15, Pathogenic	-	Trials: 1 Phase 2 2 Phase 1
FLT3: p.D835Y, Pathogenic	Gilteritinib Midostaurin	**Resistance: pexidartinib, quizartinib, sorafenib** Trials: 3 Phase 3 4 Phase 1/Phase 2 2 Phase 1
FLT3: p.K602_W603insGAFREYEYDLK, Pathogenic	Gilteritinib Midostaurin	Trials: 3 Phase 3 3 Phase 1/Phase 2 2 Phase 1
NPM1: p.W259fs*?, Pathogenic	-	Trials: 1 Phase 1
1 Variant of biological significance, Tier 3	10 Variants of uncertain significance, Tier 3	
LUC7L2: p.E250fs*34, Likely Pathogenic		

Figure 9. Partial view of QCI Interpret for Oncology final report. Users can generate a custom final report that is patient-specific and includes clinically relevant variants, interpretations, and references specified throughout the assessment process.

To date, QCI Interpret has been used to analyze and interpret more than 3 million NGS test cases and is used by leading laboratories worldwide, including LabCorp, Quest Diagnostics, the Danish National Genome Center, and Munich Leukemia Laboratory.

References

1. McClure RF et al. Clinical Significance of DNA variants in chronic myeloid neoplasms: A report of the Association for Molecular Pathology. *J Mol Diagn*. 2018;20(6):717-737
2. Jakobsen JS et al. Mutant *CEBPA* directly drives the expression of the targetable tumor-promoting factor CD73 in AML. *Sci Adv*. 2019;5(7):eaaw4304.
3. Constantinescu SN, Vainchenker W, Levy G, Papadopoulos N. Functional consequences of mutations in myeloproliferative neoplasms. *Hemasphere*. 2021;5(6):e578.

Ordering Information

Product	Contents	Cat. no.
QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel (12) (PHS-003Z 12)	Kit containing ALL reagents (except indices) for targeted DNA sequencing	333651
QIAseq Targeted DNA Pro Human Myeloid Neoplasms Research Panel (96) (PHS-003Z 96)	Kit containing ALL reagents (except indices) for targeted DNA sequencing	333655
QIAseq Targeted DNA Pro UDI (12)	Box containing unique dual-indexed primers for indexing up to 12 samples for QIAseq Targeted DNA Pro Panel sequencing on Illumina platforms	333441
QIAseq Targeted DNA Pro UDI Set A (96)	Box containing unique dual-indexed primers for indexing up to 96 samples for QIAseq Targeted DNA Pro Panel sequencing on Illumina platforms; 1st of 4 sets required for multiplexing up to 384 samples	333455
QIAseq Targeted DNA Pro UDI Set B (96)	Box containing unique dual-indexed primers for indexing up to 96 samples for QIAseq Targeted DNA Pro Panel sequencing on Illumina platforms; 2nd of 4 sets required for multiplexing up to 384 samples	333465
QIAseq Targeted DNA Pro UDI Set C (96)	Box containing unique dual-indexed primers for indexing up to 96 samples for QIAseq Targeted DNA Pro Panel sequencing on Illumina platforms; 3rd of 4 sets required for multiplexing up to 384 samples	333475
QIAseq Targeted DNA Pro UDI Set D (96)	Box containing unique dual-indexed primers for indexing up to 96 samples for QIAseq Targeted DNA Pro Panel sequencing on Illumina platforms; 4th of 4 sets required for multiplexing up to 384 samples	333485
QIAseq Targeted DNA Pro Index L (12)	Box containing oligos for indexing up to 12 samples for QIAseq Targeted DNA Pro Panel sequencing on Ion Torrent platforms	333491
QIAseq Targeted DNA Pro Index L (24)	Box containing oligos for indexing up to 24 samples for QIAseq Targeted DNA Pro Panel sequencing on Ion Torrent platforms	333492

Scan the QR code or use the link below to learn more.

www.qiagen.com/qiaseq-targeted-pro-myeloid-neoplasms

The QIAseq and QCI Interpret are intended for Molecular Biology Applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.