Application Note

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

Myeloid neoplasms is a group of diseases characterized by a wide range of mutations across a large number of genes, including oncogenes and tumor suppressor genes. Genes commonly mutated in myeloid neoplasms include *CALR* and *CEBPA* for acute myeloid leukemia (AML), and *TP53 or RB1* for 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.

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 due to several reasons including low allele frequency of variants, high GC content and low enrichment of target DNA.

The Human Myeloid Neoplasms QIAseq® Targeted DNA Panel is a complete Sample to Insight NGS solution for myeloid neoplasms analysis. This targeted enrichment panel overcomes many of the challenges associated with myeloid neoplasms analysis (see Table 1).

Highlights of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel are:

- High sensitivity of <1% variant allele frequency (VAF) using unique molecular indices (UMIs)
- Library representing original sample complexity by single primer extension (SPE) enrichment
- 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

High sensitivity using unique molecular indices

The QIAseq panel incorporates UMIs to reduce false positive rates which increases 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 (Figure 1). ▷

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

Challenge	QIAseq solution
Detection of low allele frequency variants	Incorporation of UMIs to reduce false positives
Low enrichment and sequencing uniformity	Utilization of SPE approach for target enrichment
Incompatibility with GC-rich regions	Optimized chemistry that enriches GC-rich regions
Low complexity of amplicon-based libraries	Utilization of SPE 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 required
Mechanical shearing	Enzymatic fragmentation in a single reaction
Long turnaround time	DNA to library in a single day
Low-throughput sample processing	Automation-friendly workflow for high-throughput applications
Multiple primer pools for enrichment	Very high primer multiplexing capabilities; up to 20,000 primers in a single pool
Limited sample multiplexing capabilities	Dual sample multiplexing approach; up to 384 sample indices for Illumina® platforms; up to 96 for Ion Torrent™
Hotspot coverage only	Flexibility in primer design for genome-wide coverage
Limited ability to increase panel content	SPE offers the flexibility to easily increase the content of any panel
Inefficient customization of panels	Robust primer design algorithms

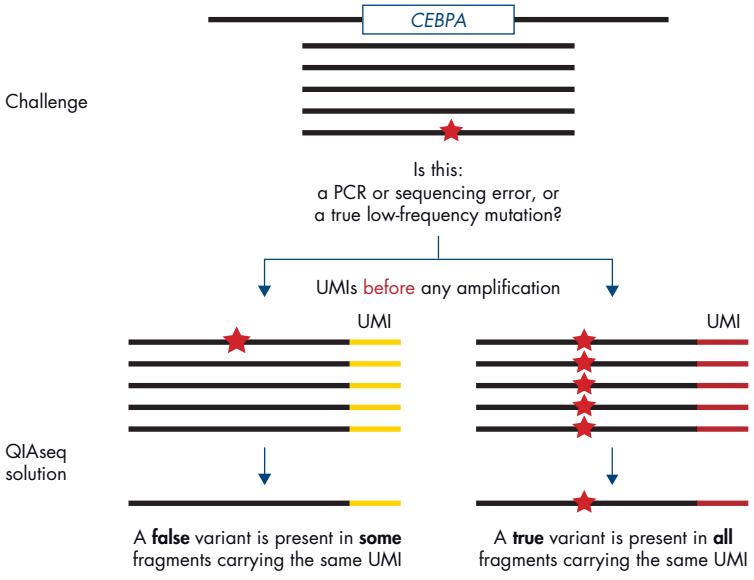


Figure 1. Mechanism of UMIs.

Overcoming enrichment challenges using single primer extension

Amplicon sequencing, a technique that transformed genomic profiling, uses PCR to enrich regions of interest for NGS. Earlier forms of amplicon NGS relied upon a two-primer or nested PCR protocol. This approach has several limitations; it is not suitable for difficult regions of the genome, inserts PCR and amplification artifacts and has only a limited ability to customize a panel after

it has been manufactured. QIAGEN's single primer extension technology improves these three attributes. SPE uses a single primer to define a genomic target, thus reducing the risk of primer dimers and dropouts. On the other hand, amplicon-based enrichment uses a universal primer that binds to library adapter sequences (Figure 2).

Further benefits of SPE include:

- Reduced number of primers
- Increased enrichment and sequencing uniformity
- Enhanced flexibility to increase panel content
- Superior library complexity compared to 2-primer amplicon designs (Figure 3)

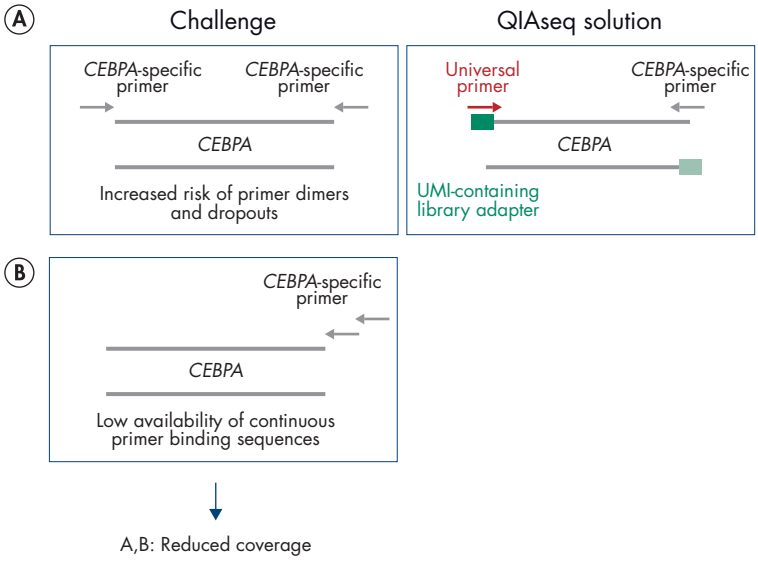


Figure 2. SPE approach utilizing only one target-specific primer. SPE overcomes the challenges of **A** 2-primer amplicon and **B** nested PCR designs.

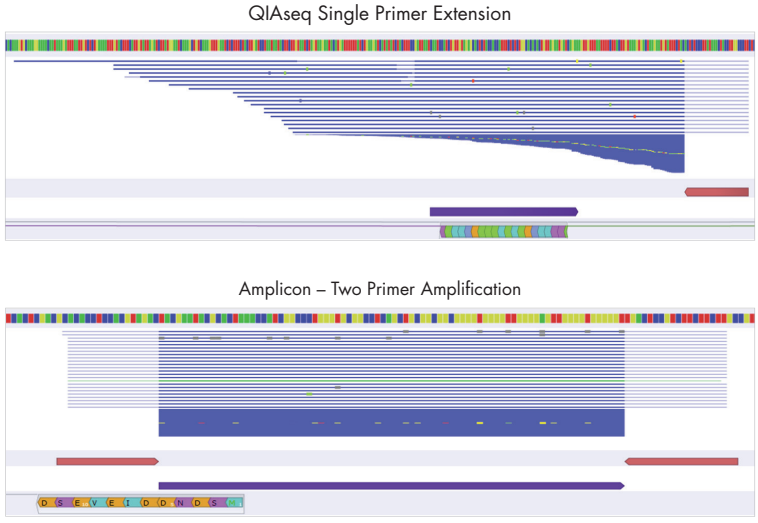


Figure 3. Complex libraries with SPE. Using one target-specific primer results in library fragments with a defined start position and a random stop position. This increases library complexity and produces library fragments of up to 300 bp. The 2-primer amplicon approach, on the other hand, produces library fragments with lower complexity since fragments have a defined start and stop positions.

The Human Myeloid Neoplasms QIAseq Targeted DNA Panel: Specifications

The Human Myeloid Neoplasms QIAseq Targeted DNA Panel is used to enrich genes and construct libraries for NGS analysis of 141 genes commonly mutated in myeloid neoplasms. This panel narrows the focus to the most relevant variants in myeloid neoplasms using a variety of resources such as recent whole genome/exome sequencing studies from scientific networks, including the Cancer Genome Atlas (TCGA), and curated databases like the Cancer Gene Census and Catalogue of Somatic Mutations in Cancer (COSMIC). When combined with sophisticated UMI-aware data analysis pipelines and a powerful knowledge base for interpretation, the panel delivers a complete Sample to Insight solution for myeloid neoplasms analysis. The simplicity of QIAseq DNA Panel target enrichment is ideal for routine detection of known and novel myeloid neoplasms mutations in any research laboratory with access to NGS platforms from Illumina or Ion Torrent. Tables 2, 3 and 4 outline the performance specifications, coverage and sample multiplexing of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel.

Table 2. Performance specifications

Attribute	Specification
DNA input	As little as 10 ng DNA
Targeted region size (bp)	436,672
Targeted regions	Exonic regions* and 5–10 bases of intron/exon junctions
Number of genes	141
Primers	5887
Number of primer pools	1
Types of variants called	SNVs, Indels, CNVs†
Enrichment technology	Single primer extension (SPE)-based with UMI-containing adapters
Amplicon size	An average of 150 bp
Sample multiplexing level	384 (Illumina), 96 (Ion Torrent)
Total workflow time	8–9 hours
Number of libraries per sample	1
Sequencer compatibility	Illumina and Ion Torrent platforms
Variant allele frequency called	<1%
Specificity (on-target reads)	95.3%
Uniformity (0.2x mean coverage)	99.7%
Design coverage	99.9%

* Check design bed file for full details of coverage.

† Depends on secondary analysis pipeline; the Biomedical Genomics Workbench enables detection of SNVs, Indels and CNVs.

Table 3. Coverage

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

Table 4. Sample multiplexing

			Number of samples	
Platform, sequencing chemistry and chip	**Read length**	**Output – number of reads (millions)**	**1% VAF 40 ng DNA**	**5% VAF 10 ng DNA**
MiniSeq® Mid Output	2x150	16	0	0
MiniSeq High Output	2x150	50	0	1
MiSeq® V2	2x150	30	0	1
MiSeq V3	2x300	50	0	1
MiSeq V2 Micro	2x150	8	0	0
MiSeq V2 Nano	2x150	2	0	0
NextSeq® 500 Mid Output	2x150	260	2	6
NextSeq 500 High Output	2x150	800	5	19
HiSeq® 2500 Rapid run SBS Kit V2, Dual FC (2 lanes/FC)	2x150	1200	8	28
HiSeq 2500 Rapid run SBS Kit V2, Single FC (2 lanes/FC)	2x150	600	4	14
HiSeq 2500 High run HiSeq SBS V4, Dual FC (8 lanes/FC)	2x125	8000	53	189
HiSeq 2500 High run HiSeq SBS V4, Single FC (8 lanes/FC)	2x125	4000	27	94
HiSeq 2500 High run TruSeq SBS V3, Dual FC (8 lanes/FC)	2x125	6000	40	142
HiSeq 2500 High run TruSeq SBS V3, Single FC (8 lanes/FC)	2x125	3000	20	71
NovaSeq® 6000 S1	2x150	1600	11	38
NovaSeq 6000 S2	2x150	3300	22	78
NovaSeq 6000 S4	2x150	10,000	66	236
Ion PGM™ 314 Chip v2	1x200	0.550	0	0
Ion PGM 316 Chip v2	1x200	3	0	0
Ion PGM 318 Chip v2	1x200	5.5	0	0
Ion Proton™ PI V3 Chip	1x200	80	1	2
Ion S5 510 Chip	1x200	3	0	0
Ion S5 520 Chip	1x200	6	0	0
Ion S5 530 Chip	1x200	20	0	0
Ion S5 540 Chip	1x200	80	1	2
Ion S5 XL 510 Chip	1x200	3	0	0
Ion S5 XL 520 Chip	1x200	6	0	0
Ion S5 XL 530 Chip	1x200	20	0	0
Ion S5 XL 540 Chip	1x200	80	1	2

Coverage of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel

The Human Myeloid Neoplasms QIAseq Targeted DNA Panel covers exonic regions of genes and +/– 5–10 bases of exon/intron boundaries. Table 5 details design coverage of genes based on library fragments of 250 bp produced from high-quality DNA samples. Coverage bed files should be consulted for full coverage details.

Table 5. Design coverage details

Gene symbol	Region-of-interest (ROI) in bp	Base pairs covered by fragments ≤ 250 bp	Percent coverage by fragments ≤ 250 bp	Base pairs not covered by fragments ≤ 250 bp
ABL1	3649	3649	100.0%	0
ADA	1278	1278	100.0%	0
ANKRD26	6679	6661	99.7%	18
ASXL1	4772	4772	100.0%	0
ASXL2	4438	4438	100.0%	0
ATM	9791	9791	100.0%	0
ATRX	7889	7889	100.0%	0
BCL6	2201	2201	100.0%	0
BCOR	5408	5408	100.0%	0
BCORL1	5488	5488	100.0%	0
BCR	4046	3926	97.0%	120
BIRC3	1895	1895	100.0%	0
BLM	4464	4464	100.0%	0
BRAF	2660	2660	100.0%	0
BRCA1	5888	5888	100.0%	0
BRCA2	10,517	10,517	100.0%	0
C17orf97	1295	1267	97.8%	28
CALR	1344	1344	100.0%	0
CARD11	3770	3770	100.0%	0
CBL	2881	2881	100.0%	0
CBLB	3342	3342	100.0%	0
CBLC	1547	1547	100.0%	0
CDKN2A	1124	1124	100.0%	0
CEBPA	1087	1087	100.0%	0
CHEK2	1911	1911	100.0%	0
CREBBP	7639	7639	100.0%	0
CRLF2	852	852	100.0%	0
CSF1R	3129	3129	100.0%	0
CSF3R	2857	2857	100.0%	0
CTCF	2284	2284	100.0%	0
CUX1	6118	6118	100.0%	0
DAXX	2356	2356	100.0%	0
DDX41	2039	2039	100.0%	0
DNM2	3062	3062	100.0%	0

Gene symbol	Region-of-interest (ROI) in bp	Base pairs covered by fragments ≤ 250 bp	Percent coverage by fragments ≤ 250 bp	Base pairs not covered by fragments ≤ 250 bp
DNMT1	5504	5504	100.0%	0
DNMT3A	3104	3104	100.0%	0
EED	1535	1535	99.7%	18
EGFR	4254	4254	100.0%	0
ELANE	854	854	100.0%	0
EP300	7555	7555	100.0%	0
ETNK1	1692	1692	100.0%	0
ETV6	1439	1439	100.0%	0
EZH2	2480	2480	100.0%	0
FAM154B	1417	1417	100.0%	0
FAM47A	2386	2386	100.0%	0
FAM5C	2385	2385	100.0%	0
FAS	1098	1098	100.0%	0
FBXW7	2758	2758	100.0%	0
FLRT2	1993	1993	100.0%	0
FLT3	3222	3222	100.0%	0
GATA1	1292	1292	100.0%	0
GATA2	1493	1493	100.0%	0
GJB3	823	823	100.0%	0
GNAS	4186	4186	100.0%	0
HNRNPK	1615	1615	100.0%	0
HRAS	730	730	100.0%	0
IDH1	1325	1325	100.0%	0
IDH2	1469	1469	100.0%	0
IKZF1	1654	1654	100.0%	0
IKZF3	1610	1610	100.0%	0
IL7R	1464	1464	100.0%	0
JAK1	3705	3705	100.0%	0
JAK2	3629	3629	100.0%	0
JAK3	3744	3744	100.0%	0
KAT6A	6175	6175	100.0%	0
KCNA4	1972	1972	100.0%	0
KCNK13	1247	1247	100.0%	0
KDM6A	4662	4662	100.0%	0
KDR	4371	4371	100.0%	0
KIT	3144	3144	100.0%	0
KLHDC8B	1136	1136	100.0%	0
KLHL6	1936	1936	100.0%	0
KMT2A	12,283	12,283	100.0%	0
KMT2C	15,568	15,568	100.0%	0
KRAS	737	737	100.0%	0
LRRC4	1972	1972	100.0%	0

Gene symbol	Region-of-interest (ROI) in bp	Base pairs covered by fragments ≤ 250 bp	Percent coverage by fragments ≤ 250 bp	Base pairs not covered by fragments ≤ 250 bp
LUC7L2	1409	1409	100.0%	0
MAP2K1	1316	1316	100.0%	0
MLH1	2461	2461	100.0%	0
MPL	2028	2028	100.0%	0
MSH2	3107	3107	100.0%	0
MSH6	4183	4183	100.0%	0
MYC	1395	1395	100.0%	0
MYD88	1004	1004	100.0%	0
NBN	2425	2425	100.0%	0
NF1	9300	9300	100.0%	0
NOTCH1	8008	8008	100.0%	0
NPAT	4464	4464	100.0%	0
NPM1	1014	1014	100.0%	0
NRAS	610	610	100.0%	0
NSD1	8311	8311	100.0%	0
NTRK3	2999	2999	100.0%	0
OR13H1	937	937	100.0%	0
OR8B12	943	943	100.0%	0
P2RY2	1144	1144	100.0%	0
PAX5	1427	1427	100.0%	0
PCDHB1	2467	2467	100.0%	0
PDGFRA	3601	3601	100.0%	0
PHF6	1293	1293	100.0%	0
PML	4038	4038	100.0%	0
PMS2	2739	2491	90.9%	248
PRAMEF2	1455	1386	95.3%	69
PRF1	1688	1688	100.0%	0
PRPF40B	2939	2939	100.0%	0
PRPF8	7428	7428	100.0%	0
PTEN	1302	1302	100.0%	0
PTPN11	1936	1936	100.0%	0
RAD21	2026	2026	100.0%	0
RB1	3057	3057	100.0%	0
RELN	11,033	11,033	100.0%	0
RUNX1	1649	1649	100.0%	0
SETBP1	5040	5040	100.0%	0
SF1	2652	2652	100.0%	0
SF3A1	2542	2542	100.0%	0
SF3B1	4195	4195	100.0%	0
SH2B3	2067	2067	100.0%	0
SH2D1A	427	427	100.0%	0
SMARCB1	1302	1302	100.0%	0

Gene symbol	Region-of-interest (ROI) in bp	Base pairs covered by fragments ≤ 250 bp	Percent coverage by fragments ≤ 250 bp	Base pairs not covered by fragments ≤ 250 bp
SMC1A	4005	4005	100.0%	0
SMC3	3944	3944	100.0%	0
SRP72	2568	2568	100.0%	0
SRSF2	686	686	100.0%	0
STAG2	4137	4137	100.0%	0
STAT3	2951	2951	100.0%	0
STXBP2	2005	2005	100.0%	0
SUZ12	2380	2380	100.0%	0
TAL1	1026	1026	100.0%	0
TERC	461	461	100.0%	0
TERT	3585	3585	100.0%	0
TET2	6188	6188	100.0%	0
TNFRSF13B	1028	1028	100.0%	0
TP53	1383	1383	100.0%	0
TPMT	818	818	100.0%	0
TUBA3C	1403	1403	100.0%	0
U2AF1	880	880	100.0%	0
U2AF2	1548	1548	100.0%	0
WAS	1629	1629	100.0%	0
WRN	4639	4639	100.0%	0
WT1	1674	1674	100.0%	0
XPO1	3496	3496	100.0%	0
ZRSR2	1559	1559	100.0%	0

Table 6 lists reasons for non-covered bases. Non-covered bases are regions that lack primer coverage because the design algorithm could not design primers to target them. Table 7 groups covered genes into functional disease groupings.

Table 6. Reasons for non-covered bases

Gene symbol	Reason(s) for no coverage
ANKRD26	High genome frequency, simple tandem repeat
C17or f97	High genome frequency, simple tandem repeat
BCR	Not unique in genome – same as *BCRP3, BCRP4*
PMS2	Not unique in genome – same as *PMS2CL*
PRAMEF2	Not unique in genome – similar to *PRAMEF1, PRAMEF14, PRAMEF13*

Table 7. Gene list by functional disease groupings

Disease	Genes covered
Acute lymphoblastic leukemia (ALL)	*ASXL2, ATM, BRAF, CALR, CDKN2A, CREBBP, CRLF2, CSF3R, CTCF, DNM2, EGFR, EP300, FBXW7, GATA2, HNRNPK, HRAS, IKZF3, IL7R, KDM6A, KDR, KMT2C, LRRC4, MAP2K1, MLH1, MSH2, MSH6, NOTCH1, NTRK3, PAX5, PDGFRA, PMS2, PRAMEF2, PTEN, RELN, SMARCB1*
Acute myeloid leukemia (AML)	*ANKRD26, ASXL1, ATM, BCOR, BCORL1, BIRC3, BRAF, C17orf97, CALR, CARD11, CBLC, CDKN2A, CEBPA, CHEK2, CREBBP, CSF1R, CSF3R, CTCF, DAXX, DDX41, DNM2, DNMT1, ELANE, EP300, FLRT2, FLT3, GATA1, GATA2, HNRNPK, IDH1, IDH2, IKZF1, IL7R, JAK1, JAK3, KDM6A, KDR, KIT (CD117), KMT2A, KMT2C, KRAS, LRRC4, MAP2K1, MPL, MSH6, MYC, NBN, NOTCH1, NPM1, NRAS, NSD1, NTRK3, OR13H1, OR8B12, P2RY2, PCDHB1, PDGFRA, PHF6, PRAMEF2, PRPF8, PTEN, PTPN11, RAD21, RUNX1 (AML1), SF1, SF3A1, SMARCB1, SMC1A (SMC1L1), SMC3, SRP72, SRSF2, STAG2, STXBP2, U2AF1, U2AF2, WT1*
Chronic lymphocytic leukemia (CLL)	*ADA, BIRC3, BLM, BRAF, CALR, CHEK2, CSF3R, KCNA4, KLHL6, KMT2C, MAP2K1, NBN, NPAT, NTRK3, OR13H1, OR8B12, PRAMEF2, SRP72, TAL1, TERT, TUBA3C, WAS, WRN*
Chronic myeloid leukemia (CML)	*ABL1, CALR, CDKN2A, CEBPA, CREBBP, CSF1R, CSF3R, FBXW7, GATA2, KDM6A, MSH2, MSH6, RB1, SMC1A (SMC1L1), TP53*
Chronic myelomonocytic leukemia (CMML)	*CALR, CEBPA, CSF1R, CSF3R, HRAS, KMT2C, LUC7L2, SRSF2*
Chronic neutrophilic leukemia (CNL)	*CALR, CSF3R*
Multiple myeloma	*ATM, BCL6, BCR, BIRC3, BRAF, CDKN2A, CEBPA, EGFR, FBXW7, GJB3, HRAS, KDM6A, MYC, NOTCH1, PTEN, SH2D1A, SMARCB1*
Myelodysplastic syndromes (MDS)	*ATRX, CALR, CDKN2A, CEBPA, CSF1R, CSF3R, EP300, ETNK1, GNAS, HRAS, KDM6A, KMT2A, KMT2C, RAD21, RB1, SETBP1, SF1, SF3A1, SMC3, SRSF2, STAG2, U2AF1, U2AF2, XPO1, ZRSR2*
Myeloid malignancies	*CBL, CBLB, DNMT3A, EED, ETV6, EZH2, PRPF40B, SUZ12, TET2, TP53*
Myeloproliferative neoplasm (MPN)	*ABL1, ASXL1, CALR, CSF1R, JAK2, JAK3, KAT6A (MYST3), KRAS, MPL, NF1, NRAS, RB1, SETBP1, SF3B1, SH2B3, SRSF2, STAG2.*
Myelofibrosis (MF)	*CALR, CHEK2, IDH1, IDH2, CSF1R, SRSF2*
Other myeloid neoplasms	*BRAF, CDKN2A, CEBPA, FBXW7, HRAS, IKZF3, KLHDC8B, KMT2C, MSH6, NTRK3, PTEN, SRP72, TPMT*
Other myeloid neoplasm genes	*BRCA1, BRCA2, BRINP3, CUX1, FAM47A, FAS, KCNK13, MYD88, PML, PRF1, SAXO2, STAT3, TERC, TNFRSF13B*

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

Assay workflow

The workflow of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel is simple and can be finished in one day with minimal hands-on time (Figure 4). The workflow can be easily automated on liquid handlers for high-throughput applications.

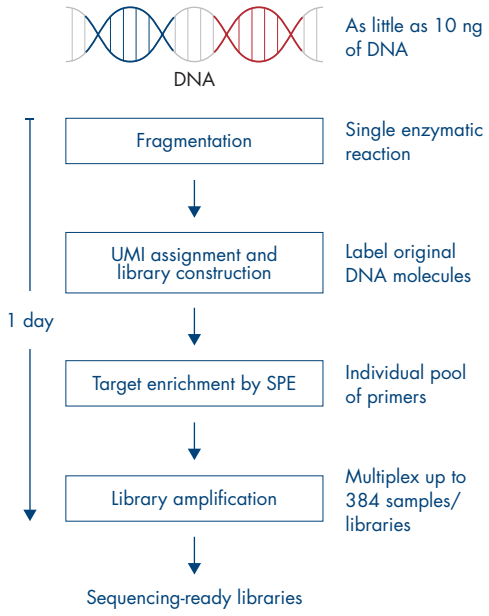


Figure 4. Workflow of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel.

Full coverage of the *CEBPA* gene

CEBPA, a putative tumor suppressor, is mutated in patients with acute myeloid leukemia. It encodes a transcription factor called CCAAT enhancer-binding protein alpha, involved in granulocyte differentiation. *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 Human Myeloid Neoplasms QIAseq Targeted DNA Panel has overcome these challenges allowing NGS analysis of *CEBPA*. Its optimized chemistry facilitates full coverage of *CEBPA* (Figure 5) enabling accurate mutant calling within this GC-rich gene (Figure 6).

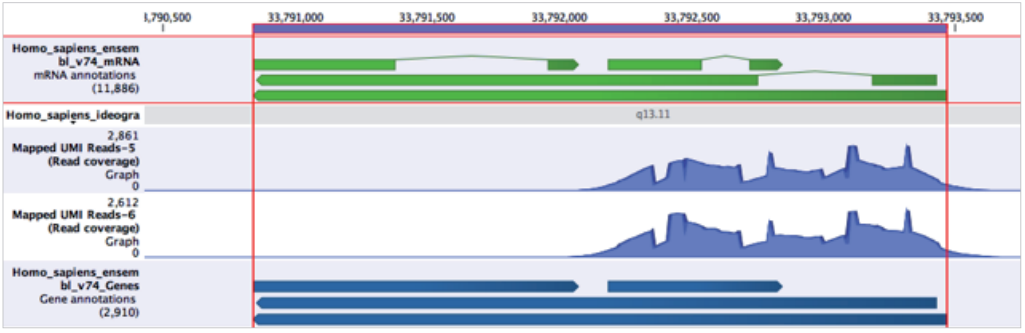


Figure 5. Coverage plot from the Biomedical Genomics Workbench showing the coverage of every exonic base within *CEBPA* in two samples known to harbor *CEBPA* mutations (the coverage is summarized over a small window chosen by the user, light blue represents minimum value in the window, dark blue maximum value).

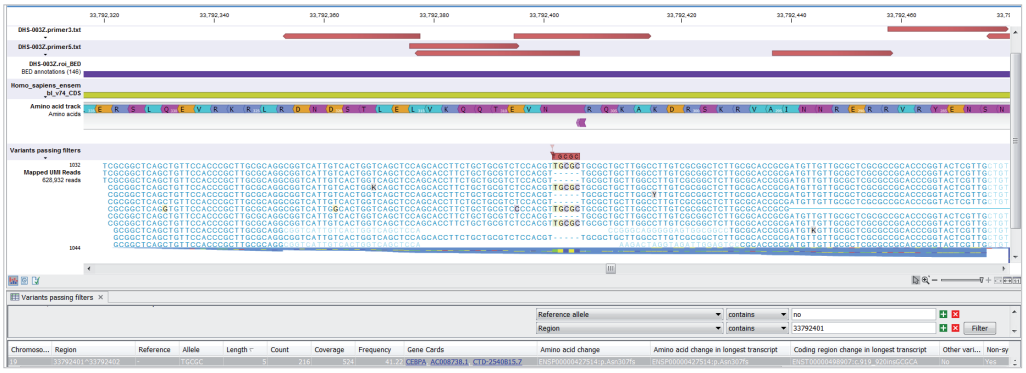


Figure 6. Analysis plot from the QIAseq Targeted Panel Analysis plugin within the Biomedical Genomics Workbench. The plot shows the presence of a biologically-relevant deletion in *CEBPA*.

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 which can progress to acute leukemia. *CALR* sequencing is challenging due to the presence of low complexity regions making the detection of insertions and deletions difficult.

The optimized chemistry of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel facilitates uniform and robust coverage of all *CALR* exons (Figure 7). Additionally, the powerful algorithms in the QIAseq Targeted Panel Analysis plugin of the Biomedical Genomics Workbench enable precise detection of *CALR* deletions (Figure 8).

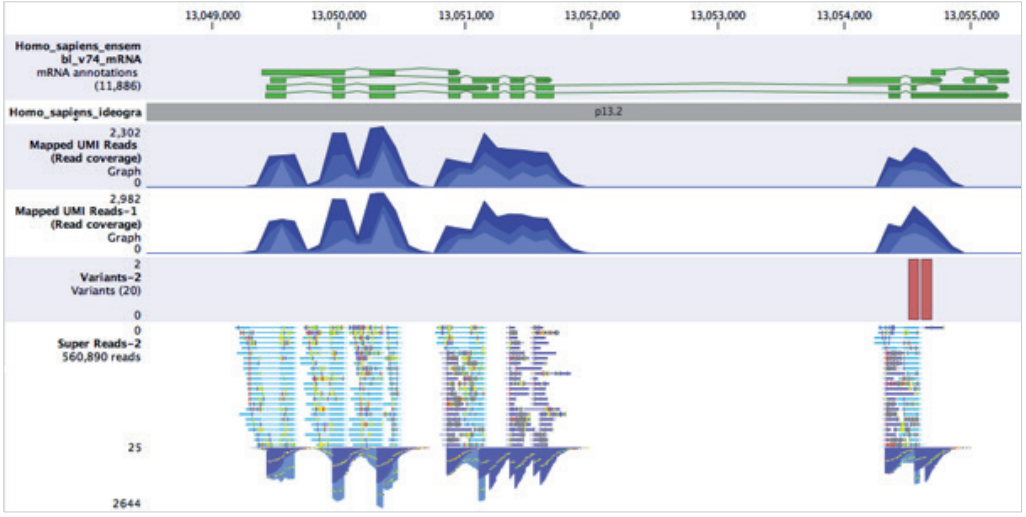


Figure 7. Coverage plot showing uniform and robust coverage of *CALR* exons. The plot also shows variants (base-colored variation) and "super reads", i.e. reads that have been grouped based on UMIs.

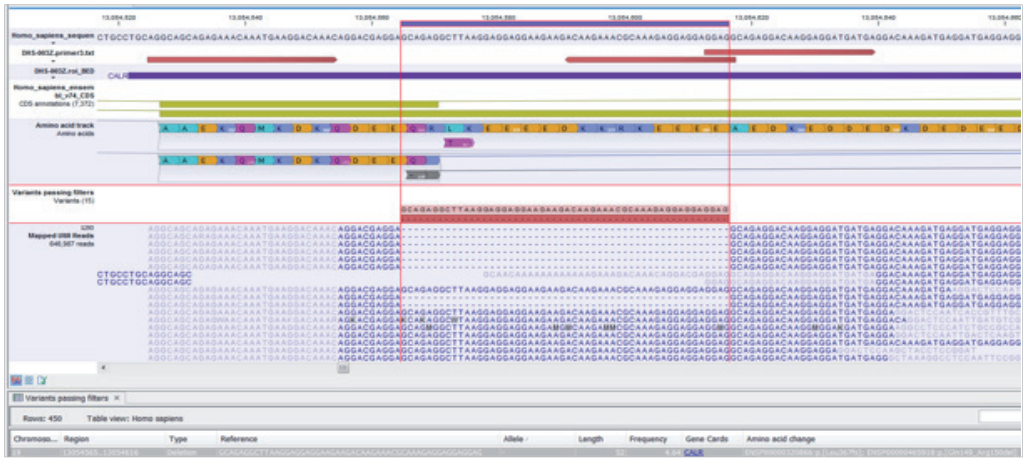


Figure 8. The QIAseq Targeted Panel Analysis plugin of the Biomedical Genomics Workbench accurately and confidently calls a 52 bp deletion in *CALR*.

Secondary data analysis: Pipelines and interpretation

The combined solution of the Human Myeloid Neoplasms QIAseq Targeted DNA Panel and the QIAseq Targeted Panel Analysis plugin within the Biomedical Genomics Workbench detects difficult variants, such as *CALR* deletions, and calls variants below 1% VAF (Figure 9).

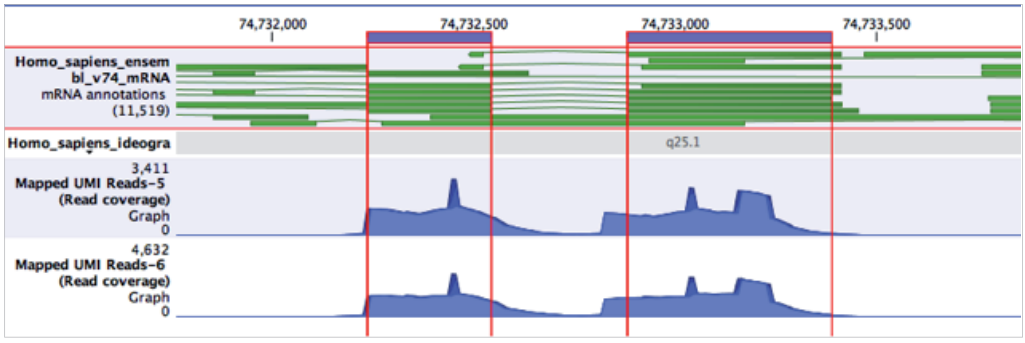


Figure 9. Coverage of SRSF2. Coverage plot showing the amount of coverage achieved using the Human Myeloid Neoplasms QIAseq Targeted DNA Panel and analyzed using the Biomedical Genomics Workbench (maximum coverage in the two samples are 3411X and 4,632X); the coverage is summarized over a small window chosen by the user; light blue represents minimum value and dark blue maximum value. Coverage is sufficient to call variants below 1% VAF.

Interpretation: Ingenuity Variant Analysis™

The Biomedical Genomics Workbench not only enables accurate variant detection but also makes it easy to explore variants down to the read level. Once the variants have been detected using any QIAseq DNA Panel and the QIAseq Targeted Panel Analysis plugin in the Biomedical Genomics Workbench, the user can easily export these variants for further biological exploration in Ingenuity Variant Analysis (IVA) (Figure 10).

IVA is a secure (HIPAA- and Safe Harbor-compliant) web platform for annotating and comparing comprehensively sequenced human genomes. IVA can quickly shortlist candidate variants in studies of matched or unmatched tumors, disease kindreds, single- or multi-proband sets or large case-control cohorts. Integration with IVA enables the user to characterize the identified variants with valuable clinical insight, leveraging the QIAGEN Knowledge Base. A few simple questions are asked at the start of analysis, after which the platform will sensibly parameterize filters for finding credibly rare, appropriate and functionally relevant variants, based on study design, focus and assumptions. Spotting putative disease-causing drivers requires sensible filters to accurately answer three key questions about each putative variant: Is it real? How common is it among other tumors and in the world at large? And how might it affect physiology, through gene sequence or/and expression? IVA uses a sensible, default-configured, yet customizable series of filters to answer these questions and shortlist candidate variants, genes and pathways (see **www.qiagenbioinformatics.com/products/ingenuity-variant-analysis/**) (Figures 10, 11 and 12).

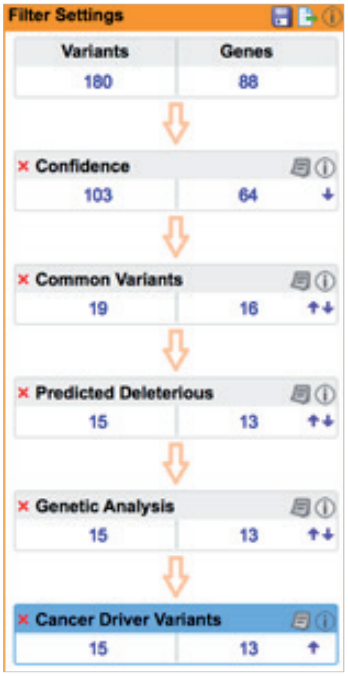


Figure 10. Screenshot from the IVA software depicting the cascade of filter setting used to shortlist the candidate variants in the Human Myeloid Neoplasms QIAseq Targeted DNA Panel.

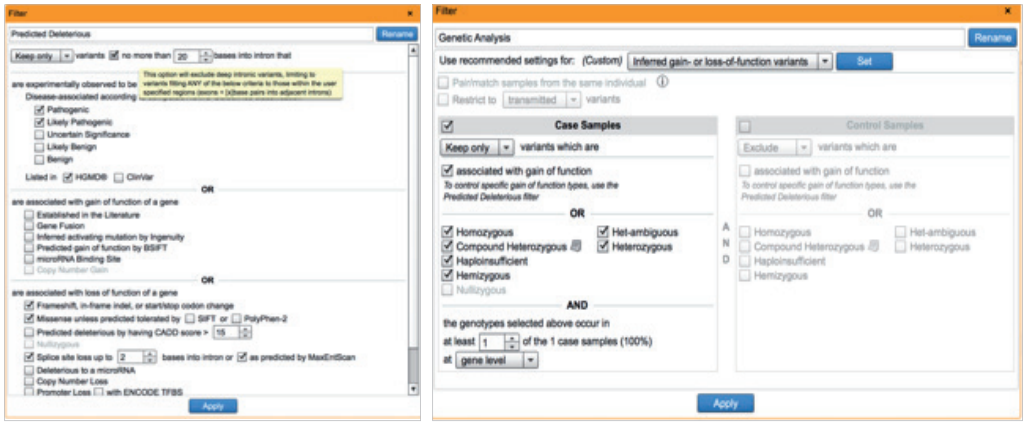
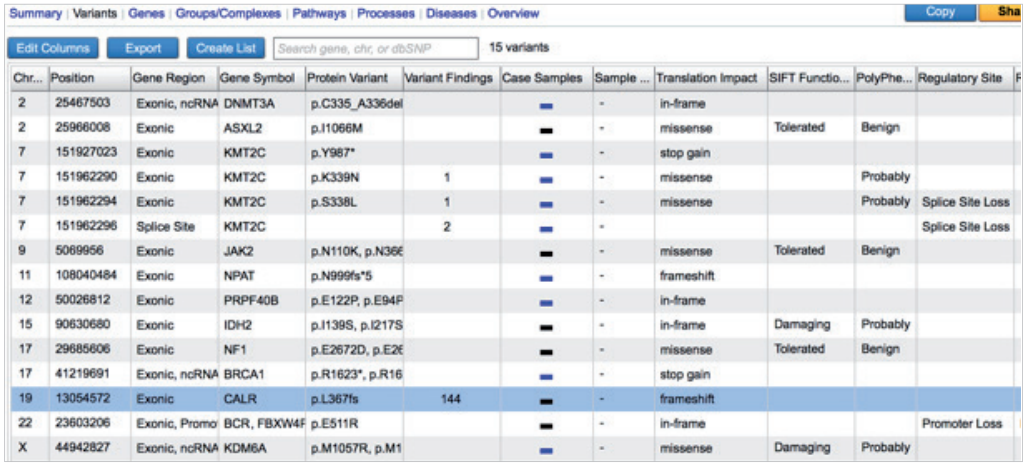


Figure 11. Screenshot from the IVA software depicting the range of settings available for the predicted deleterious and genetic analysis filters.



Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Variant Findings	Case Samples	Sample ...	Translation Impact	SIFT Functio...	PolyPhe...	Regulatory Site
2	25467503	Exonic, ncRNA	DNMT3A	p.C335_A336del			-	in-frame			
2	25966008	Exonic	ASXL2	p.I1066M			-	missense	Tolerated	Benign	
7	151927023	Exonic	KMT2C	p.Y987*			-	stop gain			
7	151962290	Exonic	KMT2C	p.K339N	1		-	missense		Probably	
7	151962294	Exonic	KMT2C	p.S338L	1		-	missense		Probably	Splice Site Loss
7	151962296	Splice Site	KMT2C		2		-				Splice Site Loss
9	5069956	Exonic	JAK2	p.N110K, p.N36			-	missense	Tolerated	Benign	
11	108040484	Exonic	NPAT	p.N999fs*5			-	frameshift			
12	50026812	Exonic	PRPF40B	p.E122P, p.E94P			-	in-frame			
15	90630680	Exonic	IDH2	p.I139S, p.I217S			-	in-frame	Damaging	Probably	
17	29685606	Exonic	NF1	p.E2672D, p.E2			-	missense	Tolerated	Benign	
17	41219691	Exonic, ncRNA	BRCA1	p.R1623*, p.R16			-	stop gain			
19	13054572	Exonic	CALR	p.L367fs	144		-	frameshift			
22	23603206	Exonic, Promo	BCR, FBXW4F	p.E511R			-	in-frame			Promoter Loss
X	44942827	Exonic, ncRNA	KDM6A	p.M1057R, p.M1			-	missense	Damaging	Probably	

Figure 12. Screenshot of the IVA results table view showing one of the variants, *CALR* (highlighted in blue) in the sample (also see Figure 7).

The results can be exported from IVA or from the Biomedical Genomics Workbench to QCI-I (QIAGEN's interpretation platform) for further interpretation. QCI-I classifies variants based on the latest guidelines (Figure 13).

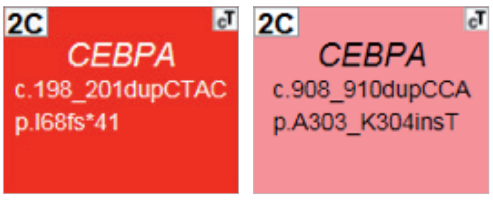


Figure 13. QCI-I-guided classification of *CEBPA* variants in biological samples. Variant analysis was performed using the Biomedical Genomics Workbench. The resultant vcf file was fed into QCI-I for further interpretation of variants.

Ordering Information

Product	Contents	Cat. no.
QIAseq Targeted DNA Panel (12) (DHS-003Z-12)	Kit containing ALL reagents (including beads; excluding indices) for targeted DNA sequencing; enough for 12 samples	333502
QIAseq Targeted DNA Panel (96) (DHS-003Z-96)	Kit containing ALL reagents (including beads; excluding indices) for targeted DNA sequencing; enough for 96 samples	333505
QIAseq 12-Index I (48)	Kit containing UMI-based library adapters, enough for a total of 48 samples, for indexing up to 12 samples for targeted panel sequencing on Illumina platforms	333714
QIAseq 96-Index I Set A (384)	Kit containing UMI-based library adapters, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; one of four sets required for multiplexing 384 samples	333727
QIAseq 96-Index I Set B (384)	Kit containing UMI-based library adapters, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; two of four sets required for multiplexing 384 samples	333737
QIAseq 96-Index I Set C (384)	Kit containing UMI-based library adapters, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; three of four sets required for multiplexing 384 samples	333747
QIAseq 96-Index I Set D (384)	Kit containing UMI-based library adapters, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; four of four sets required for multiplexing 384 samples	333757
QIAseq 12-Index L (48)	Kit containing UMI-based library adapters, enough for a total of 48 samples, for indexing up to 12 samples for targeted panel sequencing on Ion Torrent platforms	333764
QIAseq 96-Index L (384)	Kit containing UMI-based library adapters, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Ion Torrent platforms	333777

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