SureSeq

Using a Targeted NGS Approach to Detect Copy Number Alterations and Gene Variants in Chronic Lymphocytic Leukemia

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What binds us, makes us.

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Introduction

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by monoclonal B cell proliferation. It is the most common adult leukemia in Western populations and comprises 25 to 30% of leukemias in the United States.

A wide variety of genomic variations are associated with CLL, ranging from single nucleotide variants (SNVs) and insertions/deletions (indels) to complex chromosomal rearrangements such as copy number alterations (CNAs) of part or whole chromosome. Chromosomal abnormalities are very common in CLL, the most frequent being del(13q), trisomy 12, del(11q), del(17p) and del(6q).

Comprehensive genetic research into CLL requires multiple testing strategies with high associated costs. In this study, we tested the capability of OGT's NGS panel, SureSeq™ CLL + CNV V3, to detect SNVs and indels as well as chromosomal aberrations in CLL research samples, allowing for the detection of CLL variants in a cost efficient and timely manner.

Methods

Workflow

The SureSeq hybridisation–based enrichment approach and OGT's Universal Complete NGS Workflow solution were used throughout this study (Figure 1).

Panel

The SureSeq CLL + CNV V3 Panel can be used for detection of SNVs and indels in 16 genes and somatic CNAs in 5 critical regions.

Variant type	No. Genes/ Regions	Gene/region details
SNVs/Indels	16 genes	Whole genes: ATM, BIRC3, BTK, KRAS, MYB, MYD88, NRAS, SAMHD1, SF3B1 and TP53. Partial genes: BCL2 (exons 1-2), BRAF (exons 11-18), CXCR4 (exon 1), XPO1 (exons 15-16), NOTCH1 (exon 34 and 3'UTR), PLCG2 (exons 19, 20, 24, 27, 30).
CNAs	5 regions	17p (covering TP53), 11q (covering ATM), 13q (covering RB1/DLEU2/DLEU7), 6q (6q23.2-6q23.3 covering MYB) and Trisomy 12.

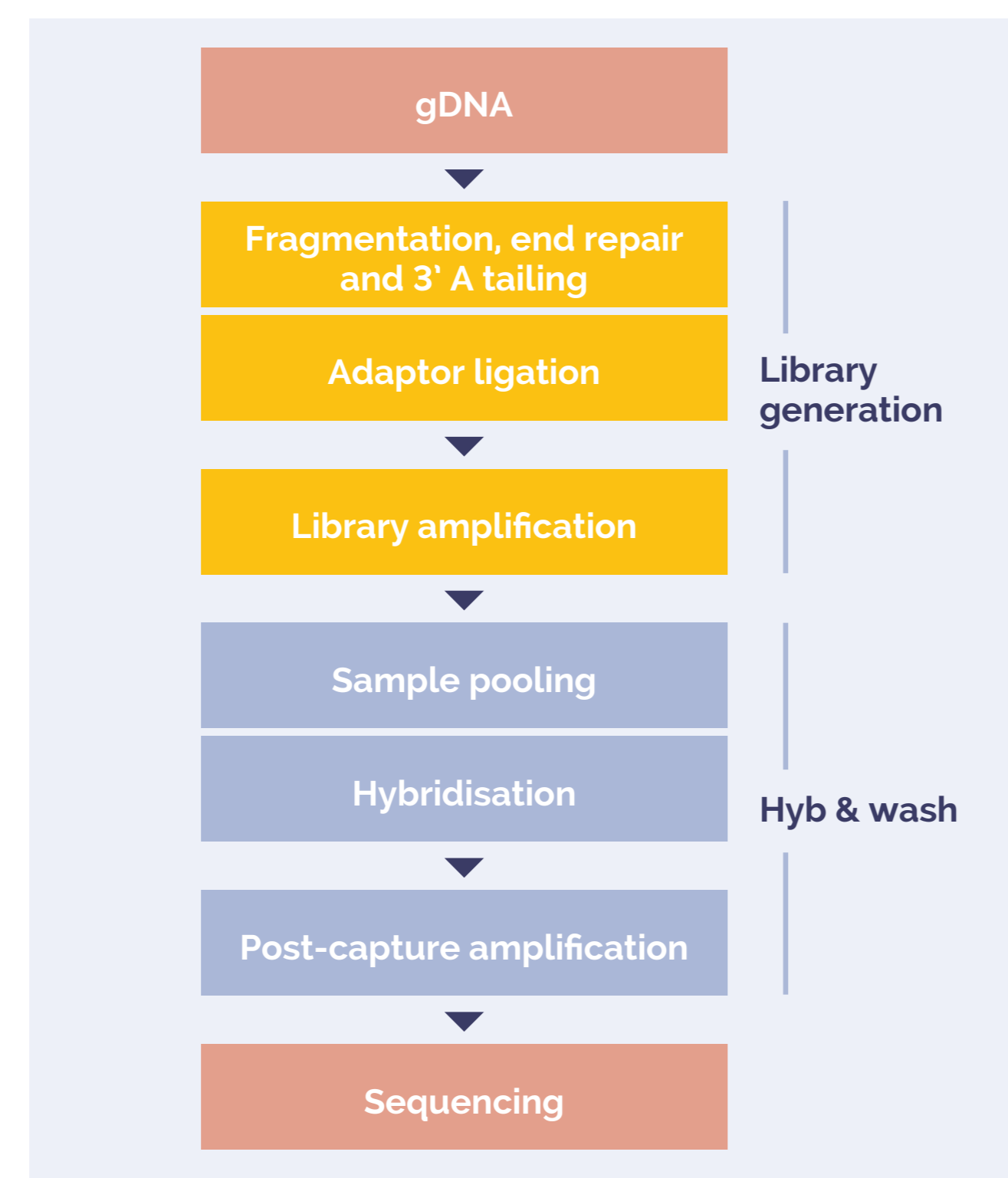Table 1: SureSeq CLL + CNV V3 Panel content.

Internal Reference for CNAs calling

For each set of 8 samples, one Reference DNA (negative for aberrations in the critical regions) was hybridised and sequenced with the research samples. Information from reference DNA samples are used in the analysis for CNAs detection.

Sequencing

Sequencing was conducted using 2 x 150 bp reads on an Illumina MiSeq® V2 300.

Samples

The workflow and the panel were tested on reference DNA (Coriell Institute, Horizon) to validate the overall performance. CNAs capability was demonstrated by using 21 research samples with known CNAs in the regions of interest previously characterised by FISH.

To mimic CNAs at low tumor content, 11 research samples with known aberrations were diluted in order to create CNA carriers with 20–40% tumor content.

Bioinformatic Analysis

Sequencing data analysis including CNA detection was performed using OGT's Interpret NGS Analysis Software.

gDNA
↓
Fragmentation, end repair and 3' A tailing
↓
Adaptor ligation → Library generation
↓
Library amplification
↓
Sample pooling
↓
Hybridisation → Hyb & wash
↓
Post-capture amplification
↓
Sequencing

Figure 1: Universal NGS Workflow DNA to sequencer in 1.5 days with minimal handling time.

Results I

Detection of SNVs and indels in 16 genes

High depth and uniform of coverage was achieved for all targeted genes and genomic regions allowing for confident detection of low frequency gene specific SNVs and indels.
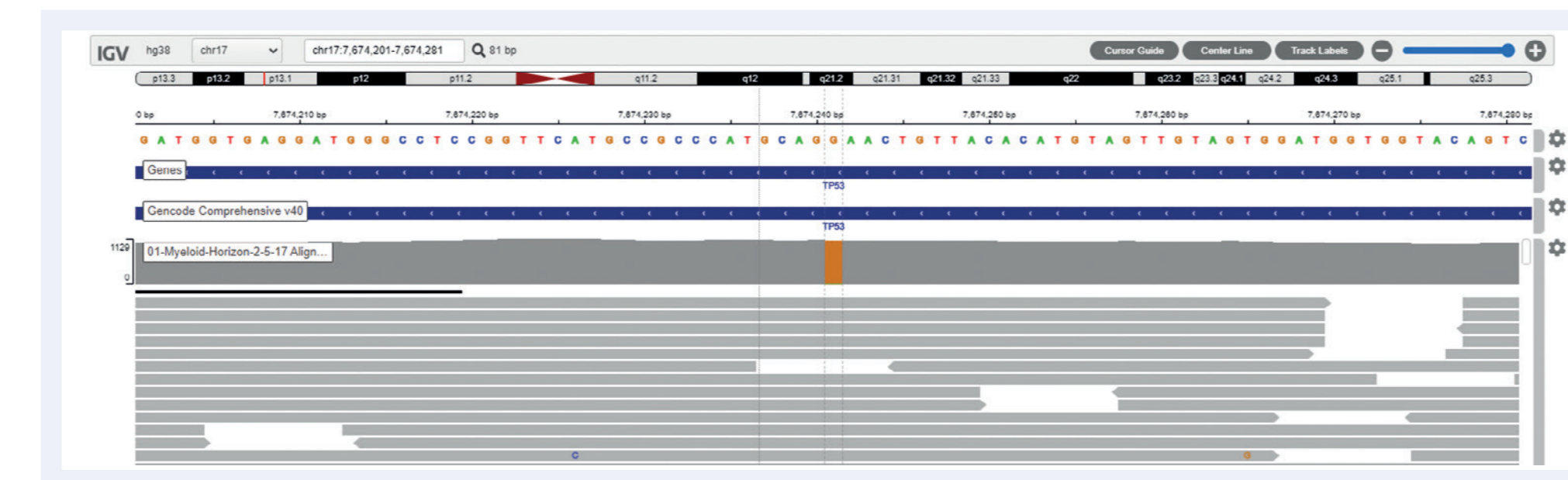
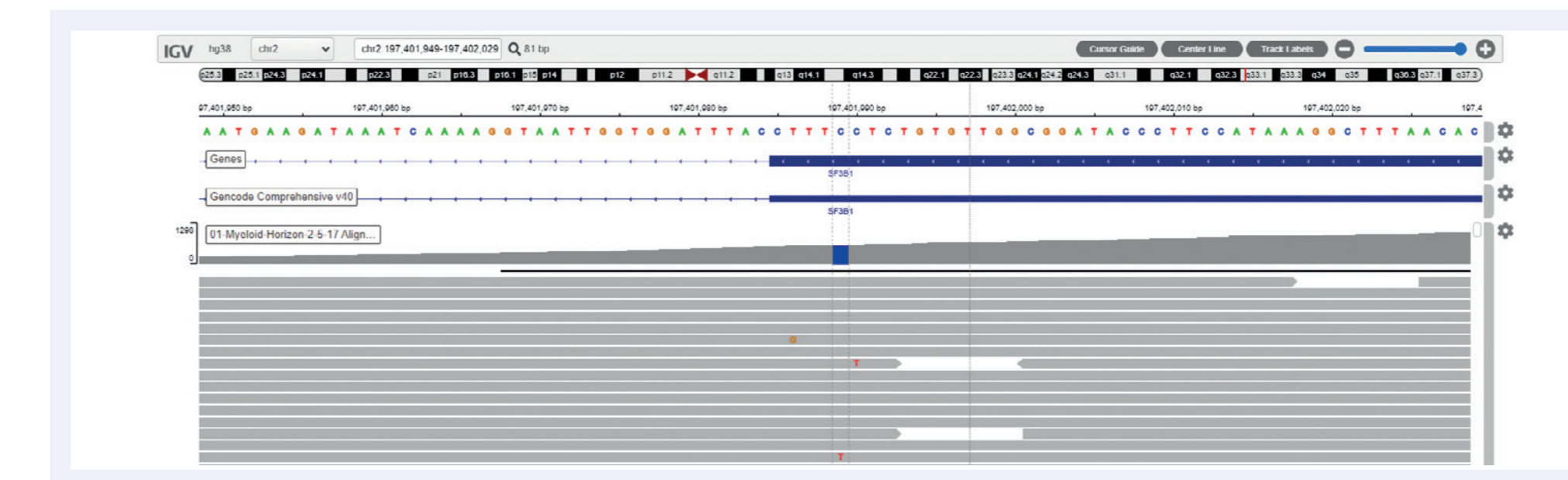Figure 2: Example of *TP53* c.722C>T estimated at 1.62% VAF.

Figure 3: Example of *SF3B1* c.2219G>A estimated at 1.04% VAF.

Gene	Variant (AA)	CDS mutation	Expected VAF	Observed VAF
KRAS	G13D	c.38G>A	20%	14.35%
NRAS	Q61L	c.182A>T	5%	4.04%
SF3B1	G740E	c.2219G>A	2.50%	1.75%
TP53	S241F	c.722C>T	2.50%	1.92%

Table 2: Variants present in the Myeloid DNA Reference Standard (Horizon) and detected by the SureSeq CLL + CNV V3 Panel.

Results II

Detection of CNAs in the critical regions

Data presented here are from 21 research samples that were processed using the OGT workflow in combination with OGT's Interpret NGS Analysis Software.

- Table 3 lists the range of CNAs detected in the 21 research samples. These include CNAs on chromosomes 17p, 11q, 13q and trisomies 12. One sample was identified as negative by FISH and confirmed by NGS.

Sample	Region	Region size	Type	Copy number ratio (log2 scale)	Tumor content NGS (%)	Aberration	Abnormal nuclei by FISH (%)
1	13q	29 Mb	deletion	-0.70	76%	del(13q)	79%
			Not detected by NGS*			del(11q)	28%
2	13q	13.2 Mb	deletion	-0.94	43%	del(13q)	57%
3	13q	2.9 Mb	deletion	-0.38	64%	del(13q)	32%
4			No CNA detected			No CNA detected	
5	13q	9.5 Mb	deletion	-0.85	70%	Not detected by FISH‡	
	11q	57.4 Mb	deletion	-0.82	79%	del(11q)	83%
6	13q	17.1 Mb	deletion	-1.00	76%	del(13q)	94%
	11q	51 Mb	deletion	-1.00	78%	del(11q)	94%
7	13q	9.3 Mb	deletion	-0.72	27%	del(13q)	20%
	17p	16 Mb	deletion	-1.00	100%	del(17p)	97%
8	13q	3.02 Mb	deletion	-0.25	NA	del(13q)	40%
9	13q	46 Mb	deletion	-0.14	NA	del(13q)	NA
10	12	132 Mb	trisomy	0.51	54%	T12	43%
11	12	128 Mb	trisomy	0.59	84%	T12	58%
12	12	132 Mb	trisomy	0.38	63%	T12	79%
	11q	37.1 Mb	deletion	-1.00	77%	del(11q)	89%
13	12	38.4 Mb	duplication	0.74	NA	T12	35%
	17p	18.1 Mb	deletion	-0.92	55%	del(17p)	82%
14	12	132 Mb	trisomy	0.60	92%	T12	76%
15	12	30.8 Mb	duplication	1.01	84%	dup(12)	NA
16	17p	22.4 Mb	deletion	-0.74	45%	del(17p)	63%
17	17p	22.4 Mb	deletion	-1.00	52%	del(17p)	79%
18	17p	22.4 Mb	deletion	-0.19	57%	del(17p)	30%
19	11q	14.2 Mb	deletion	-1.00	100%	del(11q)	88%
20	11q	14.93 Mb	deletion	-0.16	68%	del(11q)	30%
21	11q	480 Kb	deletion	-0.60	68%	del(11q)	35%
	12	122 Mb	trisomy	0.46	74%	T12	65%

Table 3: Data generated using the SureSeq CLL + CNV V3 Panel in combination with the OGT's Universal Complete NGS Workflow and OGT's Interpret NGS Analysis Software. *Not enough material to perform orthogonal tests. Suspected over-estimation of tumor content by FISH, likely to be below the level of detection of the assay (20%). ‡Not enough material to perform orthogonal tests. Highly confident variant, log2 ratio=-0.85.

- To demonstrate the capability of CNAs detection at low tumor content rate, 11 samples were further diluted using a reference DNA and analysis was performed using the OGT's complimentary CNA detection software (Table 4).

Sample	Abnormality	Expected tumor content NGS (%)	Variant detection
2	del(13q)	28.50%	YES
5	del(11q)	27.60%	YES
6	del(11q)	47%	YES
	del(13)	47%	YES
9	del(13q)	30%	YES
10	T12	21.50%	YES
13	del(17p)	41%	YES
	T12	17.50%	YES
14	T12	25.30%	YES
16	del(17p)	31.50%	YES
17	del(17p)	26.30%	YES
18	del(17p)	20%	YES
20	del(11q)	30%	YES

Table 4: Data generated diluting 11 research samples for low tumor content CNAs detection.

- Data generated by using the SureSeq CLL + CNV V3 Panel showed very high concordance (93%) with independent findings by FISH.

- After dilution, 100% of CNAs with estimated tumor content ranging from 18% to 47% were detected.

- No additional CNAs were identified in these samples, resulting in 100% specificity.

Figure 4: Example of a 16Mb deletion covering *TP53* gene (del17p).

Figure 5: Example of a trisomy 12.

Figure 6: Example of a 3Mb deletion covering *RB1* and *DLEU1* genes (del13q).

Conclusions

- High depth and uniform of coverage was achieved for all targeted genes and genomic regions allowing for confident detection of low frequency gene specific SNVs and indels.

- We have demonstrated that the SureSeq CLL + CNV V3 Panel in combination with OGT's Interpret NGS Analysis Software can be reliably used to detect the most common CNAs in CLL for tumor content as low as 20%.

- Our approach allows for the simultaneous evaluation of numerous chromosomal and gene-specific aberrations using a NGS single assay, thus enabling researchers to streamline their research to deliver a comp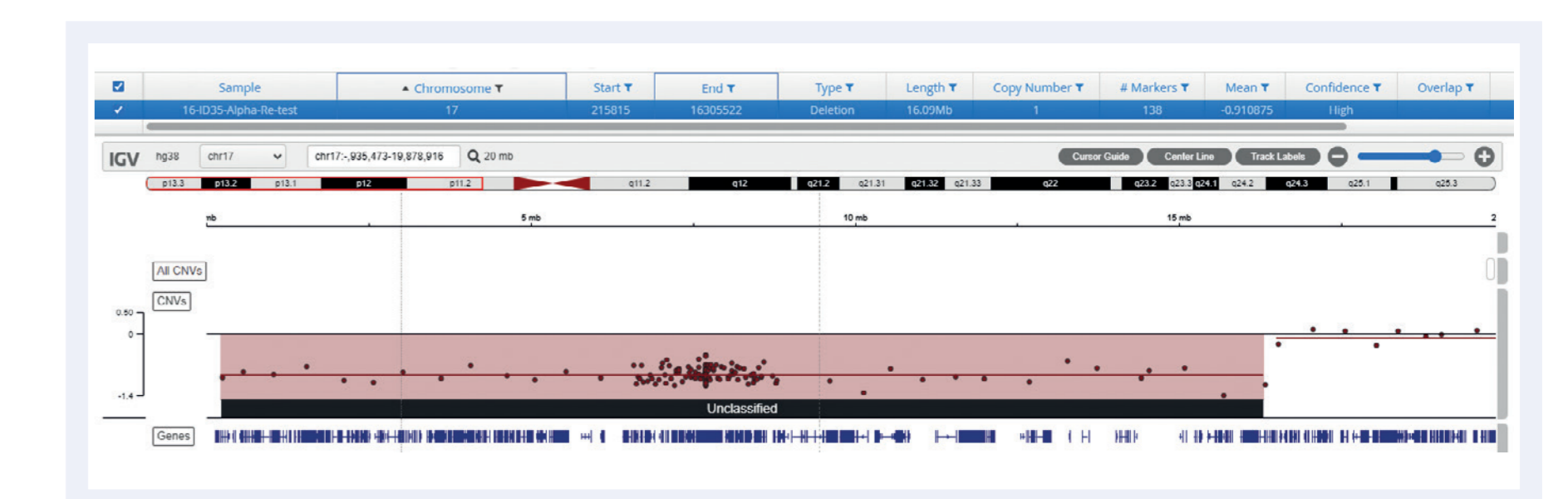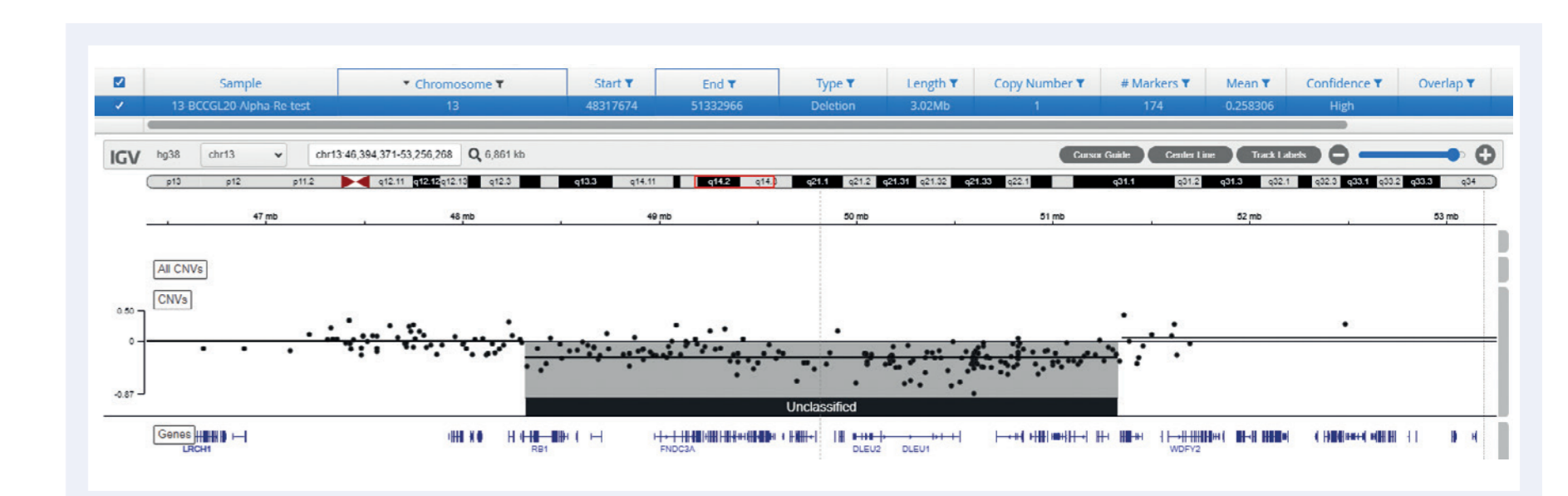rehensive genomic picture of CLL.