SureSeq

Concurrent detection of somatic copy number alterations and gene variants (SNV/indels) in CLL samples using a targeted NGS panel

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What binds us, makes us.

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Introduction

A wide variety of chromosomal abnormalities are associated with Chronic Lymphocytic Leukaemia (CLL). Currently, comprehensive genetic research into CLL requires multiple testing strategies with high associated costs.
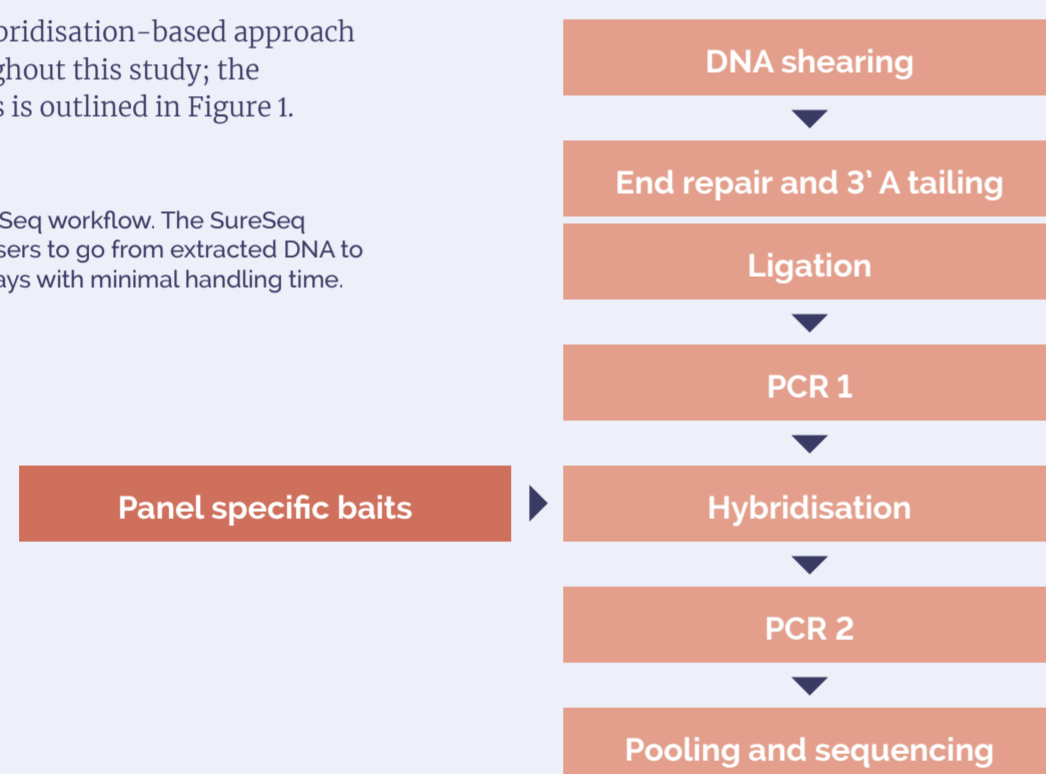
Somatic mutations (Single Nucleotide Variants (SNVs) and insertion/deletions (indels)) can be identified by next generation sequencing (NGS), but copy number alterations (CNAs) currently require additional cytogenetic methods including karyotyping, fluorescence *in situ* hybridisation (FISH) and microarrays. For a more complete picture, follow-up assays are required to determine the trigger(s) behind the malignant transformation and to characterise the genetic profile to aid research into prognosis and disease management.

In this study, we tested the capability of SureSeq™ NGS panel to overcome the challenges with detecting CNAs currently experienced and provide a possible future single test to be developed for CLL.

Methods

The SureSeq hybridisation-based approach was used throughout this study; the workflow of this is outlined in Figure 1.

Figure 1: OGT SureSeq workflow. The SureSeq workflow allows users to go from extracted DNA to sequencer in 1.5 days with minimal handling time.

DNA shearing
↓
End repair and 3' A tailing
↓
Ligation
↓
PCR 1
↓
Panel specific baits ▶ Hybridisation
↓
PCR 2
↓
Pooling and sequencing

We utilised a SureSeq CLL CNV - 14 gene panel and associated library preparation kit to determine whether this approach can be used for detection of somatic CNAs as well as SNVs and indels.

CLL CNV - 14 gene panel can be used for:

1. Detection of SNV and indels in 14 genes - *ATM*, *PLCG2*, *BIRC3*, *BRAF*, *TP53*, *XPO1*, *SF3B1*, *KRAS*, *MYD88*, *SAMHD1*, *NOTCH1*, *BTK*, *CXCR4* and *SRY*.

2. Detection of somatic CNAs within 5 chromosomal regions − 17p (covering *TP53*), 11q (covering *ATM*), 13q (covering *RB1/DLEU2/DLEU7*), 6q (covering *MYB*) and Trisomy 12.

We used a hybridisation-based enrichment approach for library preparation and analysed 15 research samples* with known CNAs. We also analysed 24 samples (Coriell, Camden, NJ) with no known CNVs in the regions of interest which were used as control/reference samples. The resulting libraries were sequenced using a 2x150 bp read length protocol on an Illumina MiSeq®. All research samples were also processed on the Cytoscan™ HD microarray and associated software (Affymetrix®) allowing for comparison of findings of copy number alterations in each sample.

Bioinformatics Analysis

Data sequencing analysis including CNA detection was performed using Interpret, OGT's complimentary gene variants and CNV detection software.

Results I

Confident detection of SNV and indels in 14 genes

We achieved high depth (>2000x) and excellent uniformity of coverage across the targeted 14 genes which enabled the confident detection of low frequency gene specific SNVs and indels.

Figure 2: Example of *SF3B1* exon 15 hotspot variant Lys700Glu with frequency 4.8%. Data generated with OGT SureSeq protocol averaging ~2000x deduplicated coverage. Depth of coverage per base (grey).
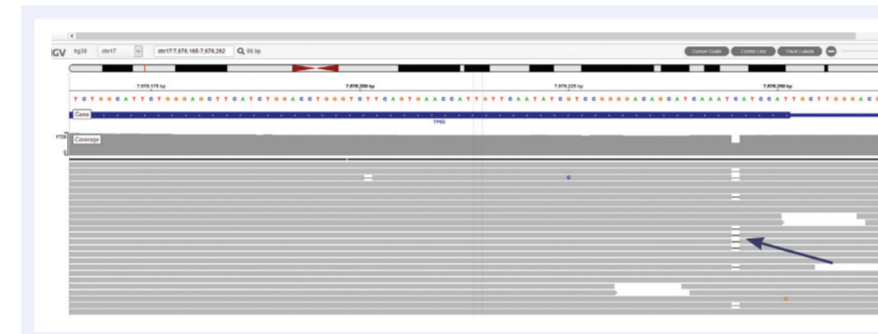

Figure 3: Example of *TP53* exon 4 frameshift deletion (*TP53* c.124del) with frequency 38.9%. Data generated with OGT SureSeq protocol averaging ~2000x deduplicated coverage. Depth of coverage per base (grey).

Validation of the CLL CNV - 14 gene panel and enhanced CNA detection software with 15 research samples

· Data presented here are from 15 research samples (1 control with no CNAs in the regions of interest) that were processed using the OGT workflow in combination with OGT's complimentary gene variant and CNV detection software.

· Table 1 details the range of CNAs detected from 15 research samples. These include CNAs on chromosomes 17p, 11q and 13q. No CNAs were identified in the control sample.

· CNA events were reported in samples with predicted tumour content as low as 25%.

| Sample	Region of aberration	CNA calls by SNP Array				CNA calls by NGS			
		Position (hg19)	Size (Mb)	Type	% Cells	Position (hg19)	Size (Mb)	Type	Copy number ratio (log scale)
1	11q	chr11:84748415-135068576	50.3	del	unknown	chr11:78784668-134585879	55.8	del	-0.58
2	13q	chr13:30818277-56270736	25.4	del	60%	chr13:31231652-56200534	25.0	del	-0.43
3	11q	chr11:78714880-134500539	55.9	del	80%	chr11:84672154-134455774	49.8	del	-0.51
4	17p	chr17:159683-17332764	17.2	del	70%	chr17:65607-16934510	6.9	del	-0.60
5	11q	chr11:103932028-117102219	13.2	del	70%	chr11:103960595-116913934	13.0	del	-0.37
	13q	chr13:49991845-50928235	0.9	del	80%	chr13:50646193-51491987	0.8	del	-0.73
6	13q	chr13:48410813-48591913	0.18	del	70%	chr13:48989061-49149794	0.20	del	-3.60
7	13q	chr13:33933290-66645972	32.7	del	55%	chr13:35270684-66902754	31.6	del	-0.29
	17p	chr17:7505268-7799401	0.29	del	50%	chr17:7572892-7579950	0.1	del	-0.45
8	13q	chr13:32213738-51880962	19.7	del	40%	chr13:33000556-52263527	19.3	del	-0.25
9	13q	chr13:48296646-51220419	2.9	del	40%	chr13:48891811-51782999	2.9	del	-0.38
10	11q	chr11:82432986-122087779	39.8	del	35%	chr11:84672154-121867725	37.2	del	-0.24
11	17p	chr17:150732-21415511	21.3	del	25%	chr17:65607-22072006	22.0	del	-0.16
12	17p	chr17:150732-19240663	19.1	del	unknown	chr17:215815-19020899	18.8	del	-0.69
		chr17:19240830-22763679	3.5	dup		chr17:19565067-23871234	4.3	dup	0.58
13	17p	chr17:150208-19019419	18.1	del	unknown	chr17:215815-19565117	19.3	del	-0.76
		chr17:19240662-22763679	3.5	dup		chr17:20134934-23871234	3.7	dup	0.63
14	13q	chr13:48296646-51220419	2.9	del	unknown	chr13:49749072-51802126	2.1	del	-0.39
15		No CNVs expected				No CNVs detected			

Table 1: Data generated with the CLL CNV - 14 gene panel using a combination of OGT workflow and enhanced CNV detection software was 100% concordant with independent findings (West Midlands Regional Genetics Laboratory – Birmingham, UK).

Results II

Confident detection of Copy Number Alterations

Using the OGT workflow we were able to reliably detect somatic copy number alterations in 15 research samples. For all samples, predicted CNAs were found to be 100% concordant with the reported events with the array data.
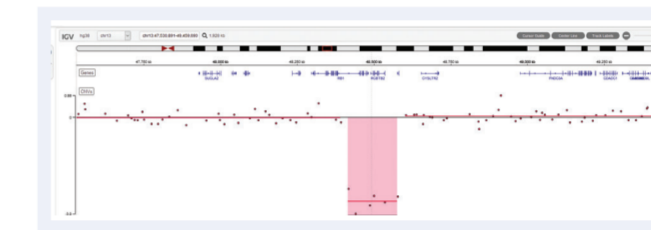
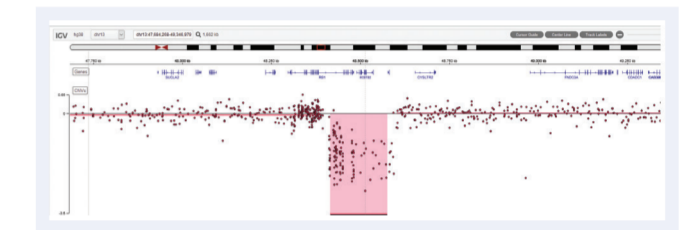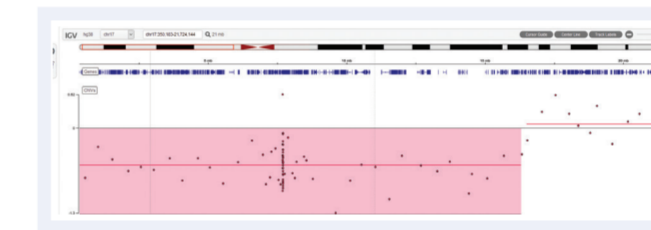**NGS**   **Array**

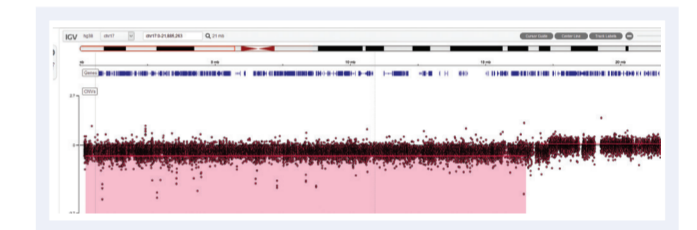Figure 4: 181kb biallelic deletion within 13q14.2 including RB1.

NGS   **Array**

Figure 5: 17.2 Mb deletion of 17pter to p11.2.

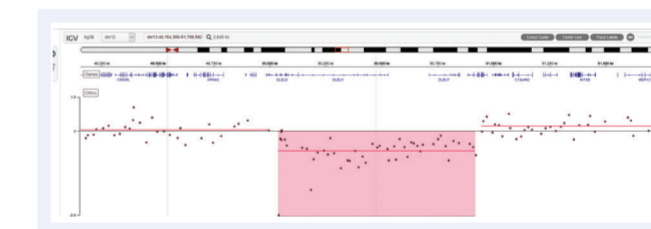**NGS**   **Array**
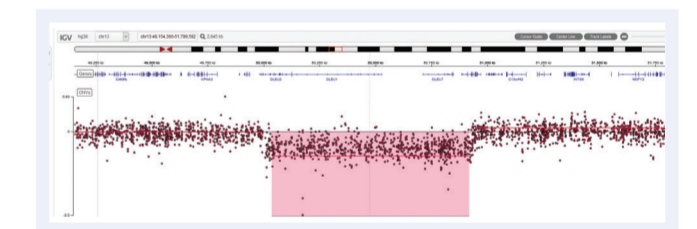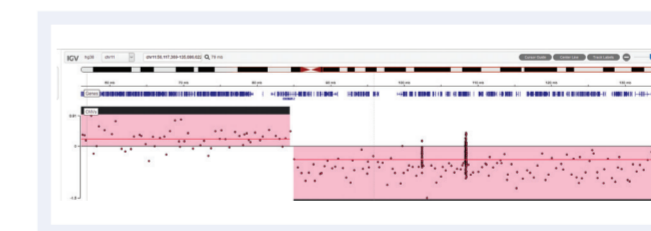

Figure 6: 11 Mb deletion of 13q14.2q14.3, including *DLEU2* and *DLEU7* genes.

NGS   **Array**
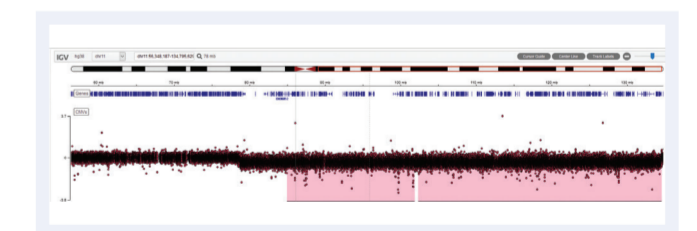

Figure 7: 56 Mb deletion of 11q14.1 to 11q25.

Conclusions

· Superior uniformity of coverage from a hybridisation-based enrichment using the SureSeq CLL CNV - 14 gene panel allowed simultaneous detection of SNVs, indels as well as larger structural alterations in a single assay.

· We have demonstrated the capability of a SureSeq CLL CNV - 14 gene panel to detect complex rearrangements, ranging from a single gene (10 kb deletion covering *TP53*) to whole arm somatic deletions in samples with tumour content as low as 25%.

· Our approach allows for the simultaneous evaluation of numerous chromosomal and gene-specific aberrations using a single assay.