CytoSure

A Sysmex Group Company

Constitutional NGS

Features

The ability to detect CNV, SNV, indel, LOH and mosaicism

- Everything you get from arrays and NGS in a single assay

Advanced panel design and software

- Robust single-exon CNV calling unlike other large targeted panels or exomes

The most up-to-date content for ID and DD

- Giving you the best chance to identify the aberration of interest

A targeted >700-gene panel, minimising variants of uncertain significance (VUS) detection

- Helping you to minimise analysis time

Cost-effective analysis

- Don't spend time and effort sequencing, storing and analysing data that is not relevant

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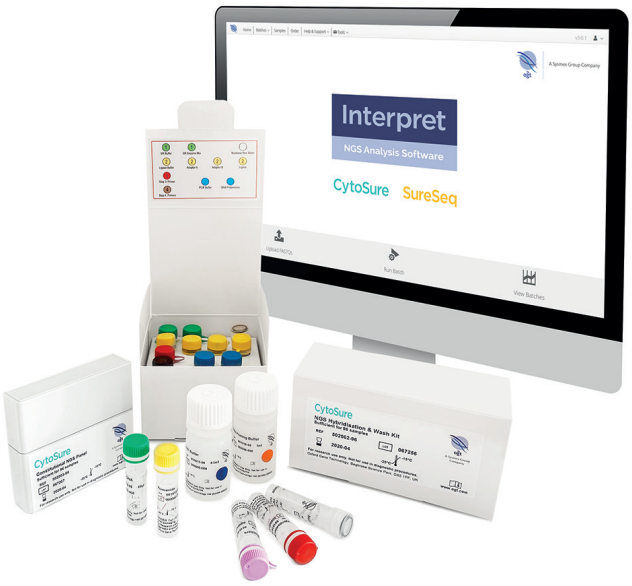
Introduction

Although array comparative genomic hybridisation (aCGH) is still regarded as the gold standard for analysis of copy number variation (CNV) in intellectual disability (ID) and developmental delay (DD) research samples, it does not provide a comprehensive view of possible aberrations associated with a given sample. Additional assays such as next-generation sequencing (NGS) have to be performed in order to obtain critical insights such as the presence of causative single nucleotide variants (SNVs) and insertion/deletions (indels), with a subsequent increase in time and cost[1].



There have been significant advances in sequencing technology and extensive developments in analysis software in recent years, but, up until now, NGS has not been able to robustly deliver single-exon CNV calling in a cost-effective manner and so has not been widely adopted in the cytogenetics research laboratory[2].

The CytoSure® Constitutional NGS solution delivers CNV analysis down to single-exon level and loss of heterozygosity (LOH) as well as SNV and indel detection all in a single assay.

The CytoSure Constitutional NGS solution includes everything you have come to rely on with the well-established CytoSure microarray brand from Oxford Gene Technology (OGT), namely, the most up-to-date ID/DD content, expert panel design, class-leading complimentary software and unparalleled support. It enables the seamless transition from microarrays to NGS, delivering a significant increase in information obtained from a single assay without extensive analysis time and costly data generation and storage.

Comprehensive aberration detection

The NGS panel is designed to cover important genes for ID/DD and also contains a backbone of baits covering common single nucleotide polymorphisms (SNPs), this allows detection of a comprehensive range of aberration types including CNVs, SNVs, indels and LOH in a single assay (Figure 1). This also includes detection within mosaic samples (Figure 2). The software user interface is conveniently arranged and also has the ability to switch between CNV/LOH calls and SNV/indel analysis, enabling a step-by-step approach to the interpretation process.

A

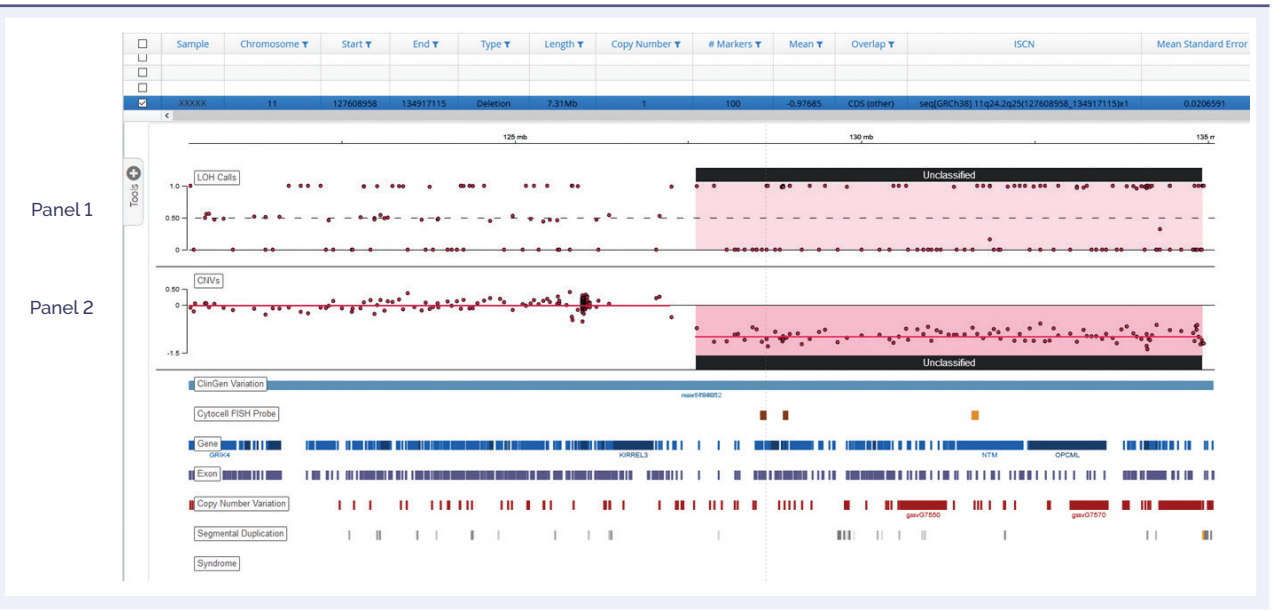


Figure 1 A : A 7.3 Mb deletion detected on chromosome 11. Panel I shows the B allele frequency or LOH, whilst panel II shows the copy number ratio change*.

B

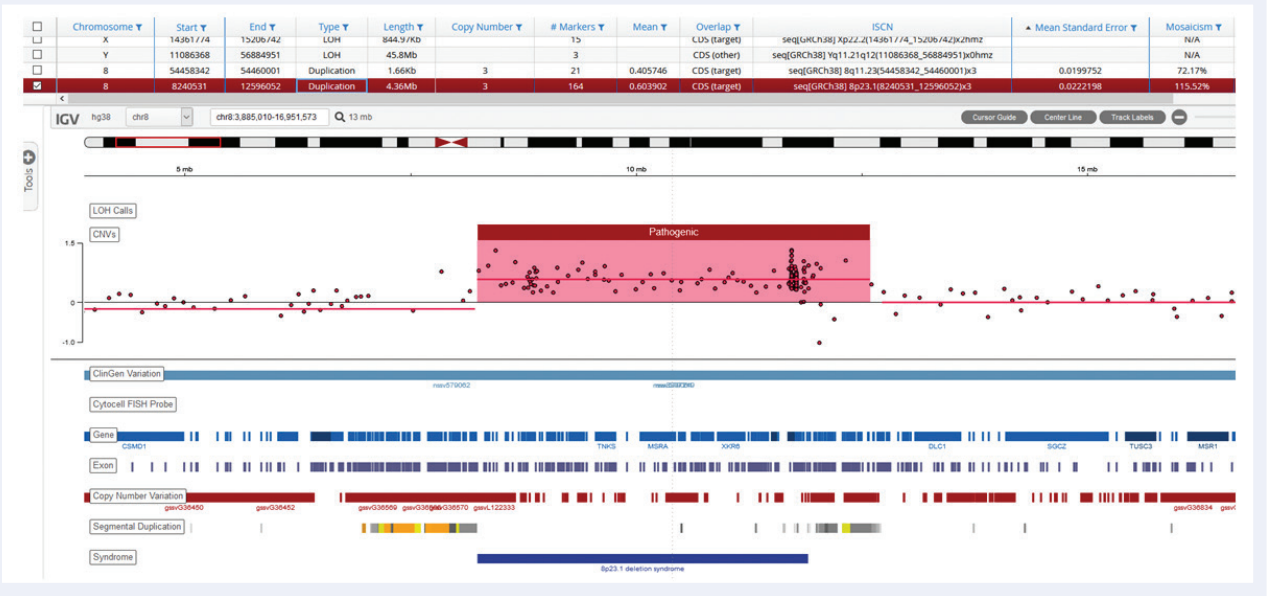


Figure 1 B : Duplication. A 4.36Mb duplication detected on chromosome 8[1].

C

Sample	True Positive	False Positive	False Negative	Precision	Sensitivity
12878_A1	2139	12	36	0.9944	0.9836
12878_A2	2139	11	36	0.9949	0.9836
12878_B1	2130	12	45	0.9944	0.9795
12878_B2	2133	13	42	0.9939	0.9809
12878_B3	2132	11	43	0.9949	0.9804

Figure 1 C : Table shows reproducibility of 5 different runs of the Genome in a Bottle (GIAB) sample, the data demonstrates robust SNV detection achieved using the CytoSure Constitutional NGS Panel. Many of the false negatives fall in highly repetitive regions (e.g. homopolymer regions) and appear to be artifacts present in the GIAB WGS. These kinds of regions are highlighted in the software.

D

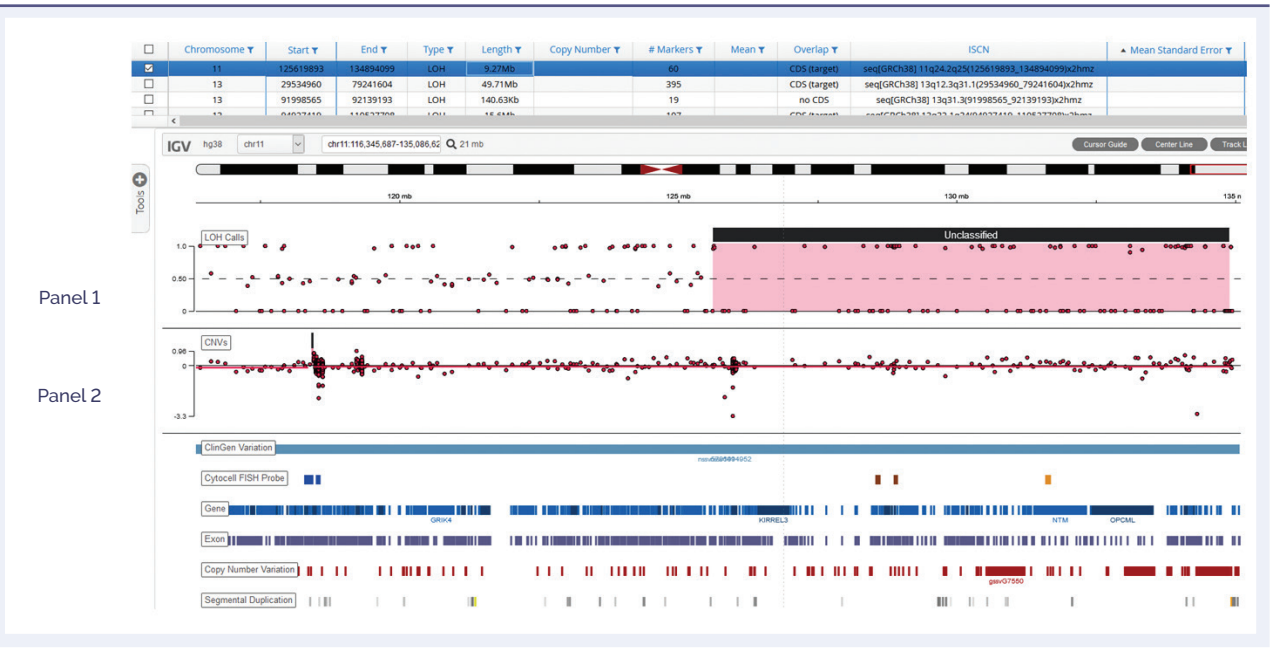


Figure 1 D : A Consanguineous sample with a stretch of copy neutral LOH on chromosome 11. Panel I shows the B allele frequency whilst Panel II displays the copy number ratio[i].

E

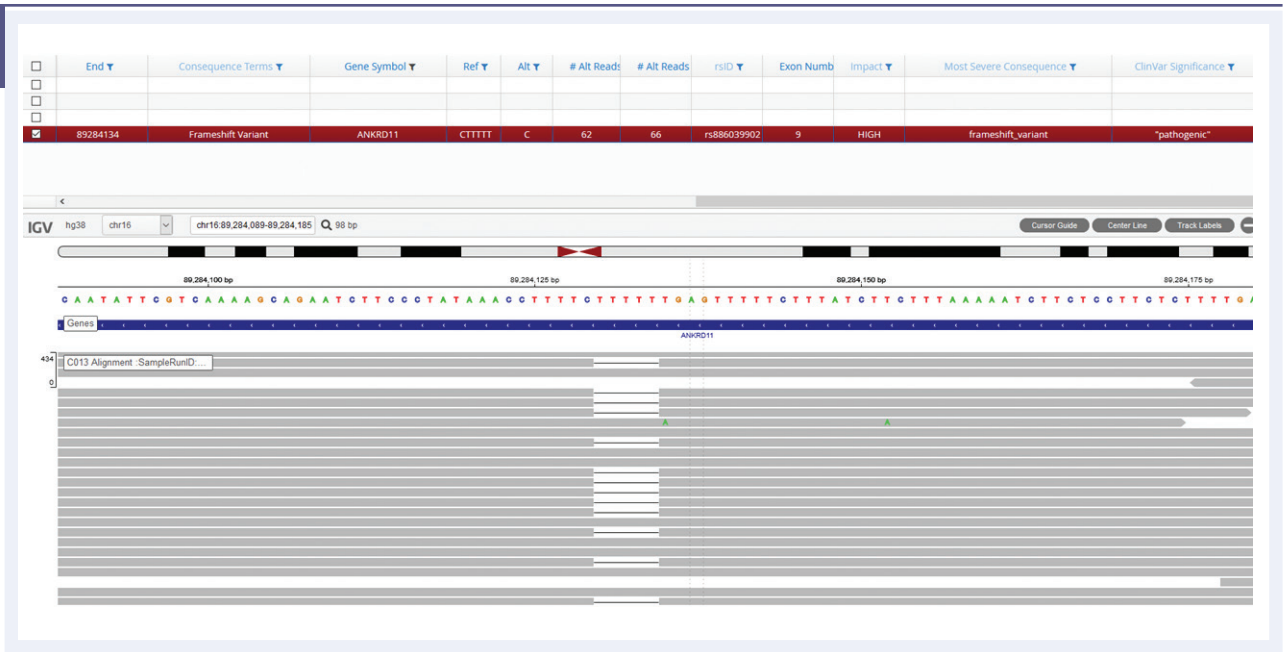


Figure 1 E : Sample showing an indel. A c.2408_2412del (p.Lys803Argfs*5) indel detected within the ANKRD11 gene*.

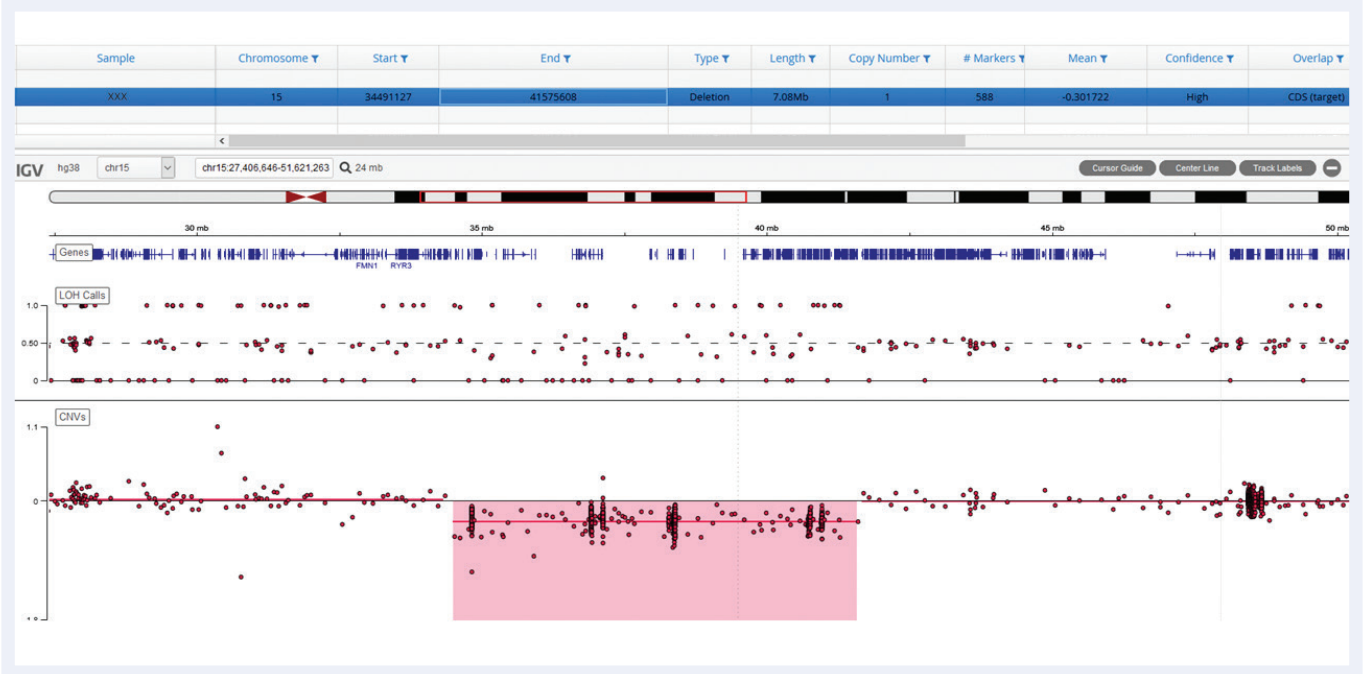


Figure 2: Sample with a 7Mb deletion at 15q14q15.1 within a mosaic sample*.

Advanced panel design and software for robust single-exon CNV calling

A key requirement in enabling the transition from a microarray-based technology to NGS for CNV detection is the ability to ensure that CNV data from the NGS panel is concordant with that from microarrays, particularly for small, sub-gene duplications and deletions. OGT's expertise in bait design ensures uniform sequencing coverage of the desired regions. This, coupled with a proprietary CNV calling algorithm, allows robust detection of even the smallest CNVs (Figure 3).

A

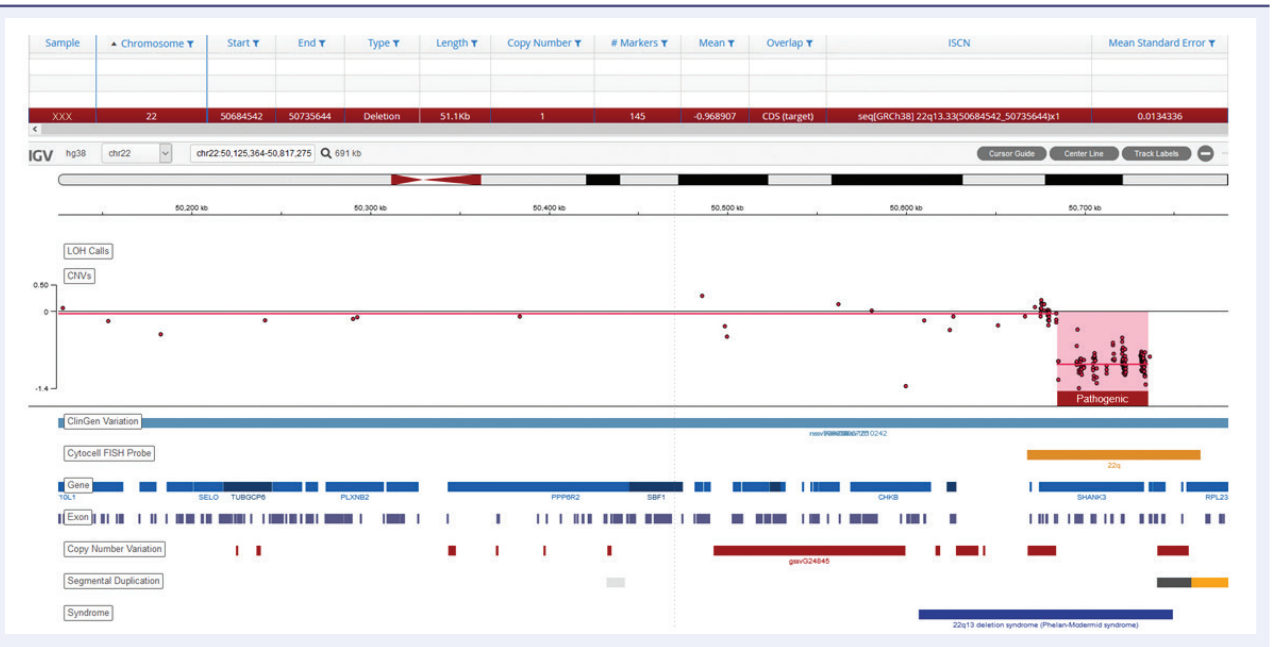


Figure 3 A : Detection of a 51kb deletion within the SHANK3 gene on chromosome 22*.

B

Figure 3 B : A 2.42Mb duplication on chromosome 22†.

The most up-to-date content for Intellectual Disability and Developmental Delay

It is important to ensure that the tools used in the laboratory are as up-to-date and comprehensive as possible. The CytoSure Constitutional NGS panel has been designed with input from leading experts in cytogenetics research as well as accessing information in international databases such as ClinGen[3] and Deciphering Developmental Disorders (DDD)[4]. The panel contains over 700 targeted ID and DD genes including all exons and UTRs. Both the 5' and 3' UTRs are targeted in order to provide comprehensive coverage of the genes and to allow detection of any SNVs which may be relevant in these regions. The extensive set of backbone baits also enables detection of large CNV and LOH regions with a resolution of <5Mb (Figure 4).

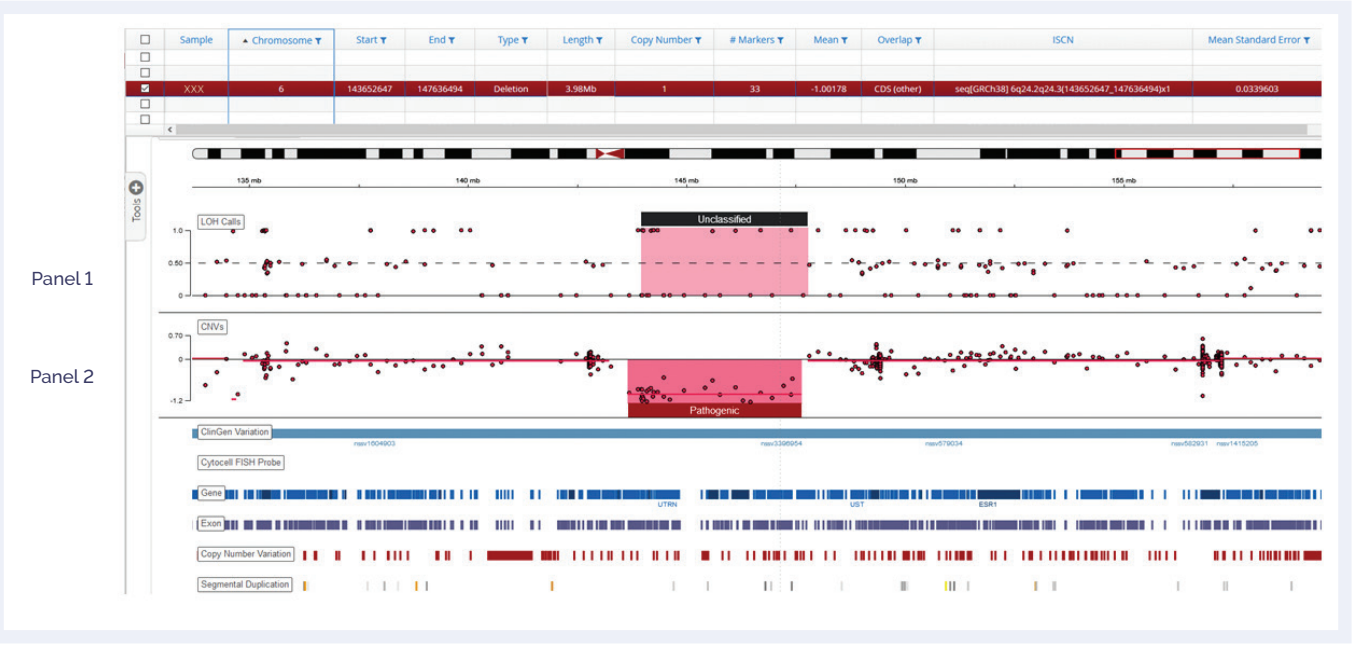


Figure 4: A 3.98Mb deletion on chromosome 6. The B allele plot (Panel 1) and the CNV ratio result (Panel II) are both shown*.

Analysis of a targeted >700-gene panel minimises VUS detection and controls costs

One of the main challenges with exome or medical/clinical exome sequencing is the large amount of data generated per sample. This has obvious implications, not only on the cost to sequence individual samples at sufficient depth for robust variant calling, but also the costs and time associated with data storage and analysis. By focussing the panel on only the content required, it is possible to minimise costs for sequencing and data storage as well as analysis time. The ID/DD-focussed content approach reduces the number of aberrations called compared to, for example, exome sequencing and, in particular, reduces the number of calls that would be classified as VUS, often requiring lengthy review and analysis.

The Interpret software offers standard variant and call attributes, which facilitate rapid data review (Figure 5), as well as numerous sophisticated filtering options (Table 1). The software also contains links to external databases for variant annotation.

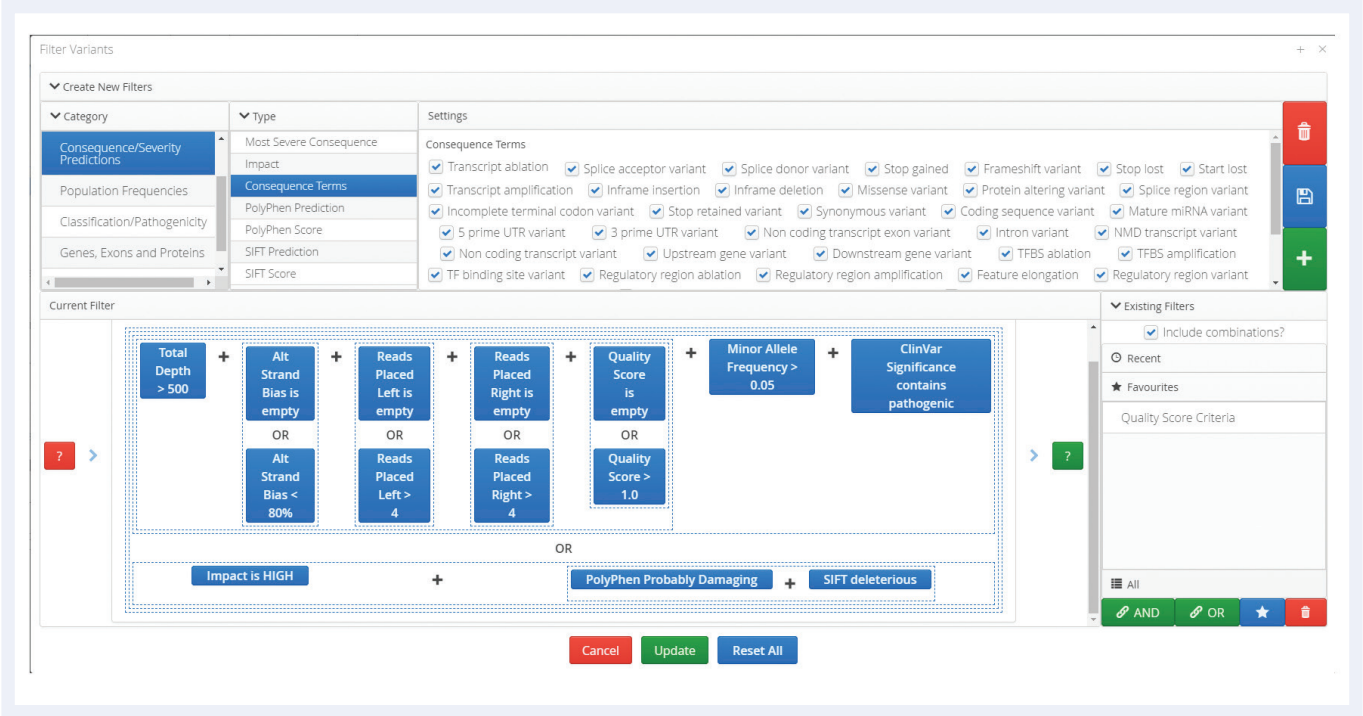


Figure 5: An example of some of the filtering options available in the Interpret software.

Consequence/ Severity Predictions	Population Frequencies	Classification/ Pathogenicity	Genes, Exons and Proteins	Region/ Variant Lists
Most Severe Consequence	rsID	ClinVar Significance	Gene ID	Overlap with user-defined Region List(s)
Impact	Minor Allele Frequency		Gene Symbol	Overlap with user-defined Variant List(s)
Consequence Terms	Minor Allele		Transcript ID	
PolyPhen Prediction			Protein ID	
PolyPhen Score			Exon ID	
SIFT Prediction			Exon Number	
SIFT Score				

Table 1: Variant filtering options.

Technical Specifications	
Targeted ID/DD Genes	707
CNV Resolution	Targeted Genes (707)- Exon and UTR level resolution Backbone Genes (723) - whole gene level resolution
SNVs and Indels	Targeted Genes (707) - Exon and UTR level resolution
LOH Resolution	> 5Mb
Mean Target Coverage	>250x
Recommended DNA Input	1000 ng
Panel Size	8.5Mb
Recommended Samples per Nextseq® Run	24

Ordering information

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Product	Contents	Cat. No.
CytoSure Constitutional NGS Solution (24)	Bundle of 1x CytoSure Constitutional NGS Panel (24), 1x CytoSure NGS Library. Preparation Kit (24) and 1x CytoSure NGS Hybridisation & Wash Kit (24)	502005-B24
CytoSure Constitutional NGS Solution (96)	Bundle of 1x CytoSure Constitutional NGS Panel (96), 1x CytoSure NGS Library. Preparation Kit (96) and 1x CytoSure NGS Hybridisation & Wash Kit (96)	502005-B96

References

1. Iglesias, A., Anyane-Yeboa, K., Wynn, J. *et al.*, (2014) The usefulness of whole-exome sequencing in routine clinical practice. Genet Med 16, 922–931 doi:10.1038/gim.2014.58
2. Yao, R., Zhang, C., Yu, T. *et al.*, (2017) Evaluation of three read-depth based CNV detection tools using whole-exome sequencing data. Mol Cytogenet 10: 30. https://doi.org/10.1186/s13039-017-0333-5
3. https://clinicalgenome.org/ [Date accessed 26 September 2018]
4. https://www.sanger.ac.uk/science/collaboration/deciphering-developmental-disorders-ddd [Date accessed 26 September 2018]

* Clinical research sample provided courtesy of Centre hospitalier universitaire de Sherbrooke (CIUSSSE-CHUS)
† Clinical research sample provided courtesy of EGL Genetics

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What binds us, makes us.

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