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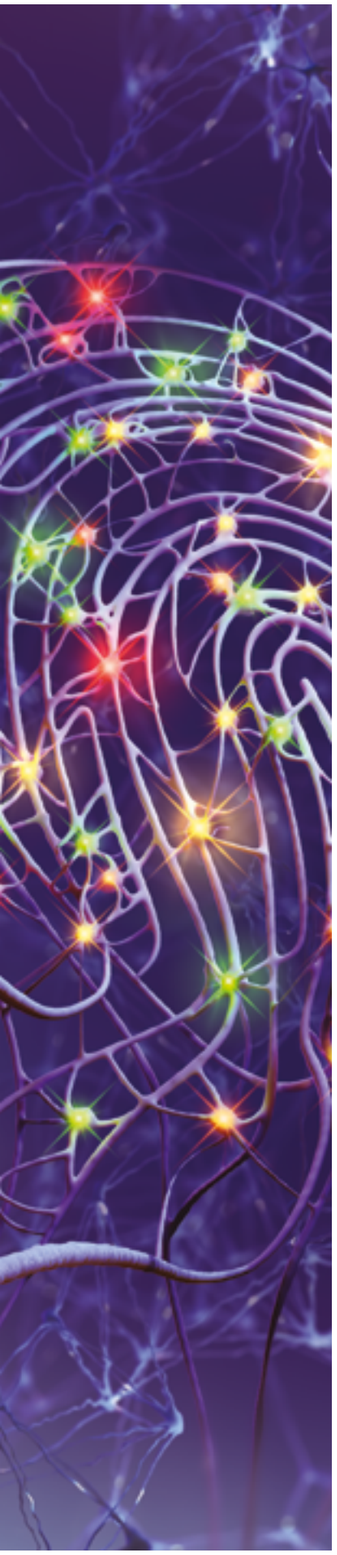
CytoSure

# Constitutional NGS



The CytoSure® Constitutional NGS Panel 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.

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 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 Panel 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 Panel includes everything you have come to rely on with the well-established CytoSure® Arrays, 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.

## 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

# 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.

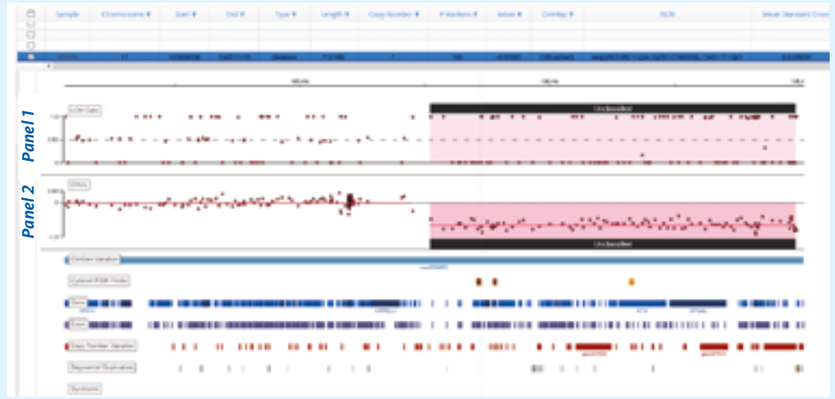



**Fig 1a:** A 7.3Mb deletion detected on chromosome 11. Panel 1 shows the B allele frequency or LOH, whilst panel 2 shows the copy number ratio change*.

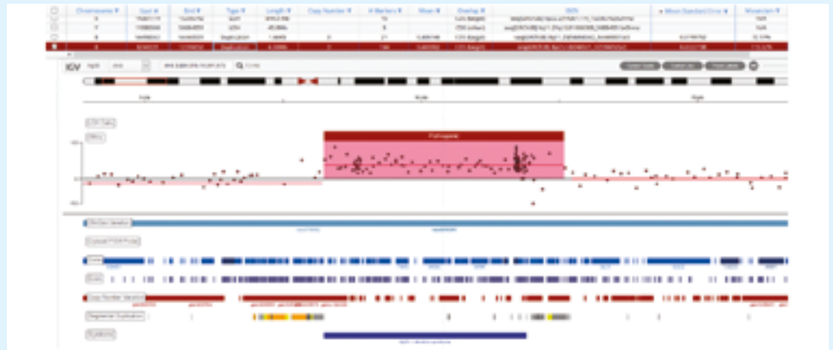


**Fig 1b:** A 4.36Mb duplication detected on chromosome 8**.

**Table 1:** 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.

| 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 |

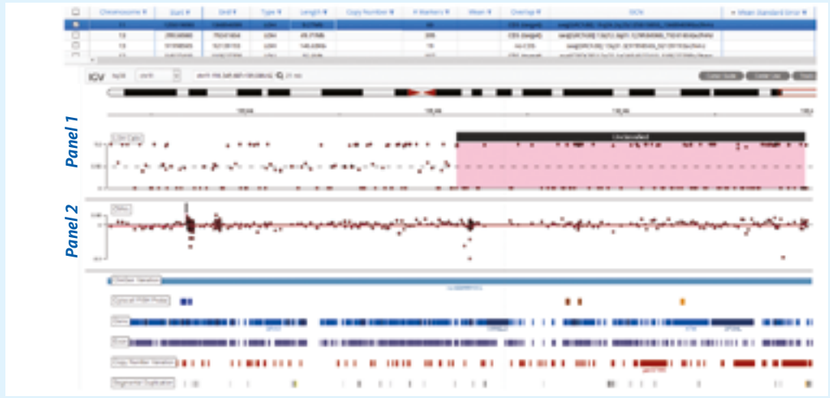



**Fig 1c:** A consanguineous sample with a stretch of copy neutral LOH on chromosome 11. Panel 1 shows the B allele frequency whilst Panel 2 displays the copy number ratio**.

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

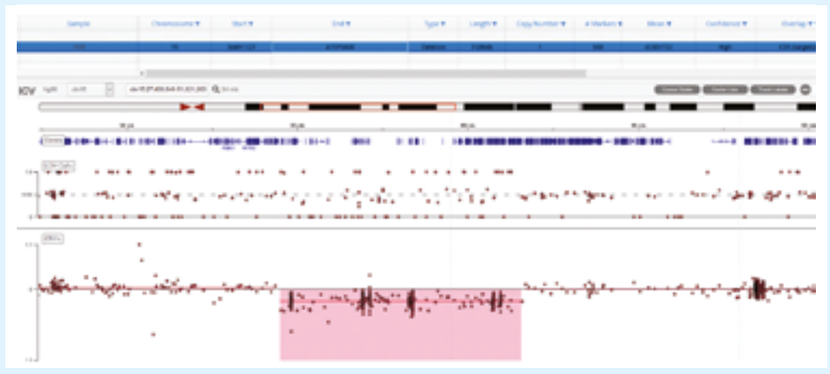


**Fig 2:** Sample with a 7Mb deletion at 15q14q15.1 within a mosaic sample*.

# 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. CytoSure's expert 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).

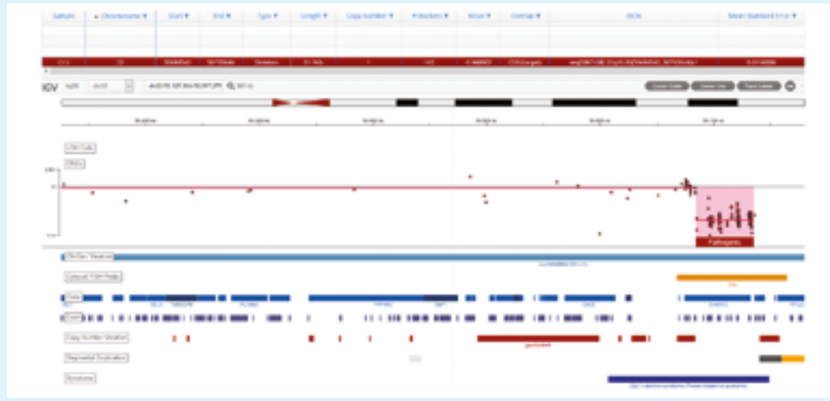


**Fig 3a:** Detection of a 51kb deletion within the SHANK3 gene on chromosome 22*.

**Fig 3b:** 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 and information from 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 CNVs and LOH regions with a resolution of > 3Mb (Figure 4).

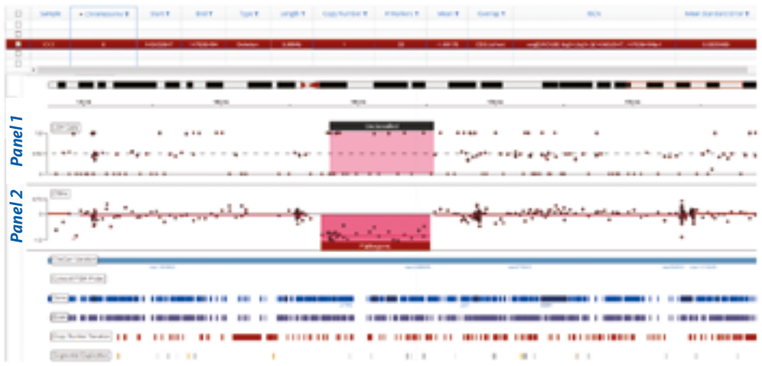



**Fig 4:** A 3.98Mb deletion on chromosome 6. The B allele plot (Panel 1) and the CNV ratio result (Panel 2) are both shown*.

## 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]




# 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 2). The software also contains links to external databases for variant annotation.

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

**Table 2:** Variant filtering options.

| 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 | | | | |

# Technical specifications

| | |
|---|---|
| **CytoSure® Constitutional NGS Complete Workflow Solution V2 (24) Cat. No. 790101-24** | Bundle of enrichment baits sufficient for 3 pools of 8 samples;<br>1 × Universal NGS Library Preparation Kit (24), containing PCR primers and enzymes,<br>1 × Universal NGS Index Adapter Kit (24),<br>1 × Universal NGS Hyb & Wash Kit (24),<br>1 × Universal NGS Bead Kit (24) |
| **CytoSure® Constitutional NGS Complete Workflow Solution V2 (96) Cat. No. 790101-96** | Bundle of enrichment baits sufficient for 12 pools of 8 samples;<br>1 × Universal NGS Library Preparation Kit (96), containing PCR primers and enzymes,<br>1 × Universal NGS Index Adapter Kit (96),<br>1 × Universal NGS Hyb & Wash Kit (96),<br>1 × Universal NGS Bead Kit (96) |
| **Targeted ID/DD genes** | 707 |
| **CNV resolution** | Targeted genes (707) – exon and UTR level resolution<br>Backbone genes (723) – whole gene level resolution |
| **SNVs and indels** | Targeted genes (707) – exon and UTR level resolution |
| **LOH resolution** | > 5 Mb |
| **Mean target coverage** | > 250 × |
| **Recommended DNA input** | 1000 ng |
| **Panel size** | 8.5 Mb |
| **Recommended samples per Nextseq® Run** | 24 |

* 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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