CytoSure

A New Next Generation Sequencing (NGS) Assay for Detecting the Aberrations in Intellectual Disability and Developmental Delay Samples

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What binds us, makes us.

Introduction

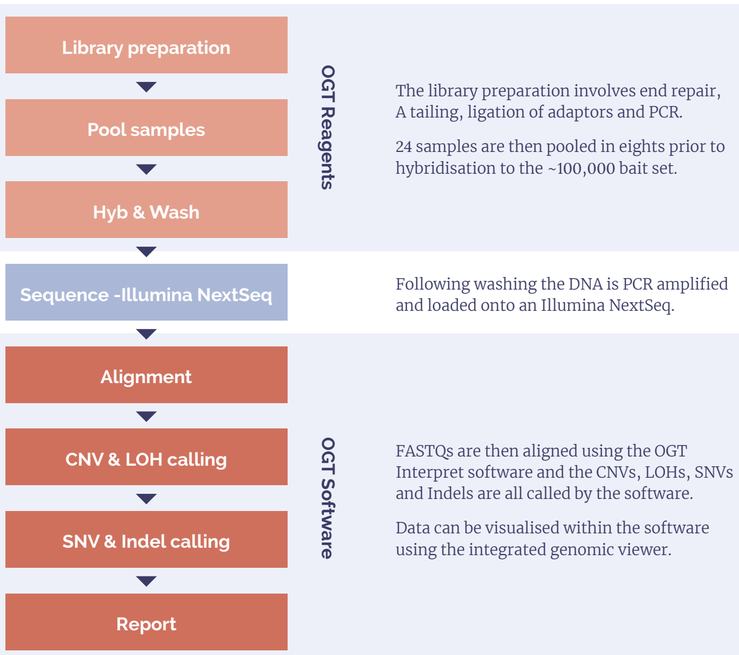
Array comparative genomic hybridisation (aCGH) has been used extensively in research to determine the causative copy number variation (CNV) in Intellectual Disability (ID) and Development Delay (DD) cases[2]. Microarrays have a limitation in that they cannot detect single nucleotide variations (SNVs), which may be causative. Conversely, robust small CNV calling via other genetic techniques, e.g. exome sequencing has remained challenging. To overcome these issues, we have designed an NGS assay and supporting analytical software that reliably detects a wide range of aberration types, including:

- **SNVs within 700 targeted ID and DD genes, exons and UTRs**
- **Small (single exon) CNVs within the targeted 700 ID and DD genes**
- **Larger CNVs (>190kb) across the genome**
- **Loss of Heterozygosity regions with a resolution of <5Mb**

The 700 targeted genes known to be important in ID and DD were carefully selected on the basis of ClinGen[3] and DDD[4] guidelines. The ability to detect these 4 different types of aberration significantly improves the likelihood of detecting causative aberrations compared to aCGH alone.

Outlined here is the design and testing of the assay using research samples obtained from cytogenetic laboratories. A particular focus of the testing has been confirmation that the CNV detection capabilities of the assay are as good as those obtained with aCGH (Figures 1-7). This has included testing with mosaic samples. We have demonstrated excellent concordance between the results obtained with the new assay and aCGH.

Methods



Results I

Small CNV

Figure 1: A ~80kb duplication detected on chromosome 2 with both CytoSure Constitutional NGS and B CytoSure Constitutional v3 4x180k array.

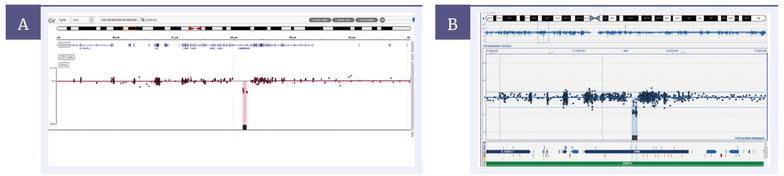


Figure 2: A Single exon deletion in a female DMD sample detected with CytoSure NGS and B CytoSure Constitutional v3 4x180k array.

Large CNV

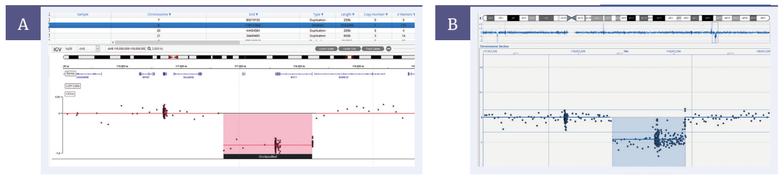


Figure 3: A 753kb deletion detected on chromosome 8 on CytoSure Constitutional NGS and B on a 4x180k CytoSure Constitutional v3 array CGH.

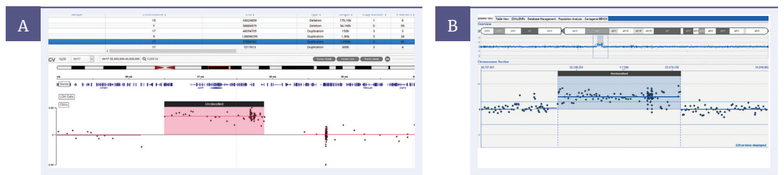


Figure 4: A 1.4Mb duplication on chromosome 17 shown with the Interpret software on CytoSure Constitutional NGS. B The matching array result with CytoSure Interpret software using a CytoSure 4x180k v2 ISCA array.

LOH

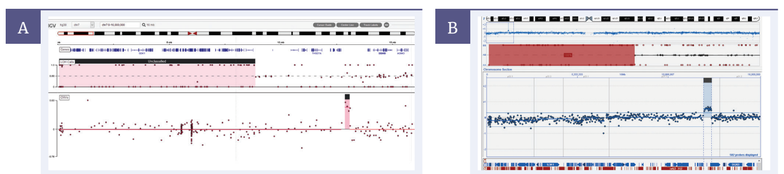


Figure 5: A 8.8Mb stretch of LOH on chromosome 7 with a 213kb duplication on CytoSure Constitutional NGS. B Equivalent 4x180k CytoSure Constitutional custom array.

Results II

Indel

Figure 6: Detection of an indel within the NDP gene on chromosome X

SNV

Figure 7: Detection of an SNV in the MYBPC3 gene

Overall Results

	Number tested	Number called	%
Controls	38	38	100
Large CNVs	24	24	100
Small CNVs (<2Mb)	33	33	100
All CNVs	57	57	100
SNVs/Indels	10	10	100
LOH (<5Mb)	62	60	97
Mosaic (>5MB)	3	3	100

Figure 8: Summary of results from overall study

Conclusions

We have demonstrated that CytoSure® Constitutional NGS was able to detect CNVs with excellent concordance to microarrays, but has the added benefit of SNV/Indel and LOH calling. We were able to call all the CNVs from our research sample trial at an independent lab providing confidence in the NGS solution. We were also able to call the SNV aberrations from our research samples, providing a rich new source of genetic information in comparison to microarray alone.

References

2. Sharp *et al.*, Nature Genetics 38 p1038
3. https://clinicalgenome.org/
4. https://www.sanger.ac.uk/science/collaboration/deciphering-developmental-disorders-ddd

Acknowledgements

Centre hospitalier universitaire de Sherbrooke, Quebec, Canada
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