Unravel the exome odyssey with the CytoScan XON Suite

Introduction

Copy number variations (CNVs) are well-recognized genomic structural variants associated with genetic disorders. Chromosomal microarray analysis (CMA) successfully detects submicroscopic CNVs, and since 2010, is used as a first-tier test for the detection of CNVs related to intellectual disability, developmental delay, autism spectrum disorders, and congenital abnormalities [1].

In addition to the CNVs involving whole-genomic regions as routinely detected by CMA, several clinical research studies have investigated CNVs involving single or multiple exonic deletions and duplications, and identified correlation to neurodevelopmental delay [2], blindness [3], and deafness [4], among others. Additionally, intragenic CNVs are more prevalent than previously suspected in Mendelian disorders, and should be considered when analyzing these samples in a clinical research setting [5]. Today, we know that up to 40% of intragenic mutations can involve only one or two exons, so it is imperative for genomic technologies to maximize coverage within a gene [6].

With the advent of next-generation sequencing (NGS) in particular, the increasing use of whole-exome sequencing to assess single-nucleotide variants (SNVs) has shown successful detection of small indels, and methods to call copy number changes from NGS data have emerged. As adoption and data analysis of whole-exome sequencing evolves in the clinical research setting, it is critical to detect both haplo-insufficient mutation aberrations as well as aberrations in genes associated with recessive disorders for which a mutation is identified in only one allele. Thus, current NGS tools exhibit significant false-positive rates and calls must be verified using orthogonal methodologies [7].

The Applied Biosystems™ CytoScan™ XON Suite is an exon-level copy number assay providing the sensitivity and flexibility required to improve and complement the analysis of these significant variants for clinical research. Designed to cover the whole genome, with increased coverage in 7,000 clinically relevant genes, the CytoScan XON Suite provides CNV data that works as a strong complement to mutation analysis performed by NGS.

With the CytoScan XON Suite, you can:

- Comprehensively detect single-exon deletions and duplications in a cost-effective manner
- Complement NGS mutation analysis with reliable exon-level deletion and duplication detection
- Confirm CNV findings from alternative technologies
- Simplify and streamline sequence variant analysis

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Sensitive exon-level copy number and superior coverage across the whole genome

Current NGS gene panels vary widely from a handful of genes to a few thousand. Comprehensive and effective detection of deletions and duplications to match these panels can be challenging from a cost and lab efficiency perspective when using multiplex ligation-dependent probe amplification (MLPA™) assays or exome arrays for medical research. Additionally, custom-made deletion and duplication arrays lack the flexibility to include any new gene of interest that is relevant to a particular disease and creates increased burden for verification and analytical validation of the new content.

The CytoScan XON Suite is a microarray solution which detects single-exon deletion and duplication events with high sensitivity in key relevant genes in addition to providing whole-genome coverage (Figure 1). The single-nucleotide polymorphism (SNP) probes allow sample tracking, duo-trio, and loss of heterozygosity (LOH) analysis.

Smart design improves resolution in key genes

The CytoScan XON Suite content was designed through empirical selection from a pool of over 49 million probes for copy number responses across the genome. The best performing probes were selected to cover key relevant genes and the exons within them. Any gaps were filled with additional probes flanking 500 bp from the targeted region, ensuring each exon contains enough probes to make a reliable call (Figure 2).

Reporting flexibility with gene panel or gene-level tier analysis

The flexibility of Chromosome Analysis Suite (ChAS) data analysis software enables easy viewing and summarizes the exon-level CNV results in various ways based on your specific clinical research needs.

Gene panel analysis

Simplify result reporting by utilizing your preferred gene list or panel in the intuitive ChAS software. This analysis feature allows you to obtain exon-level CNV results limited to the gene panel you upload and view only the genes of interest with restriction mode enabled (Figure 3).

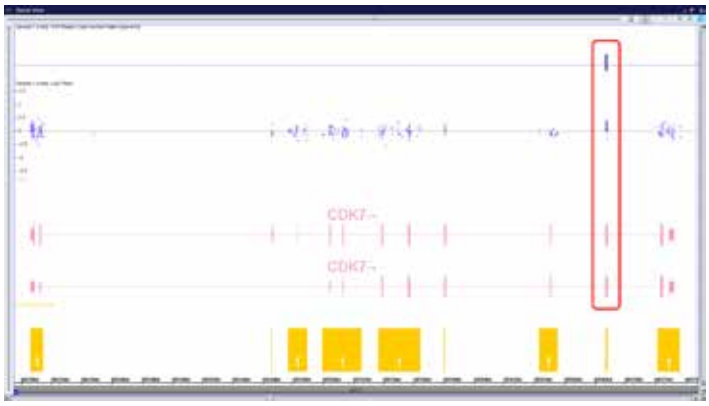

Figure 1. Detailed view of ChAS data analysis software, displaying a single duplication of exon 11 in the *CDK7* gene.

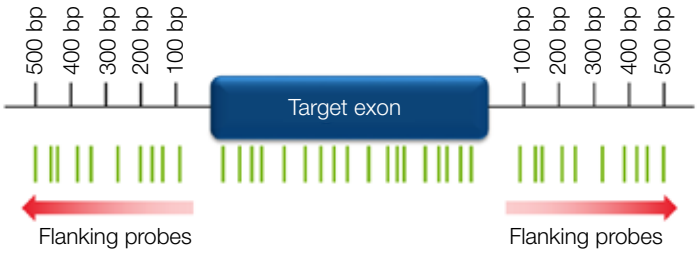

Figure 2. Design strategy for probe coverage of the key genes and exons within.

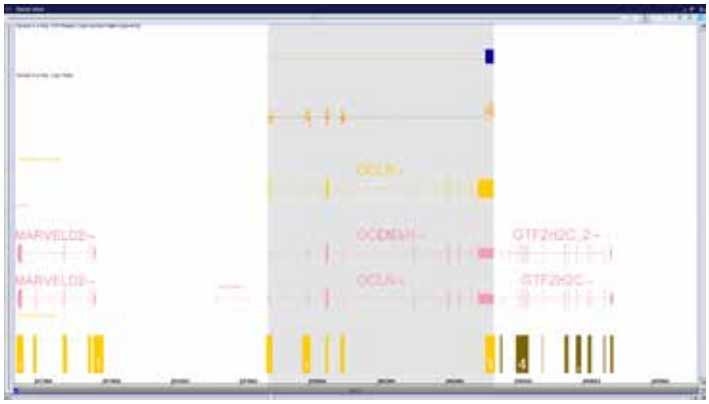

Figure 3. Detailed view of ChAS displays targeted gene panel analysis with restriction mode enabled, such that no data outside the gene(s) of interest is viewed.

Gene-level tier analysis

Gene-level tier analysis is incorporated into the user-friendly ChAS software, providing a novel and flexible approach, where the software displays results by gene-level tiers based on clinical research relevance. This unique functionality allows visualization of CNVs for a targeted number of clinically relevant genes (Level 1), or expands the gene-level tiers for a whole-genome view (Table 1). Thus, the user determines the gene-level tiers of interest for analysis and reporting.

Table 1. Description of gene-level tiers.

Level	Number of genes	Description
1	7,003	Includes genes with the strongest relevance to clinical research; for example, genes related to intellectual disability, developmental delay, autism spectrum disorder, congenital anomalies, epilepsy, primary immunodeficiencies, hereditary cancer, as well as cardiac, metabolic, neuromuscular, eye, and hearing disorders, among others
2	3,813	Includes ClinVar genes not covered in Level 1
3	5,817	Includes OMIM® genes not covered in Levels 1 and 2
4	9,347	Includes RefSeq genes not covered in Levels 1, 2, and 3

ChAS software workflow

Detect single- or multiple-exon level events with a novel and advanced calling algorithm that incorporates probe performance metrics for increased sensitivity.

Visually inspect sample quality control metrics, such as SNPQC, MAPD, and waviness-SD, quickly and easily.

Simplify results to meet your specific clinical research needs. Input a preferred gene list or panel from your NGS design or use the clinically relevant gene-tier approach.

Easily export data to create a report suited to your specific needs.

Technical specifications of the CytoScan XON Suite

Specifications	Details
Content	6.85 million empirically selected probes for whole-genome coverage including: • 6.5 million copy number probes • 300,000 SNP probes for LOH analysis, duo-trio assessment, and sample tracking
Sensitivity	95% sensitivity for the detection of exon-level CNVs*
Coverage	Total number of genes with coverage: 25,980 • Full coverage: 21,844 • Partial coverage: 4,136 • Exome genes for medical research (including cancer genes): 7,003 • Exon-level CNV detection with an average of 15 probes per call

* Sensitivity calculated from gene-level tier 1.

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Overcome the challenges of the exome odyssey with reliable single-exon deletion and duplication detection using the CytoScan XON Suite, which includes arrays, reagents, and easy-to-use software for cost-effective and streamlined analysis of exon-level CNVs.

Ordering information

Product	Cat. No.
CytoScan XON Assay Kit bundle - Arrays and reagents sufficient for 24 reactions	931311
CytoScan XON Training Kit - Arrays and reagents for 24 reactions plus training materials	931312

References

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4. Ji H et al. (2014) Combined examination of sequence and copy number variations in human deafness genes improves diagnosis for cases of genetic deafness. *BMC Ear Nose Throat Disord* 14:9.
5. Aradhya S, Lewis R, Tahrra B et al. (2012) Exon-level array CGH in a large clinical cohort demonstrates increased sensitivity of diagnostic testing for Mendelian disorders. *Genet Med* 14:594-603.
6. Retterer K, Scuffins J et al. (2015) Assessing copy number from exome sequencing and exome array CGH based on CNV spectrum in a large clinical cohort. *Genet Med* 17:623-629.
7. Mason-Suares H, Landry L, Lebo M (2016) Detecting copy number variation via next generation technology. *Curr Genet Med Rep* 4:74-85.

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