CytoScan cytogenetics suite

Coverage without compromise

ThermoFisher SCIENTIFIC

See what's been missing

Advanced genetic assessment technologies enable cytogenetic researchers to identify significantly more copy number variations (CNVs) and other structural alterations associated with constitutional disorders and malignancies than ever before.

Test methods such as karyotyping, fluorescent *in situ* hybridization (FISH), and low-resolution arrays have deficiencies in genomic coverage and limited resolution, limiting the number of significant variants that can be seen.

Compromising on genomic coverage, content, or resolution leads to significant aberrations being missed, necessitating further analysis, which can delay results and increase costs.

Whole-genome microarrays that cover both polymorphic (e.g., single-nucleotide polymorphisms or SNPs) and nonpolymorphic regions of the genome can be used to assess DNA copy number alterations at a much higher resolution than conventional cytogenetic analysis to support the assessment of potentially causative genetic alterations such as CNVs, chromosomal imbalances, and allelic imbalance indicative of absence of heterozygosity (AOH), loss of heterozygosity (LOH), or long contiguous stretches of homozygosity (LCSH).

CytoScan products help enable cytogenetic researchers to identify:

- Relevant genomic abnormalities not detectable by low-resolution or targeted techniques
- New genomic abnormalities
- Correlations between genotypic and phenotypic variants
- Patterns of inheritance or proliferation that can inform recurrence risk
- Patterns of inheritance that could convey increased risk of recessive disorders

> "Copy number variation analysis has had a major impact on the field of medical genetics, providing a mechanism to identify disease-causing genomic alterations in an unprecedented number of diseases and phenotypes."
>
> Coughlin II CR et al. (2012) [1]

Discover a faster, more reliable, and cost-effective method

For improved yield, accuracy, and efficiency, cytogeneticists should consider a technology that:

- **Covers all genes in the genome**—those established as significant today as well as those with emerging evidence, thus "future-proofing" the technology investment and eliminating revalidation burden
- **Detects as many types of chromosomal aberrations as possible at high resolution in a single test**—such as copy number gains and losses as well as copy-neutral events such as AOH
- **Provides sensitive mosaic detection**—elucidating patterns of clonal evolution, structural inconsistencies, and cellular contamination
- **Is straightforward to process and to analyze**—with no cell culture requirements
- **Is cost-effective**—enables the consolidation of multiple tests previously done sequentially onto one platform and test event

The standard for cytogenetic analysis

CytoScan cytogenetics solution

The Applied Biosystems™ CytoScan™ Array was designed by empirically selecting probes from a pool of over 20 million and then further screening them with over 3,000 samples to choose those that performed best for whole-genome cytogenetic applications.

The design is optimized for balanced whole-genome coverage, enabling high-resolution DNA copy number analysis with precise breakpoint accuracy as well as high-density SNP coverage for LOH/AOH, LCSH, and uniparental isodisomy (UPD) detection.

Our proprietary manufacturing technology produces arrays that are highly reproducible between each batch with minimal risk of the probe dropout that is inherent in some manufacturing techniques.

- **Exceptional performance**—high specificity, sensitivity, dynamic range, and resolution across the genome
- **Designed to evolve with your practice**—Covers 100% of ClinVar genes along with excellent coverage across Online Mendelian Inheritance in Man® (OMIM®), RefSeq, and the DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER)/ Developmental Disorders Genotype-to-Phenotype database (DDG2P) constitutional gene regions
- **High-density SNPs with >99% genotype accuracy**—enables low-level mosaicism visualization, AOH and acquired UPD (aUPD) detection, copy number change confirmation, triploidy detection, allelic imbalance pattern visualization, genomic contamination identification, and parent-of-origin analysis
- **Complete solution helps save time and money**—simple and robust manual or automated protocols, easy-to-use software, and self-paced or laboratory-based training
- **Streamlined data analysis**—Applied Biosystems™ Chromosome Analysis Suite (ChAS) software offers enhanced analysis features, including the ability to view and summarize chromosomal aberrations (CNVs, mosaicism, and LOH), duo-trio consistency tools, database building capabilities, and flexible export options for results
- **Robust across the broadest range of sample types**—blood, bone marrow, buccal swabs, saliva, fresh and frozen tissues, uncultured or cultured cells, and more

Future-proof your technology choice

Balanced whole-genome coverage

The high-density Applied Biosystems™ CytoScan™ HD Array includes 2.67 million markers for copy number (CN) analysis, including 750,000 SNP probes and 1.9 million nonpolymorphic probes for comprehensive whole-genome coverage.

- 100% Clinical Genome Resource (ClinGen; formerly International Collaboration for Clinical Genomics, ICCG, and International Standards for Cytogenomic Arrays Consortium, ISCA) genes
- 12,000 OMIM genes
- 36,000 RefSeq genes

Unlike other arrays, which lack the ability to deliver truly balanced whole-genome coverage due to probe density and probe placement limitations, the CytoScan HD Array offers the highest resolution gene-level coverage for constitutional, X-chromosome, and RefSeq genes.

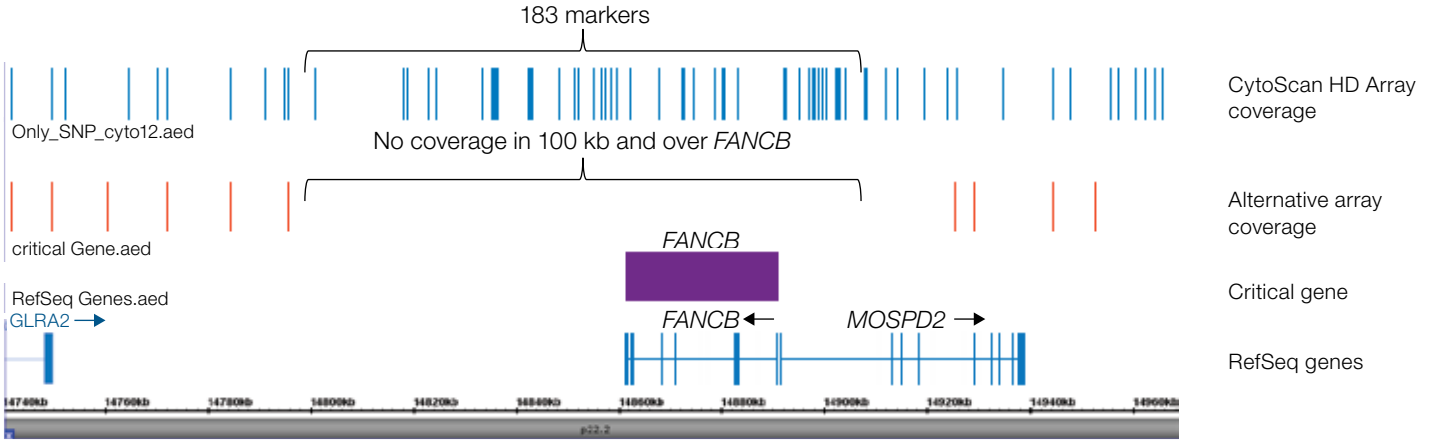


Figure 1. A balanced hybrid design with both SNPs and nonpolymorphic probes enables complete coverage. A SNP-only array will have coverage gaps when the gene, such as *FANCB*, does not contain informative SNPs.

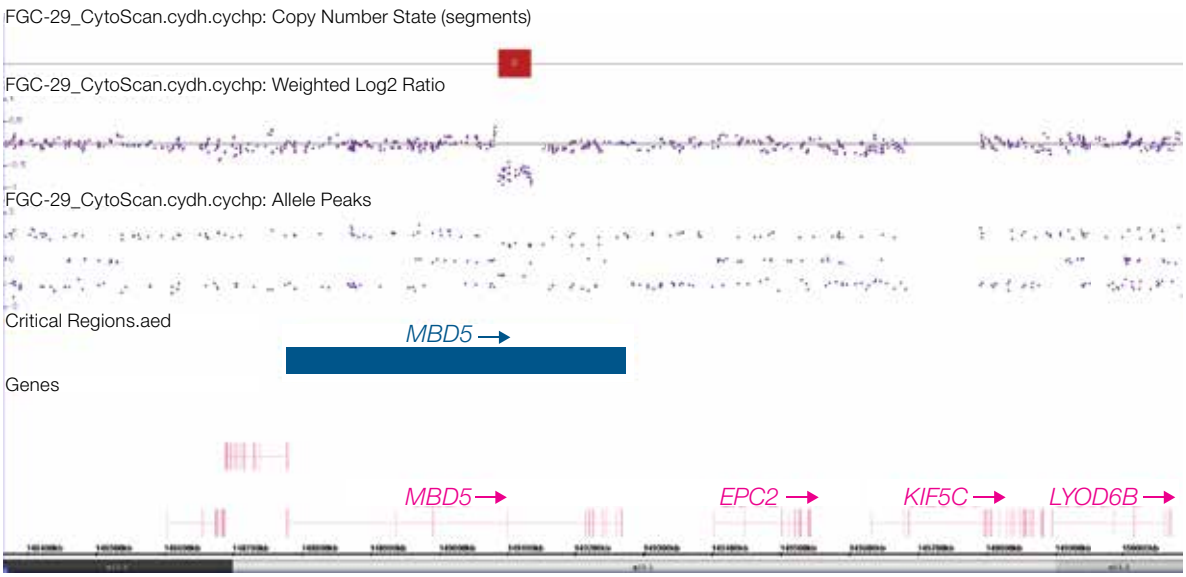


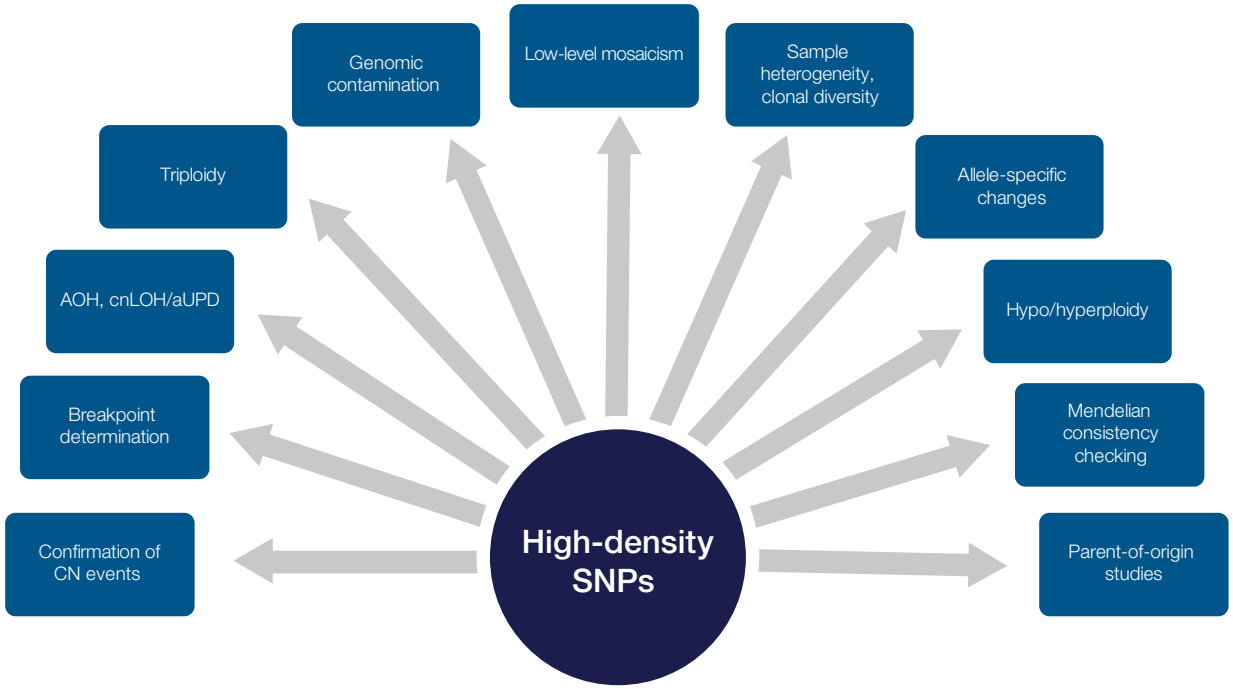
Figure 2. A gene-centric design allows the highest resolution detection of CNVs across the entire genome. This example illustrates a 41 kb single-exon deletion of the *MBD5* gene, which is not covered on common consensus array designs.

The power of SNPs

Unlock new applications

High-density SNP probes with high genotype accuracy enable confident breakpoint determination and CN change confirmation throughout the genome in addition to the detection of events such as low-level mosaicism, AOH and aUPD, triploidy, allelic imbalances, genomic contamination, and parent-of-origin analysis.

Accurate detection of regions of homozygosity



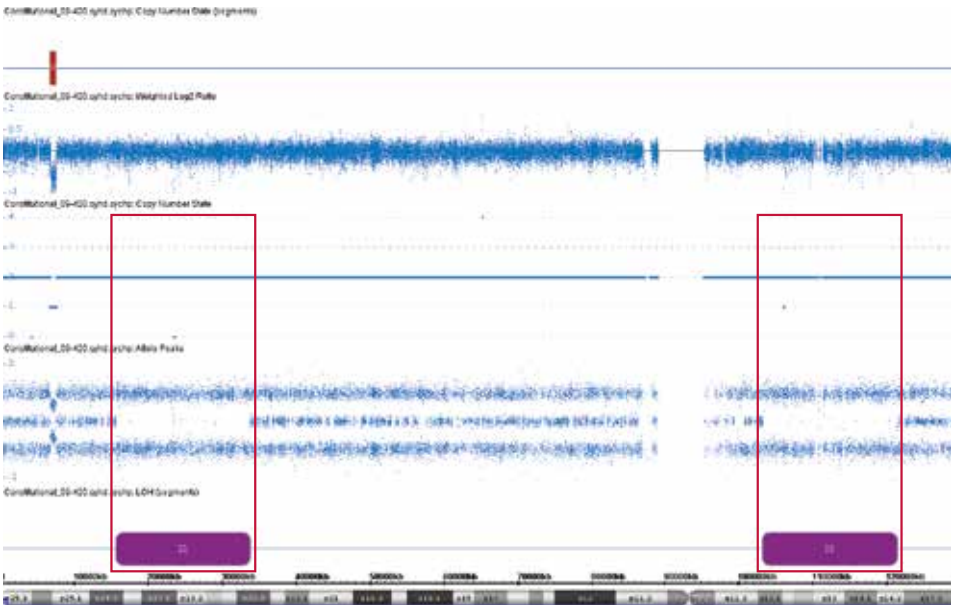


Figure 3. Identical-by-descent regions (detailed view of chromosome 2). This example illustrates 2 blocks with LOH >10 Mb on chromosome 2. The red segment illustrates an additional hemizygous loss on this chromosome.

"The low-density array called absence-of-heterozygosity regions that were not confirmed by other platforms and also overestimated the length of true absence-of-heterozygosity regions. Furthermore, the low- and mid-density platforms failed to detect some small absence-of-heterozygosity regions that were identified by the high-density platform."

Mason-Suares H et al. (2013) [2]

High-density SNPs with >99% genotype accuracy

Only highly accurate SNP genotypes allow for Mendelian consistency analysis for parent-of-origin studies.

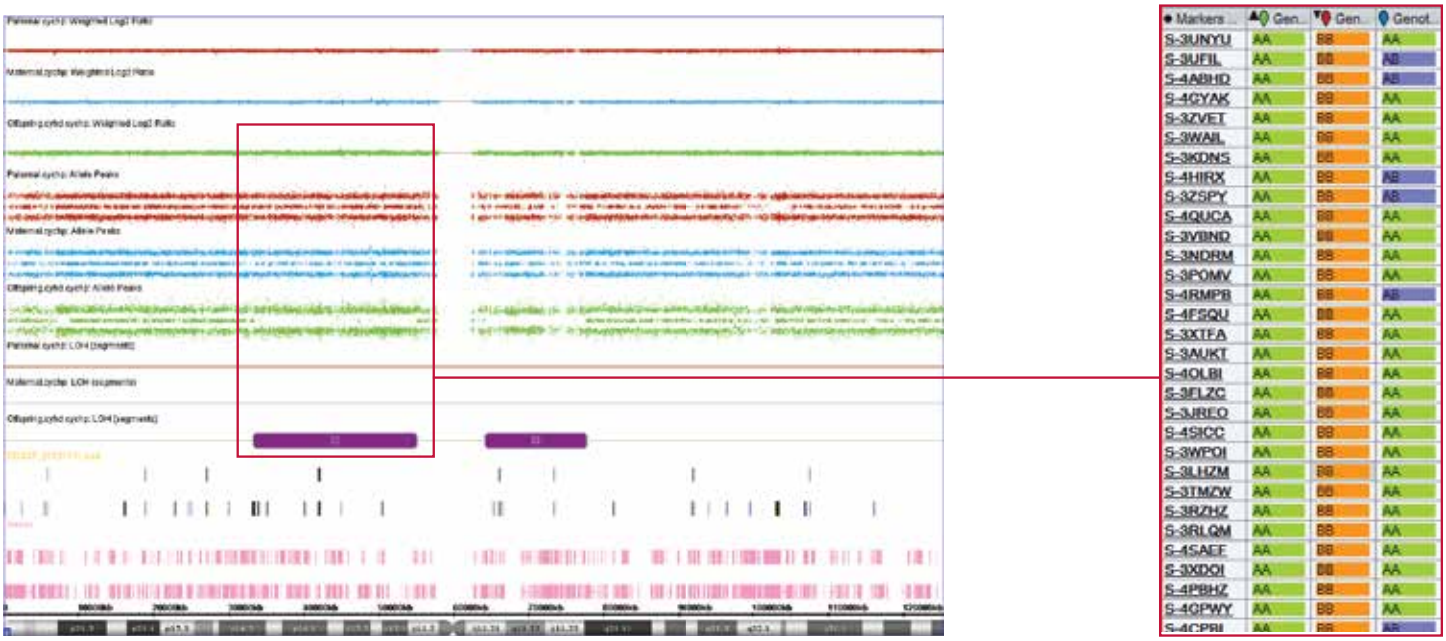


Figure 4. SNPs allow for parent-of-origin genotype analysis to detect UPD and confirm copy number changes. Maternal UPD for chromosome 7 is shown above with a corresponding trio analysis genotype summary table. New duo/trio Mendelian error check analysis for viewing sample relatedness and identifying chromosomes with higher Mendelian errors rates.

Confident breakpoint determination

High-density SNP coverage enables confident breakpoint determination and CN event-independent confirmation throughout the entire genome.

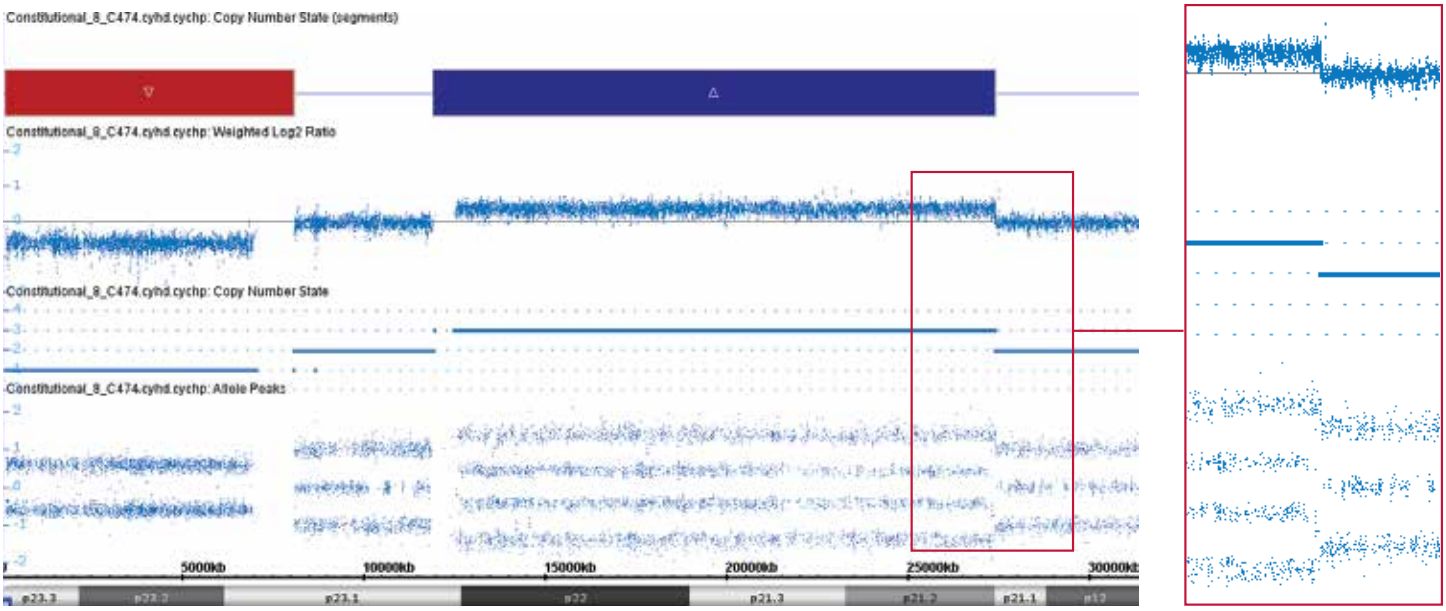


Figure 5. This example illustrates a hemizygous loss and gain on the same chromosome with the CN states 1, 2, and 3 on chromosome 8. These CN changes were all confirmed by FISH.

Triploidy detection

High-density SNPs enable the detection of triploidy, cellular contamination, and mixed cell populations.

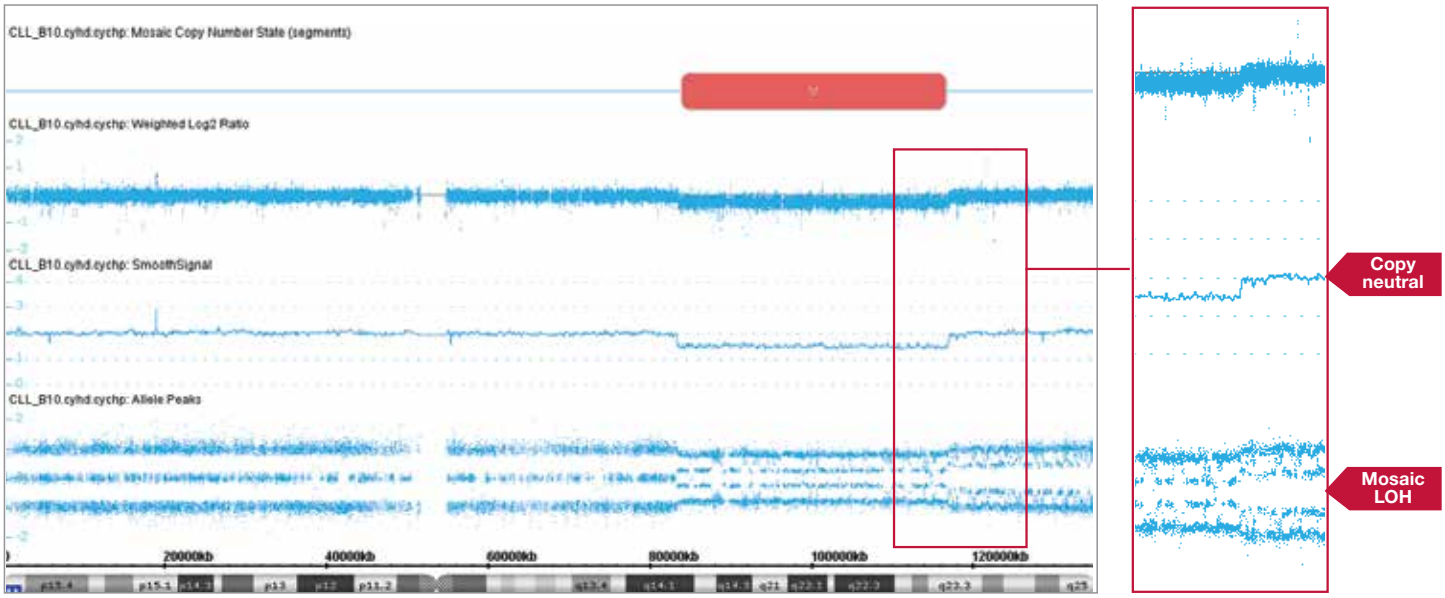


Figure 6. A representative chromosome of sample containing a triploid genome. Normal $\log_2$ ratio with 4 allelic tracks show detection of triploidy. Only the CN is normalized but not the allele track.

"... a post hoc review determined that had the SNP data been analyzed, the triploid cases would have been detected. We therefore suggest that arrays used for prenatal testing should contain SNP probes that can reliably identify triploidy."

Wapner RJ et al. (2012) [3]

Low-level mosaic detection

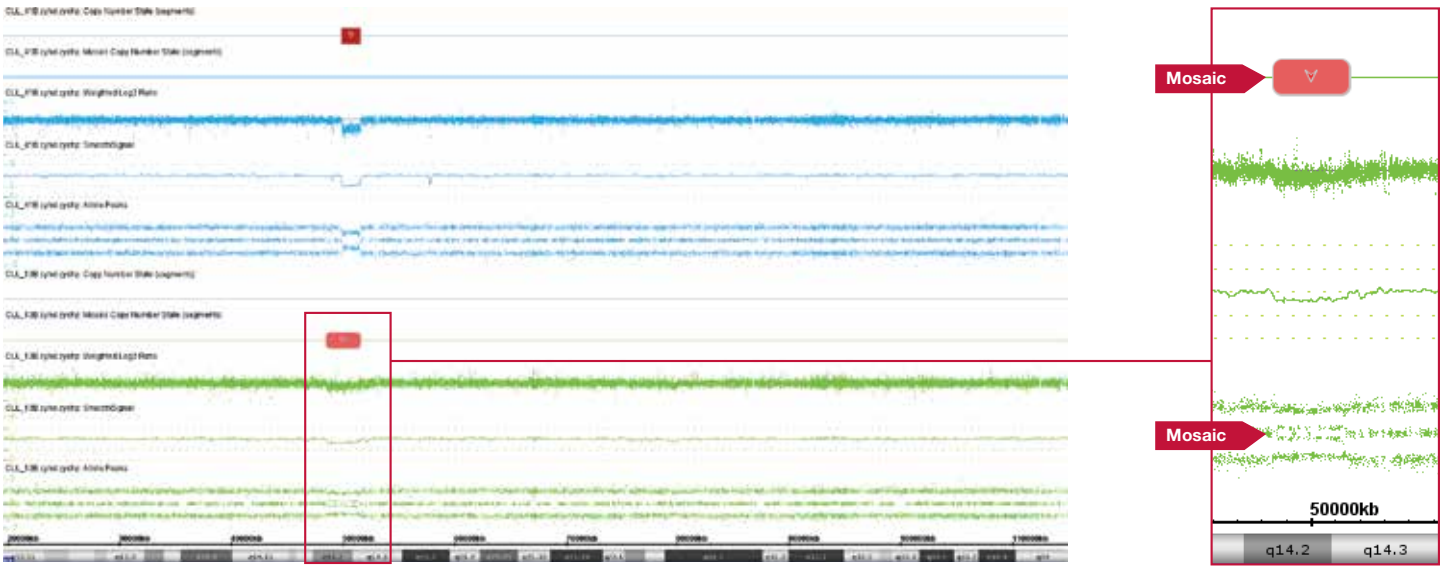


Figure 7. Two samples visualized in parallel: hemizygous loss and mosaic loss. The sample at the top represents a full hemizygous loss on chromosome 13. The sample at the bottom represents a mosaic loss in the same region that interphase FISH confirmed with the mosaic level at 20%.

Mosaic loss and mosaic copy neutral LOH

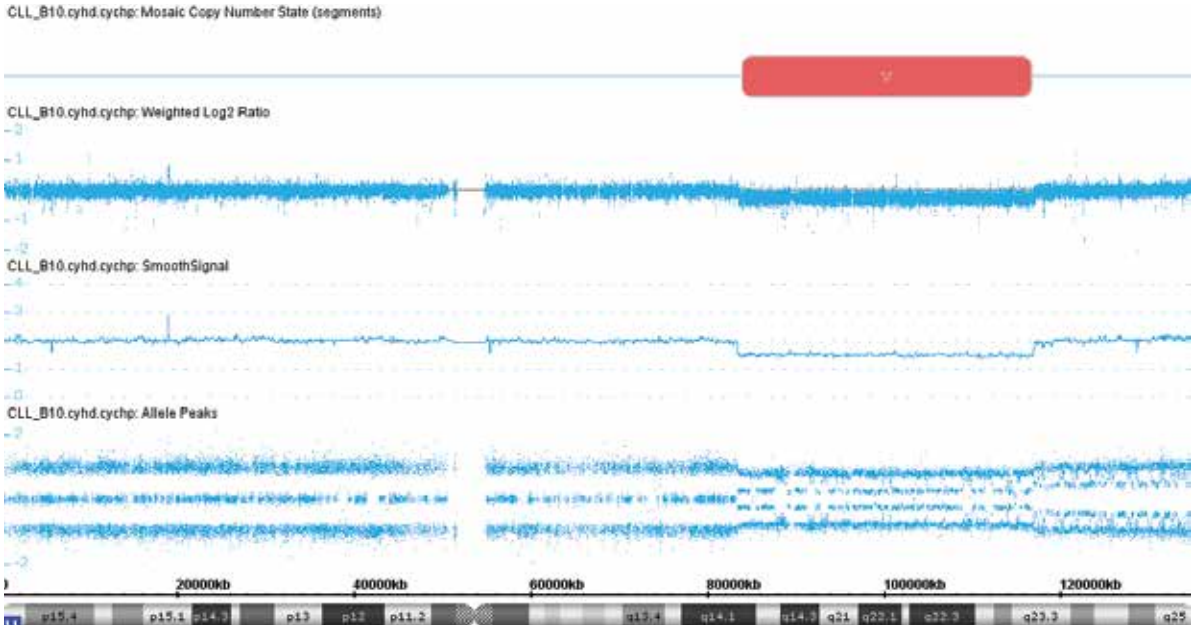


Figure 8. This sample contains multiple aberrations including mosaic loss and mosaic copy-neutral LOH. Chromosome 11 contains a mosaic loss and a mosaic copy-neutral LOH event.

> "The clinical impact of the genomic copy-number and copy-neutral alterations identified by microarray technologies is growing rapidly, and genome-wide array analysis is evolving into a diagnostic tool to better identify high-risk patients and predict patients' outcomes from their genomic profiles."
>
> Simons A et al. (2012) [4]

Allele-specific analysis

The allelic patterns elucidate genomic imbalances such as LOH, which is very common in hematological malignancies, and whether haploid/diploid and haploid-doubled events have occurred, conveying important prognostic information.

Different allelic state possibilities

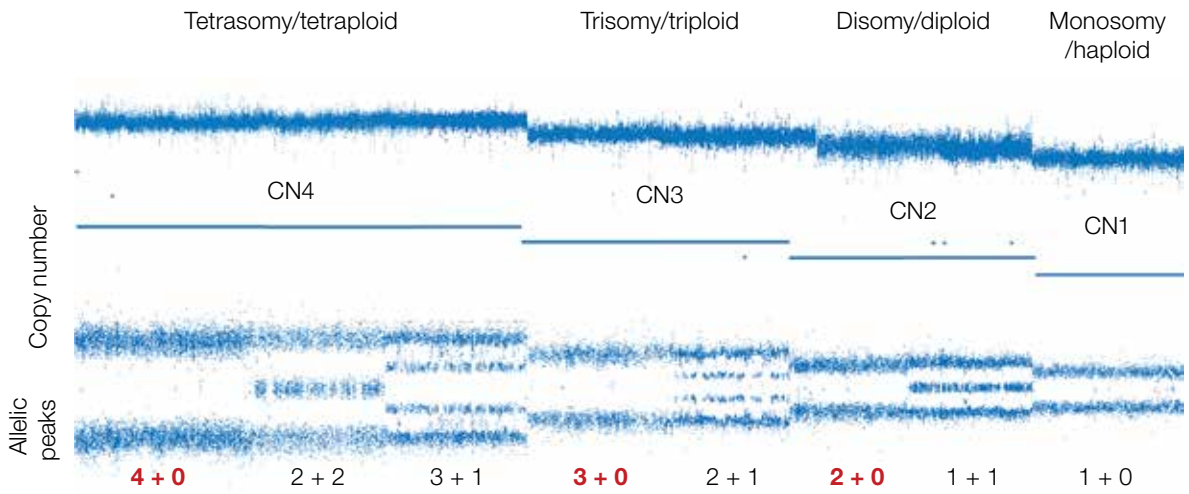


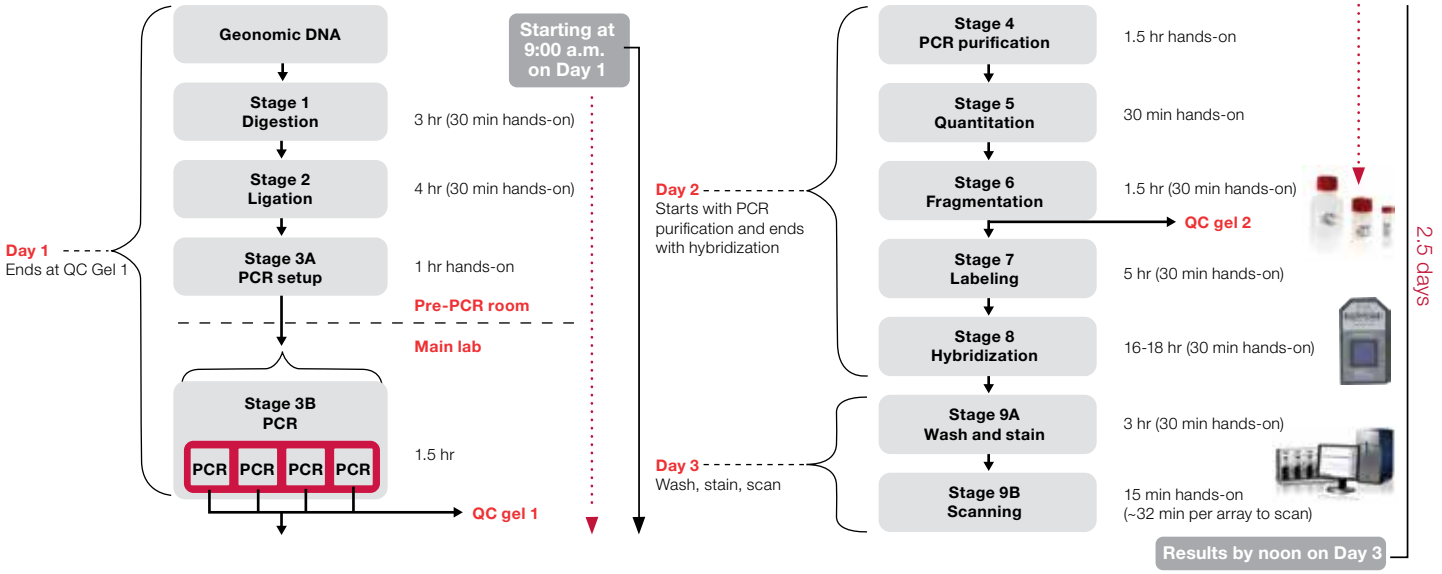
Figure 9. The CytoScan Array can accurately identify endoreduplication events in haploid genomes.

Robust manual or automated assay workflows

DNA to result in less than 3 days

The Applied Biosystems™ CytoScan™ HD Array Kit and Reagent Kit Bundle includes an optimized and streamlined assay and all-inclusive reagent kit. The assay protocol makes it easy to obtain consistent and high-quality results with processes aligned with laboratory workflow requirements. The Applied Biosystems™ CytoScan™ Reagent Kit is designed to save time and money, reduce operator error, and deliver a high level of performance.

Packaging has been designed to be ecologically friendly and is recyclable, biodegradable, resealable, and saves storage space



CytoScan automated target preparation solution

Applied Biosystems™ NIMBUS™ Target Preparation Instrument

- Accommodates 24 or 48 samples, plus a negative control
- Advanced pipetting technology for precise liquid handling
- Labware gripper arm for easy handling of microplates and pipette tips
- Laptop with intuitive software interface with visual cues for each post-PCR test step

This automated liquid-handling workstation helps reduce intra-operator variability and the labor burden associated with complex manual pipetting, helping to improve test reproducibility and laboratory efficiency. By automating much of the liquid handling associated with the CytoScan HD Array, your laboratory can increase sample processing throughput more than two-fold. The system has a small footprint with a customized deck layout designed specifically for the CytoScan HD Array.

The power of exon level resolution with the CytoScan XON Suite

Unravel the exome odyssey

CNVs are well-recognized genomic structural variants associated with genetic disorders. Chromosomal microarray analysis (CMA) successfully detects submicroscopic CNVs, and since 2010, has been used as a first-tier test for the detection of CNVs related to intellectual disabilities, developmental delays, autism spectrum disorder, and congenital abnormalities.

With the Applied Biosystems™ 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

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, blindness, and deafness, among others. Additionally, intragenic CNVs are more prevalent in Mendelian disorders than previously suspected and should be considered when analyzing these samples in a clinical research setting. Today, we know that up to 40% of intragenic mutations can involve only one or two exons, so it is imperative that genomic technologies maximize coverage within a gene.

The 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 exome, with additional coverage in 7,000 of ClinVar clinically relevant genes, the CytoScan XON Suite provides CNV data that works as a strong complement to mutation analysis performed by next-generation sequencing (NGS).

"Although NGS panels are extensively applied in clinical settings for the detection of single-nucleotide variants or small insertions and deletions, identification of deletions or duplications of whole exons, particularly single-exon CNVs, has proved problematic … We are aware that more advanced algorithms may likely have higher sensitivity and specificity. However, their use still generates a high number of false positives, and their readouts still require validation by other methods such as array [comparative genomic hybridization] CGH or [multiplex ligation-dependent probe amplification] MLPA for further mapping of breakpoints."

Giugliano Theresa et al. (2018) [11]

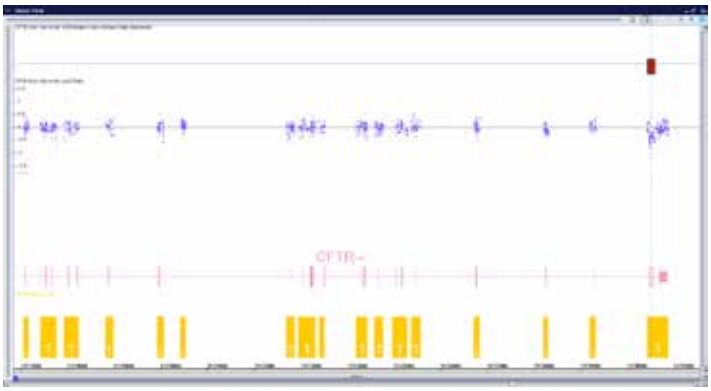


Figure 10. Detailed view of ChAS data analysis software, displaying a single duplication of exon 11 in the *CDK7* gene.

Figure 11. Design strategy for probe coverage of the key genes and exons within.

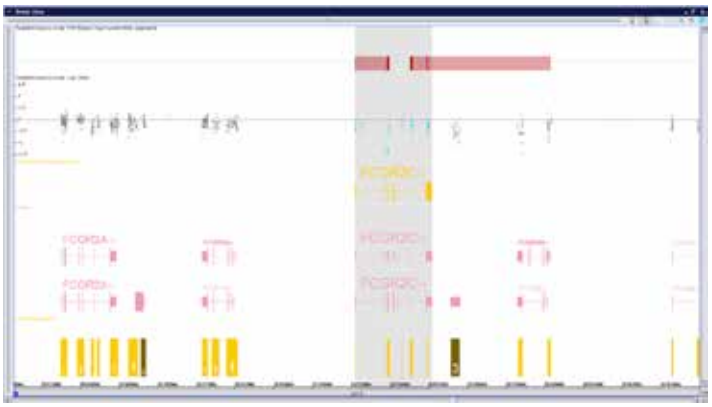


Figure 12. Detailed view of ChAS displays targeted gene panel analysis with restriction mode enabled, such that no data outside the gene(s) of interest is viewed.

Software designed for cytogenetic applications

Intuitive data analysis solutions

Chromosome Analysis Suite (ChAS) software is tailored to cytogenetic research analysis and reporting with:

- Streamlined analysis workflow
- Ability to apply customized filters to analyze the genome at different levels of resolution
- Options to create, modify, and upload annotation files and flag regions for focused analysis
- Mosaic calling and non-integer CN reporting
- Direct access to external databases such as NCBI, UCSC Genome Browser, Ensembl, and OMIM
- Database capability for storing and querying segment data and annotations
- Histogram track display of the database contents
- Mendelian error tool to check relatedness and Mendelian error rate
- Normal diploid normalization
- Enhanced reporting flexibility, including exporting as DOCX and PDF files

We are pleased to support the Cytogenomics Array Group (CAG) initiative, which was formed to facilitate sharing of microarray case information between laboratories. The Cytogenomics Array Group created a web-accessible database (CAGdb) to host cases shared in a de-identified fashion with participating laboratories.

Figure 13. ChAS karyoview screen. LCSH indicates regions that are identical-by-descent. Each LCSH segment is summarized, and individual thresholds can be selected by the user.

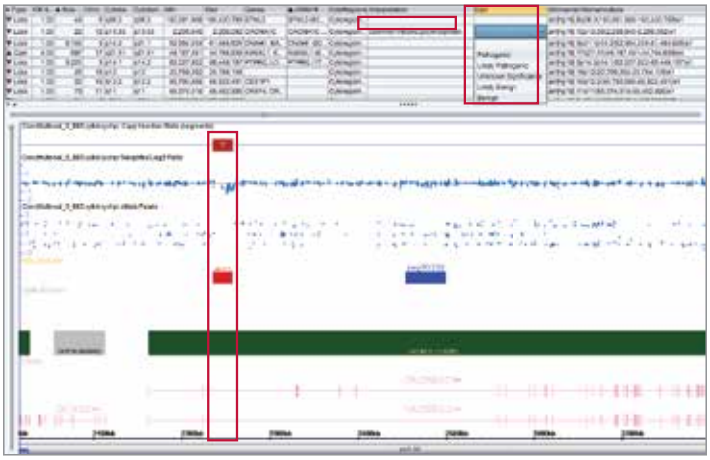


Figure 14. A common intronic deletion polymorphism on *CACNA1C* gene can be identified an easily annotated.

For more information about participation in CAG or CAGdb, email **admin@cagdb.org** or go to **cagdb.org**.

Histogram track display

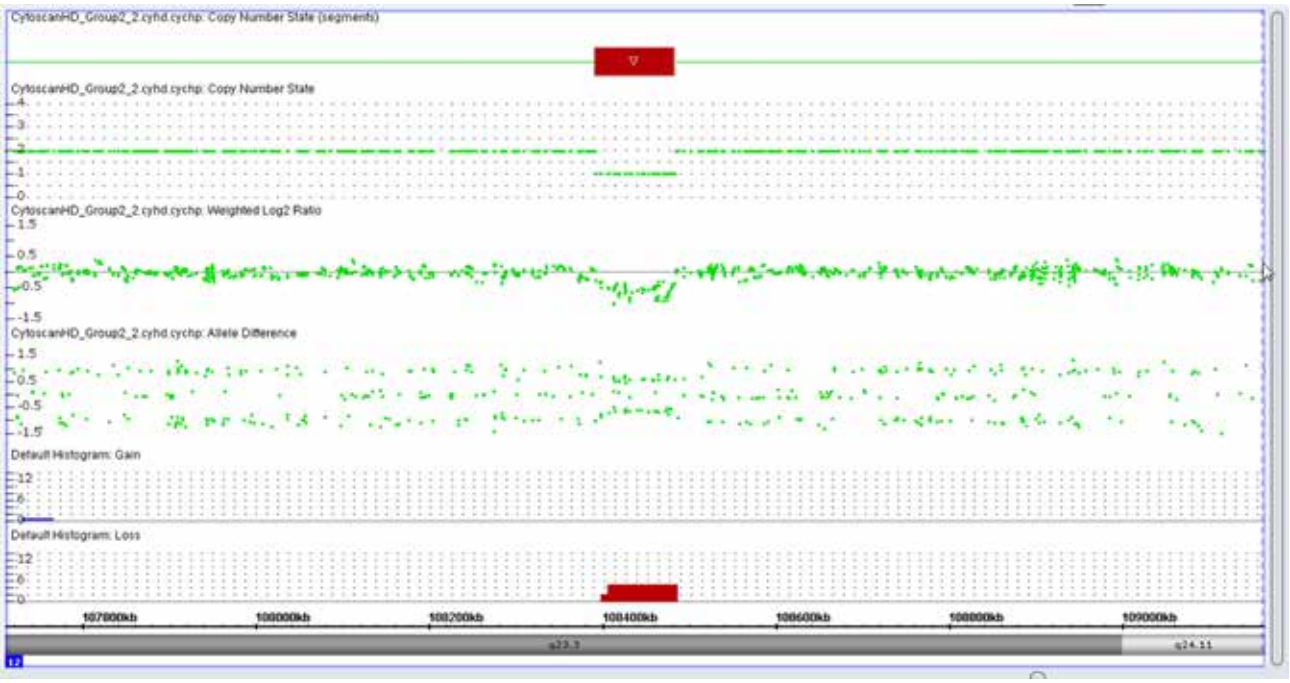


Figure 15. Histogram track display. CNVs in individual samples can be visualized and compared with data stored in the database.

Whole-genome view

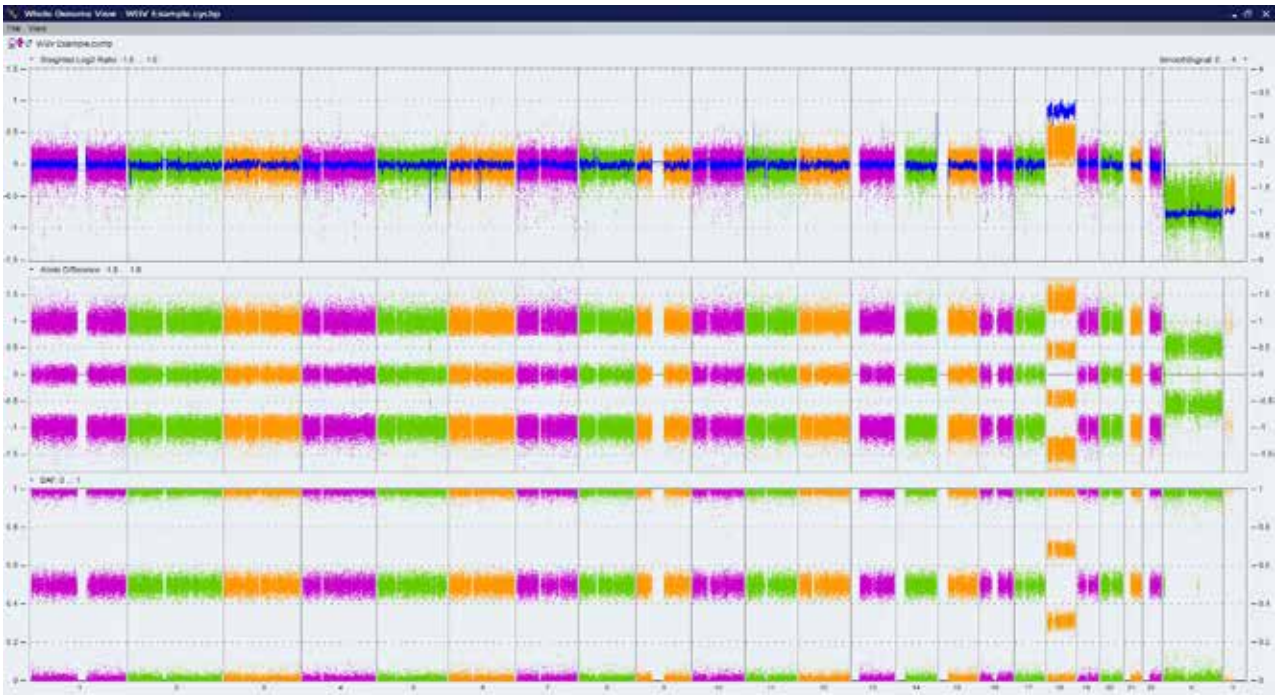


Figure 16. Whole-genome view with $\log_2$ ratio, allele difference, and B allele frequency (BAF) plots.

The GeneChip instrument platform

Flexible, proven, powerful

This superior Applied Biosystems™ GeneChip™ instrumentation system combined with innovative assays provides a complete platform for hybridizing, washing, staining, and scanning of microarrays. The CytoScan HD Array Kit and Reagent Kit Bundle may be run on either the Applied Biosystems™ GeneChip™ Scanner 3000 7G or TG Systems or the Applied Biosystems™ GeneChip™ System 3000Dx v.2, which is FDA-cleared, CE-IVD registered, and includes AutoLoaderDx, GeneChip Fluidics Station 450Dx v.2, and a workstation with Applied Biosystems™ Molecular Diagnostic Software. The Applied Biosystems™ GeneChip™ Hybridization Oven 645 is also required.

- Easy-to-use system for rapid adoption of both RNA and DNA applications
- Automated processing for increased data reproducibility and reduced hands-on time
- Cost-effective approach enabling multiple assays on a single flexible system

Table 1. GeneChip System 3000Dx v.2 assay menu

Application area	RUO*	IVD**
3´ IVT expression analysis	•	•
Whole-transcript expression analysis	•	•
Genotyping/copy number	•	
Cytogenetic analysis	•	
Drug metabolism/pharmacogenomics	•	•
miRNA gene regulation	•	
Targeted resequencing	•	
Custom assays	•	•

* Each "Research Use Only" (RUO) array requires an array-specific assay software module (ASM). A custom ASM can be developed for any GeneChip system.

** FDA-cleared, "*In Vitro* Diagnostic" (IVD)- or CE-marked test developed by a third-party company on the GeneChip Scanner 3000Dx platform.

Notes:

applied
biosystems

Ordering information

Product	Quantity	Cat. No.
CytoScan HD Array Kit and Reagent Kit Bundle	Sufficient for 24 samples	901835
CytoScan XON Assay Kit		931311

References:

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11. Giugliano Theresa et al. (2018) Copy number variants account for a tiny fraction of undiagnosed myopathic patients. *Genes* 9(11):524.

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