affymetrix
Biology for a better world

CytoScan® Cytogenetics Suite

Coverage without compromise

The ***unrivaled standard*** in cytogenetics research.

See what's been missing

The availability of advanced genetic assessment technologies enables cytogenetic researchers to identify significantly more copy number variations and other structural alterations associated with constitutional disorders and malignancies than ever before.

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

Compromising on genomic coverage, content, or resolution leads to significant aberrations being missed, which necessitates further analysis, delaying results and increasing costs.

Whole-genome microarrays that cover both polymorphic (SNPs) and non-polymorphic 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 DNA copy number variations (CNVs), chromosomal imbalances, and allelic imbalance indicative of absence of heterozygosity (AOH), loss of heterozygosity (LOH), or long contiguous stretches of homozygosity (LCSH).

Balanced whole-genome coverage enables identification of

- Pathogenic 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, C. R. II, *et al.*, Clinical impact of copy number variation analysis using high-resolution microarray technologies: advantages, limitations and concerns. *Genome Medicine* **4**:80 (2012).

Discover more in a timely and cost-effective way

For improved yield, accuracy, and efficiency, cytogenetic researchers 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 assay** – 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, heterogeneous samples, 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 into one assay

The standard for cytogenetic analysis

CytoScan Cytogenetics Suite

The CytoScan® array was designed by empirically selecting probes from a pool of over 20 million probes 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 and LCSH that can lead to uniparental disomy (UPD) detection.

Affymetrix's proprietary manufacturing technology produces arrays that are highly reproducible between each batch, with no risk of probe dropout inherent in some manufacturing techniques.

Exceptional performance

High specificity, sensitivity, dynamic range, and resolution across the genome

Designed to evolve with the community

Covers all 36,000 RefSeq genes, including 14,000 OMIM®, all ClinGen (formerly ICCG and ISCA) and Decipher** constitutional regions, and Sanger cancer genes

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 saves time and money

Simple and robust manual or automated protocols, easy-to-use software, and self-paced or laboratory-based training

Streamlined data analysis

Chromosome Analysis Suite (ChAS) is a software offering 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 results export options.

Robust across the broadest range of sample types

Blood, bone marrow, buccal swabs, saliva, fresh and frozen tissues, uncultured or cultured cells, and more

Deciphering Developmental Disorders Study, Large-scale discovery of novel genetic causes of developmental disorders. *Nature* **519(7542):223–228 (2015).

Future-proof your technology choice

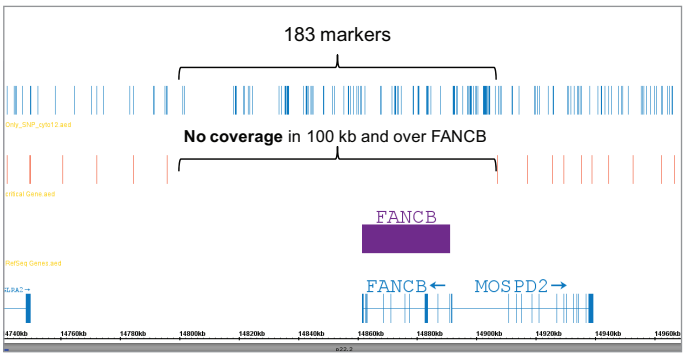
Balanced whole-genome coverage

The high-density CytoScan® array includes 2.67 million markers for copy number analysis, including 750,000 biallelic SNP probes and 1.9 million non-polymorphic probes for comprehensive whole-genome coverage.

- 100% Sanger cancer gene* coverage
- 100% ClinGen (formerly ICCG and ISCA)** constitutional gene coverage
- 14,000 OMIM genes
- 36,000 RefSeq genes

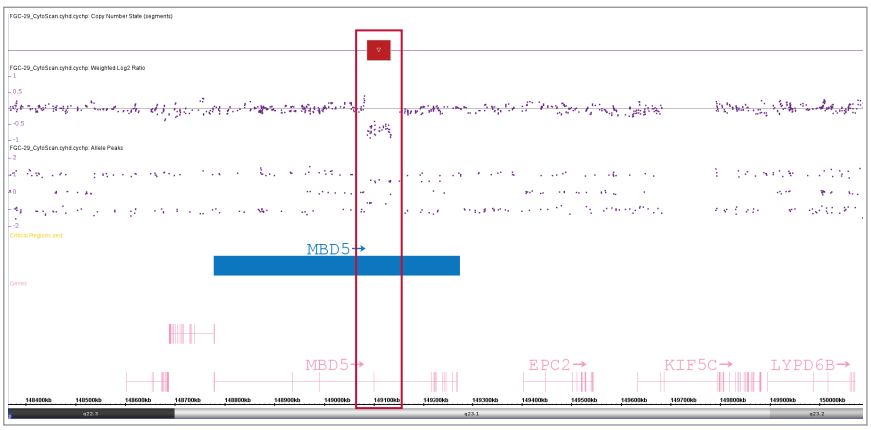
Unlike other arrays, which lack the ability to deliver truly balanced whole-genome coverage due to probe density and probe placement limitations, the CytoScan array offers the highest resolution gene-level coverage for constitutional, cancer, X-chromosome, and RefSeq genes.

Marker comparison



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

Exon-level resolution



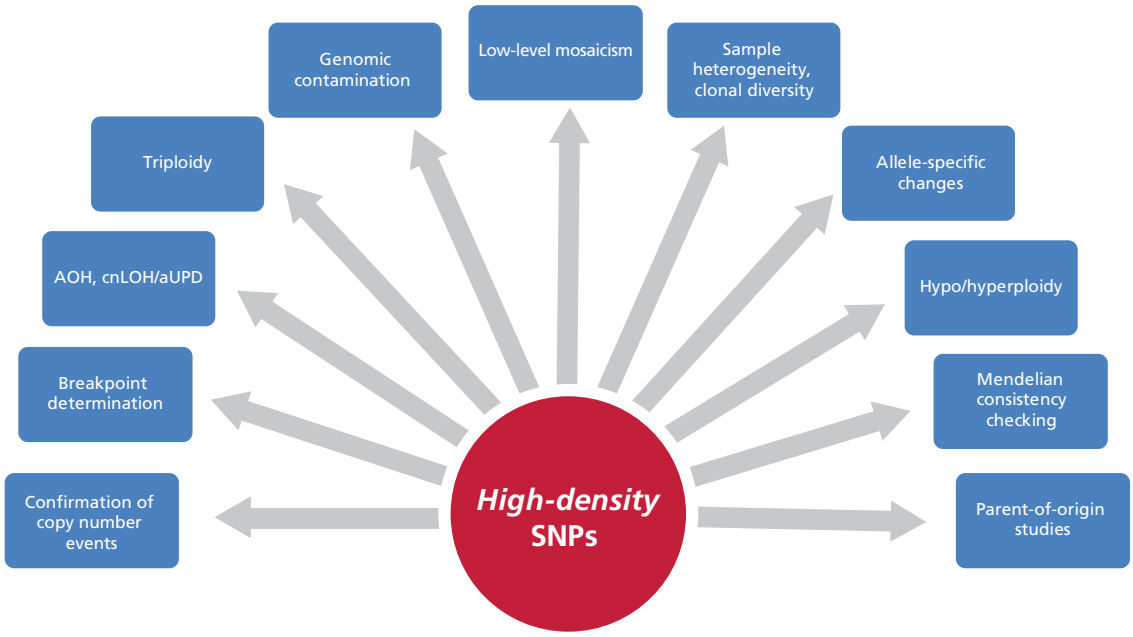
- **A gene-centric design allows the highest resolution detection of CNVs across the entire genome.**
- **This example illustrates a 41 kb single-exon deletion of the MBD5 gene – a gene not covered on common consensus array designs.**

*http://cancer.sanger.ac.uk/cancergenome/projects/census/
**http://dbsearch.clinicalgenome.org/search/

The power of SNPs

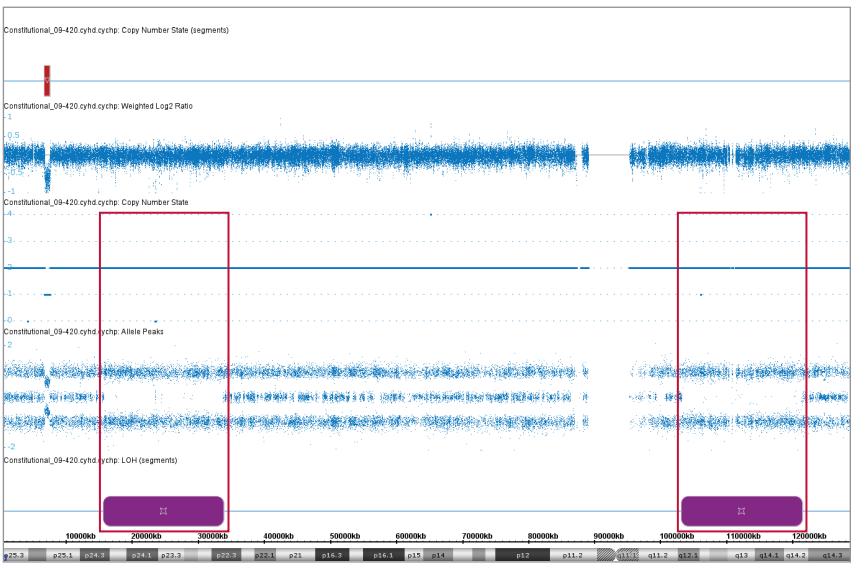
Unlock new applications

High-density biallelic SNP probes, with high genotype accuracy, enable confident breakpoint determination and copy number change confirmation throughout the genome. They also allow 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

Regions identical-by-descent (detailed view of chromosome 2)



- **This example illustrates two blocks of LOH >10 Mb on chromosome 2.**
- **The red segment illustrates an additional hemizygous loss on this chromosome.**

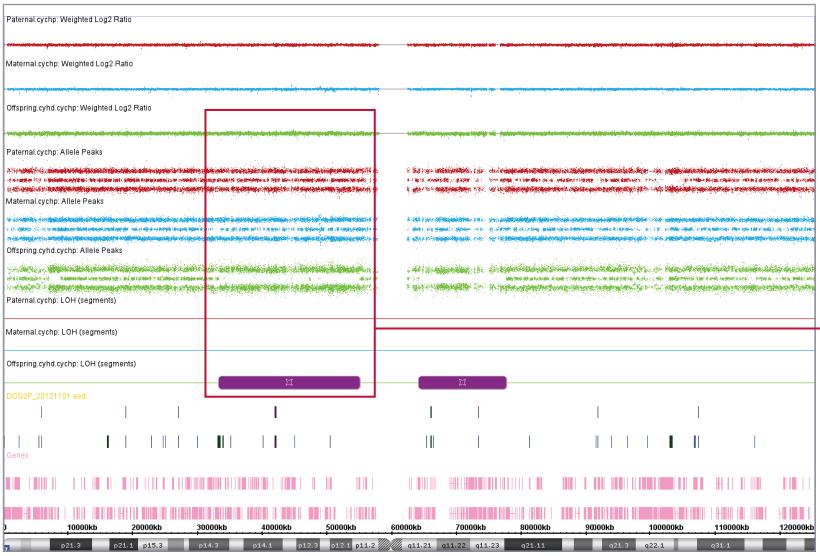
"The low-density array called absence-of-heterozygosity regions not confirmed by the other platforms and also overestimated the length of true absence-of-heterozygosity regions. Furthermore, the low- and mid-density platforms failed to detect some small absence-of-heterozygosity regions that were identified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

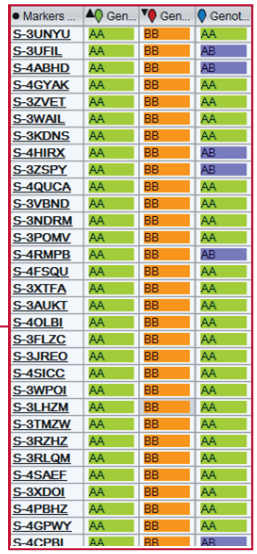
Mason-Suares, *et al*. Density matters: comparison of array platforms for detection of copy-number variation and copy-neutral abnormalities. Reprinted by permission from Macmillan Publishers Ltd: *Genetics in Medicine* **15**(9):706–712 (2013).

High-density SNPs with >99% genotype accuracy

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

Trio analysis





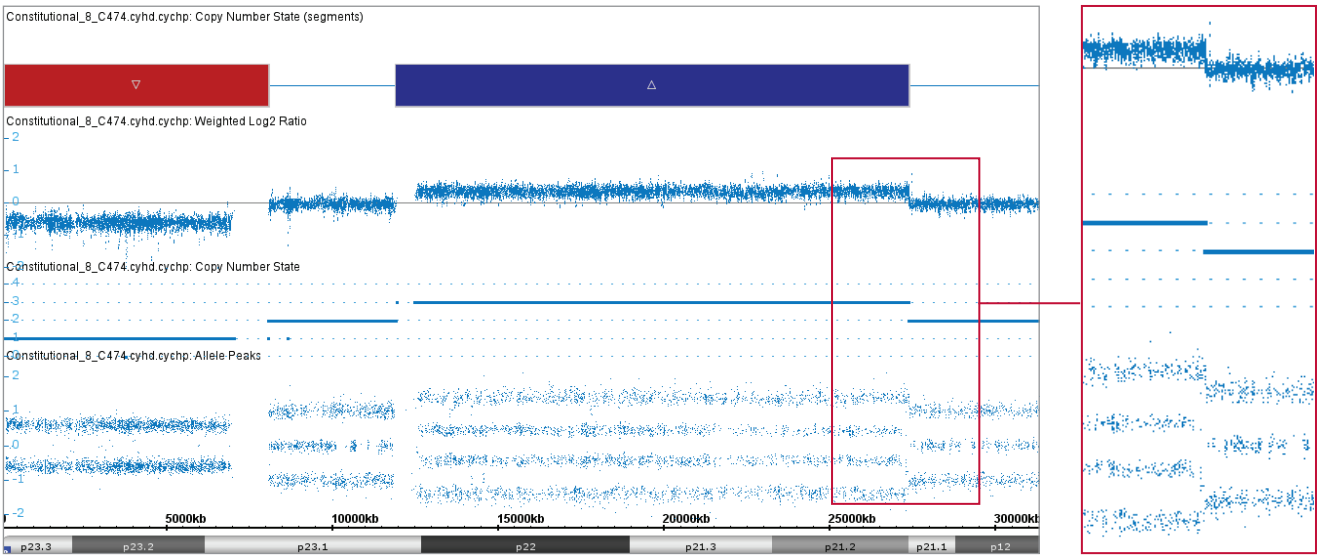
Markers ...	Gen...	Gen...	Genot...
S-3UNYU	AA	BB	AA
S-3UFIL	AA	BB	AB
S-4ABHD	AA	BB	AB
S-4GYAK	AA	BB	AA
S-3ZVET	AA	BB	AA
S-3WAIL	AA	BB	AA
S-3KDNS	AA	BB	AA
S-4HIRX	AA	BB	AB
S-3ZSPY	AA	BB	AB
S-4QUCA	AA	BB	AA
S-3VBND	AA	BB	AA
S-3NDRM	AA	BB	AA
S-3POMV	AA	BB	AA
S-4RMPB	AA	BB	AB
S-4FSQU	AA	BB	AA
S-3XTFA	AA	BB	AA
S-3AUKT	AA	BB	AA
S-4OLBI	AA	BB	AA
S-3FLZC	AA	BB	AA
S-3JREQ	AA	BB	AA
S-4SICC	AA	BB	AA
S-3WPOI	AA	BB	AA
S-3LHZM	AA	BB	AA
S-3TMZW	AA	BB	AA
S-3RZHZ	AA	BB	AA
S-3RLQM	AA	BB	AA
S-4SAEF	AA	BB	AA
S-3XDOI	AA	BB	AA
S-4PBHZ	AA	BB	AA
S-4GPWY	AA	BB	AA
S-4CPBI	AA	BB	AB

- **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 copy number event-independent confirmation throughout the entire genome.

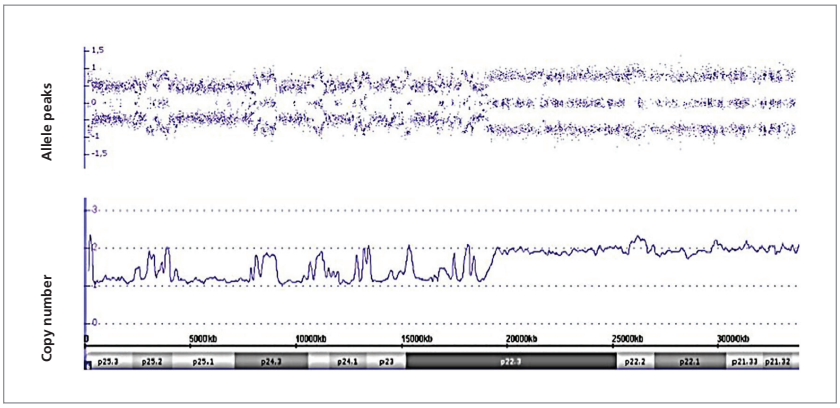
Example of a hemizygous loss and gain on the same chromosome



- **This example illustrates copy number states 1, 2, and 3 on chromosome 8.**
- **These copy number changes were all FISH confirmed.**

High-density SNPs enable the visualization of complex genomic patterns such as chromothripsis.

Example of chromothripsis in chromosome 6



- **The copy number signal in the p-arm of chromosome 6 oscillates between two states, also confirmed by changes in the allelic difference pattern. With more than 30 breakpoints, this pattern indicates chromothripsis.**

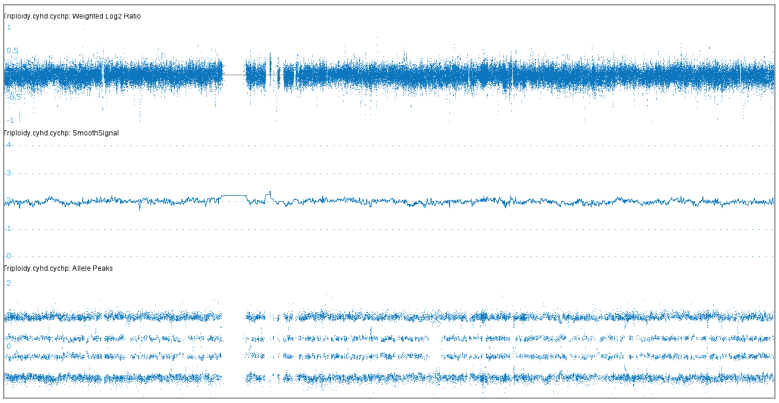
"Recently, a number of new genomic aberrations have been described in neuroblastomas, which are assumed to also have prognostic impact. The most spectacular genomic aberration to be found in a large number of tumors, including neuroblastomas, is referred to as chromothripsis. This state of genomic 'catastrophe' is assumed to be an indicator of unfavorable prognosis."

Affymetrix, Inc., Unraveling the complexities of the cancer genome to guide patient-tailored treatment strategies. [Q&A with Dr. Peter Ambros], Scientist Spotlight, P/N CL02351 Rev. 1.

Triploidy detection

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

A representative chromosome of sample containing a triploid genome



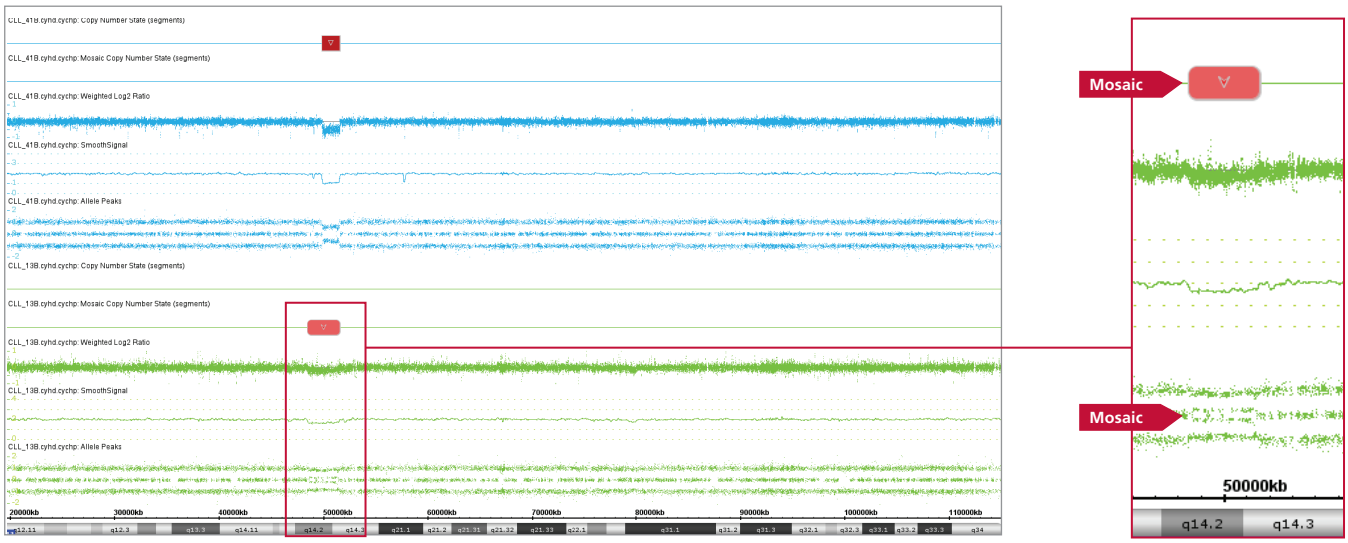
- **Normal log2 ratio + 4 allelic tracks = detection of triploidy.**
- **Copy number only is normalized but not the allele track.**

"...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 R. J., *et al.* Chromosomal microarray versus karyotyping for prenatal diagnosis. *New England Journal of Medicine* **367**(23):2175–2184 (2012).

Low-level mosaic detection

Two samples visualized in parallel: hemizygous loss and mosaic loss

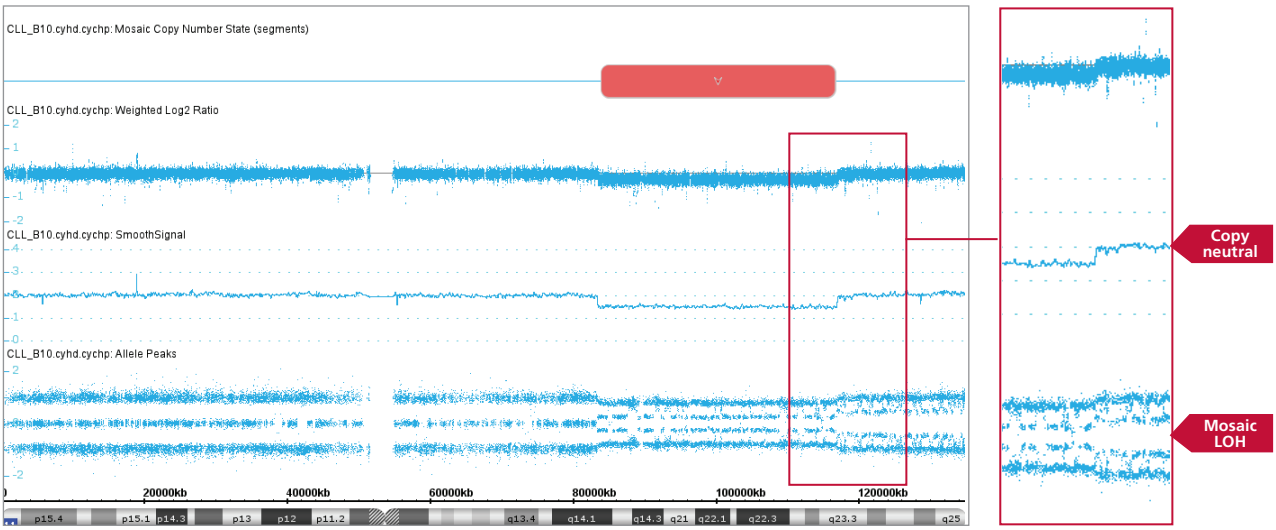


- **The sample at the top represents a full hemizygous loss on chromosome 13.**
- **The sample at the bottom represents a mosaic loss in the same region.**
- **Interphase FISH confirmed with the mosaic level at 20%.**

"...genetic complexity of cancer cells requires a sensitive genome-wide analysis, enabling the detection of small genomic changes in a mixed cell population, as well as of regions of homozygosity. The advent of comprehensive high-resolution genomic tools, such as… single-nucleotide polymorphism microarrays, has overcome many of the limitations of traditional cytogenetic techniques and has been used to study complex genomic lesions in, for example, leukemia."

Simons A., *et al.* Genome-wide arrays in routine diagnostics of hematological malignancies. *Human Mutation* **33**(6):941–948 (2012).

Mosaic loss and mosaic copy neutral LOH



- **This CLL sample contains multiple aberrations.**
- **Chromosome 11 contains a mosaic loss and a mosaic copy-neutral LOH event.**

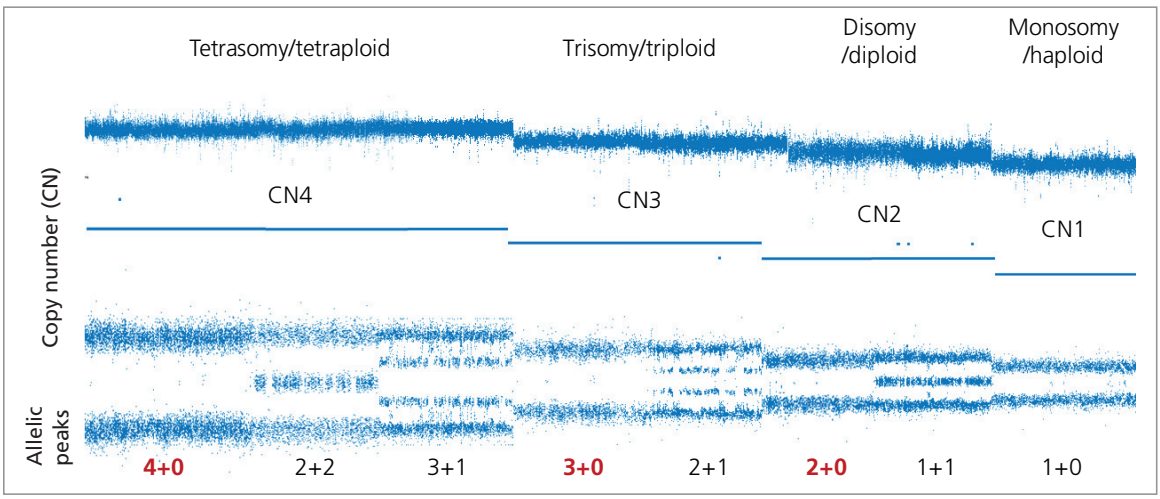
"Although FISH analysis is now widely used for the cytogenetic assessment of CLL, other approaches such as oligonucleotide-based array comparative genomic hybridization and single nucleotide polymorphism (SNP) gene chips show comparable results but also assess all chromosomal regions rather than the current standard clinical practice of identifying alterations with probes targeting only 4–5 chromosomal sites."

Pei, *et al.* Chromothripsis in a case of TP53-deficient chronic lymphocytic leukemia. *Leukemia Research Reports* **1**(1):4–6 (2012).

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



▪ **The CytoScan® array can accurately identify endoreduplication events in haploid genomes.**

"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.* Genome-wide arrays in routine diagnostics of hematological malignancies. *Human Mutation* **33**(6):941–948 (2012).

"...(SNP) microarray analysis was used to identify a hyperdiploid clone that had evolved from a presumptive, near-haploid clone. By conventional methods, this clone may have been easily misinterpreted as a common hyperdiploid clone. Given the extreme prognostic differences of the two clones, this distinction is especially critical to accurately guide therapy."

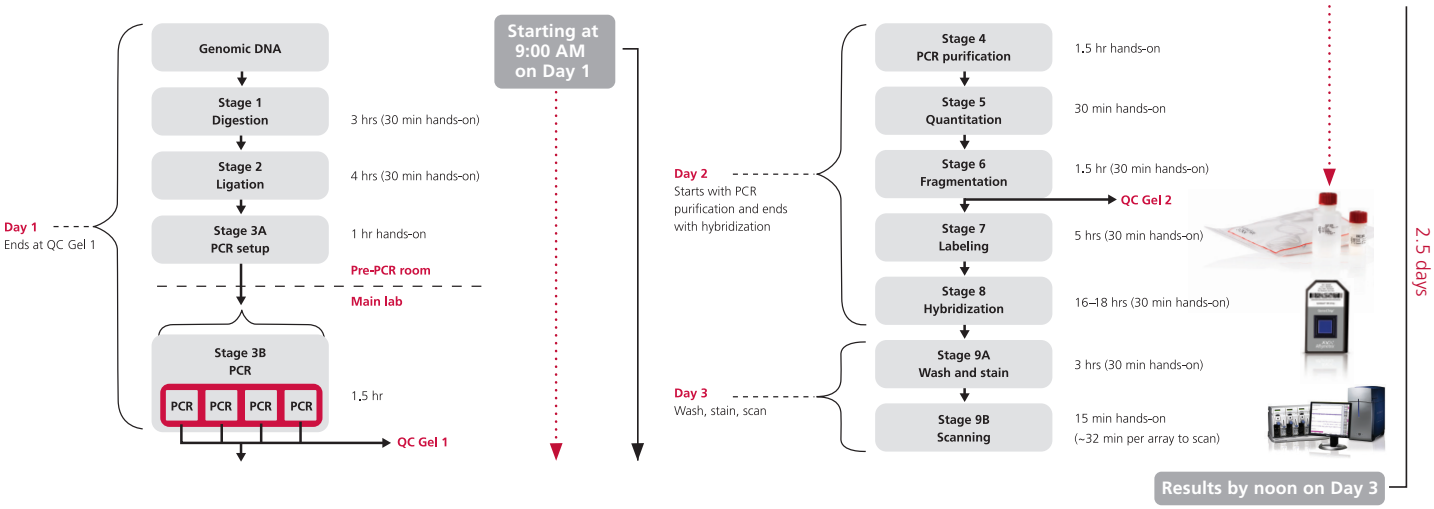
Choi S. M., *et al*. Near-haploid B lymphoblastic leukemia with an apparent hyperdiploid karyotype: the critical role of SNP analysis in establishing proper diagnosis. *Journal of Hematopathology* **7**(1):27–32 (2014).

Robust manual or automated assay workflows

DNA to result in less than 3 days

CytoScan Cytogenetics Suite 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 CytoScan® reagent kit is designed to save time and money, reduce operator error, and deliver the highest level of performance.

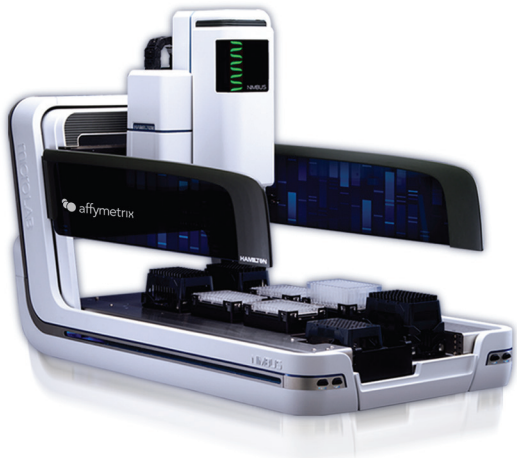
Packaging has been designed to be ecologically friendly and is recyclable, biodegradable, resealable, and saves storage space.



CytoScan® Automated Target Preparation Solution

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



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

Software designed for cytogenetic applications

Intuitive data analysis solutions

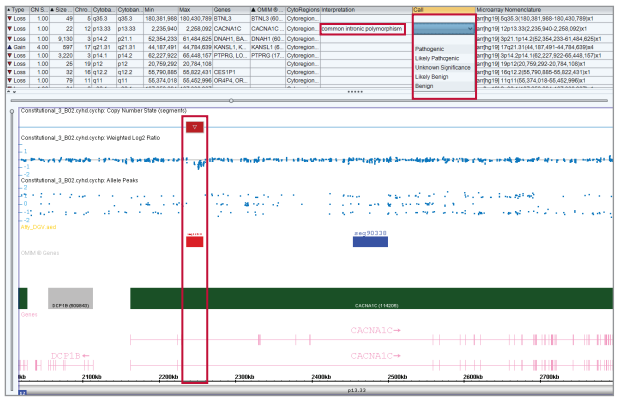
Chromosome Analysis Suite (ChAS) is tailored to cytogenetic research analysis and reporting with

- 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 copy number 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

Karyoview screen

- **Long contiguous stretches of homozygosity (LCSH) indicating regions identical-by-descent.**
- **Each LCSH segment is summarized, and individual thresholds can be selected by the user.**

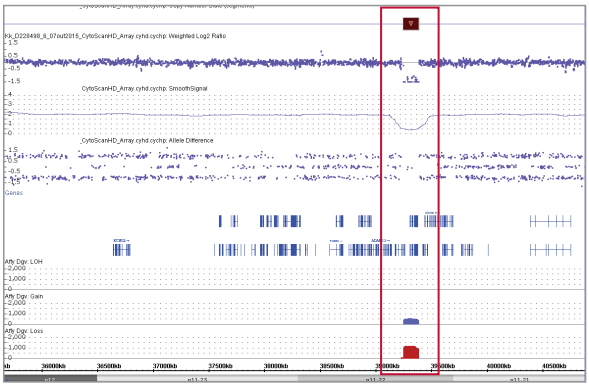
Calls and annotation for ease of interpretation



- **A common intronic deletion polymorphism on CACNA1C gene can be identified and easily annotated.**

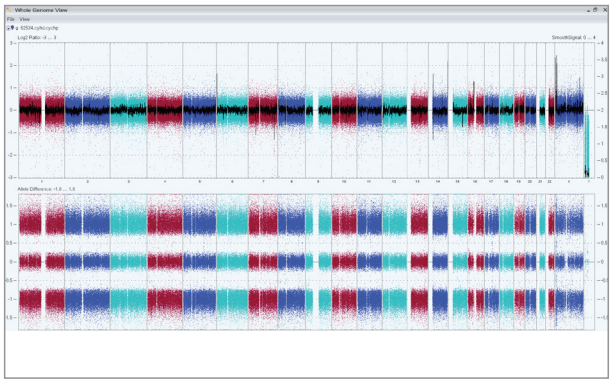
Please visit www.affymetrix.com/chas to download ChAS and all related array-specific files.

Histogram track display



- **CNVs in individual samples can be visualized and compared with data stored in the database.**

Whole Genome View



- **Whole Genome View log2 ratio and allele difference plot.**

CAGdb
Cytogenomics Array Group
CNV Database

Affymetrix is 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.

The GeneChip® System 3000 instrumentation platform

Flexible, proven, powerful

This industry-leading GeneChip® instrumentation system combined with innovative assays provides a complete platform for hybridizing, washing, staining, and scanning of microarrays. CytoScan® Cytogenetics Suite may be run on either GeneChip® System 3000 or GeneChip® System 3000Dx v.2. GeneChip® System (GCS) 3000Dx v.2 is FDA-cleared, CE-IVD registered, and includes GeneChip® Scanner 3000Dx v.2 with AutoLoaderDx, GeneChip® Fluidics Station 450Dx v.2, and Workstation with Affymetrix Molecular Diagnostic Software (AMDS). 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

Ordering information

Part number	Description	Details
901835	Cytoscan® HD Array and Reagent Kit Bundle	Sufficient for 24 samples
00-0218	GeneChip® 3000 7G with Workstation and AutoLoader	Includes ▪ GeneChip® Scanner 3000 7G with AutoLoader ▪ n2D Handheld Barcode Reader ▪ GeneChip® Fluidics Station 450 ▪ GeneChip® Hybridization Oven 645 ▪ Computer Workstation with instrument control software
00-0334	GeneChip® System 3000Dx v.2	Includes ▪ GeneChip® Scanner 3000Dx v.2 with AutoLoaderDx ▪ GeneChip® Fluidics Station 450Dx v.2 ▪ Workstation with Affymetrix Molecular Diagnostics Software (AMDS) *Requires GeneChip® Hybridization Oven 645 (see below)
00-0331	GeneChip® Hybridization Oven 645	
00-0401	Affymetrix® NIMBUS® Target Preparation Instrument	Robotics workstation and laptop

Affymetrix, Inc: (US) +1-888-362-2447, +1-408-731-5000 ▪ **(EU)** +44-(0)1628-552550 ▪ **(JP)** +81-(0)3-6430-4020 ▪ **(CN)** +86-21-63915511
eBioscience Products: (US) +1-888-999-1371, +1-858-642-2058 ▪ **(EU)** +43 1 796 40 40 305 ▪ **(JP)** +81-(0)3-6430-4020
USB Products: (US) +1-800-321-9322, +1-216-765-5000 ▪ **(EU)** +44-(0)1628-552600

www.affymetrix.com Please visit our website for international distributor contact information.
For Research Use Only. Not for use in diagnostic procedures.

P/N CL00731 Rev. 9