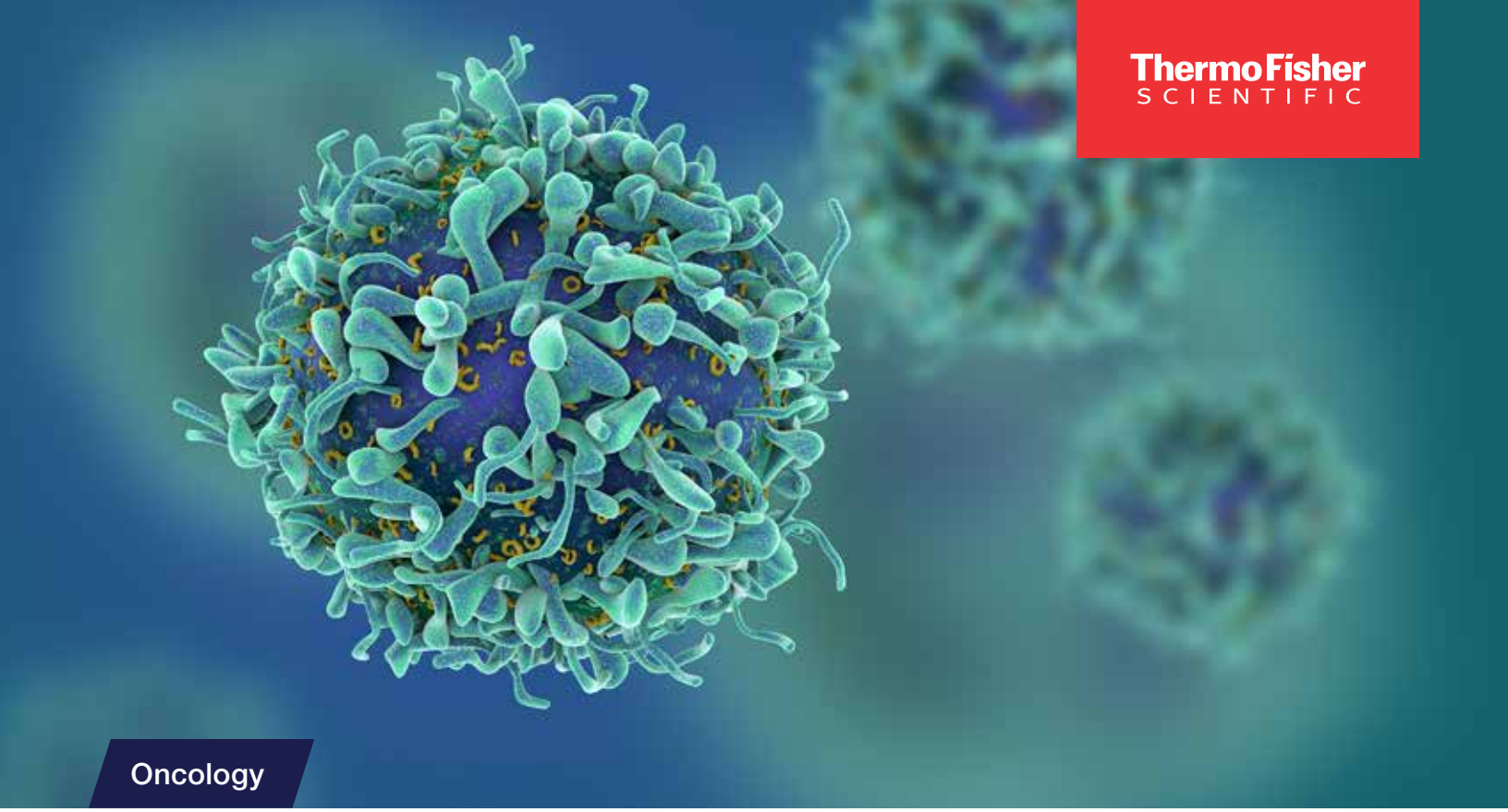


Oncology

Cutting through the complexity with chromosomal microarrays in cancer research

Decoding genetic variations with advanced microarray technology

Introduction

Cancer is a polygenic and multifactorial genetic syndrome characterized by many different forms of molecular variants, including somatic mutations and copy number variations (CNVs). CNVs are crucial for cancer research, providing a framework for exploring genetic variability in cancer and offering insights in tumor progression, susceptibility, and prognosis.[1] Yet, accurate identification of CNVs in cancer using traditional methods is a challenge.

Chromosomal microarray analysis (CMA) is a highly efficient method for identifying CNVs in cancer, offering significant advantages over traditional techniques. Unlike G-banding, which requires cell culturing, CMA eliminates this step, reducing time to results and allowing analysis of preserved samples, such as formalin-fixed paraffin-embedded (FFPE) tissue. This capability makes CMA particularly valuable for the study of archival cancer samples.

CMA works by analyzing genomic DNA from large populations of cells, which enhances its ability to detect clonality—a key factor in understanding tumor heterogeneity. CMA offers higher resolution and reliability for the detection of clonality, which is a challenge with technologies such as targeted sequencing and low-pass sequencing. The ability to work with FFPE samples, which are often difficult to analyze with other methods, further sets CMA apart from traditional techniques.

Furthermore, CMA is a cost-effective approach that offers a standardized workflow for CNV analysis. Unlike whole-genome sequencing (WGS) and optical genome mapping, which may lack the same level of standardization for CNV detection, CMA delivers consistent and reliable results for cancer research.

Applied Biosystems™ CytoScan™ Accel and Applied Biosystems™ OncoScan™ are complete microarray research solutions for oncology that leverage high-density SNP arrays to provide gene-level resolution for a variety of critical genomic analyses in just two days. These arrays enable precise identification of chromosomal changes, including CNVs, loss of heterozygosity (cnLOH), and clonality detection. The key applications of SNP-based arrays in oncology are illustrated below (Figure 1).

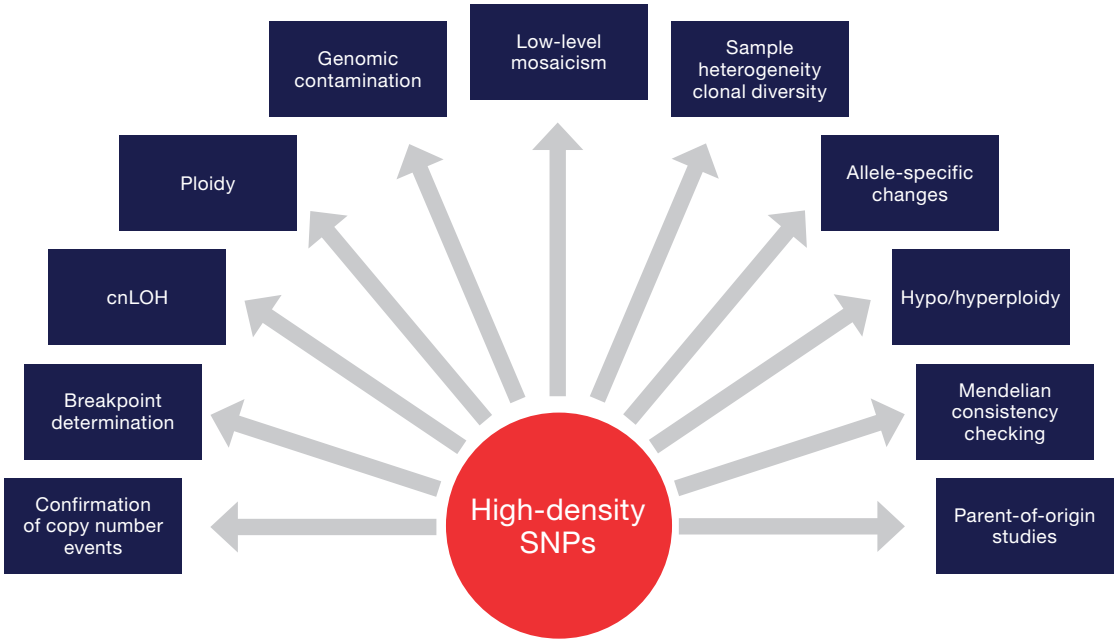


Figure 1. The power of SNP arrays. High-density SNP arrays with high genotyping accuracy enable a range of oncology research applications.

CytoScan Accel Suite for hematologic malignancies

Hematologic cancers include acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma (MM), acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Most hematologic cancers involve diverse and complex whole-genome events such as

- **Relevant submicroscopic CNVs:** Focal amplifications and deletions
- **Copy-neutral loss of heterozygosity (cnLOH):** Chromosomal regions converting to homozygosity
- **Clonality:** Variation across tumor populations
- **Chromothripsis:** Complex chromosomal rearrangements

These aberrations in blood cancers are difficult to detect and characterize using traditional methods, low-density arrays and arrays lacking high density SNP markers. The CytoScan Accel Suite offers two specialized products that are the benchmark for detecting CNV, mosaicism, copy-neutral loss of heterozygosity (cn LOH) and chromothripsis.[2]

Both of these solutions enable sample processing from DNA digestion through CMA studies in just two days.

The CytoScan Accel Array workflow takes just two days from sample to insights

Day 1 ----------> Overnight -----> Day 2

Digestion	Ligation	PCR amplification	Purification	Fragmentation	Labeling	Start hybridization	Hybridization	Washing/ staining	Scanning
0.5 hr	0.5 hr	1.5 hr	1 hr	1 hr	1 hr	0.5 hr	16 – 18 hr	2.5 hr	0.37 - 0.25 hr *
		25 cycles							
		Break			Break				

* Dependent on number of cartridges

Figure 2. CytoScan Accel workflow.

CMA use cases in hematologic malignancies

Researchers worldwide rely on the CytoScan Accel Suite to address the challenges of hematologic oncology.

Detection of aberrations missed by conventional karyotyping: In MDS samples, 73% showed abnormal karyotypes with CytoScan HD, compared to 56% with traditional methods, demonstrating its superior sensitivity.

Accurate identification of hyperdiploid and hypodiploid ALL: Hyperdiploid and hypodiploid ALL have different prognoses and treatments. SNP microarrays can identify hypodiploid doubling, which conventional and non-SNP microarrays misclassify as hyperdiploidy, leading to improper stratification and treatment.[3,4]

Identification of features of chromothripsis in AML: Chromothripsis in AML is commonly detected by FISH, but when identified with the CytoScan HD Accel Suite, several key features were observed, including higher copy number alterations, frequent chromosome breaks, and correlated with TP53 mutations.[5]

Improved detection of cnLOH in MDS: Abnormalities in key chromosomal regions, including 9p, were identified, offering valuable outlook on data outcomes.[6]

Detection of low-level clones: CytoScan reliably detects low-level clones in CLL, MDS, AML and other blood cancers.

Risk stratification in pediatric ALL: Stratify risk profiles based on CNV data, facilitating tailored therapeutic approaches.

The CytoScan HD Accel and CytoScan 750K Accel Suites are two powerful CMA solutions designed to detect chromosomal aberrations in a wide range of sample types for oncology research. CytoScan HD Accel is updated with some of the latest available content in more than 5,000 critical genome regions, while CytoScan 750K Accel offers focused, high-performance analysis optimized for specific research applications.

Key features of CytoScan HD Accel and CytoScan 750K Accel Suites are highlighted below.

Feature	CytoScan HD Accel Suite	CytoScan 750K Accel Suite
Workflow	**Fast 2-day workflow:** Complete the assay in just 2 days with minimal hands-on time (Figure 2).	
DNA input requirement	**Low DNA input:** Requires only 100 ng of genomic DNA—50% less than some other commercial CMAs.	
Marker count	**2.8 million markers** for copy number analysis, including 750,000 SNPs and 2 million nonpolymorphic probes.	**Over 950,000 markers** for copy number analysis, including 255,000 SNPs and 700,000 nonpolymorphic probes
Reference model	**Broad reference model:** Includes challenging sample types such as buccal swabs, saliva, amniotic fluid, and chorionic villus samples (CVSs), facilitating high-quality results across diverse sample types.	
Genomic analyses	**Enhanced genomic insights:** Enables advanced analyses such as breakpoint determination, gene-level homozygosity mapping, parent-of-origin analysis, and triploidy detection.	
Genomic coverage	**Comprehensive gene coverage:** High-resolution coverage of more than 5,000 regions in OMIM, RefSeq, ClinGen, and DECIPHER/DDD databases.	
SNP analysis	**High-density SNPs:** >99% genotype accuracy, enabling accurate detection of low-level mosaicism, LOH, aUPD, clonality, and more.	
Probe design	**Advanced dual-probe design:** Includes both copy number probes for optimized performance and high-density SNPs chosen for the best separation of allele tracks.	
Signal detection	**Sensitive detection:** Offers high specificity, wide dynamic range, and high resolution for comprehensive genomic profiling.	
Reproducibility	**Reliable and reproducible:** Proprietary manufacturing facilitates consistent results, with no risk of probe dropout.	
Productivity	**Maximized lab productivity:** Up to 100% improvement in efficiency compared to other microarrays.	

Learn how Dr. Madina Sukhanova, associate professor of pathology at Northwestern University, used CMA combined with NGS assays to identify and research genetic aberrations associated with specific prognoses indifferent types of cancer.

OncoScan CNV Assay for solid tumor samples

Traditional methods often fail to accurately identify CNVs in solid tumors due to the following challenges:

FFPE samples: 80% of solid tumors are FFPE, which can degrade DNA quality.

Limited sample size: Small tumor biopsies often provide insufficient DNA for testing.

Low DNA quality: FFPE processing results in fragmented DNA that is unsuitable for many assays.

High heterogeneity: Variability in fixation methods and storage complicates analysis.

Aneuploidy is used as a proxy for increased CNVs at the level of whole or partial chromosomes. Table 1 shows the prevalence of aneuploidy in common tumor types.

Table 1. High-aneuploidy tumors[5]

Tumor Type	Average aneuploidy score
Adrenocortical carcinoma	18.3
Bladder urothelial carcinoma	13.6
Breast invasive carcinoma	12.1
Colon adenocarcinoma	11.6
Lower grade glioma	3.9
Ovarian serous cystadenocarcinoma	14.0
Prostate adenocarcinoma	2.6

The OncoScan CNV and CNV Plus Assay addresses these challenges and is a powerful tool for genome-wide CN analysis. OncoScan assays use molecular inversion probe (MIP) technology, which has been proven to identify CN alterations, cnLOH/LOH, and somatic mutations. Our white paper shows how the OncoScan assay is best suited for accurately identifying CNVs in solid tumors.

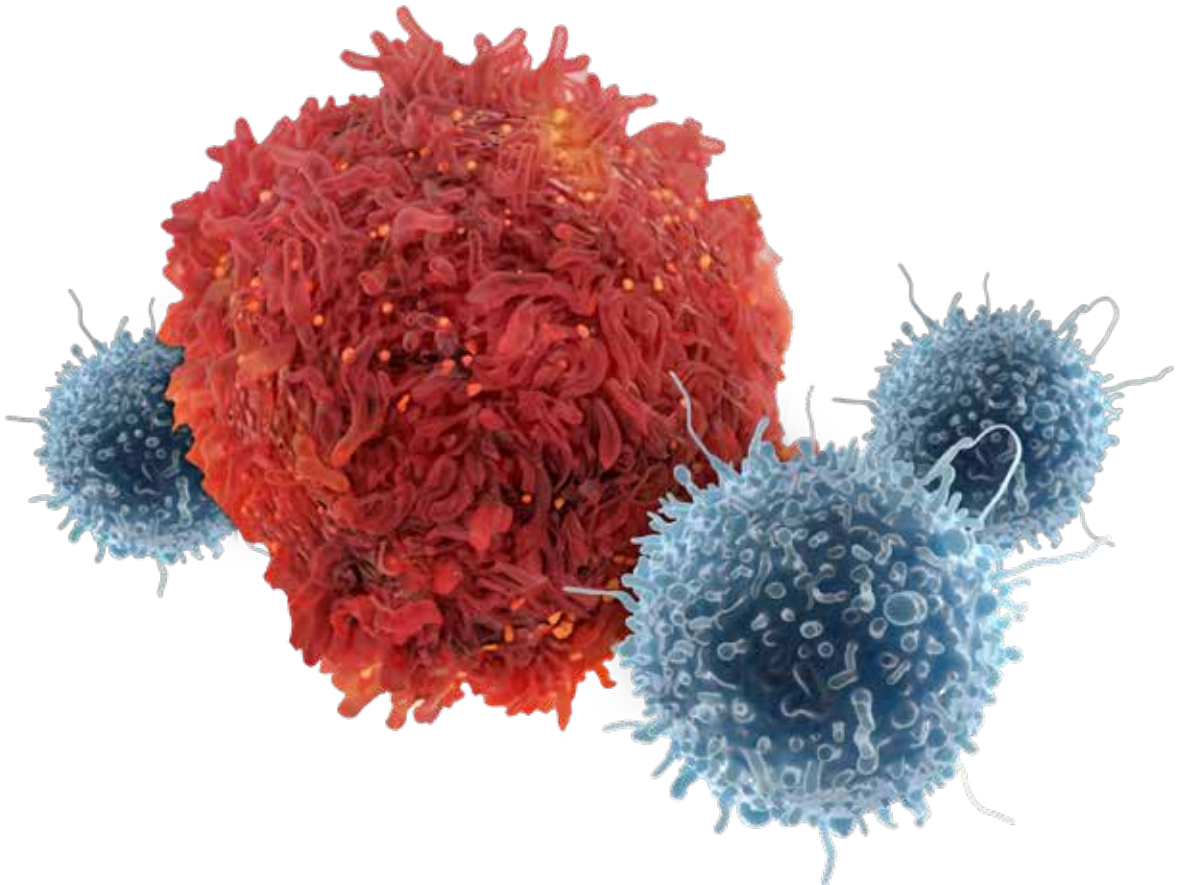
OncoScan CNV Assay use cases in solid tumors

Researchers worldwide rely on the OncoScan CNV Assay to accurately identify CNVs in solid tumor analysis:

1. **Unlocking HRD complexity with whole genome profiling**
   Homologous recombination deficiency (HRD) is critical in the stratification and selection of cases for novel treatments using PARP inhibitors in multiple cancers. OncoScan CNV and CNV Plus Assays, due to their ability for whole-genome profiling of CN and loss of heterozygosity (LOH), are among the best tools for researching the cytogenetics of HRD.

2. **Identifying biomarkers and tumor classifications**
   OncoScan CNV and CNV Plus Assays have been applied in clinical research of different types of cancer. They can detect key biomarkers important for researching tumor classification, like in pediatric brain tumor research and melanoma research, for example.

3. **Analyzing genomic variation using circulating cell-free DNA**
   Liquid biopsies are rapidly becoming an alternative to invasive tissue biopsies for analyzing genomic variation associated with tumors. Cell-free DNA (cfDNA) is also a degraded DNA, which makes the molecular inversion probes (MIPs) of OncoScan CNV and CNV Plus Assays excellent tools for researching chromosomal aberrations and CNVs in this tissue type.[7–9]



Key features of the OncoScan CNV Assay include:

- **Low sample input:** Only 80 ng of FFPE-derived DNA
- **Fast turnaround time:** Results in just 2-3 days
- **Exceptional flexibility:** Identifies copy number gain/loss, cnLOH/LOH, ploidy and more
- **Advanced analysis:** Detects clonality, clonal heterogeneity and chromothripsis
- **Broad somatic mutation panel:** Covers 64 mutations in 9 genes (*BRAF, EGFR, IDH1, IDH2, KRAS, NRAS, PIK3CA, PTEN and TP53*)
- **Easy data analysis:** Results are analyzed using Chromosome Analysis Suite (ChAS) software, which provides intuitive data visualization

Our data indicate that the OncoScan assay is a sensitive and robust method to assess CNA, allelic imbalances/losses and specific recurrent point mutations in FFPE tumor material.[9]

- **High-resolution CN detection in priority cancer genes:** Accurately identifies very small (50-125 kb) to large (Mbs) CNVs
- **Evidence-based database development:** Linking of evidence with observations for internal database management and expansion
- **Rapid analysis—included software:** Provides intuitive data visualization for hundreds of samples in minutes

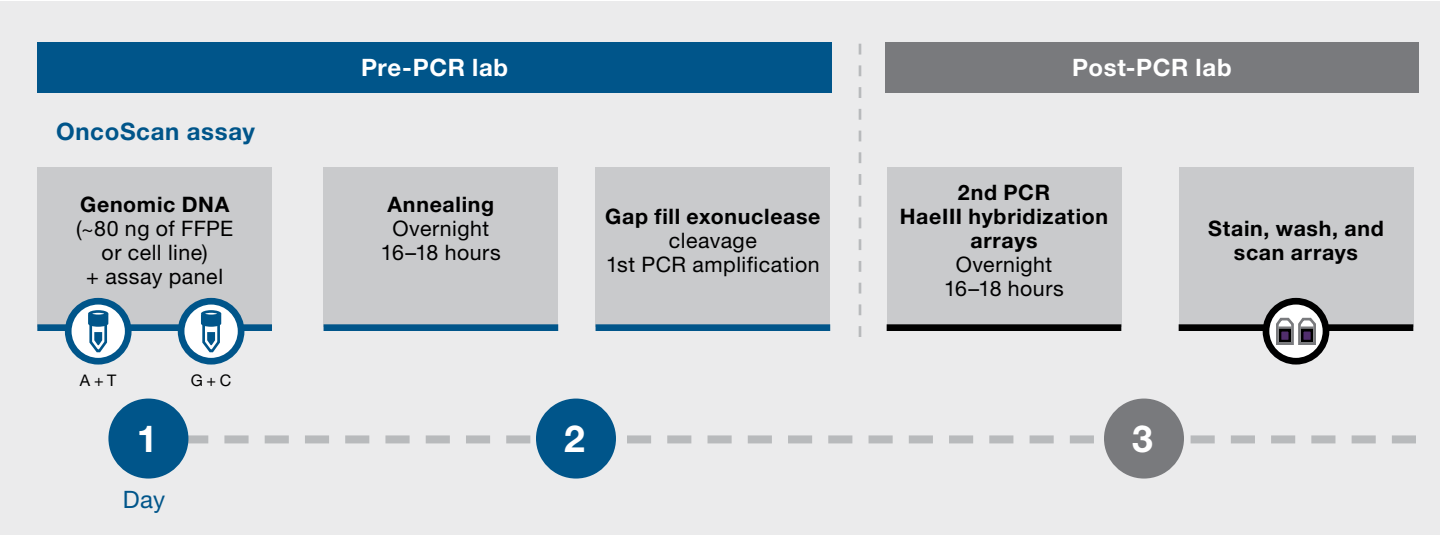


Figure 4. OncoScan CNV Assay workflow.

OncoScan CNV and CNV Plus assays:
Comprehensive research solutions for whole-genome copy number analysis of FFPE tumor samples

Learn about solid tumor genetics with whole-genome copy number analysis

Dr. Ravindra Kolhe from Augusta University Medical College explores chromosomal analysis in melanoma research, while Dr. Joanna Przybyl from Stanford University studies copy number changes in leiomyosarcoma (LMS). Both researchers explore genetic factors to understand and treat these cancers.

Streamline analysis, interpretation, and reporting for challenging genes with Chromosome Analysis Suite (ChAS) software

View and analyze chromosomal aberrations across the genome, including copy number gain or loss, LOH, and mosaicism. Developed with input from our customers and leading experts, Applied Biosystems™ Chromosome Analysis Suite (ChAS) software is designed specifically for analysis and reporting in chromosomal aberration research. Enhanced, intuitive features simplify cytogenetics investigation.

Key features

- Whole-genome support for the CytoScan suite
- CN state for the CytoScan suite
- LIMS APIs
- Analyze data at different levels of resolution
- Customize and load your own annotations and regions for focused analysis
- Store, query, and display historic sample data and annotations for streamlined analysis
- Directly access NCBI, UCSC Genome Browser, and Ensembl databases and others
- Export user-selected data in formats like browser extensible data (BED), Applied Biosystems™ Affymetrix™ extensible data (AED), and variant call format (VCF) files
- APIs to push and pull segment coordinates in and out of ChAS software
- Automatic results file generation with zero manual setup required

View ChAS training modules

Request a ChAS demo

Table 3. Specifications for sample profiling suites for oncological clinical research

	CytoScan HD Accel Suite	CytoScan 750K Accel Suite	OncoScan CNV Assay/Plus*
Research applications	Faster turnaround time and high-resolution analysis of genome-wide CNVs for oncology research	High-resolution and faster TAT with improved coverage for highest genome-wide resolution of CNVs for oncology research	High-resolution analysis across the whole genome, with focus on cancer genes, and high performance with FFPE samples
Sample types	Blood, bone marrow, and fresh and frozen tissue	Blood, bone marrow, and fresh and frozen tissue	FFPE, fresh and frozen tissue (best for solid tumors)
Input DNA	100 ng‡	100 ng‡	80 ng‡
Analytical claims (size of aberrations)**	Losses: 25 kb Gains: 50 kb LOH: 3 Mb Mosaicism† limit of detection: >15%	Losses: 100 kb Gains: 400 kb LOH: 5 Mb Mosaicism† limit of detection: >15%–20%	Losses: 50 kb Gains: 50 kb LOH: 10 Mb Mosaicism† limit of detection: >20-30%
Probe structure	2.8 million markers for whole-genome coverage 2 million non-polymorphic markers ~743,000 SNP markers for LOH analysis and sample tracking	960,755 markers for whole-genome coverage 706,054 non polymorphic markers ~255,000 SNP markers for LOH analysis and sample tracking	220,000 molecular inversion probes (MIPs) for whole-genome coverage 5,700 non-polymorphic markers 216,000 SNP markers for LOH and sample tracking
Protocol time	2 days	2 days	2–3 days

* The OncoScan CNV Plus Assay includes a somatic mutation panel covering 64 mutations in 9 genes (*BRAF, EGFR, IDH1* and *2, KRAS, NRAS, PIK3CA, PTEN,* and *TP53*).

** Size of aberration: The size of the segment call depends on the average marker spacing in the region. Best performance can be achieved in regions with higher marker coverage. Mosaicism detection may depend on the size of the altered segment and the type of aberration involved.

† Mosaicism in cancer is classified by % aberrant cells in the sample and is called in ChAS software.

‡ 80/100 ng is optimal, but users have reported success using lower input DNA.

CytoScan HD Accel Suite
Download the datasheet

CytoScan 750K Accel Suite
Download the white paper

OncoScan CNV and CNV Plus Assays for research
Download the brochure

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