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Biology for a better world

OncoScan® FFPE Assay Kit

Genome-wide copy number in 48 hours

Un*block* your view. It will take your breath away.

Why is genome-wide copy number important in the analysis of solid tumors?

- The significance of copy number aberrations is being established in an ever increasing number of genes
- The number and complexity of copy number aberrations are indicative of prognosis in many solid tumors
- Detecting aberrations in subclones and assessing clonal evolution are critical for making treatment decisions

OncoScan® FFPE Assay Kit

Finally, the ability to deliver whole-genome analysis from FFPE samples quickly, accurately, and cost-effectively

Obtaining genome-wide copy number and loss-of-heterozygosity (LOH) profiles from solid tumor samples is a significant challenge due to the difficulty of working with limited amounts of DNA from highly degraded FFPE samples.

A simple whole-genome scanning approach avoids the traditional single locus, low-resolution bottleneck of FISH and PCR. Although next-generation sequencing technologies have shown utility in mutation discovery, the requirement for target enrichment and high-depth coverage in order to obtain copy number information from heterogeneous FFPE samples remains a significant challenge.

OncoScan® FFPE Assay Kit, based on Molecular Inversion Probe (MIP) technology, offers genome-wide copy number and LOH detection, enhanced resolution in ~900 cancer genes, and the status of frequently tested somatic mutations, all from a single assay. Data can be generated from only 80 ng of FFPE-derived DNA within 48 hours.

"The Lymphoma Study Association (LYSA) trials for DLBCL (Diffuse Large B Cell Lymphoma) and MCL (Mantle Cell Lymphoma) has over 30,000 patients enrolled. The goal of the lymphoma genomic project is to predict prognosis based on copy number aberrations on every patient with DLBCL or MCL to help inform clinical trials enrollment and therapy selection. In order to do this prognostication, we need to generate whole-genome copy number profiles to identify recurrent aberrations that correlate with prognosis. The OncoScan assay is ***the only practical technology for whole-genome copy number and copy-neutral LOH analyses in FFPE****. In our initial study, we successfully generated high-quality whole-genome copy number, including aberrations expected from literature on the OncoScan FFPE Assay Kit. Once we validate the OncoScan FFPE Assay Kit in 50 samples at our lab in Nantes, we expect to use the kit to analyze large datasets with MCL and DLBCL samples from LYSA clinical trials."*

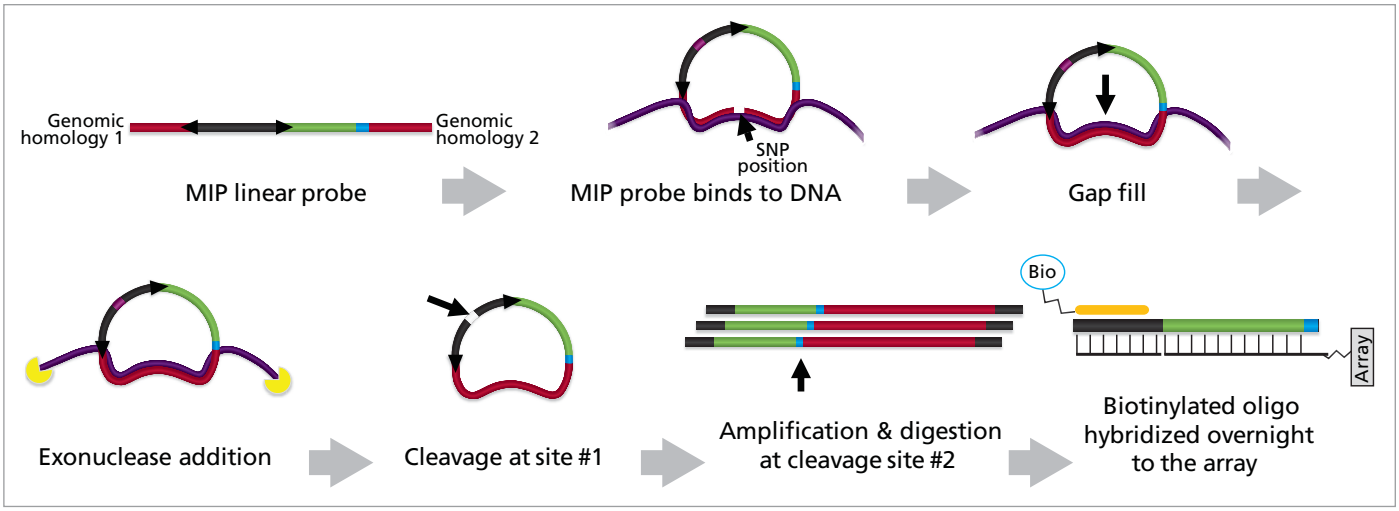
Stephane Minvielle, Director, Integrative Oncogenomics of Multiple Myeloma Pathogenesis and Progression, INSERM UMR, Nantes, France

Exceptional performance

Limited input of degraded DNA from FFPE samples

- Requires only 80 ng input DNA
- Utilizes Molecular Inversion Probe (MIP) technology, which is optimized for highly degraded FFPE samples (probe interrogation site of just 40 base pairs)
- Assay pass rate of >90% across over 7,000 samples
- Validated with archived FFPE samples (10 years or older)
- Proven compatibility with all major solid tumor tissue types

Figure 1: Molecular Inversion Probe (MIP) assay.



"Using the OncoScan® assay we were able to generate reliable, high-quality whole-genome copy number using 75 ng of DNA from highly degraded, archived FFPE samples, including samples that were at least 20 years old. The OncoScan assay ***enables highly powered, retrospective studies*** *that can accelerate new drug development if genomic information can be coupled with drug response, such as in companion diagnostic tests. It also is an incredibly useful assay for discovery and implementation of prognostic signatures in cohorts of patients with FFPE samples."*

Joshua D. Schiffman, MD, Medical Director, High Risk Pediatric Cancer Clinic Associate Professor, Pediatric Hematology/Oncology Adjunct Associate Professor, Oncological Sciences Investigator, Huntsman Cancer Institute, University of Utah

"We ran the OncoScan assay using 50 ng of DNA from 64 formalin-fixed, paraffin-embedded (FFPE) melanocytic lesions. Without microdissection, and with one tenth the quantities recommended for Agilent 180K CGH arrays, we were able to detect clear gains and losses and differentiate between malignant melanoma and benign melanocytic nevi. A major advantage of the OncoScan assay compared with FISH is the breadth and quantifiable detail of the results. A commercially available FISH panel for the diagnosis of melanoma contains 4 probes directed against 2 chromosomes. Our results suggest that this FISH panel would have missed 6 melanomas in our cohort that the OncoScan assay detected, 1 of which proved to be lethal to the patient."

Sarah South, PhD, Medical Director, Cytogenetics, Genomic Microarray, and Genetic Processing Laboratories, ARUP labs

One assay – many powerful data types

Genome-wide copy number and LOH, high resolution on cancer genes, plus somatic mutations, all from a single assay

- 50–100 kb copy number resolution in ~900 cancer genes
- 300 kb genome-wide copy number resolution outside of the cancer genes
- Genome-wide LOH detection including copy-neutral LOH detection
- High dynamic range of 10+ copies
- Demonstrated concordance with FISH-confirmed amplifications in key cancer genes including ERBB2 (Her2), EGFR, MDM2, MYC, and FGFR1
- Actionable somatic mutations

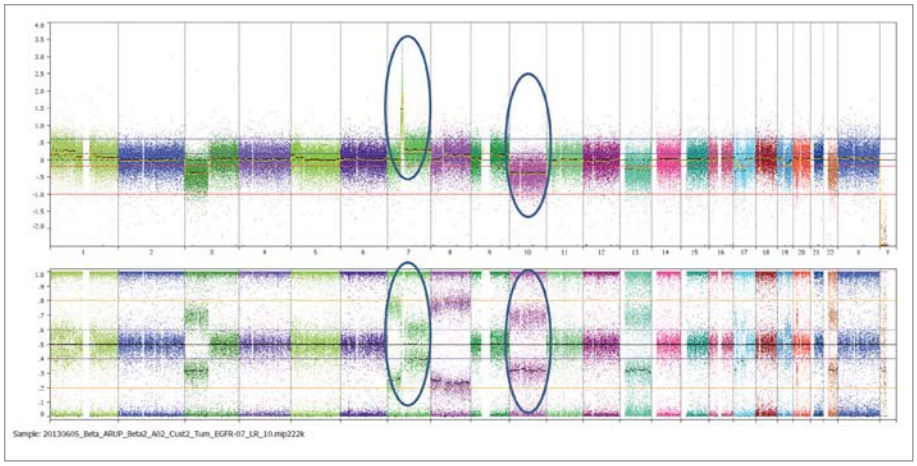


Figure 2: OncoScan® Nexus Express Software Whole-Genome View.

Top view: Total copy number view enables detection of gains and losses. ***Shown here:*** *Gain on chromosome 7 and loss on chromosome 10.*

Bottom view: B allele frequency view enables detection of low-level mosaic gains and losses, LOH, and assessment of clonal evolution.
Shown here: *Splitting of the middle B allele frequency band confirming the gain on chromosome 7 and the loss on chromosome 10.*

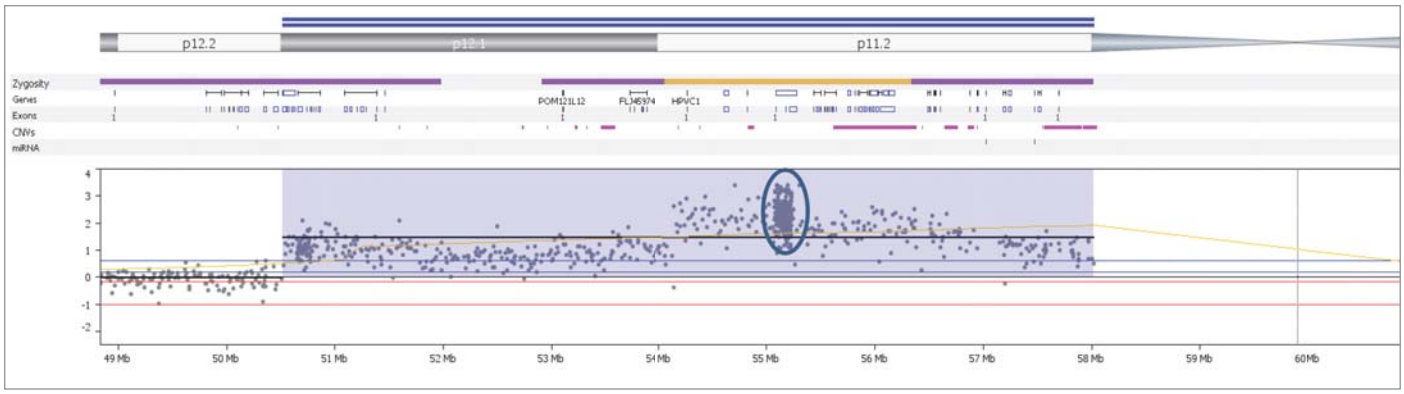


Figure 3: OncoScan Nexus Express Software Chromosome View.

Shown here: *Chromosome 7 view of sample in Figure 2 showing gain of the EGFR gene at high resolution.*

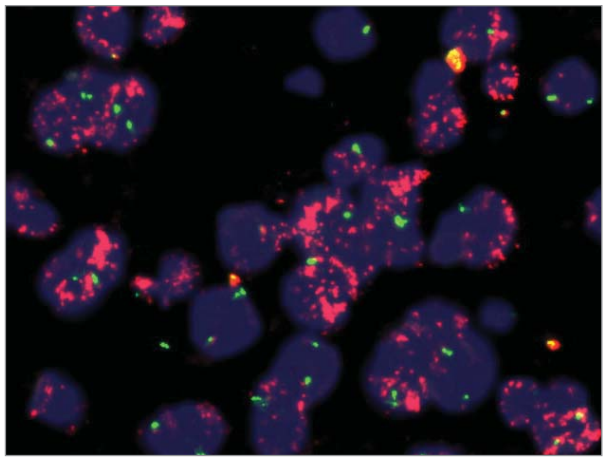


Figure 4: FISH confirmation of the EGFR gain shown in Figures 2 and 3.

Shown here: *Vysis LSI EGFR (spectrum orange) and Vysis CEP 7 (spectrum green) probes.*

"We performed beta testing of the new OncoScan® FFPE Assay Kit at ARUP. During beta testing, we ran 38 FFPE samples from multiple solid tumor tissues, including glioma, breast carcinoma, and liposarcoma, which contained FISH-confirmed aberrations. The age of the samples ranged from 6 months to 3 years since fixation. We used the recommended 80 ng of DNA from FFPE and were able to complete the OncoScan assay over a 48-hour period. 98% of the samples passed QC, and we were able to detect clear gains, losses, and LOH, including copy-neutral mosaic LOH in all the passing samples. Because OncoScan FFPE Assay Kit has whole-genome coverage, we were not only able to detect the FISH-confirmed aberrations in several cancer genes including ERBB2, MDM2, EGFR, and MYC but also obtained incremental copy number aberrations in these samples, many of which were expected aberrations for the tissue type. Our results suggest that OncoScan FFPE Assay Kit may be considered a viable higher resolution and higher specificity alternative to FISH testing for confirmation of cancer gene aberrations in solid tumor tissue."

Sarah South, PhD, Medical Director, Cytogenetics, Genomic Microarray and Genetic Processing Laboratories, ARUP Laboratories, Utah

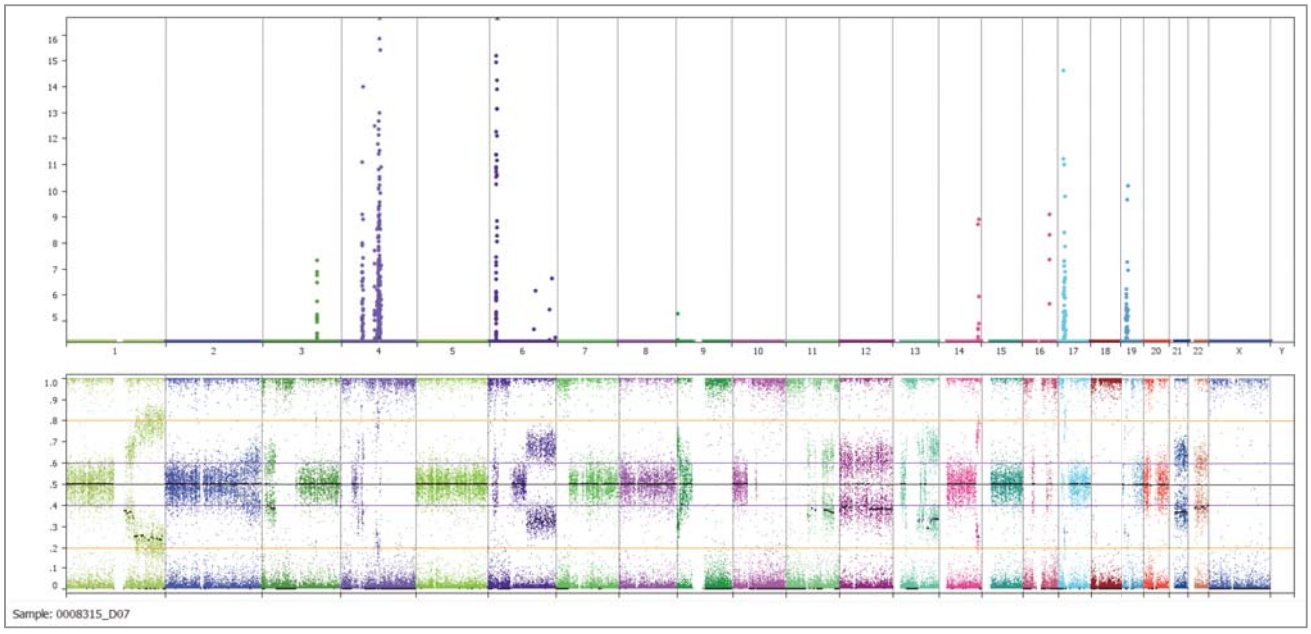


Figure 5: OncoScan® Nexus Express Software Whole-Genome View.

Top view: Total copy number view enables detection of gains and losses.
Shown here: *High copy number dynamic range on chromosome 4 and chromosome 6.*

Bottom view: B allele frequency view enables detection of low-level mosaic gains and losses, LOH, and assessment of clonal evolution.
Shown here: *Splitting of the middle B allele frequency band confirming the gain on chromosome 4 and the gain on chromosome 6.*

Detection of subclones and assessment of clonal evolution

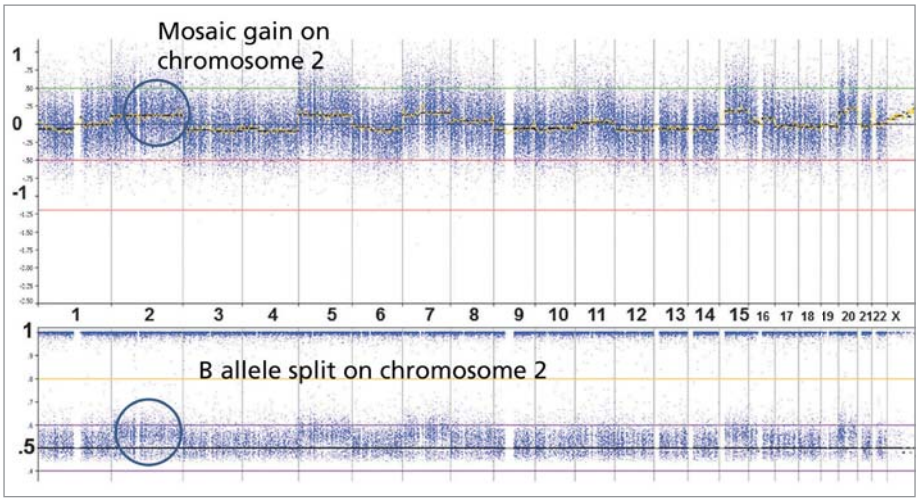


Figure 6: BioDiscovery Nexus Copy Number 6.0 Software Whole-Genome View.

Top view: Total copy number view enables detection of gains and losses.
Shown here: *A mosaic gain on chromosome 2.*

Bottom view: B allele frequency view enables detection of low-level mosaic gains and losses, LOH, and assessment of clonal evolution.
Shown here: *Splitting of the middle B allele frequency band confirming the low-level mosaic gains on chromosome 2.*

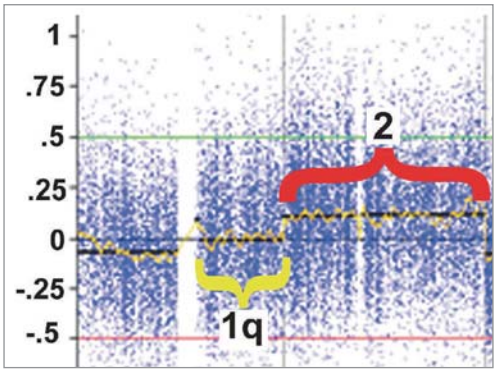


Figure 7: BioDiscovery Nexus Copy Number 6.0 Whole-Genome View.

Shown here: *Zoom-in view of mosaic gain on chromosome 2 from Figure 6. The mosaic gain appears to be present in 20% of the cells.*

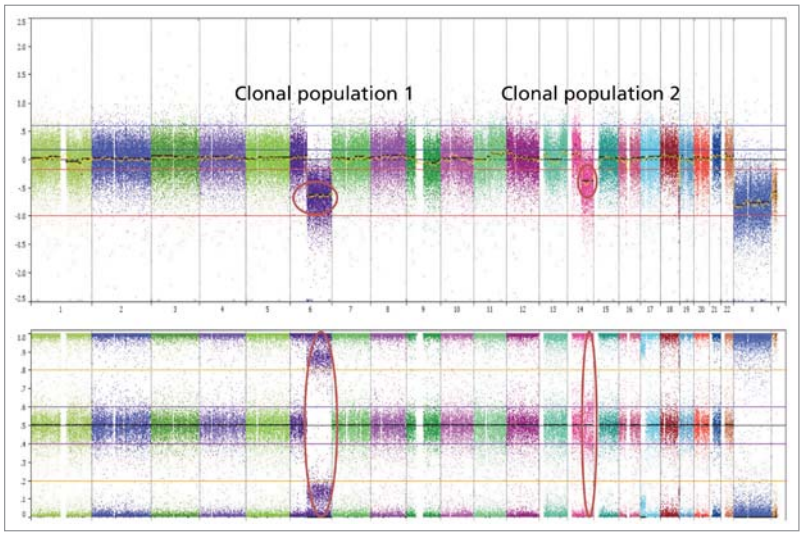


Figure 8: OncoScan® Nexus Express Software Whole-Genome View.

Top view: Total copy number view enables detection of gains and losses.
Shown here: *Two possible clonal populations with a different fraction of cells containing the aberration.*

Bottom view: B allele frequency view enables detection of low-level mosaic gains and losses, LOH, and assessment of clonal evolution.
Shown here: *B allele frequency values confirming the existence of the 2 clonal populations.*

"Using the OncoScan® assay, we could detect aberrations in subclones within a tumor. This makes the OncoScan assay a ***great platform for monitoring treatment response because we can detect aberrations found in subclones in biopsies after radiation treatment and after chemotherapy compared to the primary biopsy of the untreated patient****."*

Peter van der Spek, Professor and Department Head BioInformatics, Erasmus University Medical Center, Rotterdam, Netherlands

Rapid data analysis enables cost-effective signature discovery

From data to copy number calls for hundreds of samples in minutes using OncoScan® Nexus Express Software

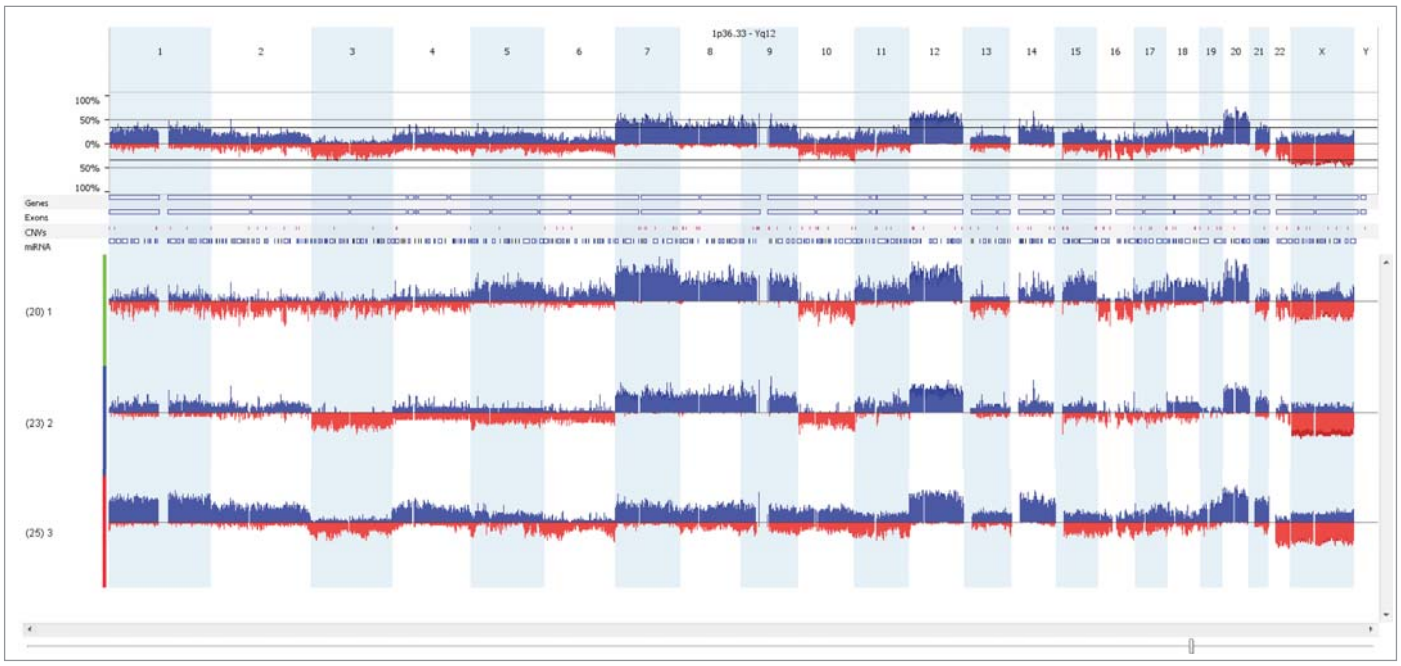


Figure 9: Multi-sample whole-genome view in OncoScan® Nexus Express Software.

Top view: Aggregate copy number gains and losses across a sample cohort.

Bottom view: Differences/similarities in gains and losses between the 3 groups. In this example, each group represents a different tumor stage.

"We ran 190 FFPE ependymoma samples on the OncoScan assay. On FISH, such samples have typical failure rates of 15–20%. On the OncoScan assay, only 8 of the samples failed, so that ***95.8% of the samples could be analyzed****.*

We were able to ***rapidly and easily analyze the whole-genome copy number data using the Nexus Software and cluster the samples into 3 distinct groups*** *based on their copy number profiles; namely, ependymomas with balanced copy number profile, a second group showing whole chromosomal gains and losses, and a third group with partial chromosomal gains and losses. We were able to correlate these groups with different survival rates. The structurally altered group was related to worse outcome, validating previous results of retrospective series. In addition, chromosome 1q gain, an established unfavorable prognostic marker, was clearly visualized on the OncoScan assay. Based on our experience, we believe that the* ***OncoScan assay is a robust platform for the rapid discovery and validation of novel prognostic copy number signatures and may also be useful in a clinical setting for the detection of 1q gain in tumors of ependymoma patients as a marker for patient stratification****."*

Professor Torsten Pietsch, MD, PhD, Institute of Neuropathology, University of Bonn, Germany

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. OncoScan® FFPE Assay Kit 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 for multiple applications on a single flexible system

GeneChip® System (GCS) 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® Array.
**FDA-cleared, IVD or CE-marked test developed by a third-party company on the Affymetrix® GCS 3000Dx v.2 platform.
***FDA-cleared CytoScan® Dx Assay for postnatal use only. Instructions for use (IFU) and assay limitations can be found at http://www.affymetrix.com/cytoscandx

OncoScan® FFPE Assay Kit includes:

OncoScan® Reagents – Molecular Inversion Probe (MIP) probe panel amplifies the precise target DNA of interest
OncoScan® Array – 2 universal tag arrays capture the amplified target DNA for a single color readout on GeneChip® Scanner 3000 or GeneChip® Scanner 3000Dx v.2, installed in over 2,000 labs globally
OncoScan® data analysis – TuScan algorithm developed for the analysis of solid tumor cancer samples and OncoScan® Nexus Express Software allow rapid, easy-to-use data analysis and visualization

Ordering information

Part number	Description	Details
902293	OncoScan® FFPE Assay Kit – Array and Reagent Kit Bundle	Sufficient for 24 samples and includes 48 OncoScan® Arrays
902305	OncoScan® Training Kit – Array and Reagent Kit Bundle	Sufficient for 18 samples and includes 36 OncoScan® Arrays

World-class support

Affymetrix offers an expanding portfolio of customer support and services—from training and instrument maintenance to consulting and compliance—led by our world-class team of multilingual technical experts, field application scientists (FAS), and regional field service engineers (FSE). For more information please visit www.affymetrix.com/service.

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