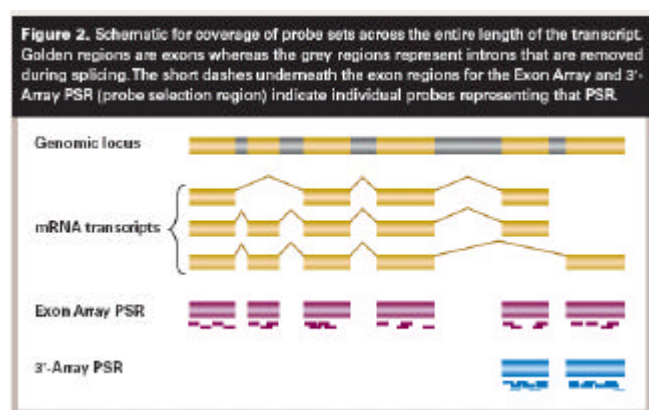


Affymetrix Exon Array

Affymetrix Exon 1.0 Array

The GeneChip® Exon 1.0 ST (sense target) Arrays combine a novel array design strategy with the highest density array manufacturing capability. For the first time, both gene-level and exon-level expression profiling can be performed on the whole-genome scale using a single array. The Exon Array system solution presents new dimensions for researchers to explore the human, mouse, and rat genome so they can investigate global expression of individual exons to uncover and document novel alternative splicing events and obtain more direct comparative genomic information with the syntenic sequences incorporated.



Features of this integrated solution include:

- Highest resolution expression analysis interrogating approximately one million exon clusters within the known and predicted transcribed regions of each genome, therefore providing the highest level of coverage at the gene level with more than 30 distinct probes representing most genes.
- Tailored GeneChip® Whole Transcript (WT) Assay and Reagents utilizing a random priming method for generating sense targets along the entire length of the transcripts.
- Flexible data analysis solutions and comprehensive annotation providing users the opportunity to explore and dissect the data in various, user-defined workflows.

GeneChip® Human Exon 1.0 ST Array Design Statistics Summary			
	Human	Mouse	Rat
Probe sets	1.4 million	1.2 million	1 million
Exon clusters	>1 million	~1 million	850,000
Supported by putative full-length mRNA	289,961 probe sets	266,200 probe sets	92,038 probe sets
Supported by Ensembl transcripts	306,583 probe sets	266,791 probe sets	195,943 probe sets
Supported by EST	665,175 probe sets	554,003 probe sets	211,451 probe sets
Supported by syntenic mRNA	220,262 probe sets ⁷	214,793 probe sets ⁴	272,061 probe sets ⁵
Supported by gene prediction	883,105 probe sets	835,897 probe sets	875,666 probe sets
Probe selection region	Along the entire length of the transcripts		
Probes/Probe selection region	4 ¹		
Background subtraction strategy	Median fluorescence intensity of up to 1,000 background probes with the same GC content		
Total features per array	> 5,500,000		
Interrogated strand	Sense ²		

Some application publications using Affymetrix Exon Array 1.0:

1) ANALYSIS/METHODS DEVELOPMENT

‡ Mao X., *et al.* Rapid high-resolution karyotyping with precise identification of chromosome breakpoints. *Genes, Chromosomes and Cancer* **46**(7):675-83 (2007). Accessible online at <http://www3.interscience.wiley.com/cgi-bin/abstract/114210044/ABSTRACT>

Key findings:

- Mao *et al.* used a combination of multiple color fluorescent in situ hybridization (M-FISH) and Affymetrix 500K Arrays for high-resolution karyotyping and identification of chromosome breakpoints in prostate cancer cell models.
- Exon arrays were used to verify translocation events that resulted in fusion genes or partial gene deletions.
- The authors demonstrated an approach that is capable of rapidly and precisely identifying most chromosomal rearrangements in individual tumors; this facilitates identification of critical genes and genetic biomarkers in tumorigenesis.

‡ Okoniewski M. J., *et al.* An annotation infrastructure for the analysis and interpretation of Affymetrix exon array data. *Genome Biology* **8**(5):R79 (2007). Accessible online at <http://genomebiology.com/2007/8/5/R79> (subscription required)

Key findings:

- Okoniewski *et al.* proposed a process to analyze exon array data through a genome-level annotation database, called “X:MAP,” and a BioConductor/R package for exon array analysis, called “Exonmap.”
- X:MAP is used to efficiently handle fine-grained mapping of Affymetrix probe set sequences from exon arrays to the genome and visualize data in a genome browser powered by a Google API interface.
- Exonmap is a Bioconductor/R package that is optimized for analysis of exon arrays, utilizing and combining data extracted from multiple tables in the database. This results in smaller data transfer overheads between client and server.

2) GENE EXPRESSION ANALYSIS ON EXON ARRAYS

‡ Huang R. S., *et al.* A genome-wide approach to identify genetic variants that contribute to etoposide-induced cytotoxicity. *PNAS* **104**(23):9758-9763 (2007). Accessible online at <http://www.pnas.org/cgi/content/full/104/23/9758>

Key findings:

- Huang *et al.* aimed to identify potentially functional SNPs and/or haplotypes associated with chemotherapeutic agent-induced cytotoxicity using genotype, gene expression and cytotoxicity data.
- Exon 1.0 ST Arrays were used to obtain whole-genome expression data to correlate using linear regression with SNPs found to be associated with etoposide cytotoxicity.
- Analysis identified 63 genetic variants that contribute to etoposide-induced toxicity through their effect on gene expression.

‡ Kapur K., *et al.* Exon array assessment of gene expression. *Genome Biology* **8**(5):R82 (2007).

Accessible online at <http://genomebiology.com/content/pdf/gb-2007-8-5-r82.pdf>

Key findings:

- Kapur *et al.* developed a strategy for estimating gene expression on Affymetrix exon arrays, a first step toward creating a baseline to judge the expression of individual exons.
- This method includes probe-specific background correction and a probe selection strategy. It is based on the MAT algorithm developed for Affymetrix tiling arrays.
- The authors propose that using exon arrays with their model (called GeneBASE) offers more accurate measurements of gene expression than using traditional 3' arrays.

‡ Okoniewski M., *et al.* High Correspondence Between Affymetrix Exon and Standard Expression Arrays. *Biotechniques* **42**(2):181-185 (2007). Accessible online at

http://www.biotechniques.com/default.asp?page=aop&subsection=article_display&display=full&id=112315

Key Findings

- The authors compared the gene expression profiles between two established cell lines and compared results obtained on exon and classical U133 Plus 2.0 Arrays.
- With three different mapping techniques, the two arrays showed a high degree of correspondence in terms of fold changes.
- The authors concluded that “since the classical microarrays have already been repeatedly validated experimentally, this provides strong evidence that exon arrays are also reliable, not only for probesets that can be successfully mapped to the existing arrays, but also for the many thousands of additional probesets that provide more detailed coverage of the transcriptome.”

ALTERNATIVE SPLICING ANALYSIS ON EXON ARRAYS

‡ Clark T. A., *et al.* Discovery of tissue-specific exons using comprehensive human exon microarrays. *Genome Biology* **8**(4):R64 (2007). Accessible online at <http://genomebiology.com/2007/8/4/R64>

Key findings:

- This paper is the original Affymetrix publication examining alternative splicing using the prototype exon array.
- The authors showed results for tissue-specific alternative splicing events as well as significant expression outside of known exons and well-annotated genes. This data is only available because of the comprehensive design of the exon arrays.
- Additionally, a Splicing Index algorithm is offered to identify alternative splicing events, whose efficacy was confirmed with RT-PCR validation on brain-enriched exons.

‡ French P. J., *et al.* Identification of differentially regulated splice variants and novel exons in glial brain tumors using exon expression arrays. *Cancer Research* **67**:5635-5642 (2007).

Key findings:

- French *et al.* set out to identify splice variants that are differentially expressed between histological subgroups of glial brain tumors.
- The results showed that using Human Exon 1.0 ST Arrays can help molecular classification of subgroups of gliomas based on their histological appearance.
- Exon-level profiling also identified more than 700 novel exons and a significant number of exons that are differentially spliced between glioblastomas and oligodendrogliomas, many of which were validated using RT-PCR.

‡ Gardina P. J., *et al.* Alternative Splicing and Differential Gene Expression in Colon Cancer Detected by a Whole Genome Exon Array. *BMC Genomics* 7(1):325 (2006). Accessible online at <http://www.biomedcentral.com/1471-2164/7/325>

Key Findings

- The authors analyzed the expression profiles of 10 colon cancer and 10 normal tissue samples with exon arrays.
- They found a correlation in gene-level signals between exon and U133 Plus 2.0 Arrays for genes that were significantly differentially expressed between tissue types.
- When reviewing differentially expressed genes between the cancer and control samples, they were able to identify 160 genes differentially expressed, and they found that almost one-third of the up-regulated genes in cancer form a part of a tightly interconnected network involved in mitosis, cell cycle control, cell proliferation, invasion, matrix remodeling and Wnt signaling.
- They also identified a number of genes that are differentially spliced between cancer and normal groups. Eleven of these events were confirmed by RT-PCR. Interestingly, out of these 11 genes, 10 are involved in the organization of the cytoskeleton, or interaction with the matrix of other cells, forming a network that is regulated by splicing. These results could contribute to better understanding of cancer etiology and may provide therapeutic targets and diagnostic markers