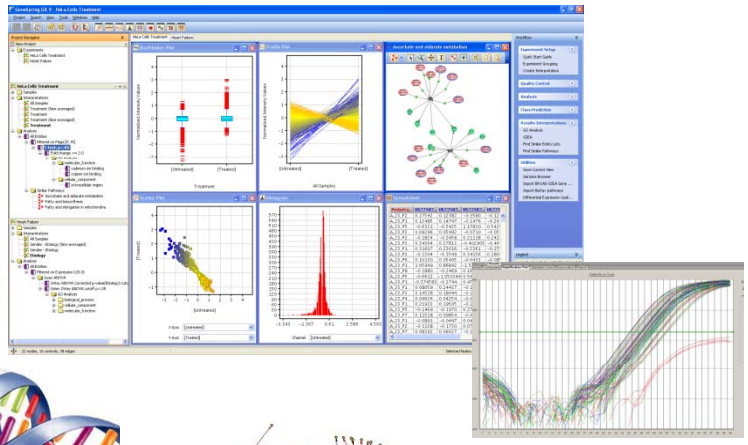
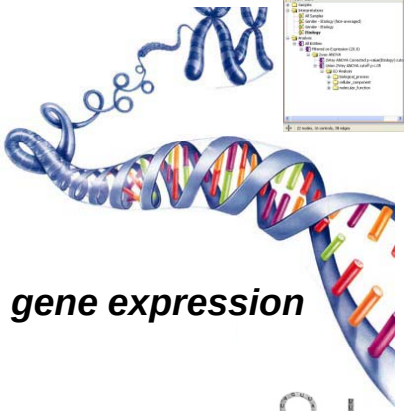
Identifying Significant Differences in Alternative Splicing Events in GeneSpring GX

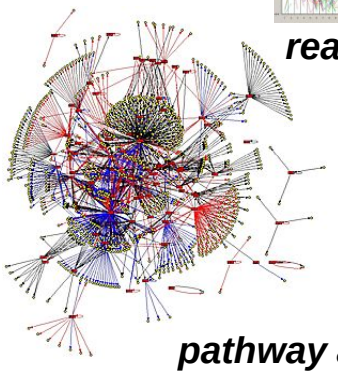
alternative splicing



real-time PCR



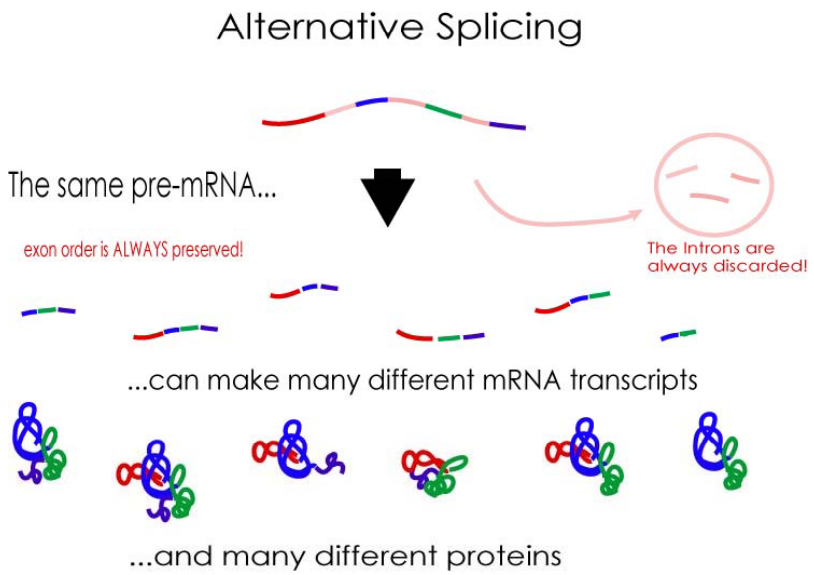
gene expression



pathway analysis

miRNA

Alternative Splicing and Human Disease

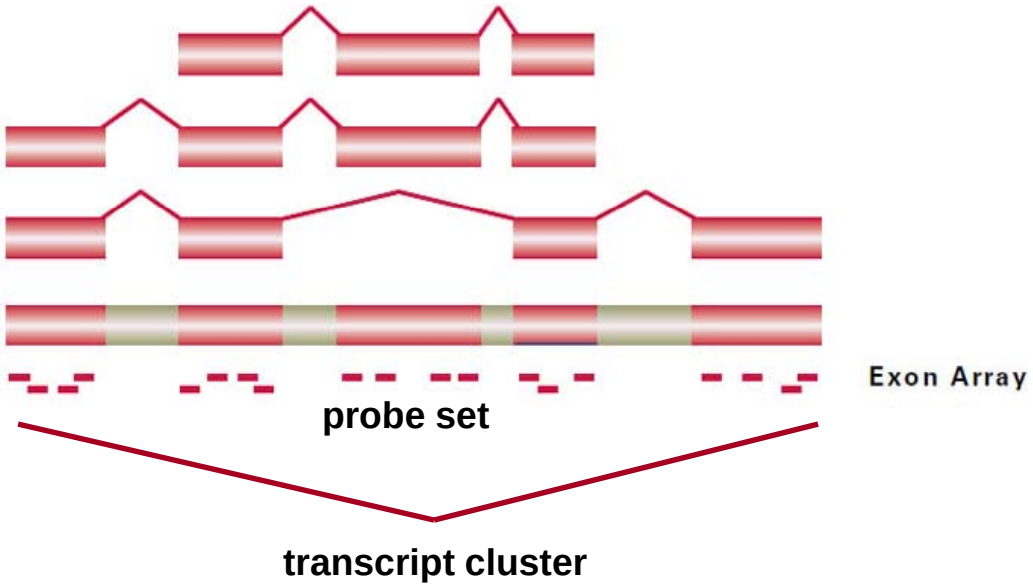


Importance of alternative splicing and human diseases:

- Approximately 75 percent of genes undergo alternative splicing
- Splice variants can produce proteins with distinct biological functions
- About 50 percent of disease-related point mutations may result in changes in splicing patterns

Thus, measuring changes in splicing patterns is integral to understanding the disease mechanism or biological process under study

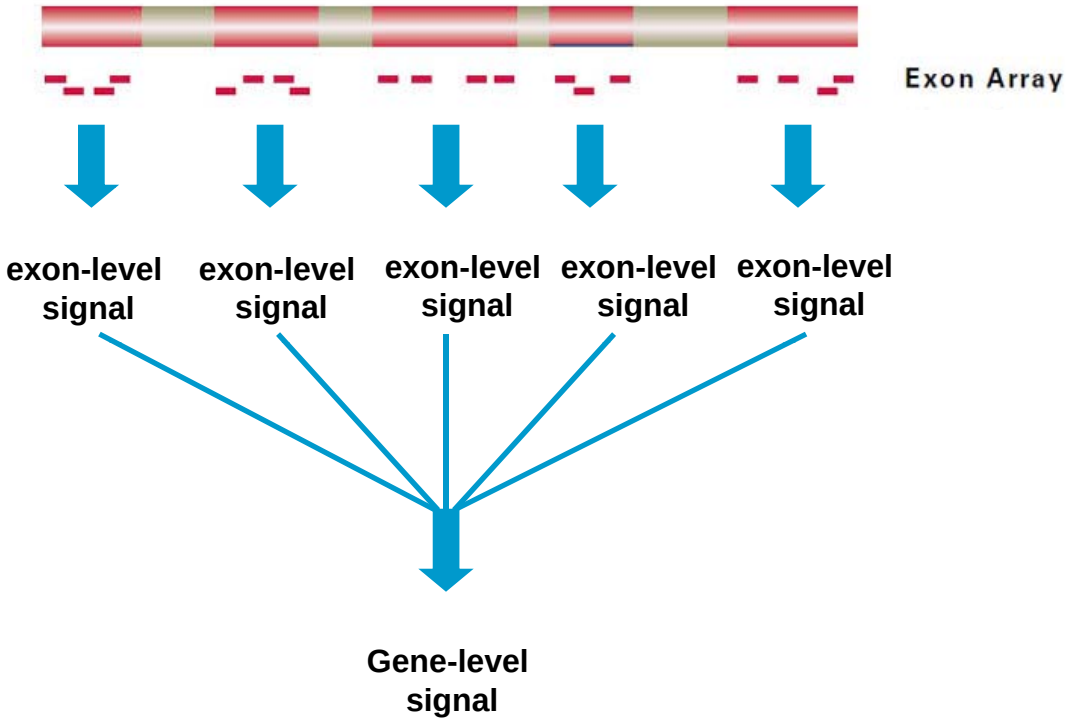
Affymetrix GeneChip Exon 1.0 ST Array



Human array contains 5.5M probes > 1.4M probe sets

- Core (17,800 transcript clusters): RefSeq and full-length GenBank mRNAs
- Extended (129K transcript clusters): core + EST and partial mRNA-based annotation
- Full (262K transcript clusters): Extended + ab-initio gene predictions

Signal Estimation Algorithms in GeneSpring GX



Exon-level Summarization:
RMA16
PLIER16
IterPLIER16

Gene-level Summarization:
RMA16
PLIER16
IterPLIER16

Values Used for Alternative Splicing Analysis in GeneSpring GX

Detection Above Background (DABG) p-value:
p-value assigned to each probe set (exon) to indicate whether signal of probe set is statistically significantly different from background signal

Gene-normalized intensity value:

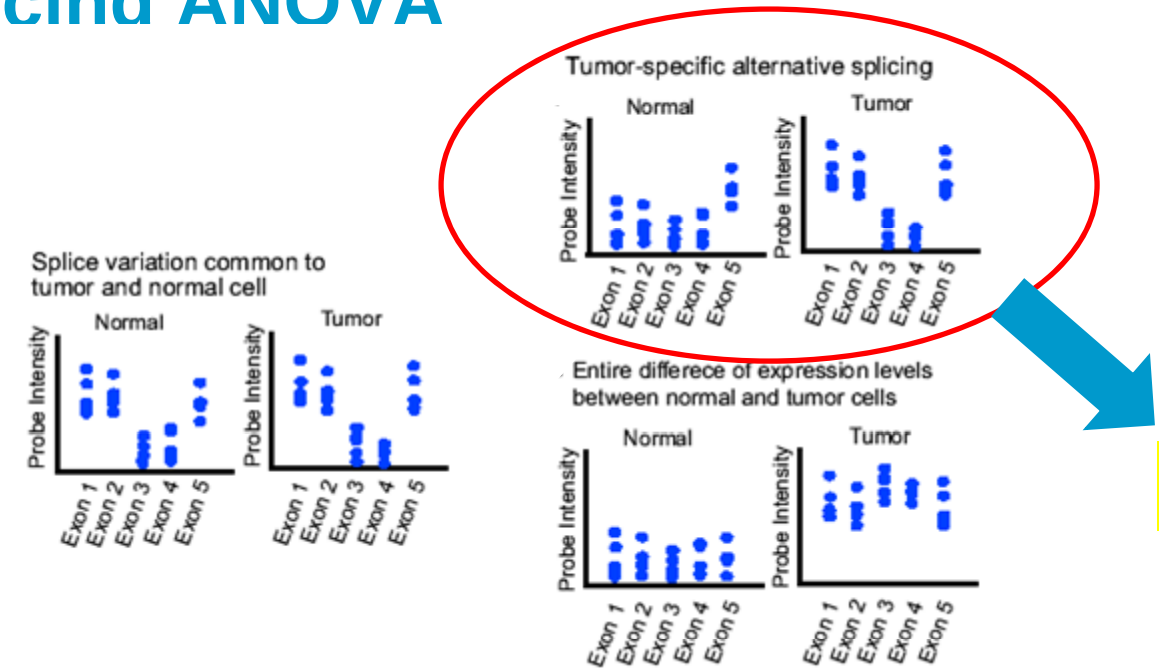
Figure 3: Gene-level normalized intensity (NI).

$$\text{Gene-level Normalized Intensity (NI)} = \frac{\text{Probe set intensity}}{\text{Expression level of the "gene"}}$$

Gene-normalized intensity in GeneSpring GX

Gene-normalized intensity values are used in multi-variate splicing ANOVA

Detection of Alternative Splicing Using Multivariate Splicing ANOVA

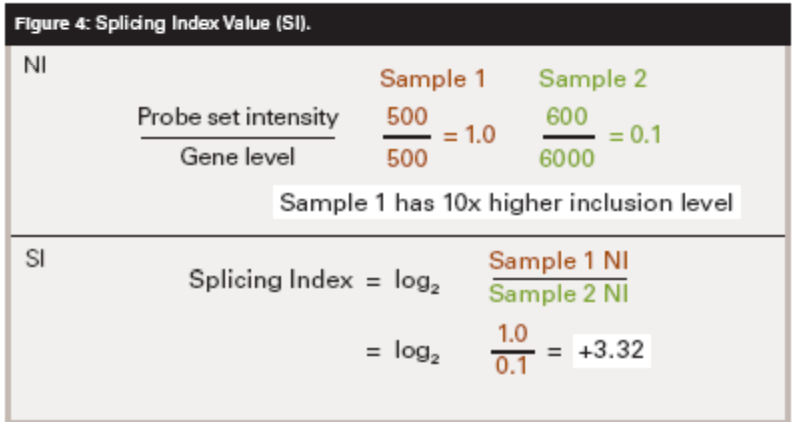


Saved results from Splicing ANOVA test in GeneSpring GX

In above case, analysis similar to 2-way ANOVA:

- Effect of parameter A- “Exon”
- Effect of parameter B- “Tissue Type”
- Effect of interaction between parameters A and B

Filter Transcripts on Splicing Index (SI)

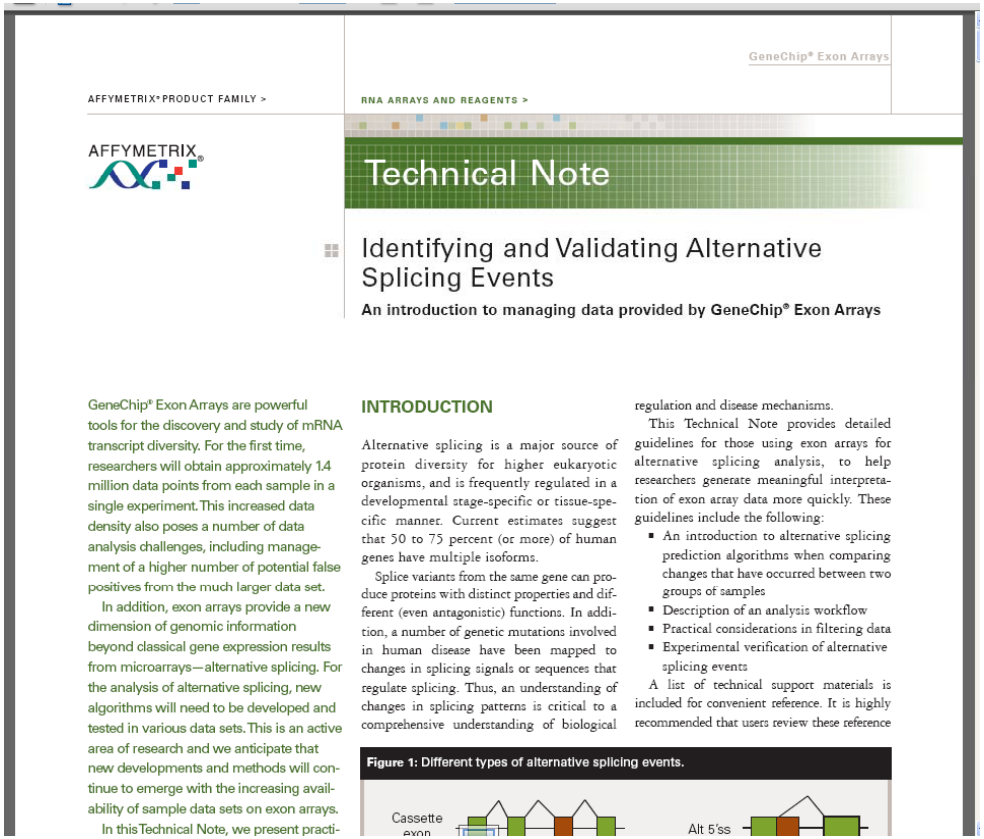


Splicing Index in GeneSpring GX

Filter on Splicing Index tool allows you to identify transcripts with a user-defined magnitude of the difference for exon inclusion between two groups of samples

- SI of 0 indicates equal inclusion rates between the two groups, positive SI value indicates enrichment of exon in Sample 1 (above example), and negative value indicates enrichment of exon in Sample 2 (above example)

GeneSpring GX's Alternative Splicing Workflow Follows Affymetrix's White Paper



GeneChip® Exon Arrays

AFFYMETRIX® PRODUCT FAMILY > RNA ARRAYS AND REAGENTS >

AFFYMETRIX®

Technical Note

Identifying and Validating Alternative Splicing Events

An introduction to managing data provided by GeneChip® Exon Arrays

GeneChip® Exon Arrays are powerful tools for the discovery and study of mRNA transcript diversity. For the first time, researchers will obtain approximately 1.4 million data points from each sample in a single experiment. This increased data density also poses a number of data analysis challenges, including management of a higher number of potential false positives from the much larger data set.

In addition, exon arrays provide a new dimension of genomic information beyond classical gene expression results from microarrays—alternative splicing. For the analysis of alternative splicing, new algorithms will need to be developed and tested in various data sets. This is an active area of research and we anticipate that new developments and methods will continue to emerge with the increasing availability of sample data sets on exon arrays.

In this Technical Note, we present practi-

INTRODUCTION

Alternative splicing is a major source of protein diversity for higher eukaryotic organisms, and is frequently regulated in a developmental stage-specific or tissue-specific manner. Current estimates suggest that 50 to 75 percent (or more) of human genes have multiple isoforms.

Splice variants from the same gene can produce proteins with distinct properties and different (even antagonistic) functions. In addition, a number of genetic mutations involved in human disease have been mapped to changes in splicing signals or sequences that regulate splicing. Thus, an understanding of changes in splicing patterns is critical to a comprehensive understanding of biological regulation and disease mechanisms.

This Technical Note provides detailed guidelines for those using exon arrays for alternative splicing analysis, to help researchers generate meaningful interpretation of exon array data more quickly. These guidelines include the following:

- An introduction to alternative splicing prediction algorithms when comparing changes that have occurred between two groups of samples
- Description of an analysis workflow
- Practical considerations in filtering data
- Experimental verification of alternative splicing events

A list of technical support materials is included for convenient reference. It is highly recommended that users review these reference

Figure 1: Different types of alternative splicing events.

Cassette exon

Alt 5'ss

https://www.affymetrix.com/products_services/arrays/specific/exon.affx

Alternative Splicing in Colon Cancer

Experimental Aim:

- To identify differential splice variation and differential gene expression that may contribute to the etiology of colon cancer

Experimental Design:

- 20 paired tumor-normal colon cancer samples (10 pairs will be used for this demonstration)
- Affymetrix GeneChip Human Exon 1.0 ST Array

Publication:

- *Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array*. P Gardina et al. BMC Genomics 2006, 7:325

Overview of Analysis Workflow in GeneSpring GX

Alternative splicing analysis

- Data import and summarization of probe sets
- QC on Samples
- QC on transcripts and probe sets
- Multi-variate splicing ANOVA to detect differential splicing
- Filter on Splicing Index
- Biological contextualization
  - GO Analysis and Find Significant Pathways