



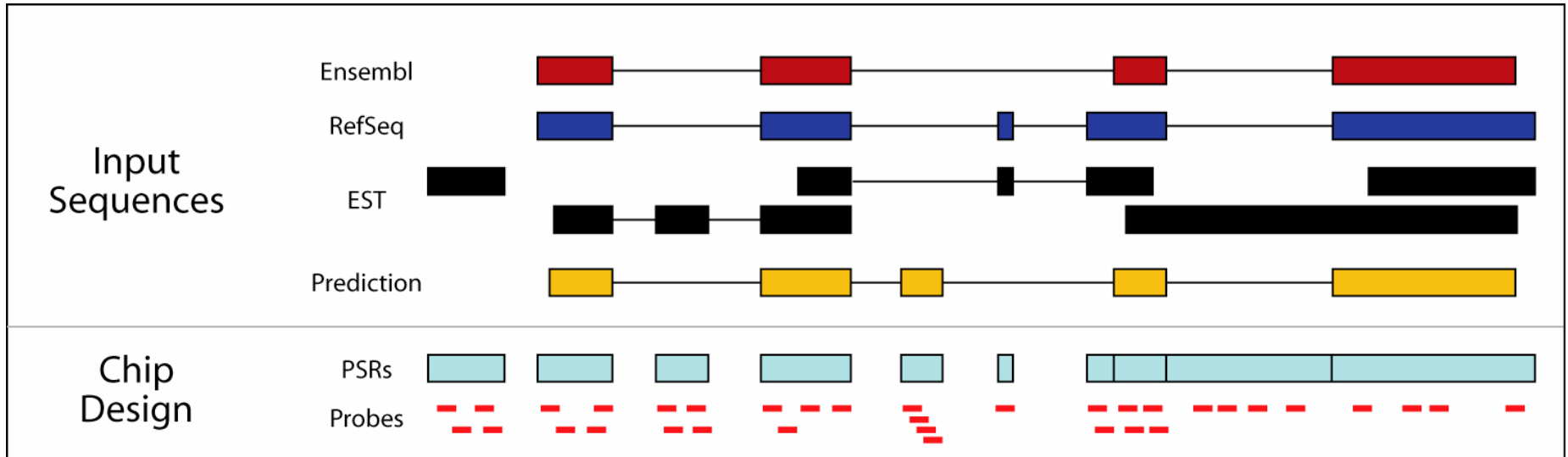
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Whole Genome Transcript Analysis with Affymetrix Exon Microarrays

Chuck Sugnet

Rocky 05 Conference

Exon Array Design Strategy



- Goal: 1 probeset per exon in genome.
- 4 Perfect match probes per probeset (no mismatch probes)
 - Average of 66 probes per RefSeq transcript
- New assay using random primers to label entire transcript



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Array Statistics

	Human Exon 1.0 ST Exon Array	Human Genome U133 2.0 Plus Array
Chips	1	1
Probes	5.3 million	1.3 million
Probe sets	1.4 million	54,000
Exon clusters	> 1 million	N/A
Probes per Probeset	4 PM Probes	11 PM/MM Probe Pairs
Background subtraction strategy	Median intensity of up to 1,000 background probes with the same GC content	Specific MM
Probeset Target	(sub) exon	3' 600 bp
Interrogated strand	Sense	Antisense
Feature Size	5 micron	11 micron

Looking at Alternative Splicing

- Between two groups of tissues: Splicing index
 - Splicing Index = Intensity of Probe Set / Intensity of Gene
 - Use t-test to see which exons are differentially included.

- Across groups of tissues: MIDAS

$$e_{i,k} = \mu \alpha_k \gamma_k \Rightarrow e_{i,k} / \gamma_k = \mu \alpha_k$$

$$\ln(e_{i,k}) - \ln(\gamma_k) = \ln(\mu) + \ln(\alpha_k)$$

where $e_{i,k}$ is the observed expression of exon i in sample k

μ is the ratio of the exon to the expression the gene if fully expressed

α_k is the fraction of expression level of the exon due to sample k

γ_k is expression of the gene in tissue k (estimated via Plier, should be robust)

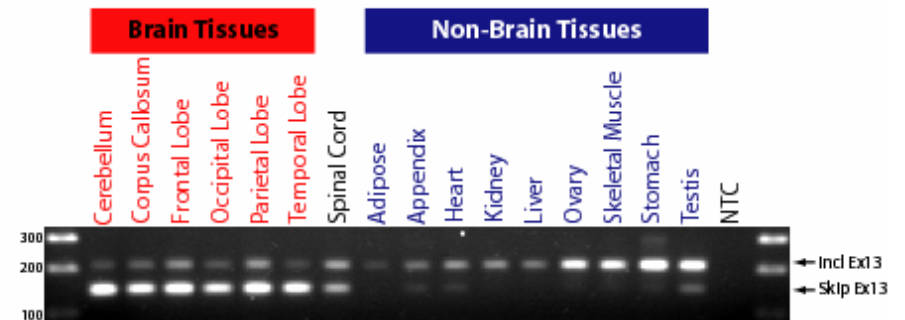
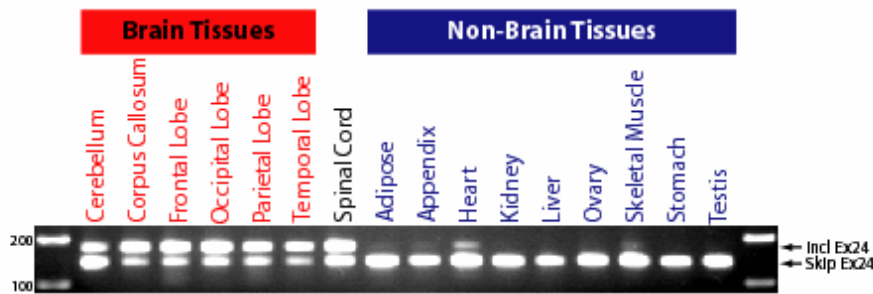
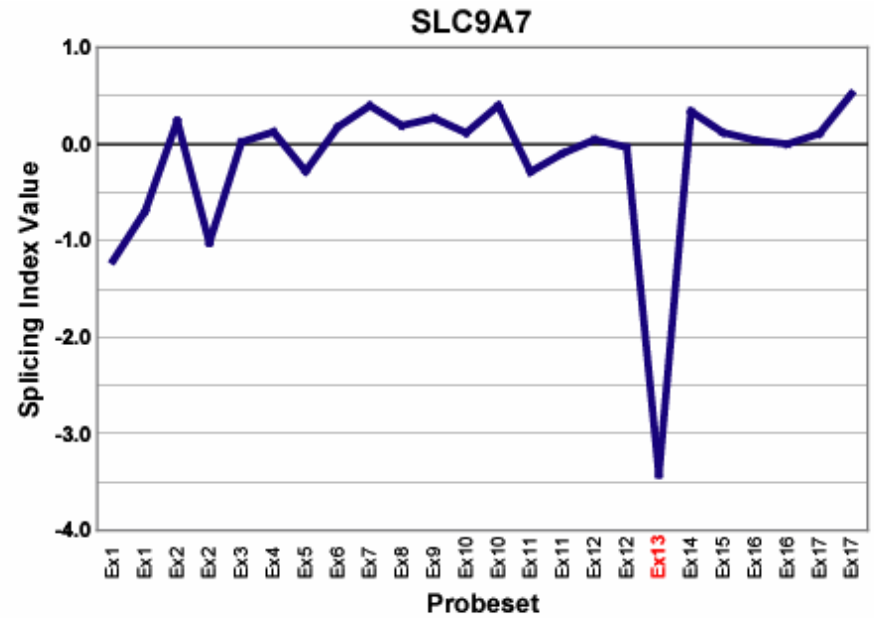
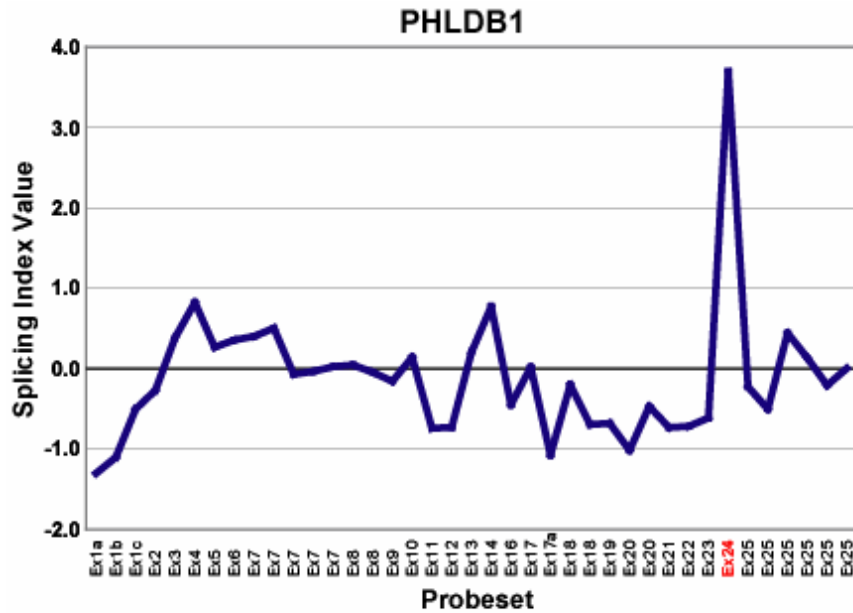
- Reduces to 1 way ANOVA :

For every k , $\ln(\alpha_k)$ should be 0 under H_0



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Splicing Index Examples



Credits:

- **Chip Design:**
 - Alan Williams, Raymond Wheeler,
- **Data Analysis:**
 - Jim Veitch, Yaron Turpaz, Melissa Cline, Hui Wang
- **Affy Labs Expression Research:**
 - Tyson Clark, Anthony Schweitzer
- **Steve Lincoln, John Blume**
- **Product Page:**
 - <http://www.affymetrix.com/products/arrays/specific/exon.affx>
- **Sample Data:**
 - http://www.affymetrix.com/support/technical/sample_data/exon_array_data.affx