

Genome Sciences Centre

BC Cancer Agency, Vancouver, BC, Canada

ALEXA- A microarray design platform for alternative expression analysis

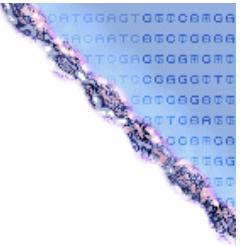
Malachi Griffith

Supervised by Marco A Marra

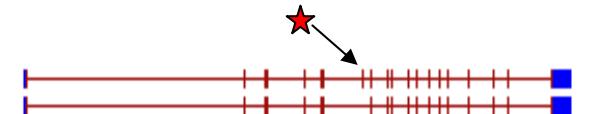
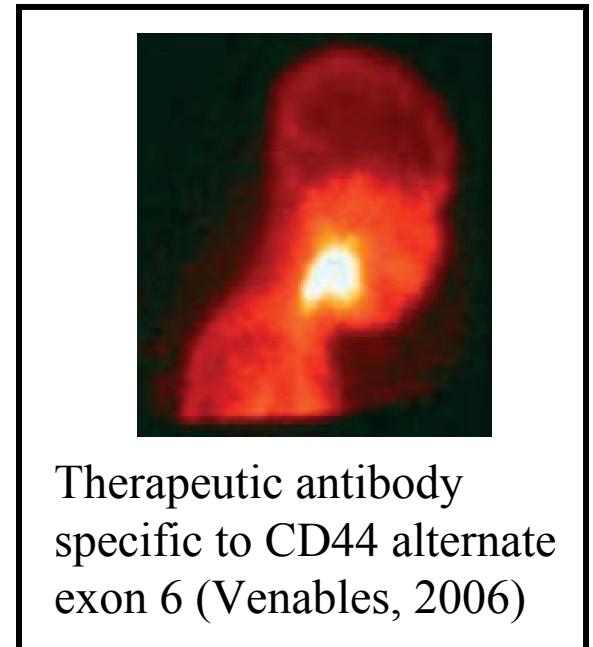
September 19 2007



Introduction



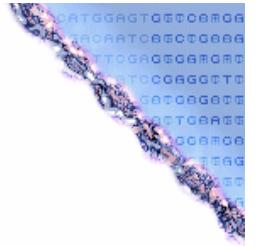
- Alternative expression generates multiple isoforms from most human loci
 - Alternative transcript initiation, splicing, and polyadenylation
- Specific isoforms may represent useful therapeutic targets or diagnostic markers
- Affymetrix Exon microarrays[†] and our own 'ALEXA' microarrays[†] are two methods for identifying such events



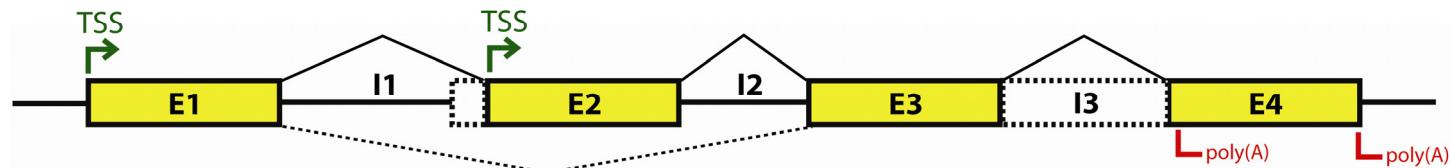
[†]Affymetrix GeneChip® Human Exon 1.0 ST vs. ALEXA arrays synthesized by NimbleGen Systems



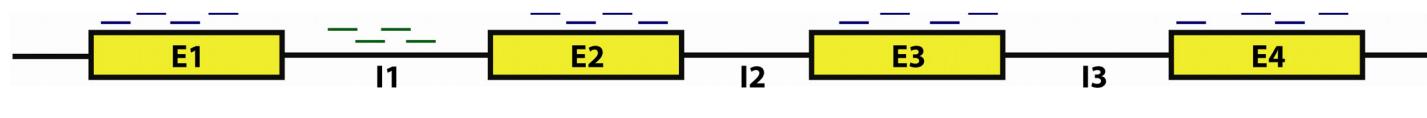
Affymetrix versus ALEXA approach



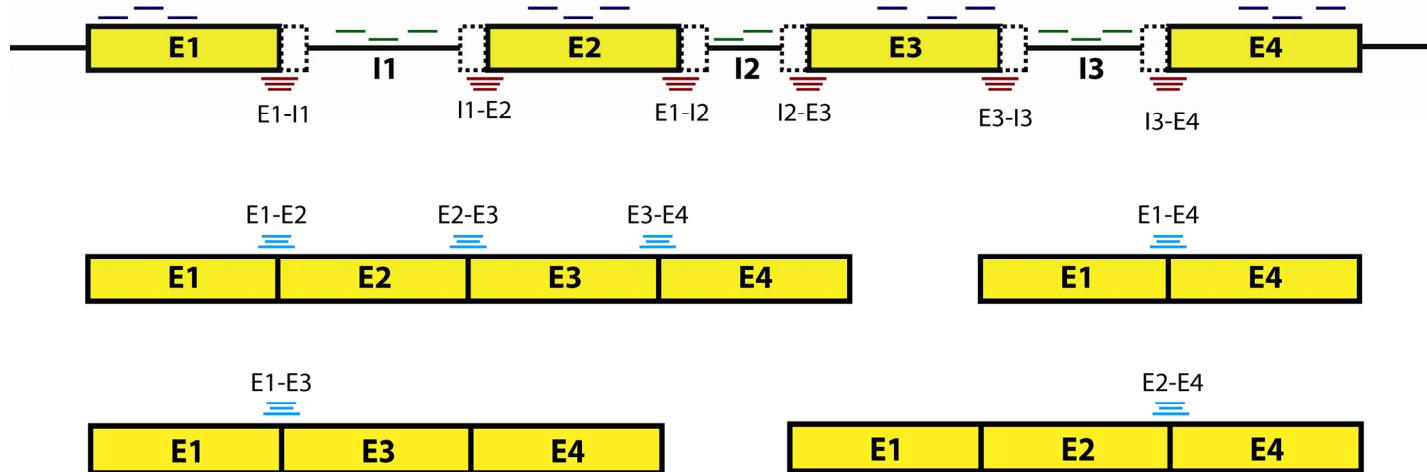
a. Alternative transcription



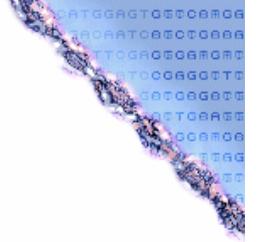
b. Affymetrix array design



c. ALEXA array design



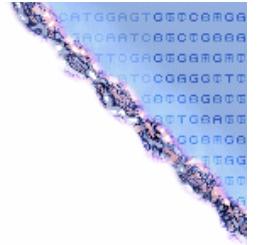
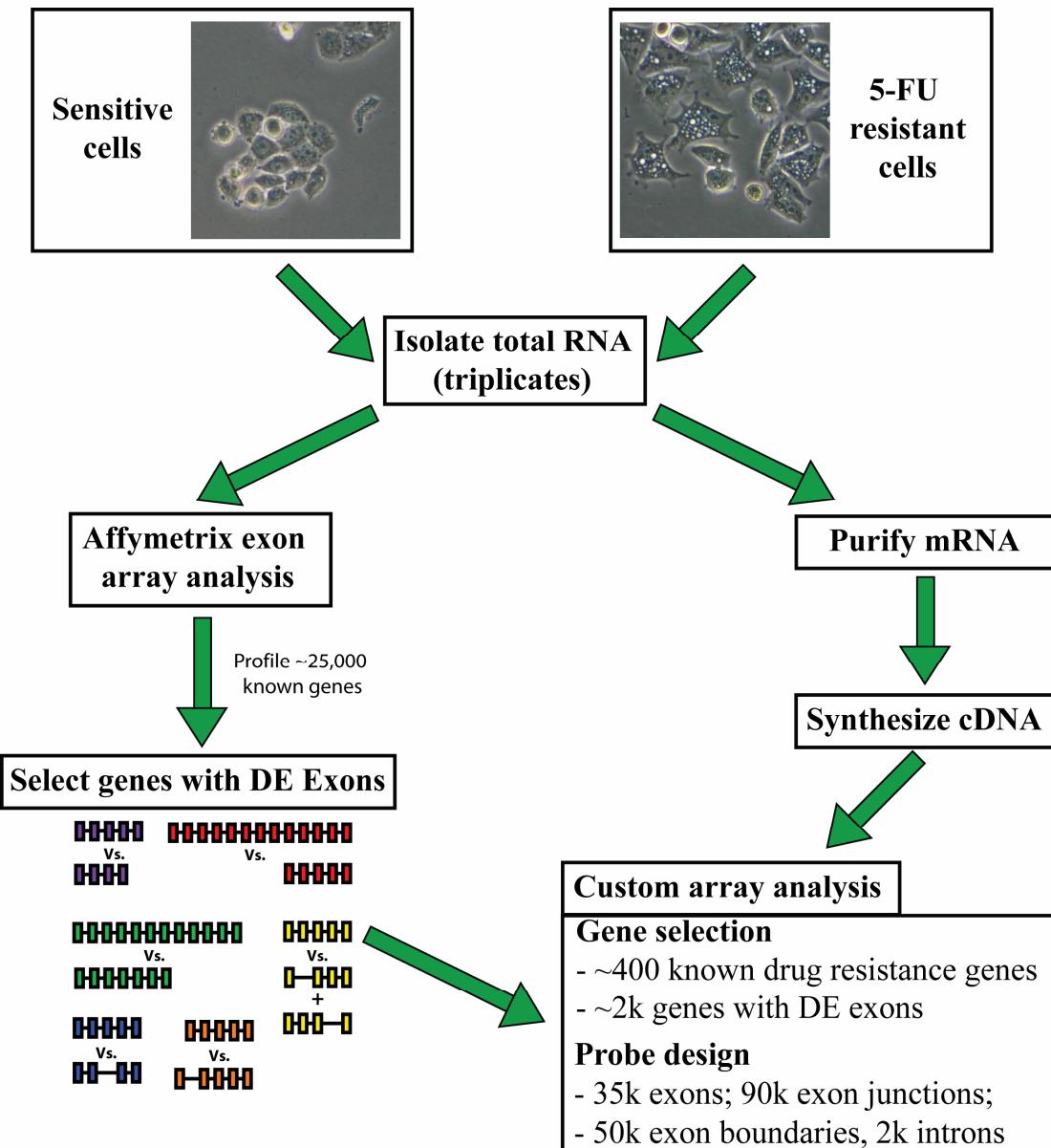
The ALEXA platform



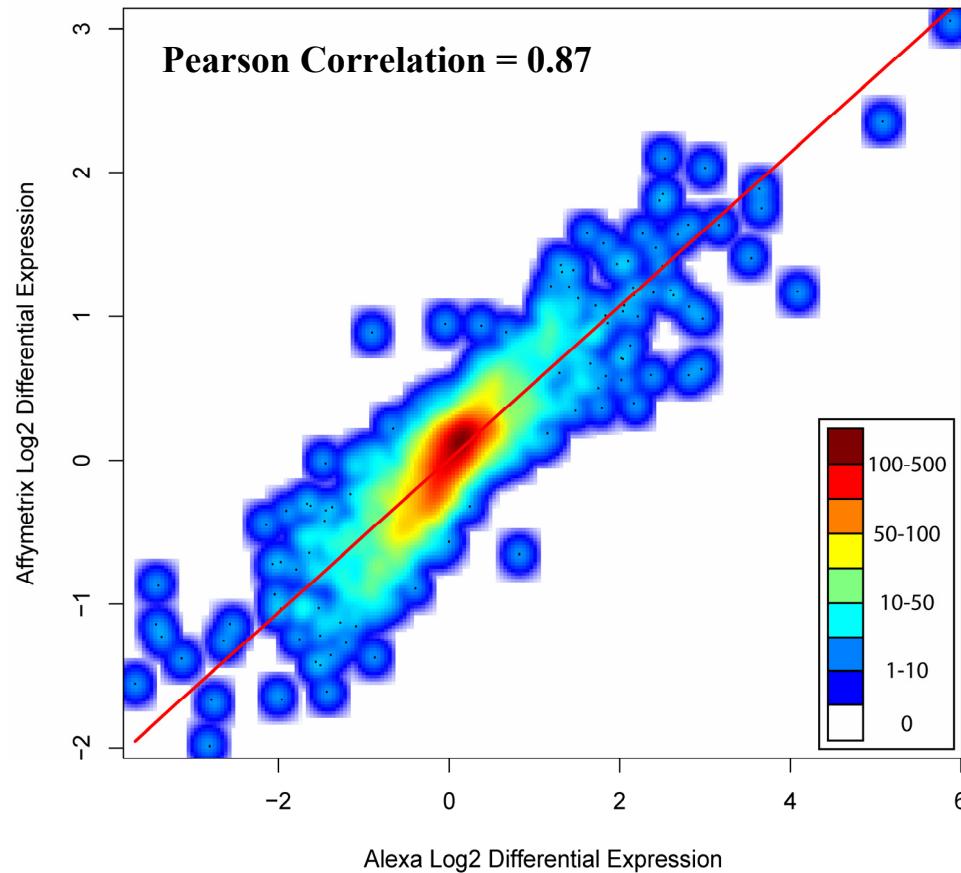
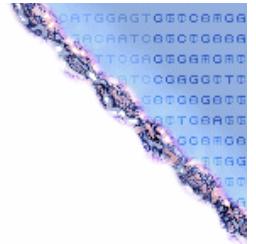
- Array design pipeline
 - Implemented in MySQL, Perl and R
 - Probe extraction, quality scoring/filtering and annotation
- Pre-computed designs for ten genomes
 - ~100 million probes total
 - Yeast, *C elegans*, Zebrafish, Fruit Fly, Chicken, Dog, Rat, Mouse, Chimp and Human
- Availability
 - Open source
 - Virtual Machine Appliance
 - www.AlexaPlatform.org



Validation of ALEXA platform



Differential gene expression results (ALEXA vs. Affymetrix)

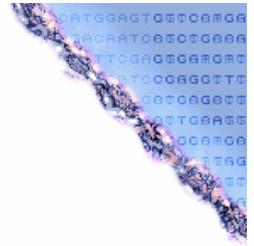


- **233** of ~2,500 genes have 2-fold change or greater
- **50** genes have a 4-fold change or greater
 - Enriched for genes involved in: ‘response to drug’, transport of phosphates, lipids and anions
- DE of known and novel isoforms
 - alternate transcription initiation, splicing and polyadenylation

$$\text{DE} = \log_2(\text{Sensitive}) - \log_2(\text{Resistant})$$



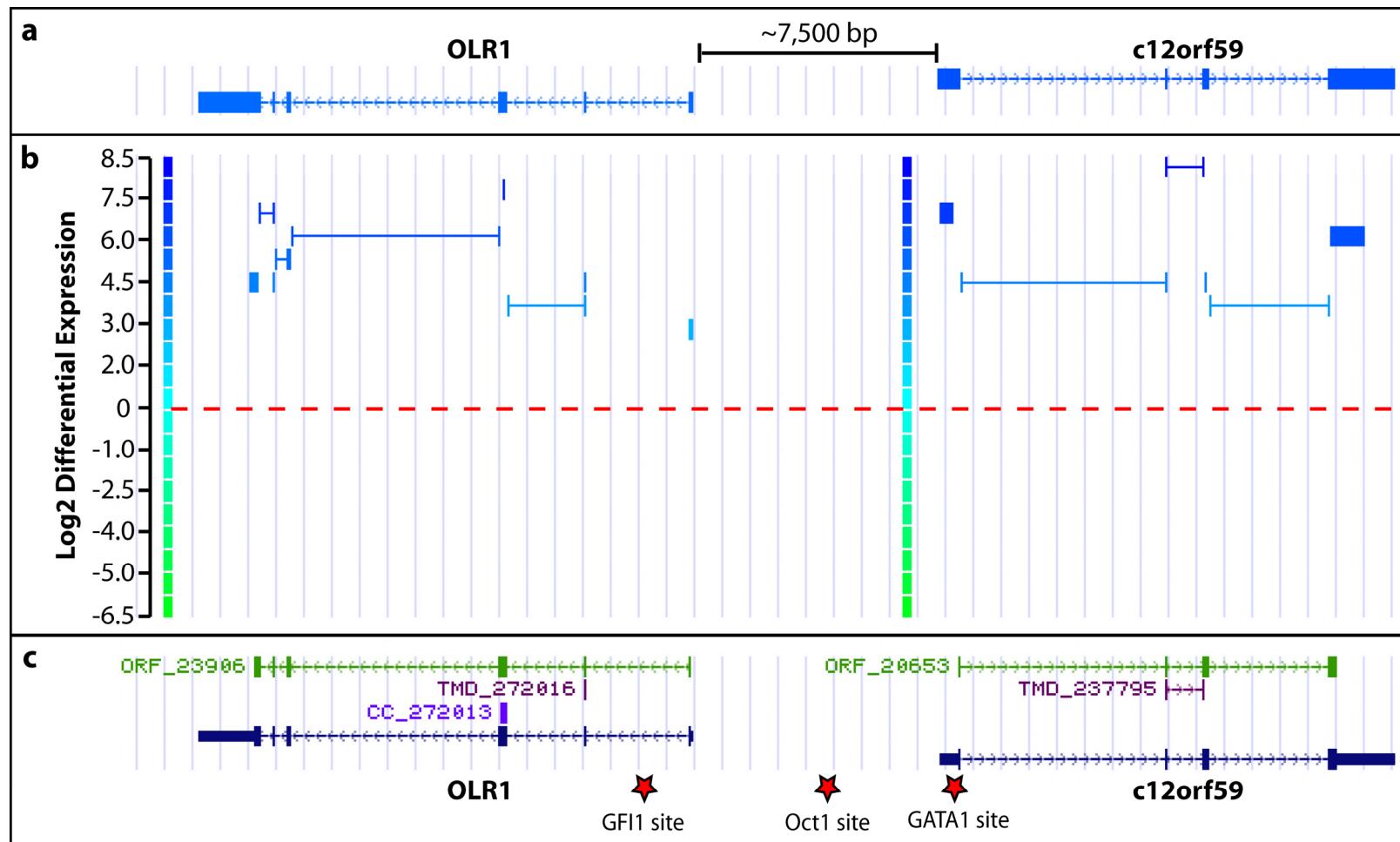
Summary of DE events



Platform	DE event type	Total events profiled	<u>Significant</u> DE events
Affymetrix	Gene-level	2,507	78
	Exon	49,681	1117
	Intron	65,327	25
	Total	117,515	1,220
ALEXA	Gene-level	2,509	233
	Exon	32,164	2,703
	Canonical junction	27,046	2,310
	Exon skip	69,761	191
	Exon boundary	52,402	253
	Intron	472	0
	Total	184,354	5690

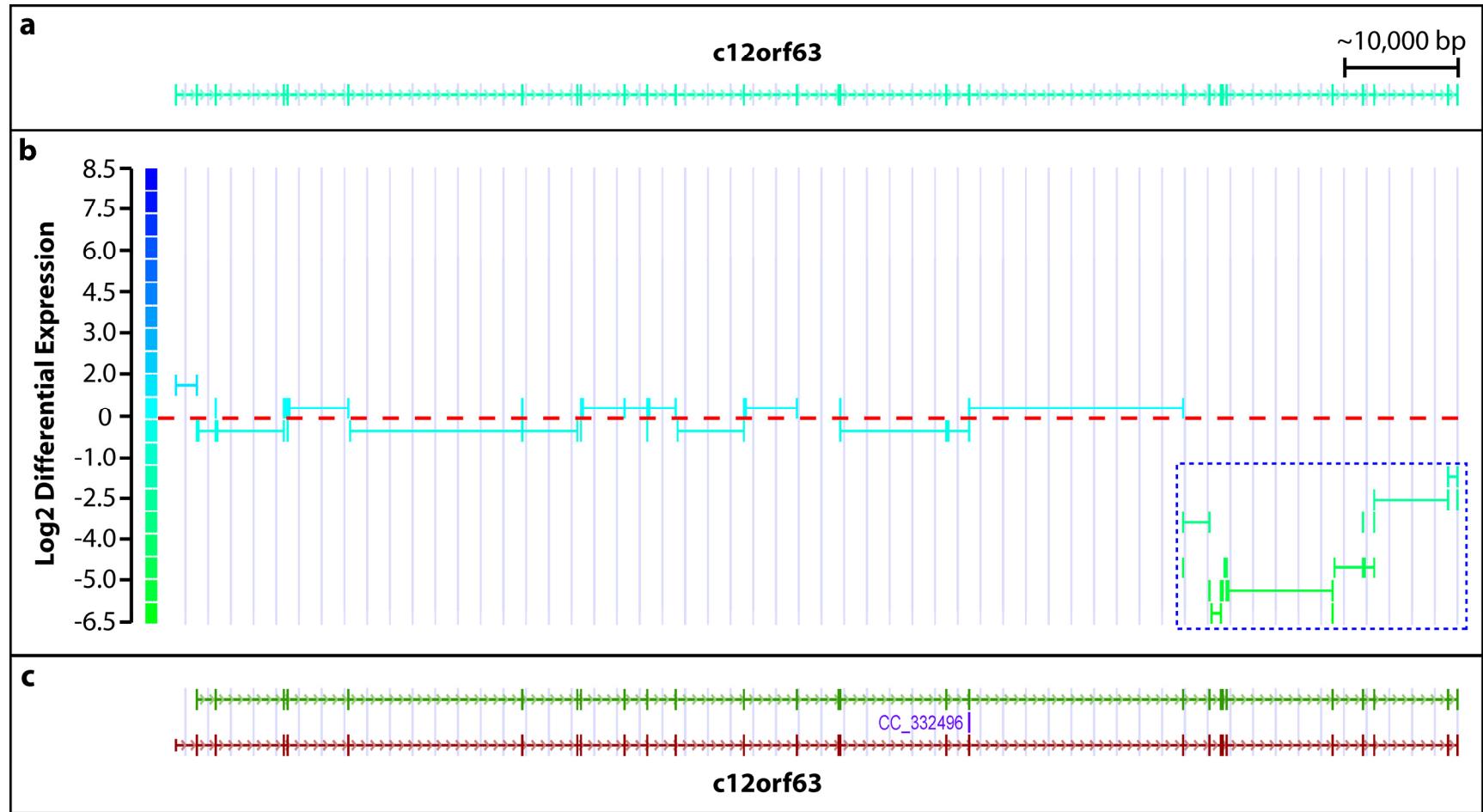
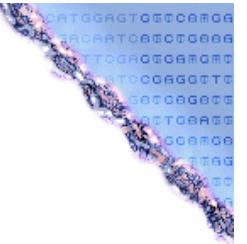


Example of a differentially expressed locus[†]



[†]Data shown is from ALEXA microarrays synthesized by NimbleGen Systems Inc.

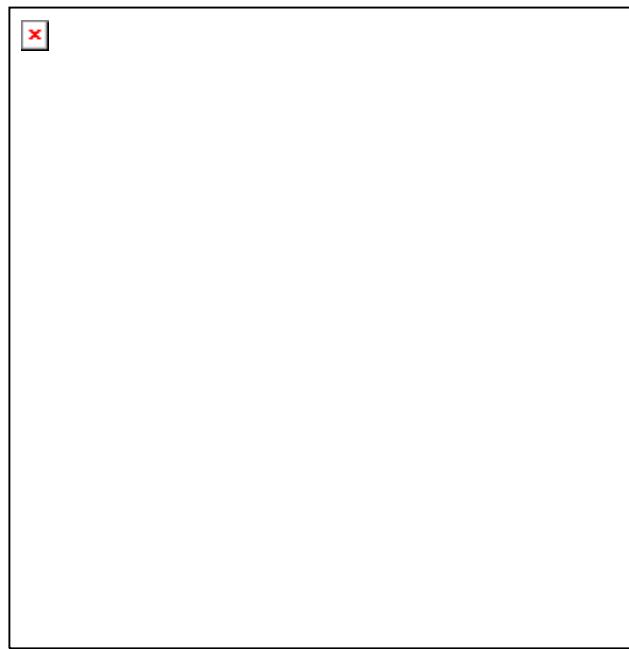
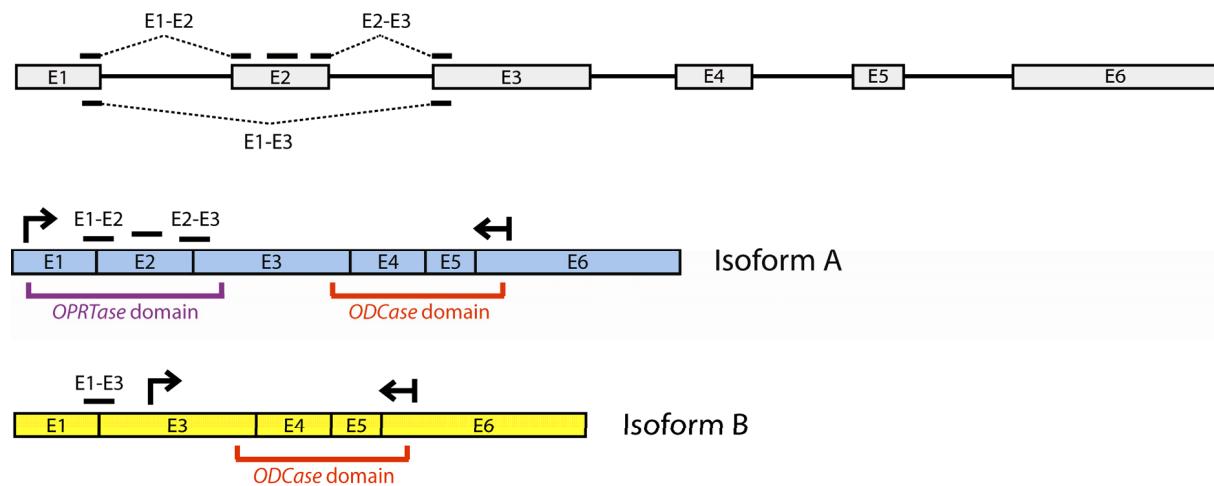
Example of a differentially expressed novel 3' isoform[†]



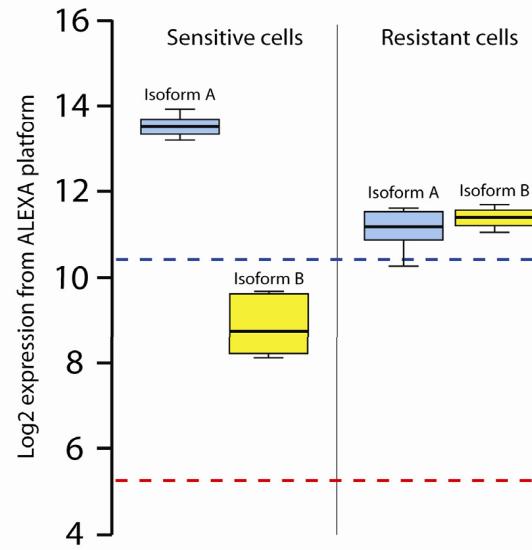
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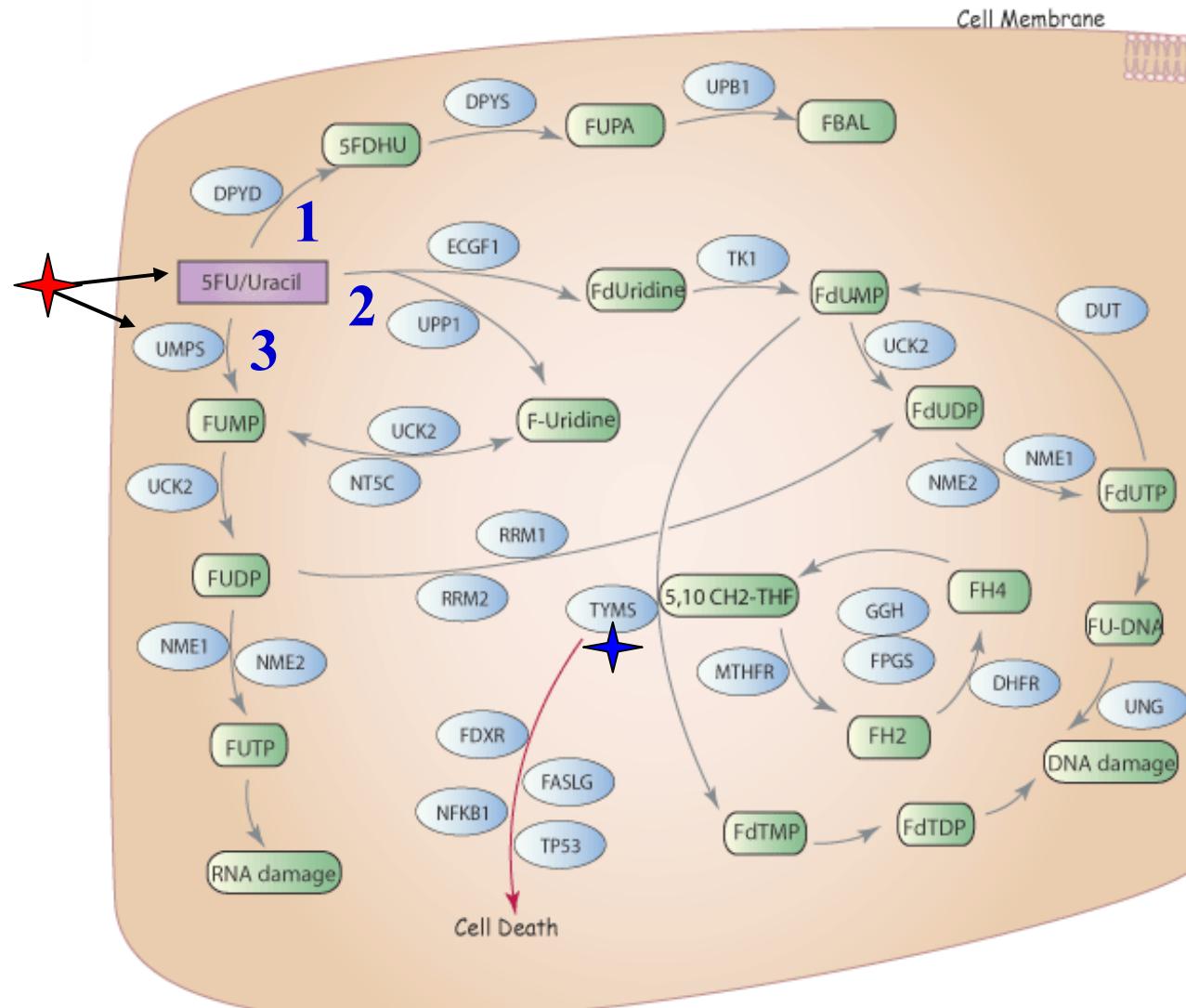
UMPS probesets and isoforms



ALEXA microarray data

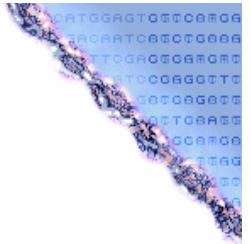


5-Fluorouracil Metabolic Pathway



Pathway diagram from PharmGKB

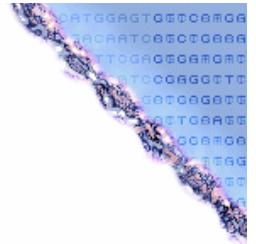
Conclusions



- The ALEXA platform
 - First open source ‘splicing’ microarray design platform
 - Compatible with any EnsEMBL annotated genome (tested on 10 species)
 - www.AlexaPlatform.org
- Experimental validation
 - Performance was comparable or superior to Affymetrix exon arrays for every metric examined
 - Sensitivity/Specificity, differential expression of exons and genes, etc.
 - Provides additional information on the connectivity and boundaries of exons
- Biological application
 - Differential expression of novel and known isoforms associated with 5-FU resistance in colorectal cancer cell lines
- Why use custom microarrays?



Acknowledgements



Supervisor

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Committee

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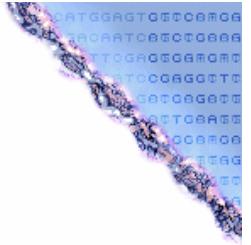
Gastrointestinal Cancer Group

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Affymetrix Group

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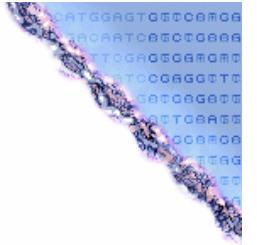
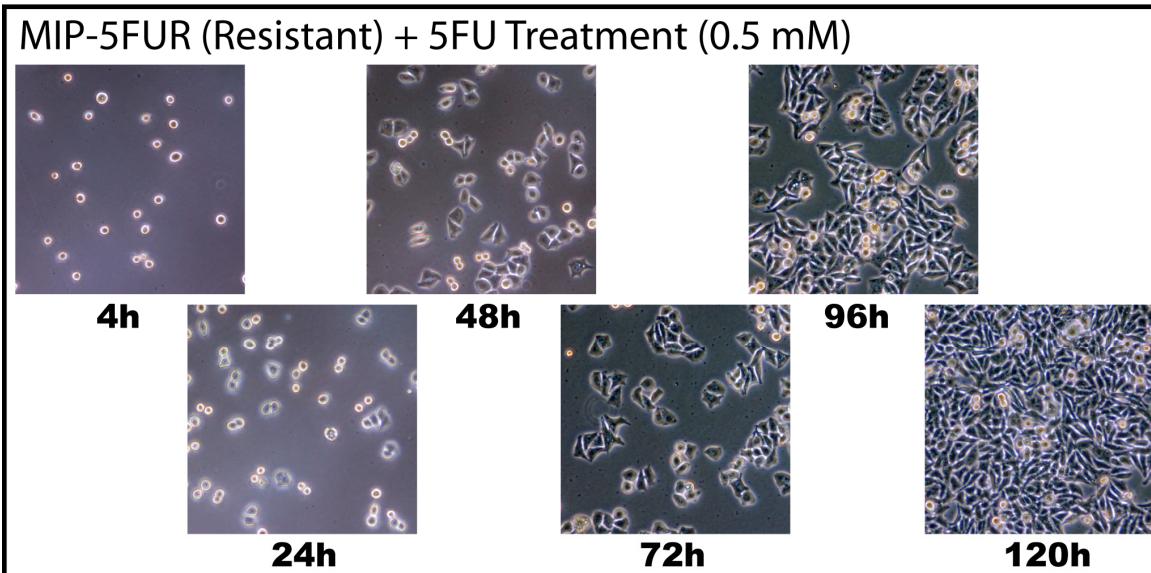
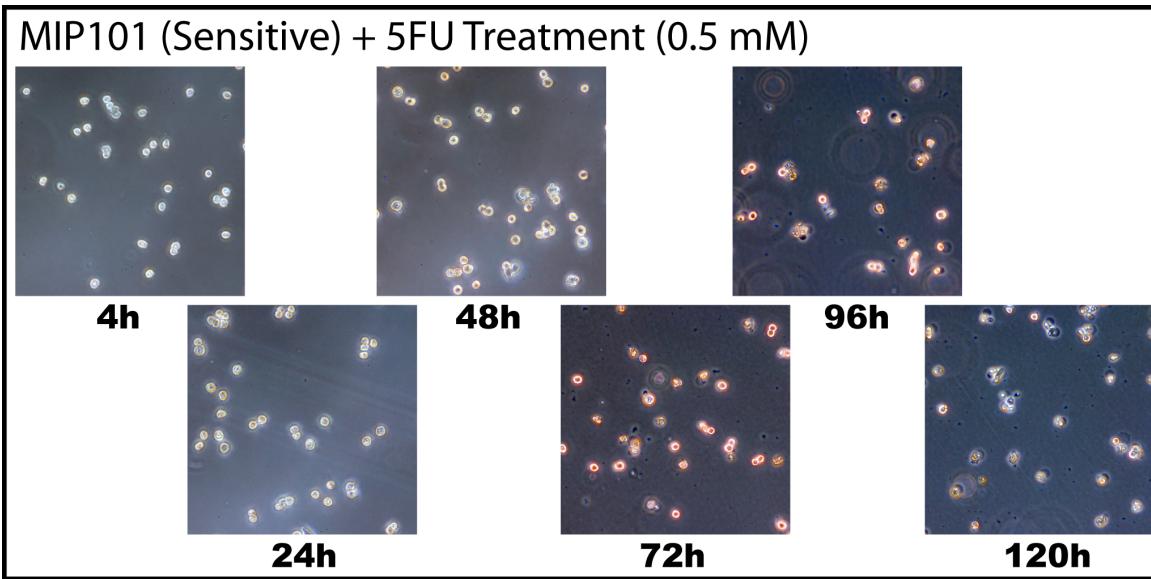
Supplementary slides



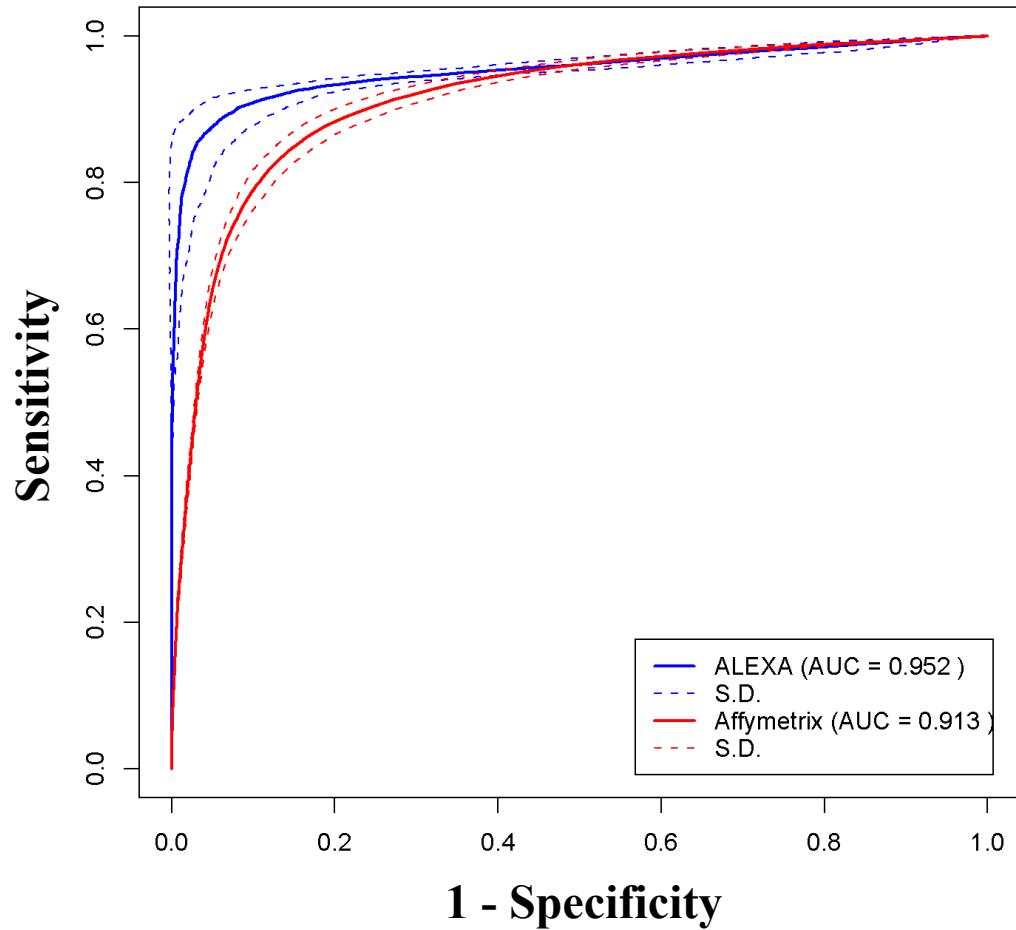
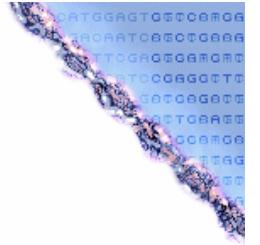
1. Experimental illustration of sensitivity and resistance to 5-FU in colorectal cancer cell lines
2. ROC curves comparing the sensitivity and specificity of Affymetrix and ALEXA microarray platforms
3. Overlap between Affymetrix and ALEXA microarray platforms for genes and exons identified as differentially expressed
4. Absolute expression values for probes specific to alternate isoforms of UMPS in 5-FU sensitive and resistant cells



Test of sensitivity/resistance



Comparison of ALEXA and Affymetrix exon microarray performance

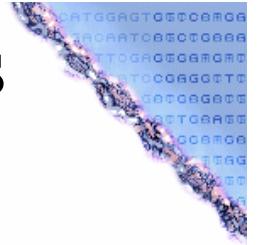


- **ROC curves**
 - Generated from expression values for +ve (exonic) & -ve (intronic) control probes of 100 housekeeping genes
- **Max. Specificity/Sensitivity**
 - ALEXA: 94.8% & 87.9%
 - Affymetrix: 85.8% & 84.2%
- **Signal-to-Noise ratio**
 - ALEXA: 56.0 ± 2.3 SD
 - Affymetrix: 20.9 ± 0.42 SD

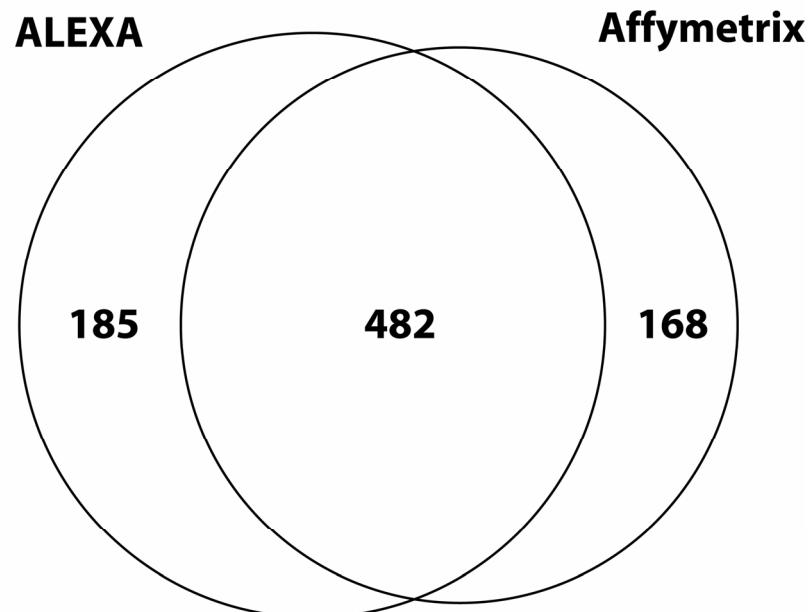
AUC: Area under curve; SD: standard deviation



Overlap between platforms for genes and exons identified as differentially expressed

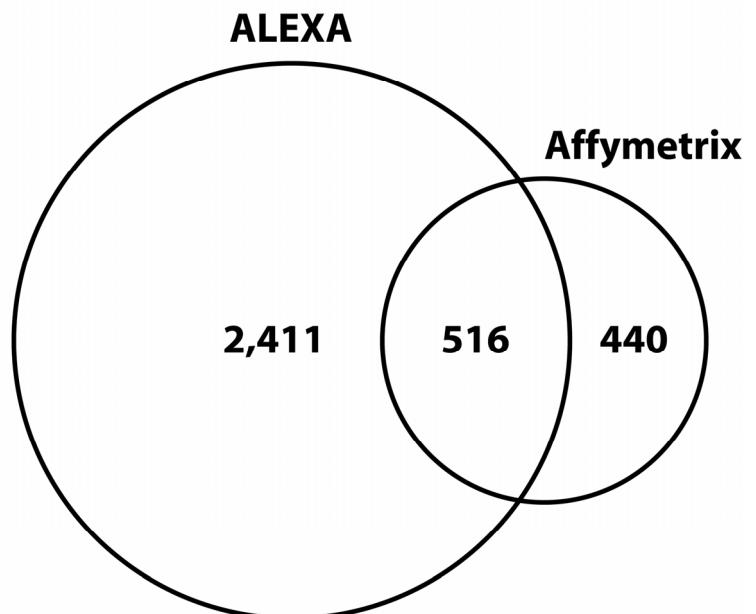


a Gene DE Overlap



835 of 2,473 genes profiled by both platforms were detected as DE (by either or both platforms)

b Exon DE Overlap



3,367 of 31,368 exons profiled by both platforms were detected as DE (by either or both platforms)

Absolute expression level of UMPS isoforms in both cell lines

