



Genome Sciences Centre

BC Cancer Agency, Vancouver, BC, Canada

ALEXA – a microarray design platform for alternative expression analysis.

Malachi Griffith

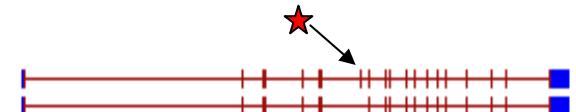
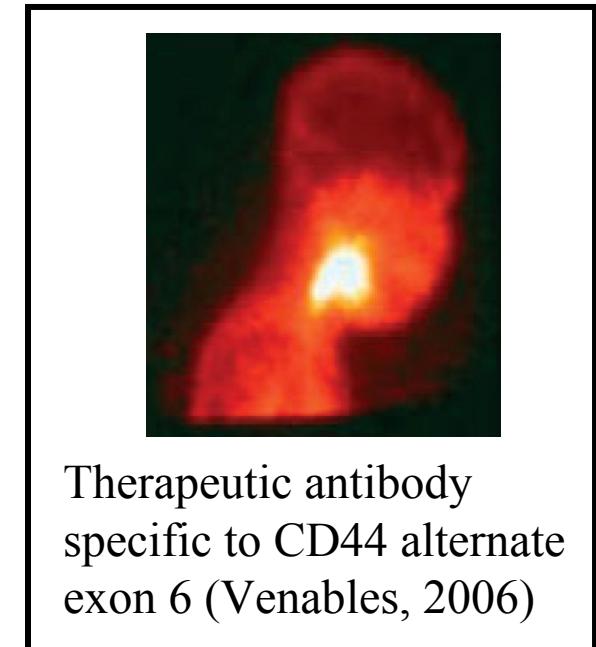
Supervised by Marco A Marra

April 10 2008



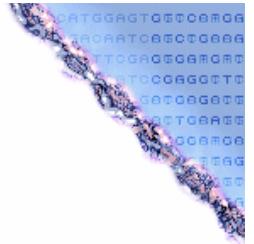
Transcript variants are a feature of many cancers

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

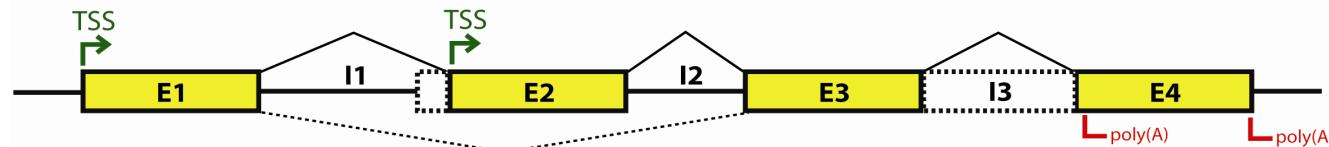


[†]Affymetrix GeneChip® Human Exon 1.0 ST vs. ALEXA arrays synthesized by NimbleGen Systems

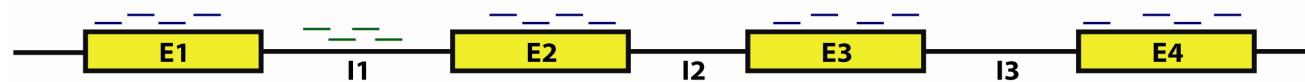
‘Splicing’ microarrays are an evolution of traditional gene expression arrays



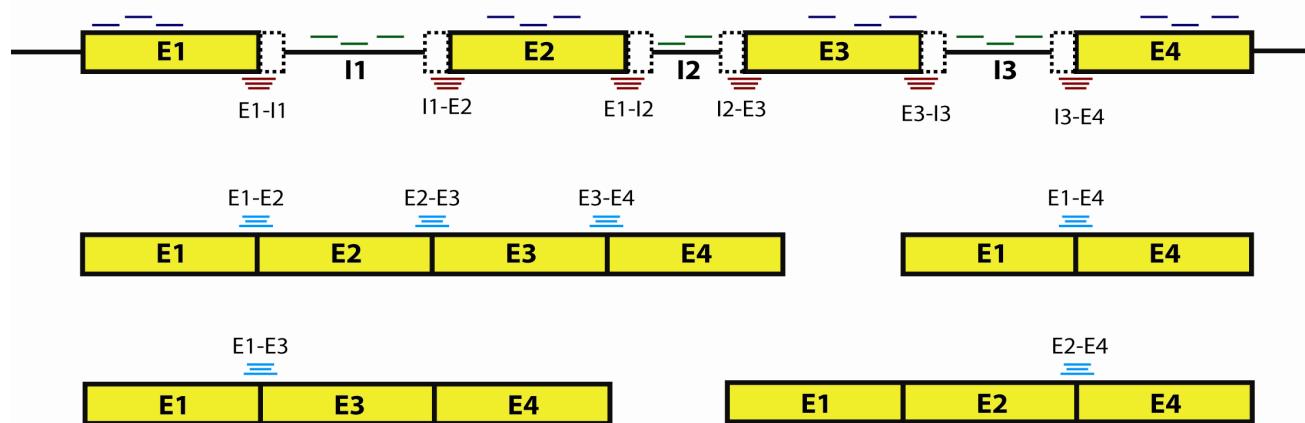
a. Alternative expression



b. Affymetrix array design



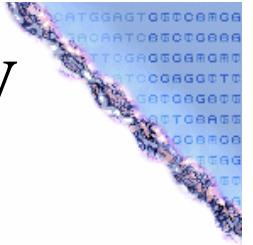
c. ALEXA array design



www.AlexaPlatform.org



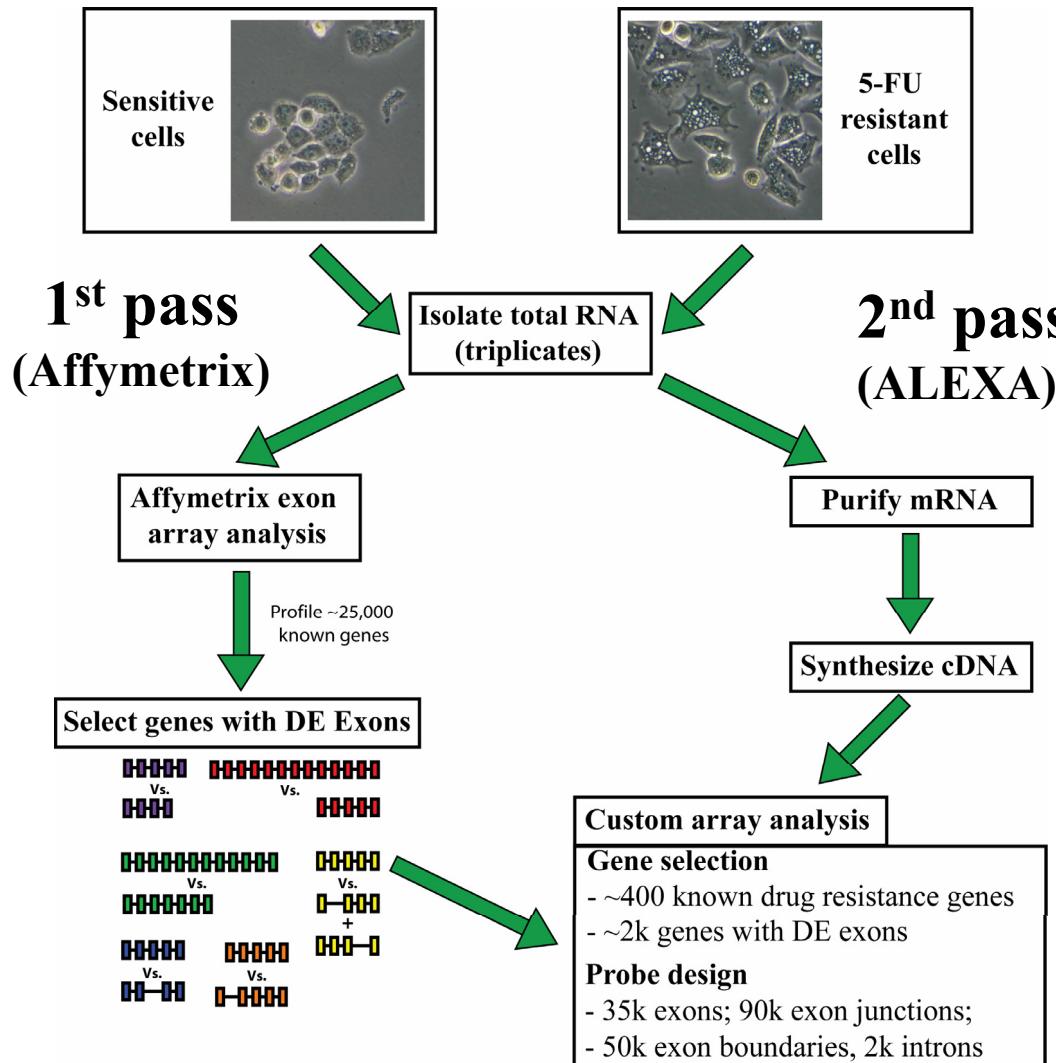
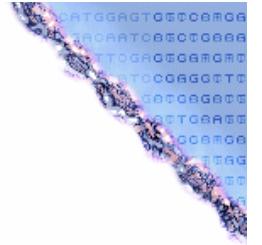
The ALEXA platform was created to allow the design of custom splicing microarrays



- 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
 - Griffith et al. 2008. *ALEXA – A microarray design platform for alternative expression analysis*. Nature Methods. Feb. 5(2):118

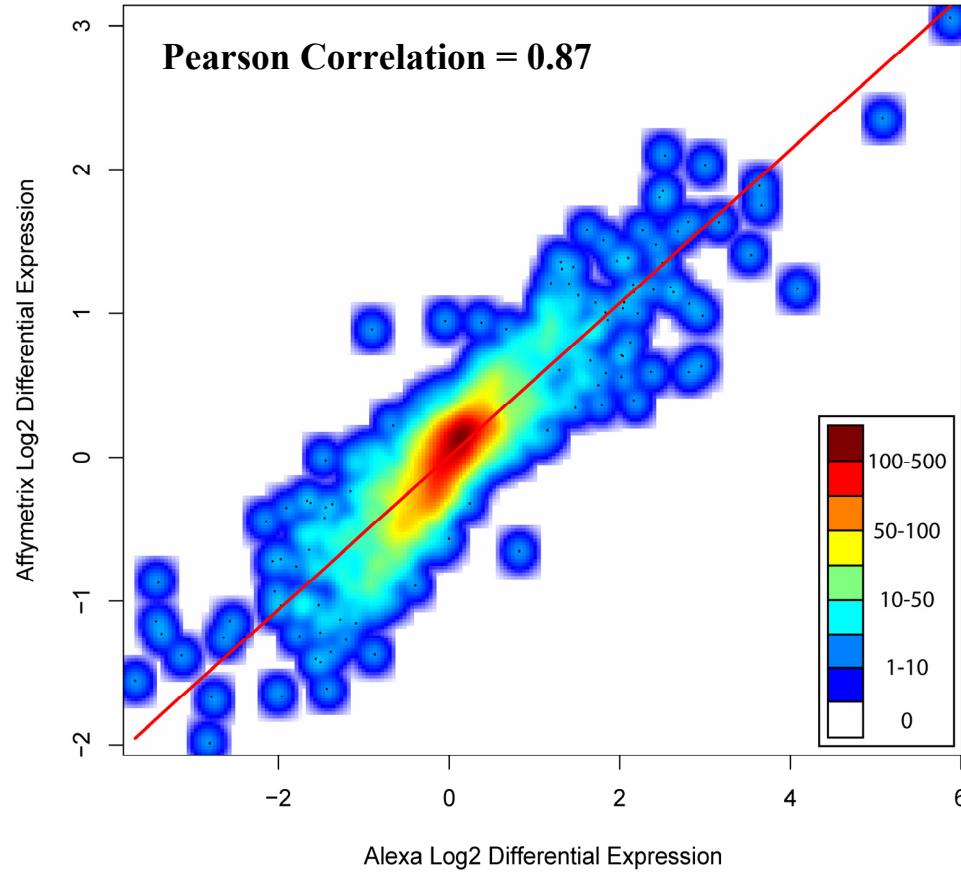


A proof-of-concept validation of the ALEXA approach was successful



www.AlexaPlatform.org

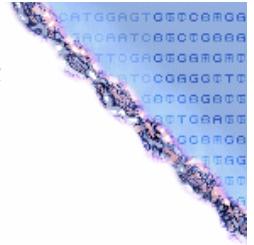
Both platforms identify differentially expressed genes



- 233 of ~2,500 genes have 2-fold change or greater
- 50 genes have a 4-fold change or greater
- DE of known and novel isoforms
 - alternate transcription initiation, splicing and polyadenylation

Griffith et al. 2008. ALEXA – A microarray design platform for alternative expression analysis. Nature Methods. Feb. 5(2):118

ALEXA provides additional information on the structure of expressed transcripts

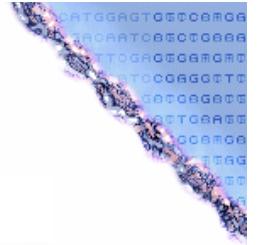


Platform	DE event type	Total events profiled	<u>Significant DE events</u>
Affymetrix	Gene-level	2,507	78
	Exon	49,681	1117
	Intron	65,327	25
	Total	117,515	1,220
ALEXA	Gene-level	2,507	233
	Exon	32,164	2,703
	Canonical junction	27,046	2,310
	Exon skip	69,761	191
	Exon boundary	52,402	253
	Intron	472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

Griffith et al. 2008. ALEXA – A microarray design platform for alternative expression analysis. Nature Methods. Feb. 5(2):118

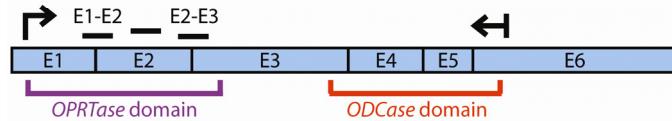


UMPS isoforms are DE in resistant cells

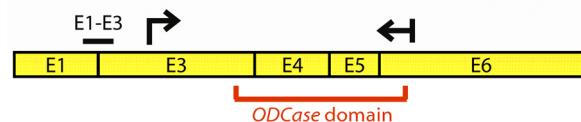


UMPS isoforms, protein domains, probesets

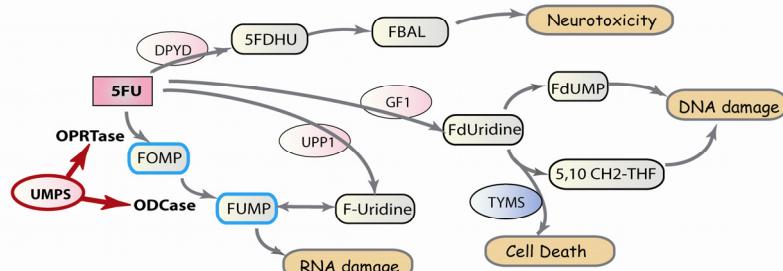
Isoform A



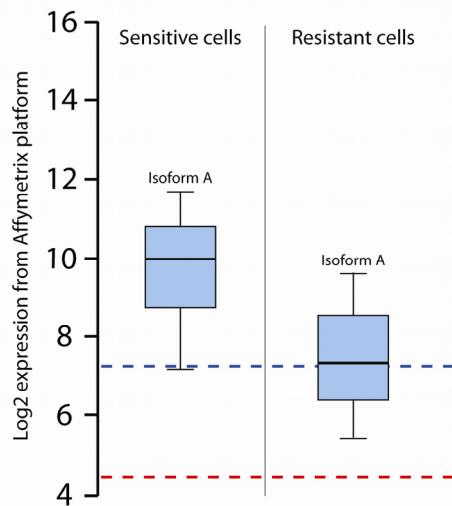
Isoform B



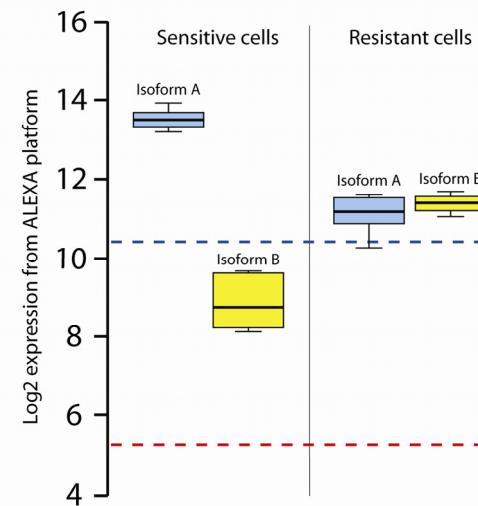
5-FU metabolism pathway



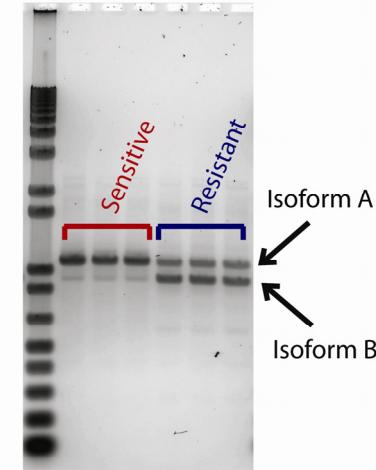
Affymetrix microarray data



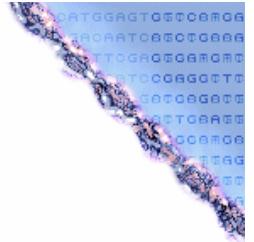
ALEXA microarray data



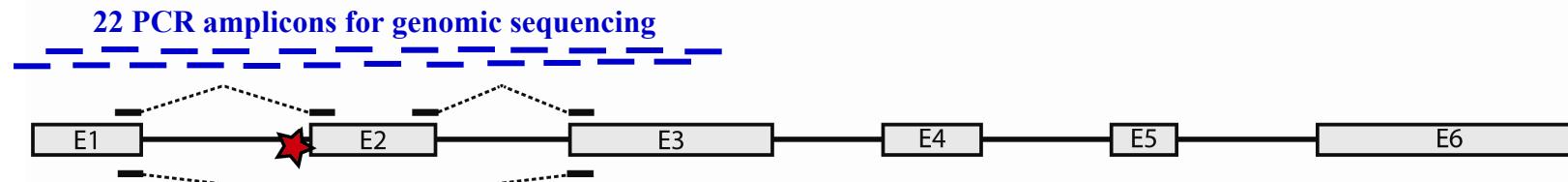
RT-PCR data



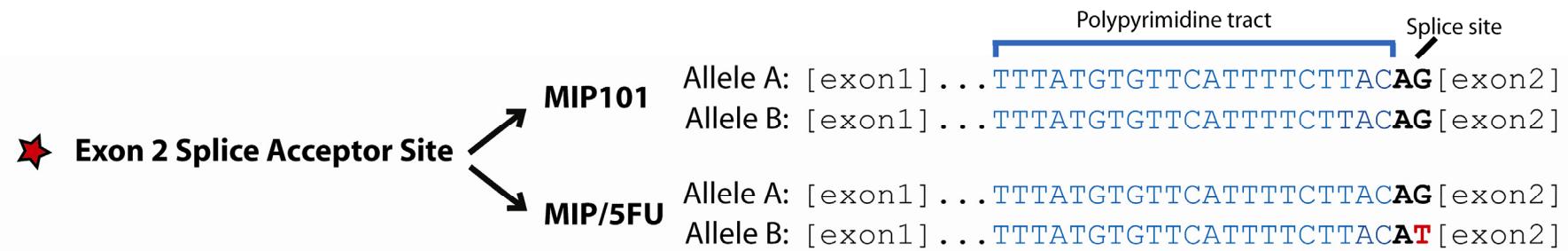
Differential expression of *UMPS* isoforms is likely caused by a splice site mutation



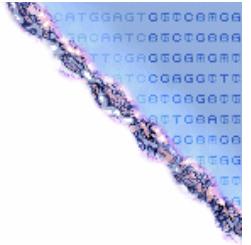
UMPS gene model showing alternative splicing of exon 2



Genomic sequence data



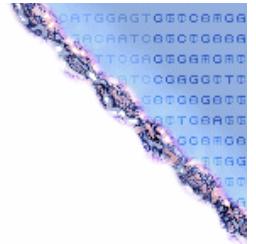
Conclusions



- The ALEXA platform
 - First publicly available ‘splicing’ microarray design platform
 - www.AlexaPlatform.org
- Experimental validation
 - Performance was comparable or superior to Affymetrix exon arrays for every metric examined
 - Provides additional information on the connectivity and boundaries of exons
 - Comparison to Illumina/Solexa whole transcriptome shotgun
- Biological application
 - Differential expression of novel and known isoforms associated with 5-FU resistance in colorectal cancer cell lines
 - Follow up validation for *UMPS* and other candidates
 - Pathway analysis, mechanism, functional assays, clinical study



Acknowledgements



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Gastrointestinal Cancer Group

- Isabella Tai, Michelle Tang

Gene Expression Group

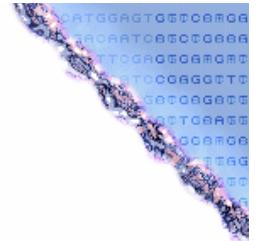
- Agnes Baross, Susanna Chan,
Jennifer Asano, Adrian Ally

Sample Preparation & Validations

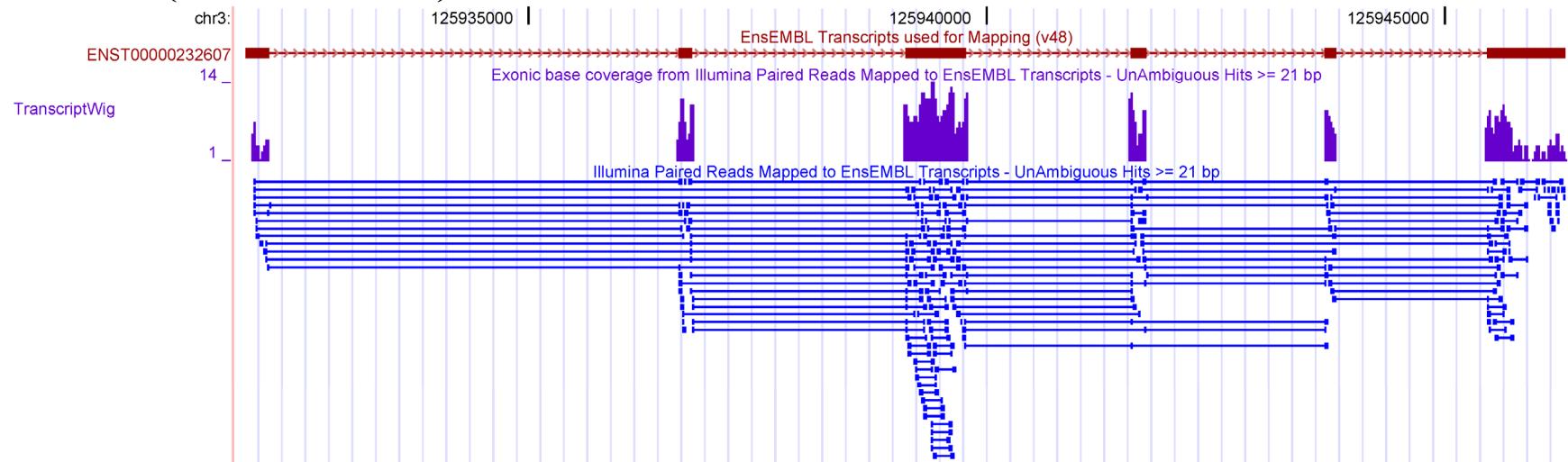
- Martin Hirst, Thomas Zeng,
Trevor Pugh, Tesa Severson,
Jaswinder Khattri



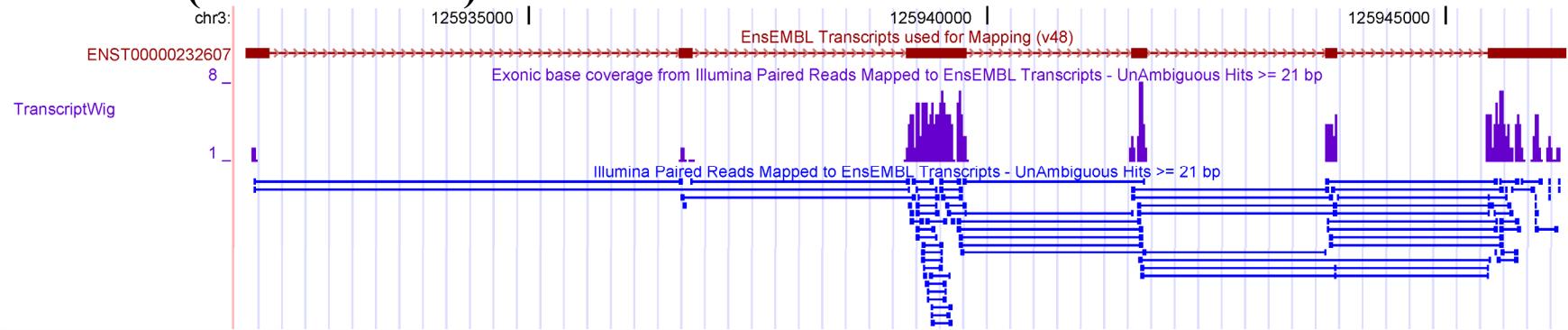
Example of Illumina transcriptome shotgun data (*UMPS* locus)



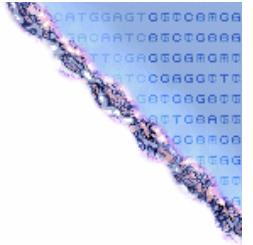
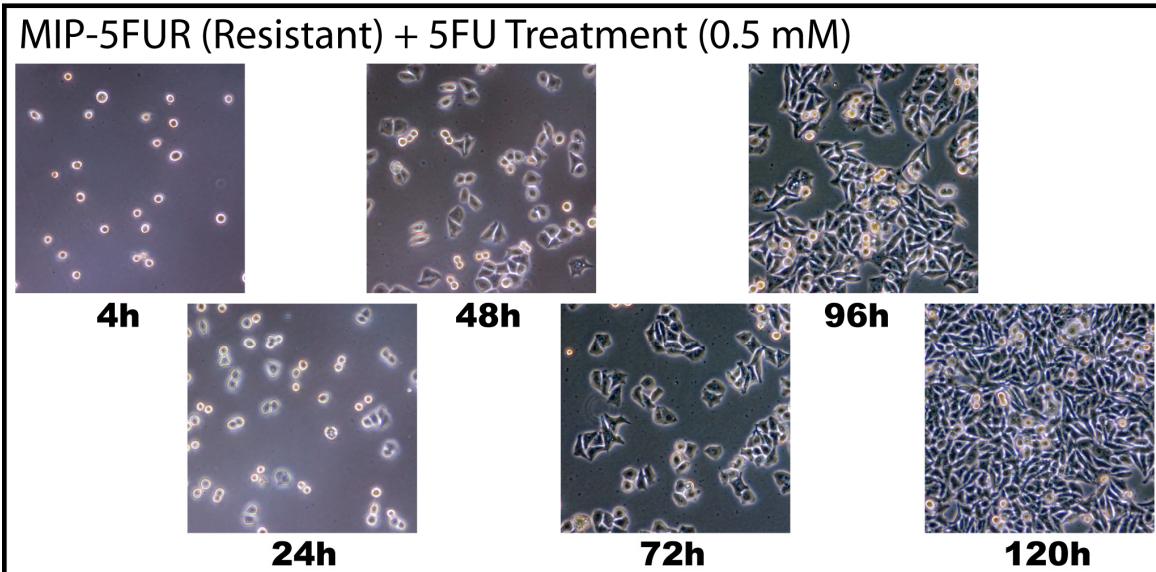
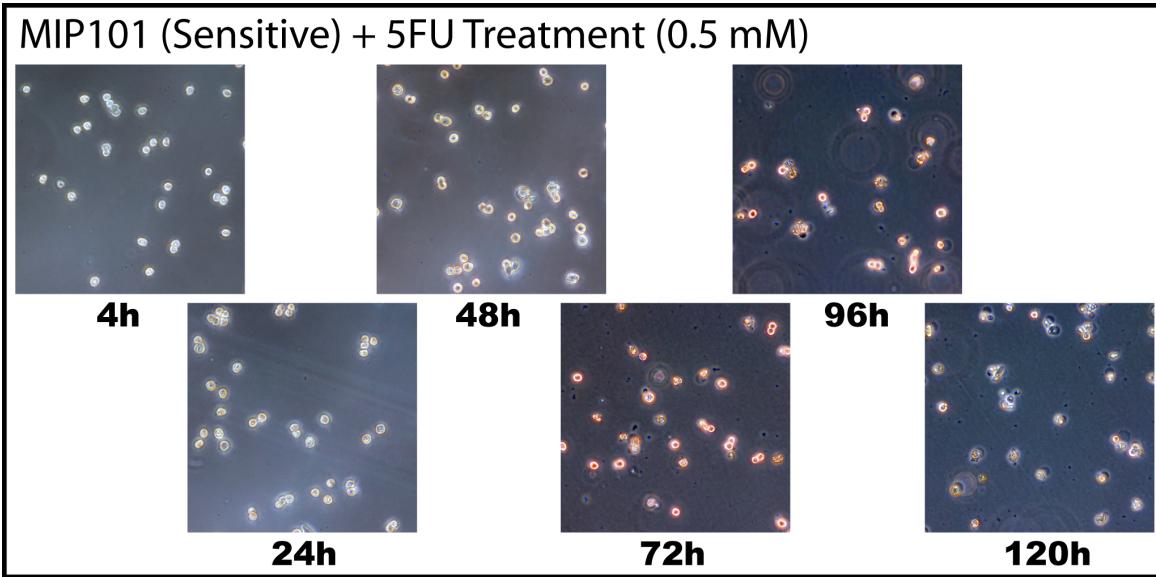
MIP101 (5-FU sensitive) – 1 lane of data



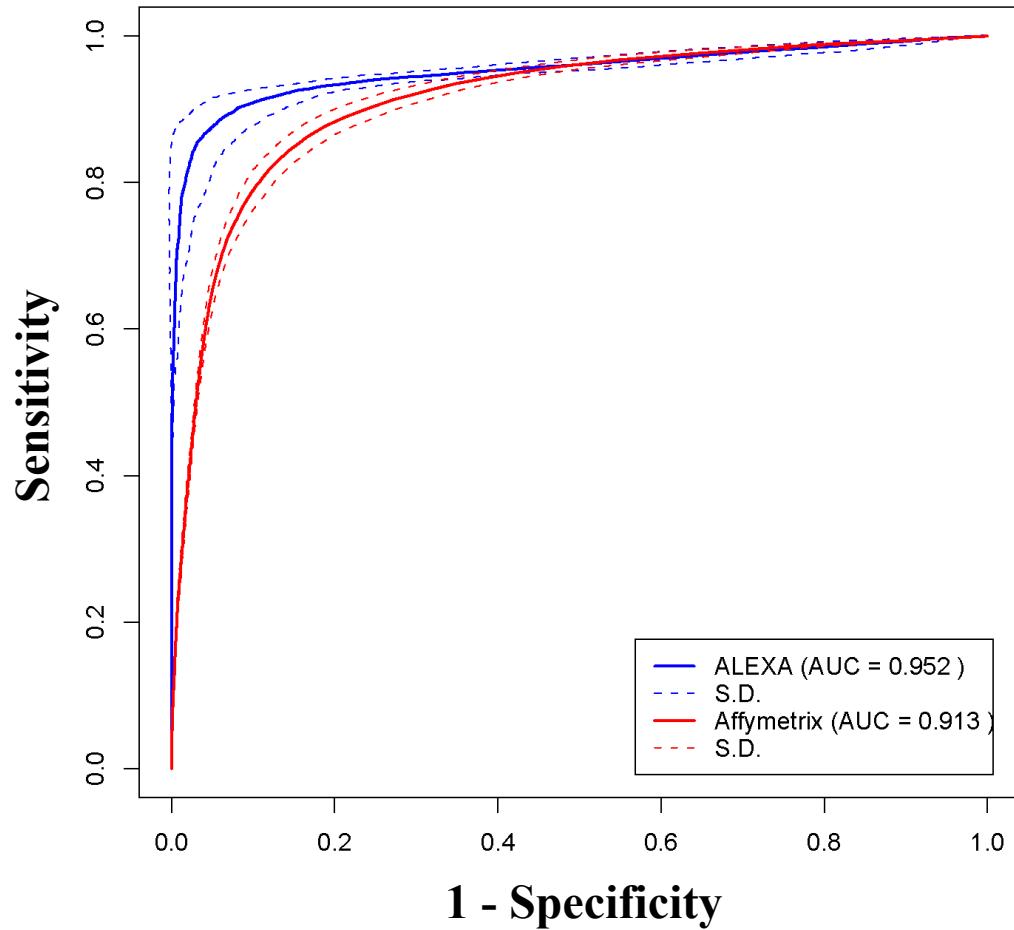
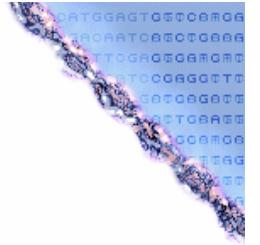
MIP/5FU (5-FU resistant) – 1 lane of data



Test of sensitivity/resistance



Comparison of ALEXA and Affymetrix exon microarray performance

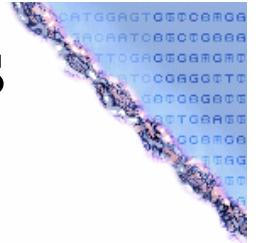


- **ROC curves**
 - Generated from expression values for +ve (exonic) & -ve (intronic) control probes of 100 housekeeping genes
- **Max. Specificity/Sensitivity**
 - ALEXA: 94.8% & 87.9%
 - Affymetrix: 85.8% & 84.2%
- **Signal-to-Noise ratio**
 - ALEXA: 56.0 ± 2.3 SD
 - Affymetrix: 20.9 ± 0.42 SD

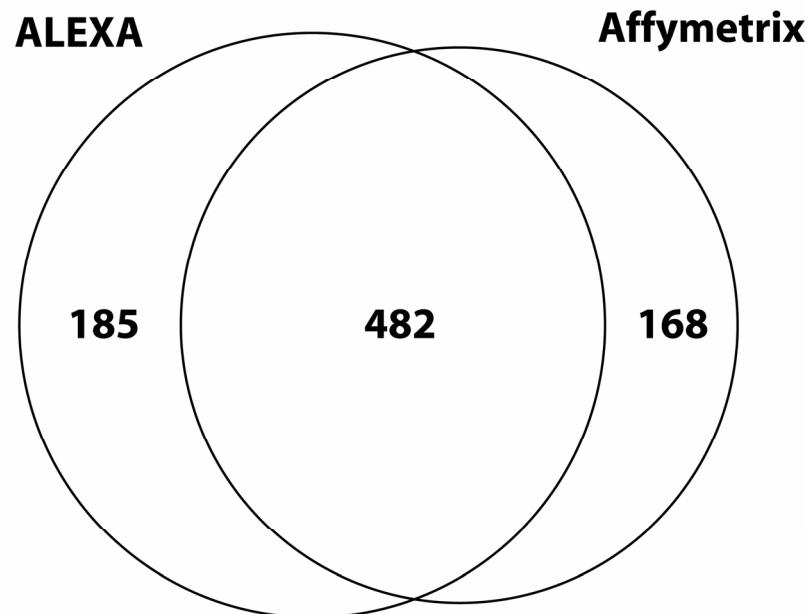
AUC: Area under curve; SD: standard deviation



Overlap between platforms for genes and exons identified as differentially expressed

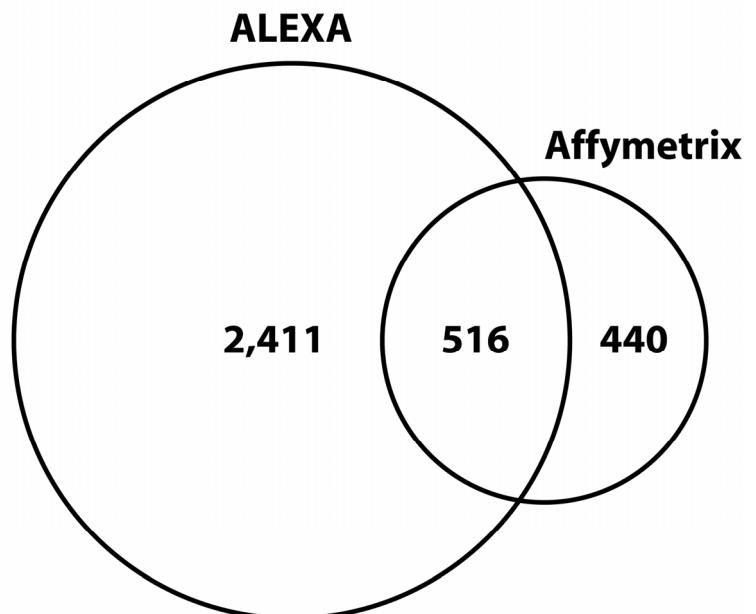


a Gene DE Overlap



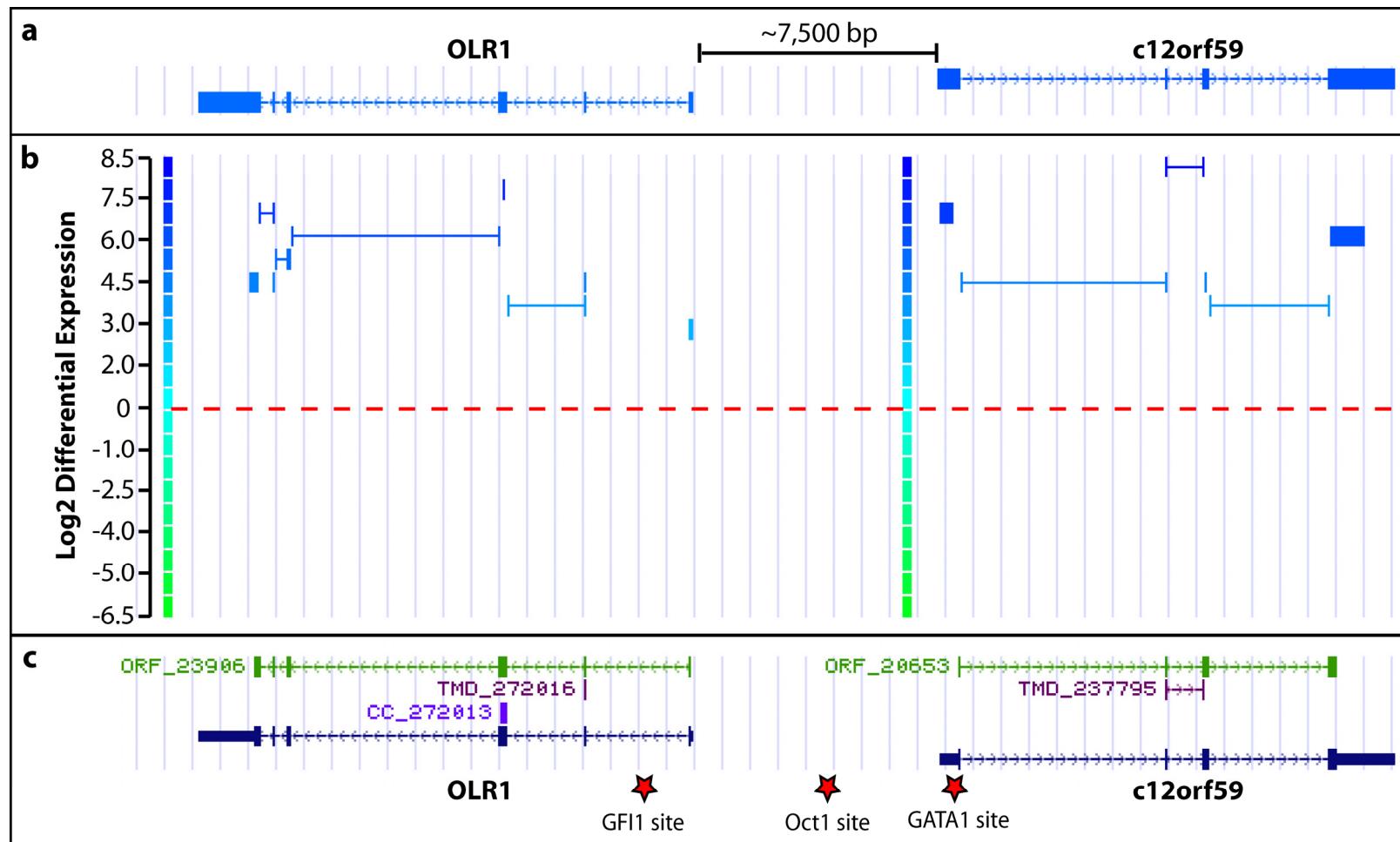
835 of 2,473 genes profiled by both platforms were detected as DE (by either or both platforms)

b Exon DE Overlap



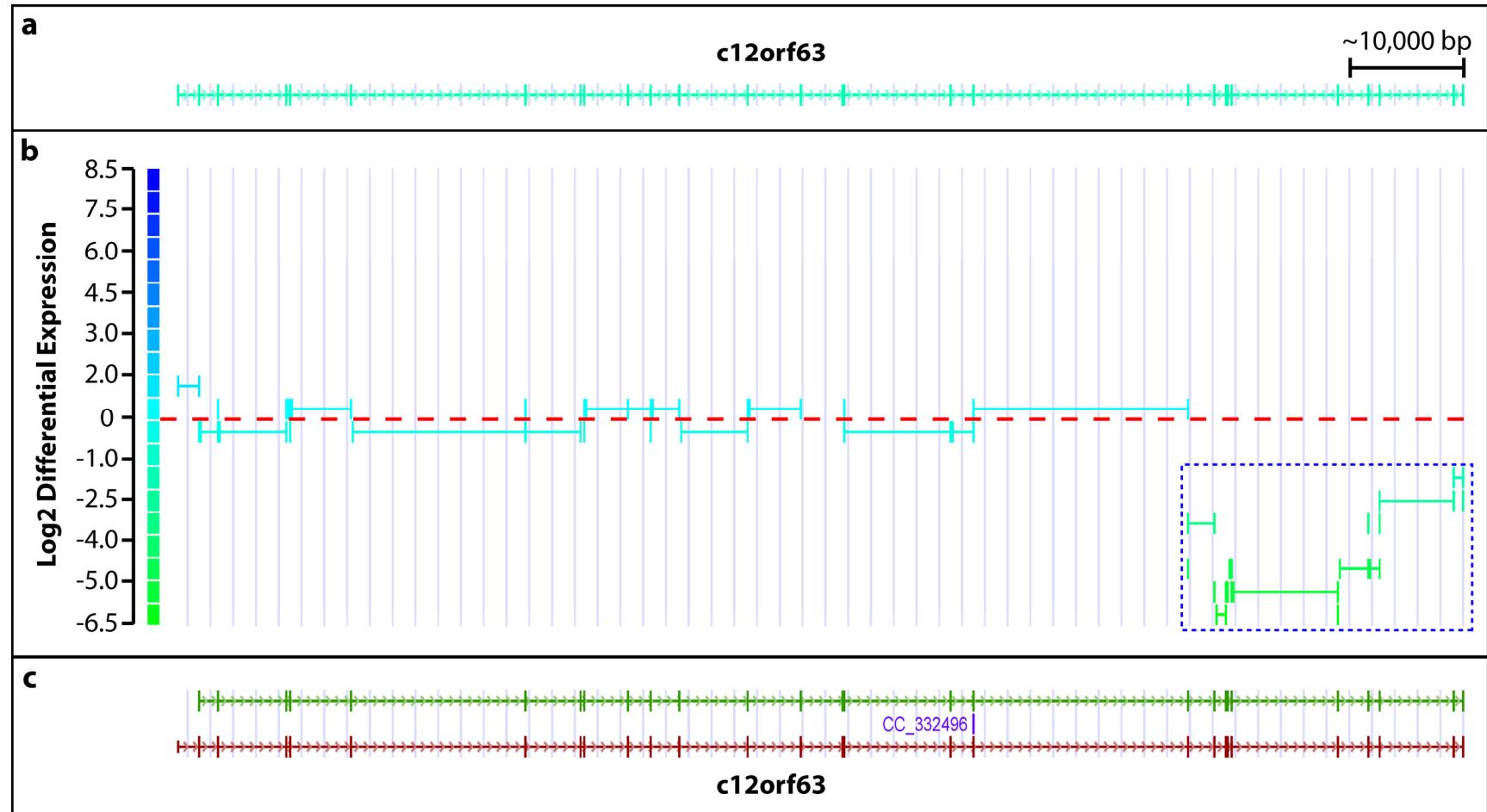
3,367 of 31,368 exons profiled by both platforms were detected as DE (by either or both platforms)

Example of a differentially expressed locus[†]



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

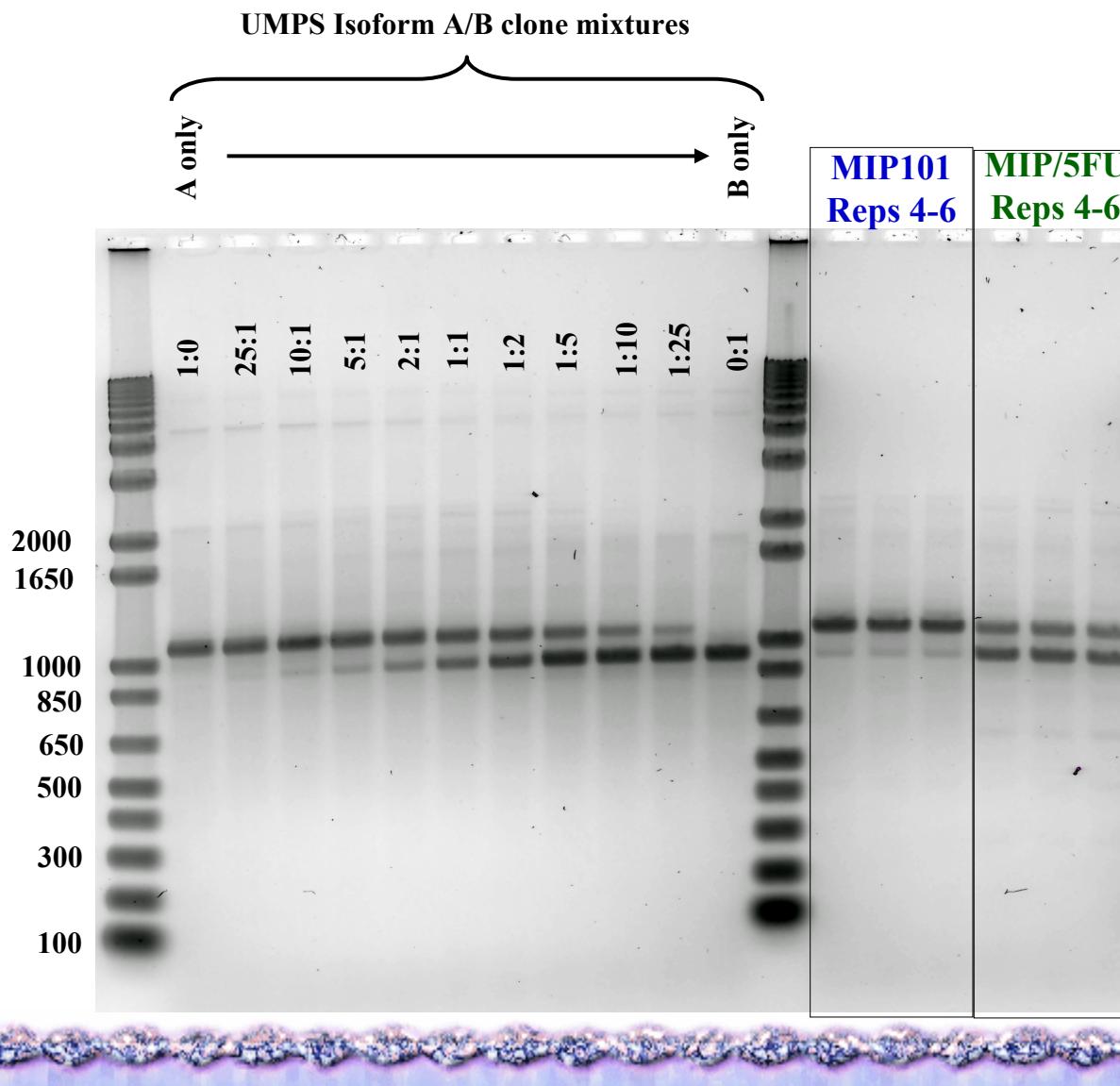
Example of a differentially expressed novel 3' isoform[†]



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

RT-PCR of UMPS isoforms

Used UMPS primers F5/R5 and template as labeled



MIP101 = 5-FU Sensitive
MIP/5FU = 5-FU Resistant

Primer design

- UMPS has six exons. Exon 2 is skipped in isoform B
- F5 is within Exon 1
- R5 is within Exon 4

Template

- Lanes 2-12 used mixtures of UMPS clones representing isoform A & B
- Lanes 14-19 used 1ul of 20ul ss-cDNA from MIP101 or MIP/5FU cell lines
- ss-cDNA was generated from 500 ng polyA+ RNA by SuperScript III using random hexamers.

PCR reaction

- 35 cycles, Pfx platinum enzyme

Expected PCR product sizes

- Isoform A = 1107 bp
- Isoform B = 953 bp

RT-PCR of UMPS isoforms

Used UMPS primers F5/R5 and template as labeled - All PCR details are as previous slide

