



Genome Sciences Centre

BC Cancer Agency, Vancouver, BC, Canada

# A microarray strategy to identify alternative splicing events specific to cancer cells

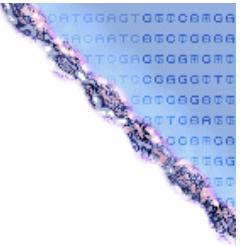
Malachi Griffith

Supervised by Marco A Marra

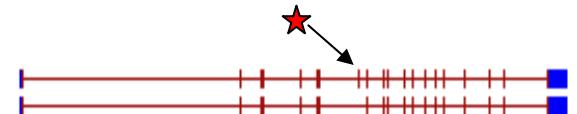
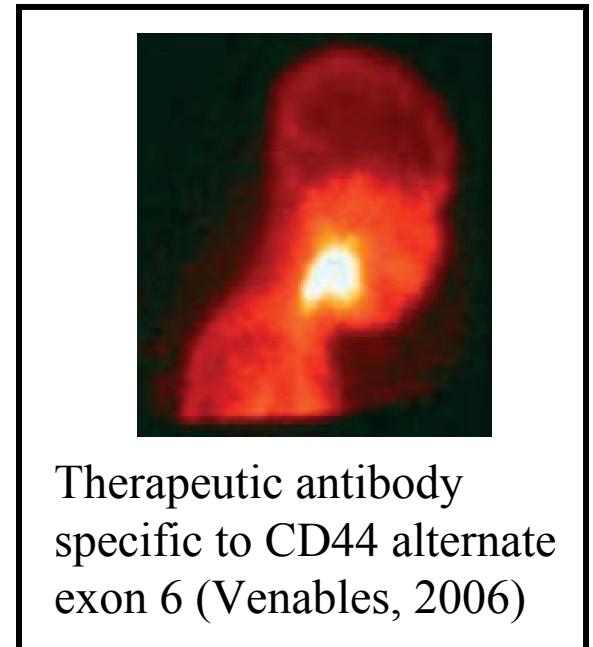
November 29 2007



# Introduction



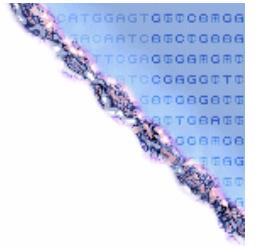
- Alternative expression generates multiple isoforms from most human loci
  - Alternative transcript initiation, splicing, and polyadenylation
- Specific isoforms may represent useful therapeutic targets or diagnostic markers
- Affymetrix Exon microarrays<sup>†</sup> and our own 'ALEXA' microarrays<sup>†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



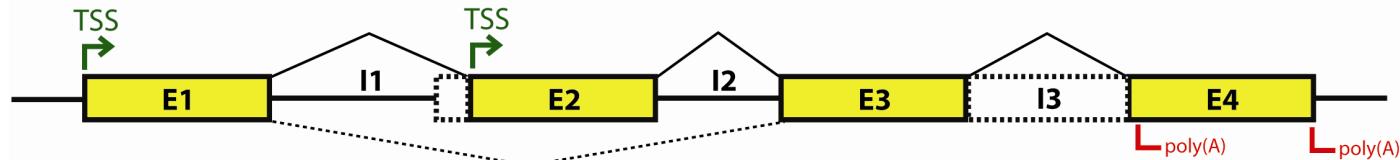
<sup>†</sup>Affymetrix GeneChip® Human Exon 1.0 ST vs. ALEXA arrays synthesized by NimbleGen Systems



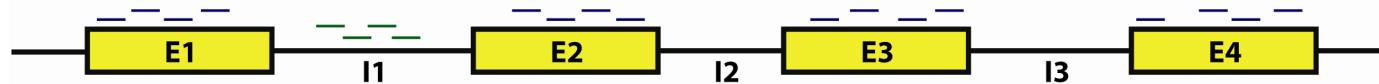
# Exon tiling and splicing microarrays



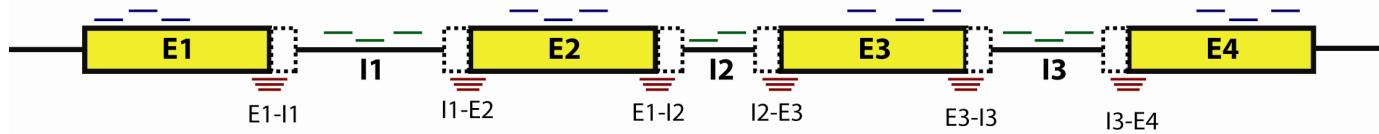
## a. Alternative expression



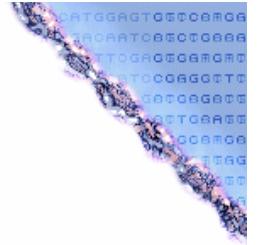
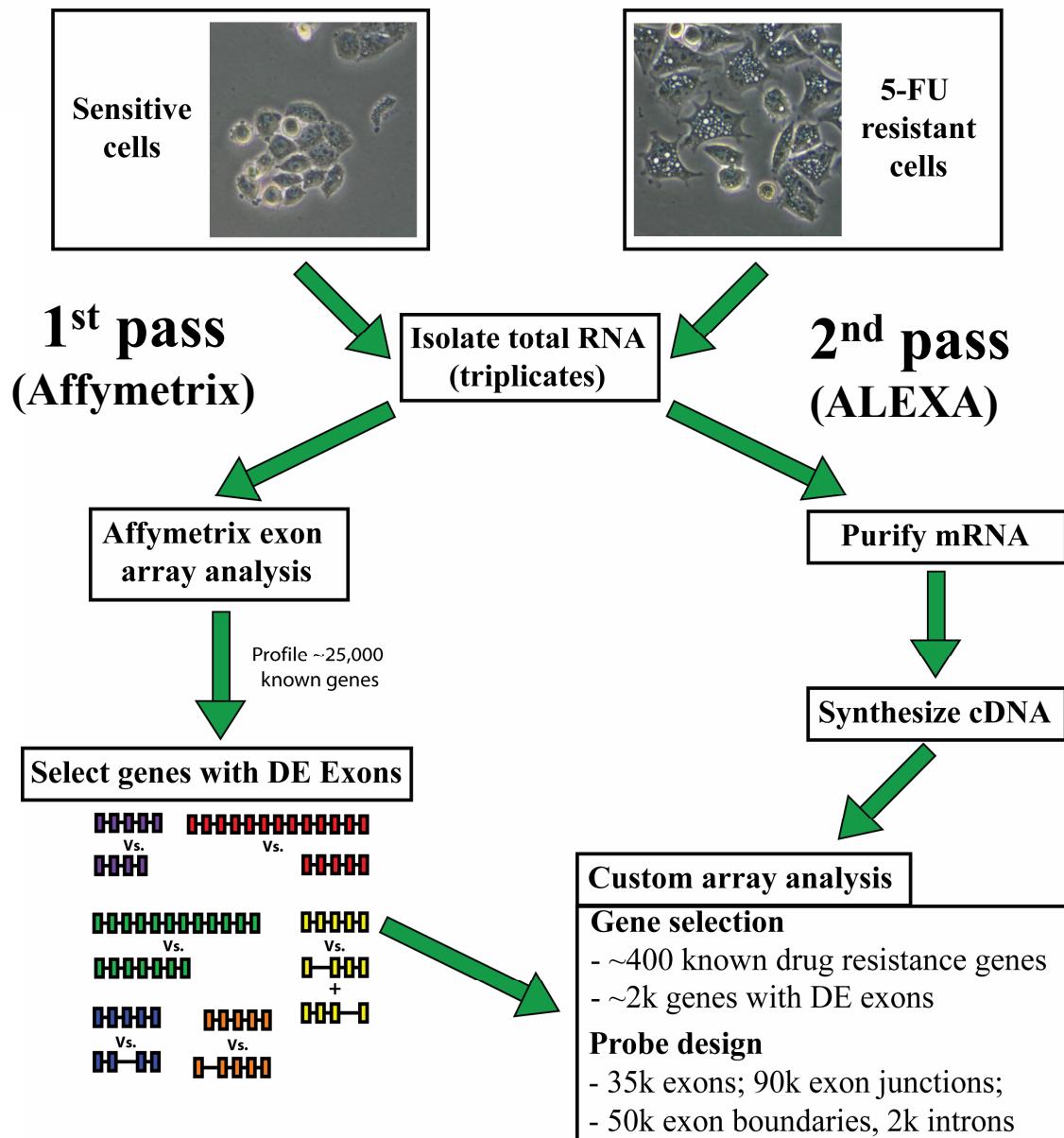
## b. Affymetrix array design



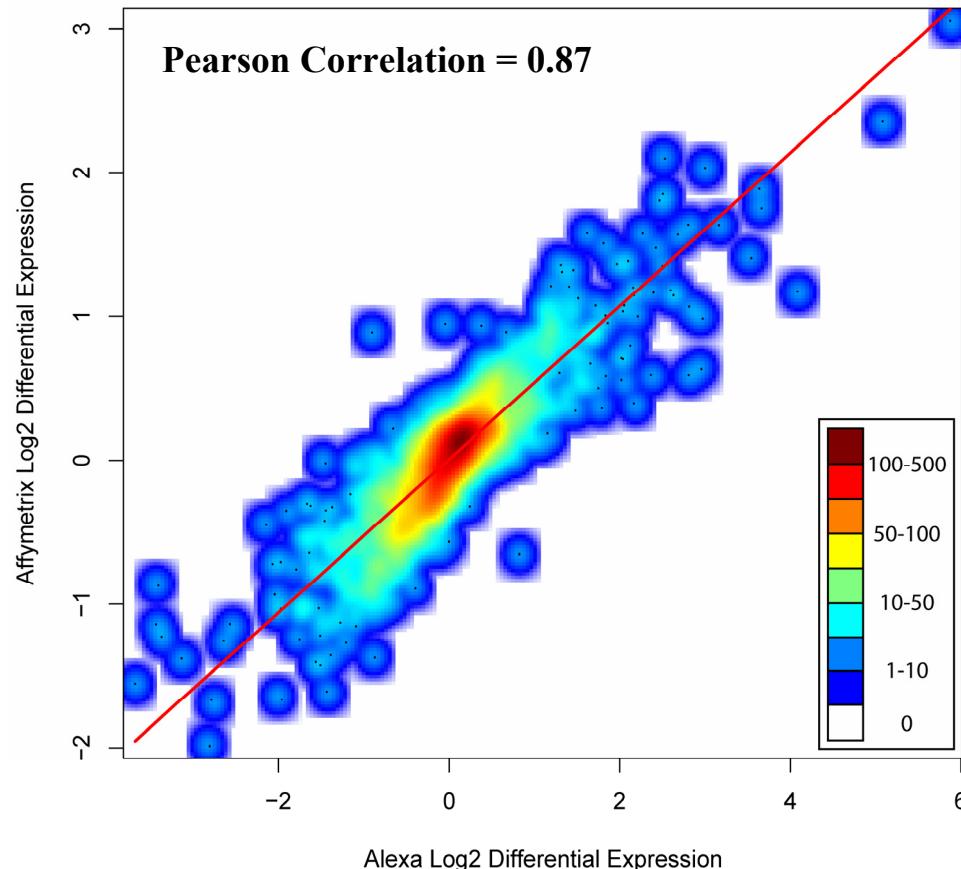
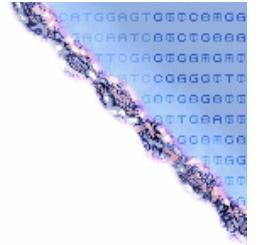
## c. ALEXA array design



# Experimental design



# Differential gene expression results (ALEXA vs. Affymetrix)

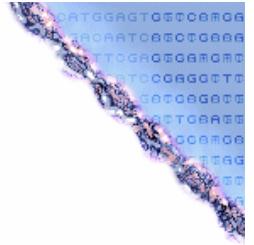


- 233 of ~2,500 genes have 2-fold change or greater
- 50 genes have a 4-fold change or greater
- DE of known and novel isoforms
  - alternate transcription initiation, splicing and polyadenylation

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



# Summary of information provided

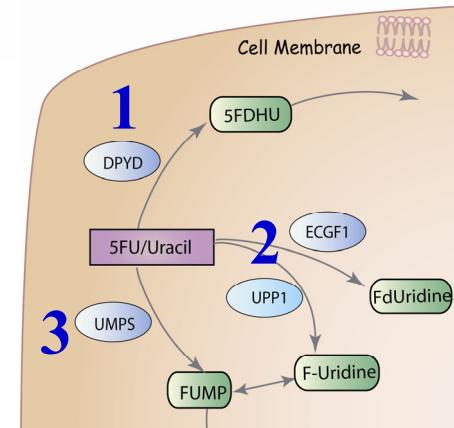
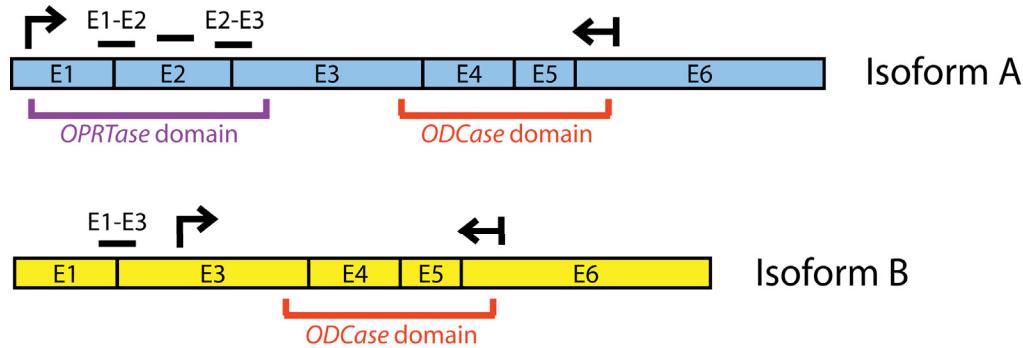


Platform	DE event type	Total events profiled	<u>Significant DE events</u>
Affymetrix	Gene-level	2,507	78
	Exon	49,681	1117
	Intron	65,327	25
	Total	117,515	1,220
ALEXA	Gene-level	2,507	233
	Exon	32,164	2,703
	Canonical junction	27,046	2,310
	Exon skip	69,761	191
	Exon boundary	52,402	253
	Intron	472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

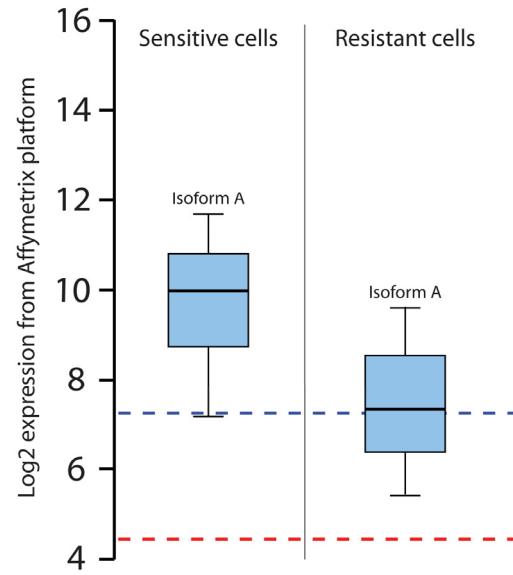


# Reciprocal DE of UMPS isoforms

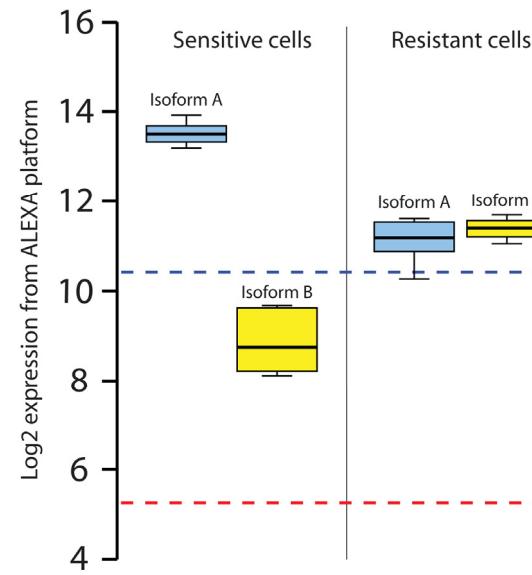
*UMPS* probesets and isoforms



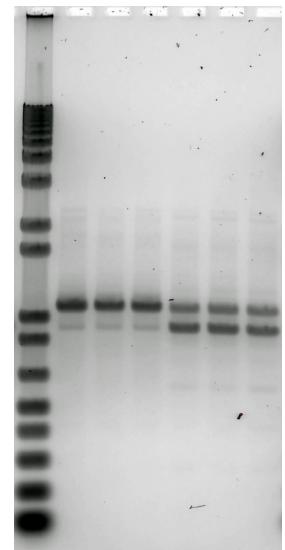
Affymetrix microarray data



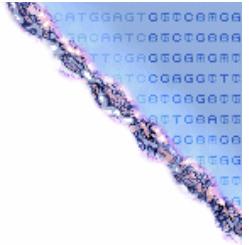
ALEXA microarray data



RT-PCR data



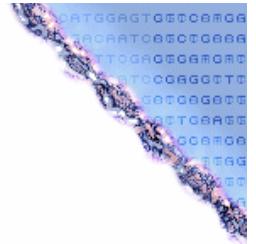
# Conclusions



- The ALEXA platform
  - First open source ‘splicing’ microarray design platform
  - [www.AlexaPlatform.org](http://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
- Biological application
  - Differential expression of novel and known isoforms associated with 5-FU resistance in colorectal cancer cell lines
  - Follow up validation
    - Pathway analysis, mechanism, functional assays, clinical study



# Acknowledgements



## Supervisor

Marco A. Marra

## Committee

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Steve Jones

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- Isabella Tai, Michelle Tang

## Gene Expression Group

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Jennifer Asano, Adrian Ally

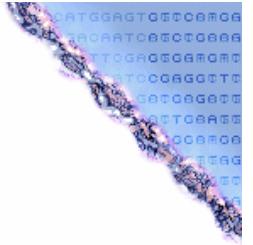
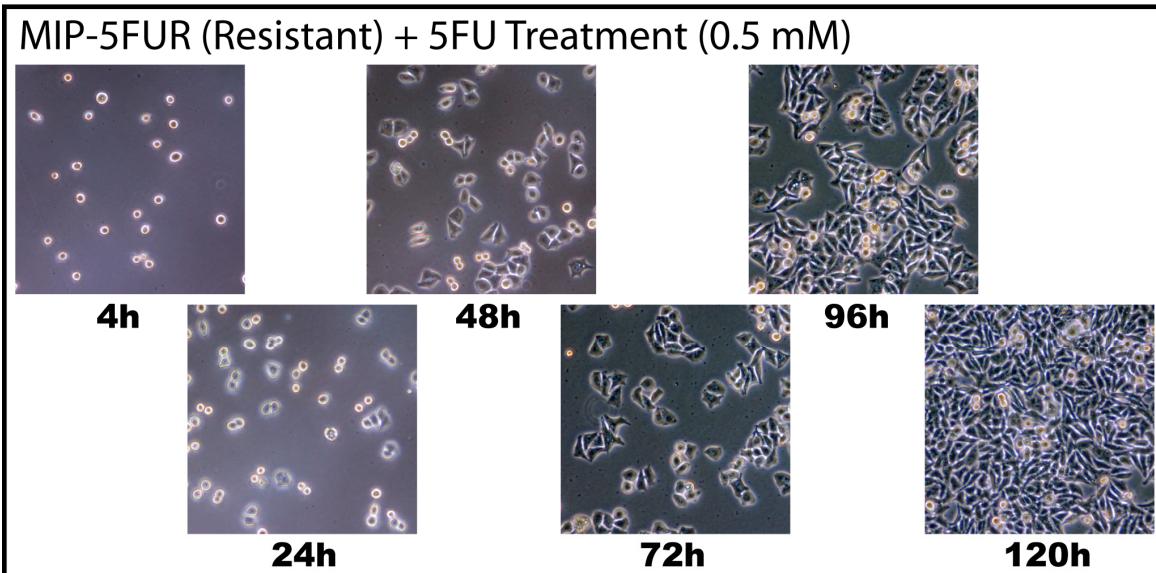
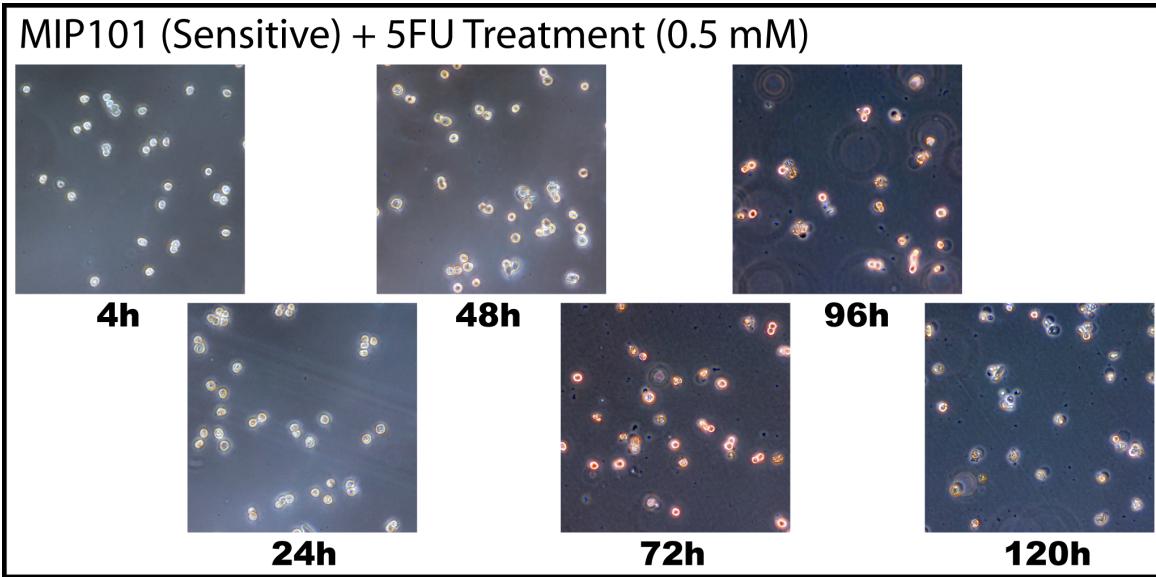
## Sample Preparation & Validations

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

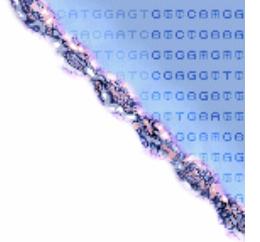




# Test of sensitivity/resistance



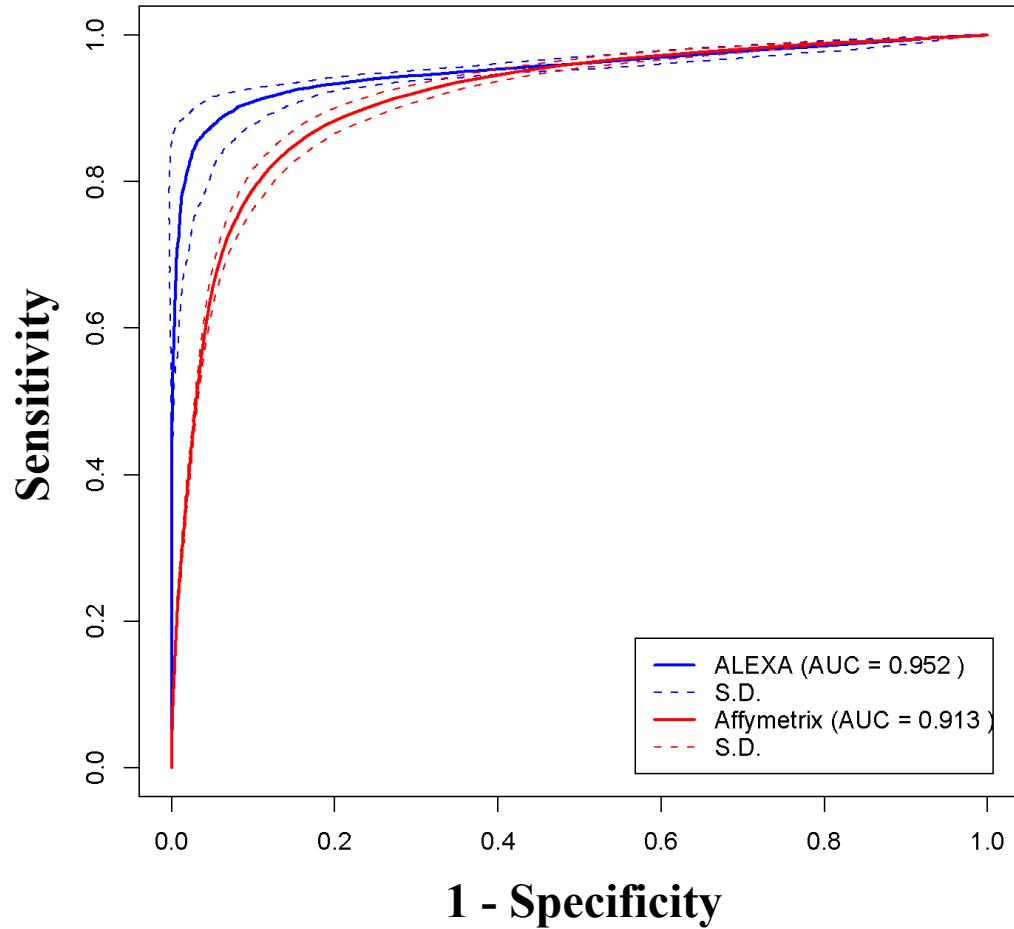
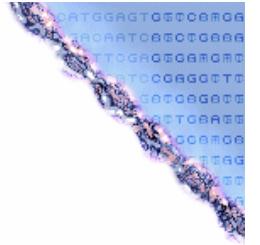
# The ALEXA platform



- 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](http://www.AlexaPlatform.org)



# Comparison of ALEXA and Affymetrix exon microarray performance

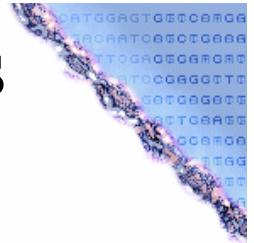


- **ROC curves**
  - Generated from expression values for +ve (exonic) & -ve (intronic) control probes of 100 housekeeping genes
- **Max. Specificity/Sensitivity**
  - ALEXA: 94.8% & 87.9%
  - Affymetrix: 85.8% & 84.2%
- **Signal-to-Noise ratio**
  - ALEXA:  $56.0 \pm 2.3$  SD
  - Affymetrix:  $20.9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

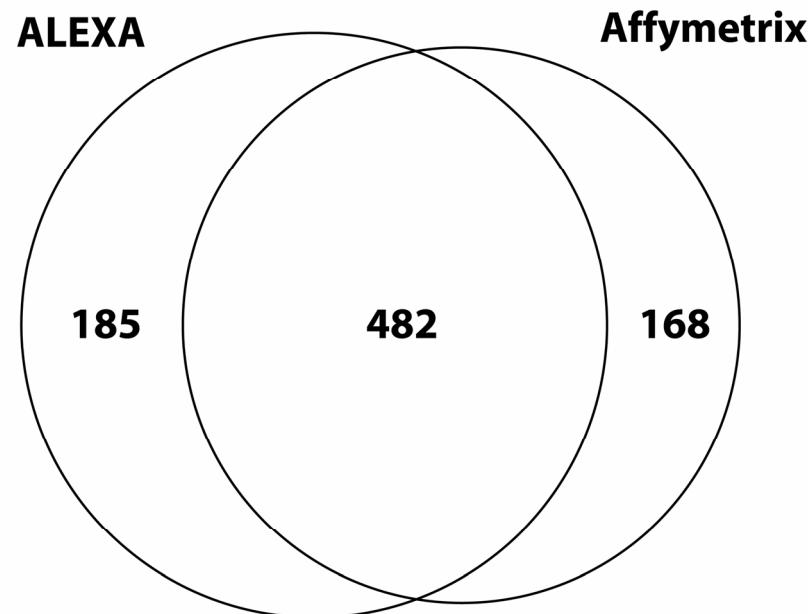
AUC: Area under curve; SD: standard deviation



# Overlap between platforms for genes and exons identified as differentially expressed

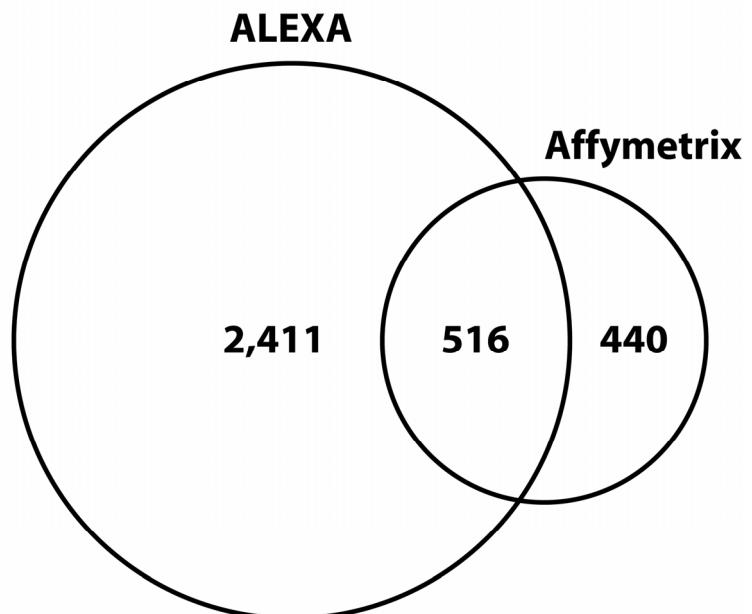


## a Gene DE Overlap



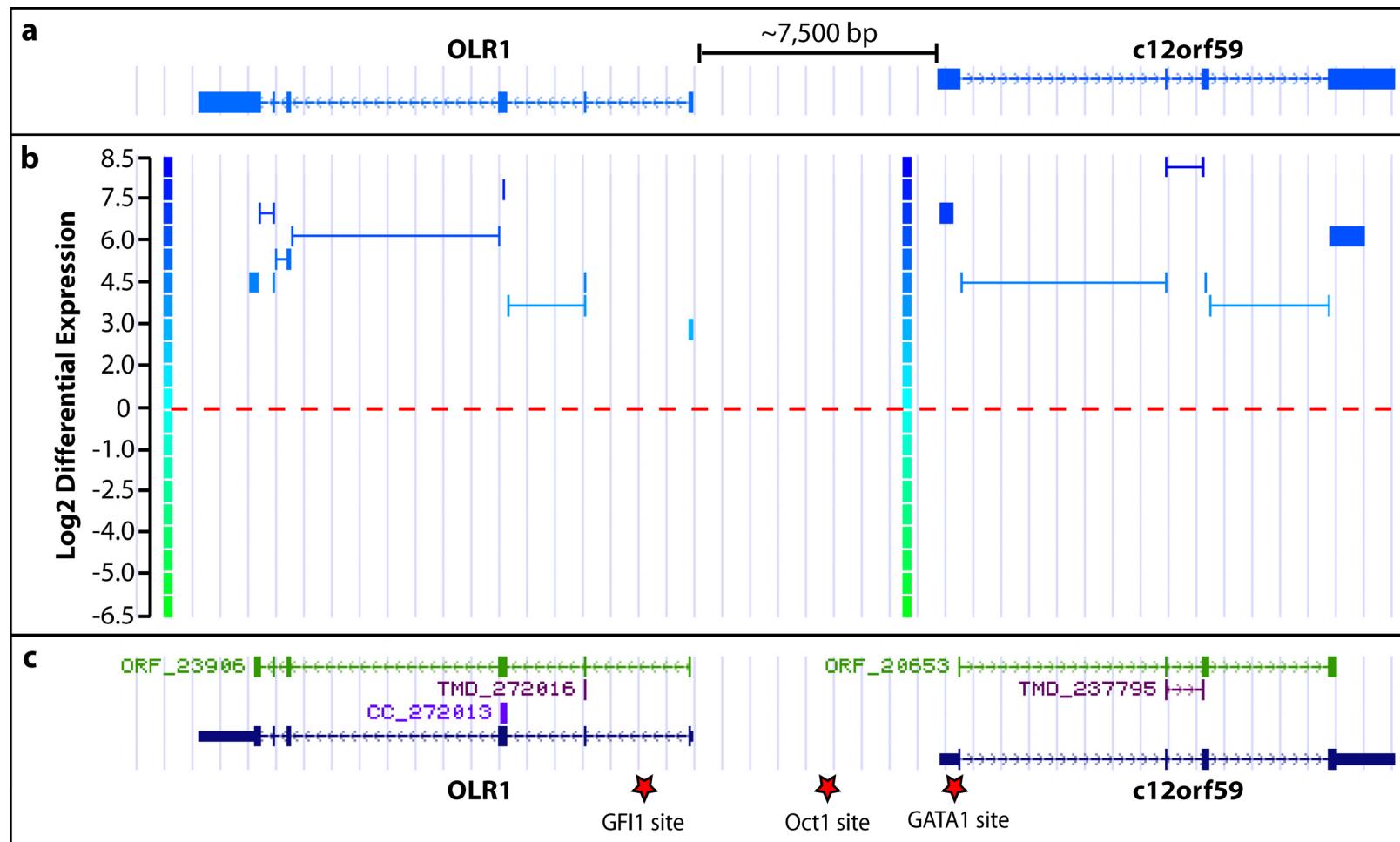
835 of 2,473 genes profiled by both platforms were detected as DE (by either or both platforms)

## b Exon DE Overlap



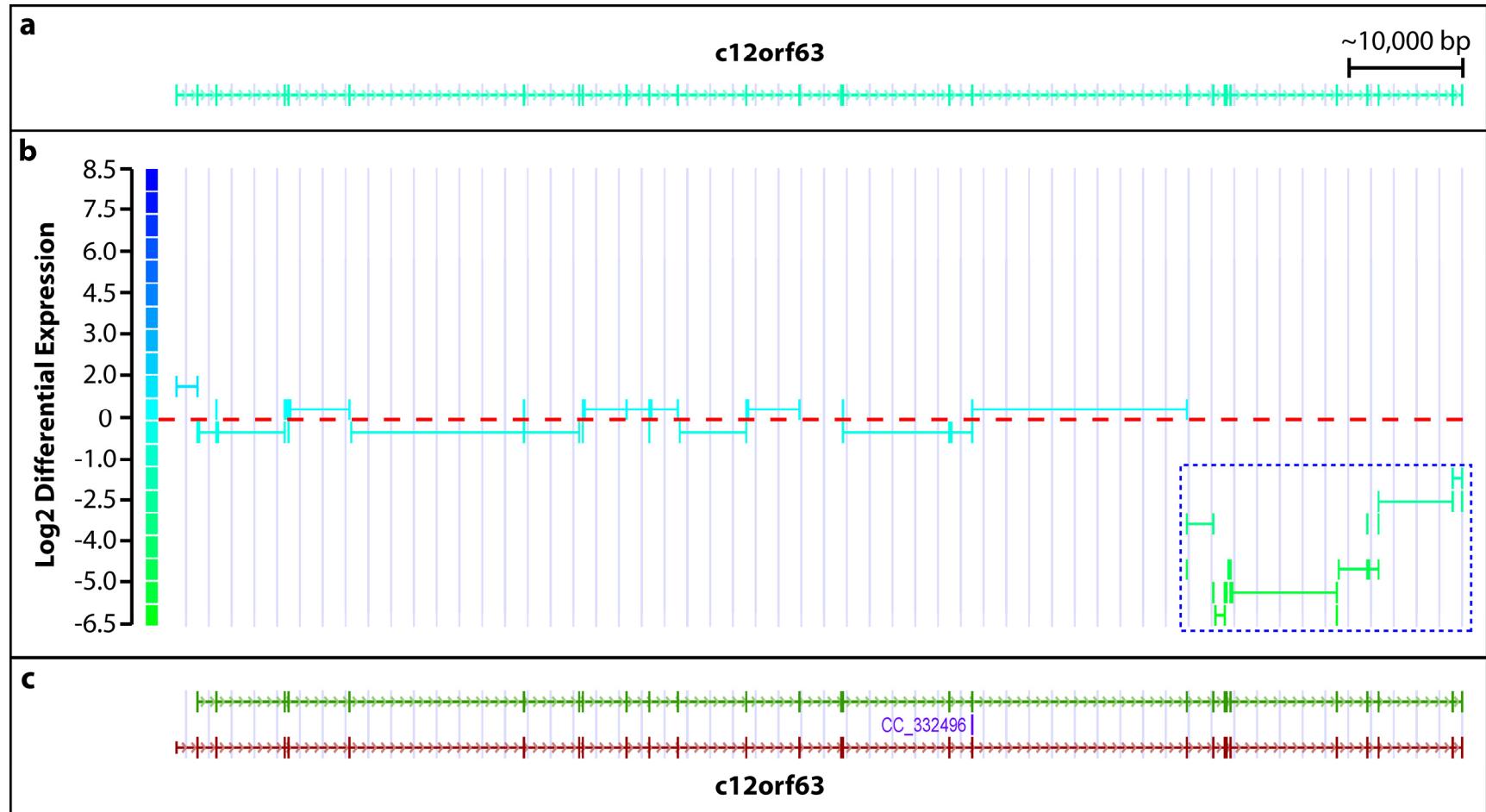
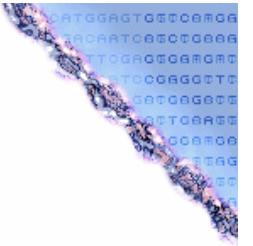
3,367 of 31,368 exons profiled by both platforms were detected as DE (by either or both platforms)

# Example of a differentially expressed locus<sup>†</sup>



<sup>†</sup>Data shown is from ALEXA microarrays synthesized by NimbleGen Systems Inc.

# Example of a differentially expressed novel 3' isoform<sup>†</sup>

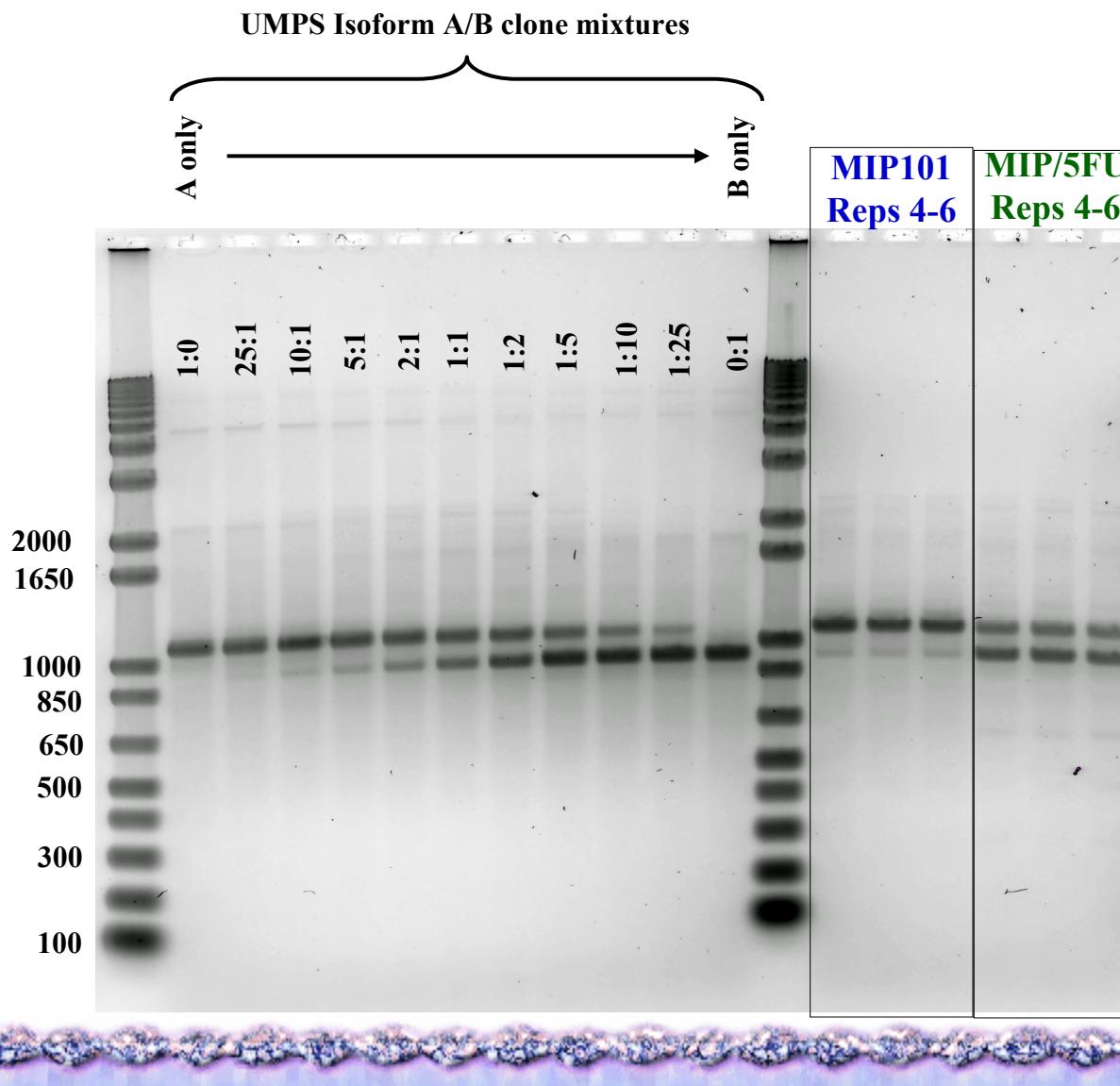


<sup>†</sup>Data shown is from ALEXA microarrays synthesized by NimbleGen Systems Inc.



# 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

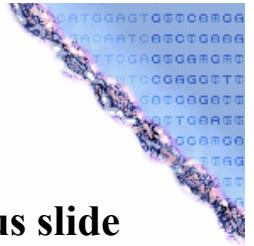
- 35 cycles, Pfx platinum enzyme

## Expected PCR product sizes

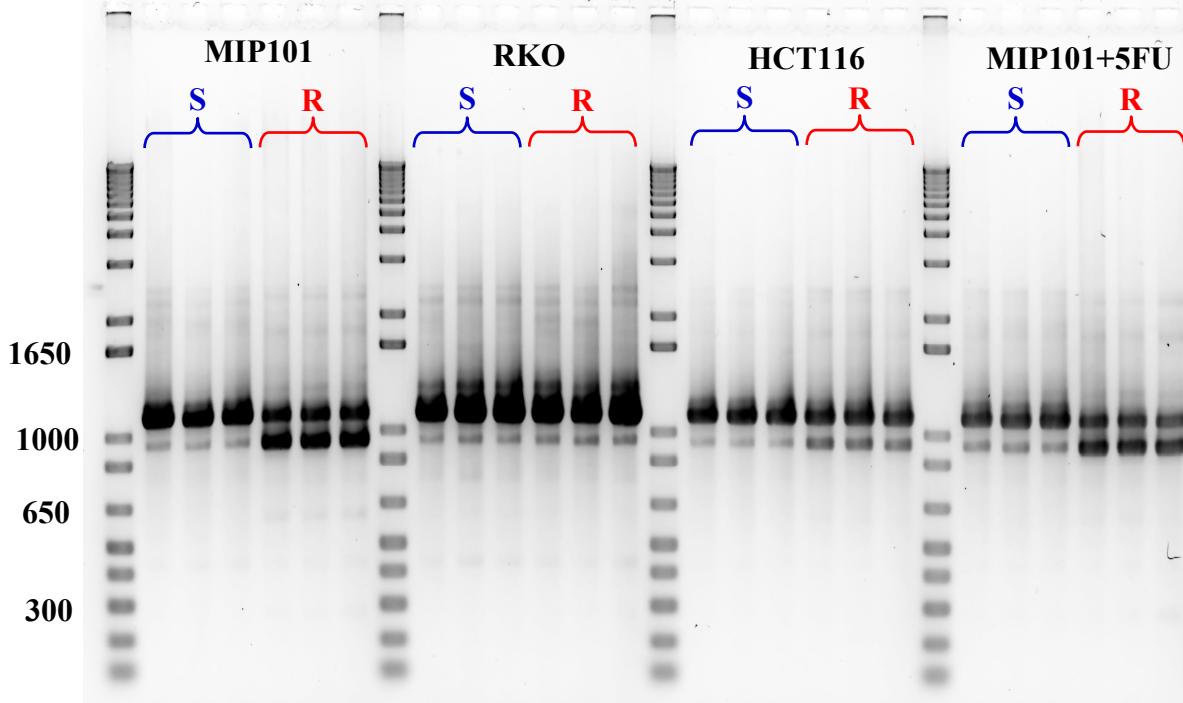
- Isoform A = 1107 bp
- Isoform B = 953 bp

# RT-PCR of UMPS isoforms

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



**S** = Replicates of sensitive version of cell line  
**R** = Replicates of resistant version of cell line



UMPS Isoform A/B clone mixtures

