Introducing the
New SurePrint G3 Exon Microarrays

Whole transcript coverage
Gene-level and exon-level analysis
Continued confidence in results

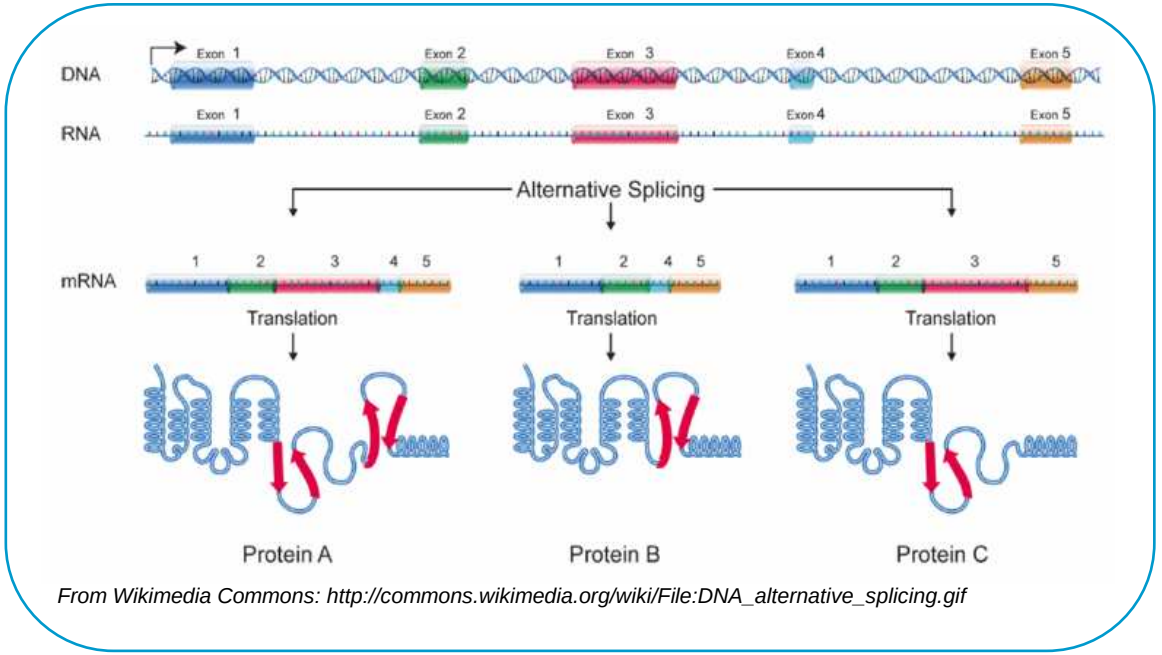
Why is Exon Analysis Important?

One gene ⇨ One transcript ⇨ One protein ***Old school thinking***

Multiple transcripts

Multiple proteins

Current understanding

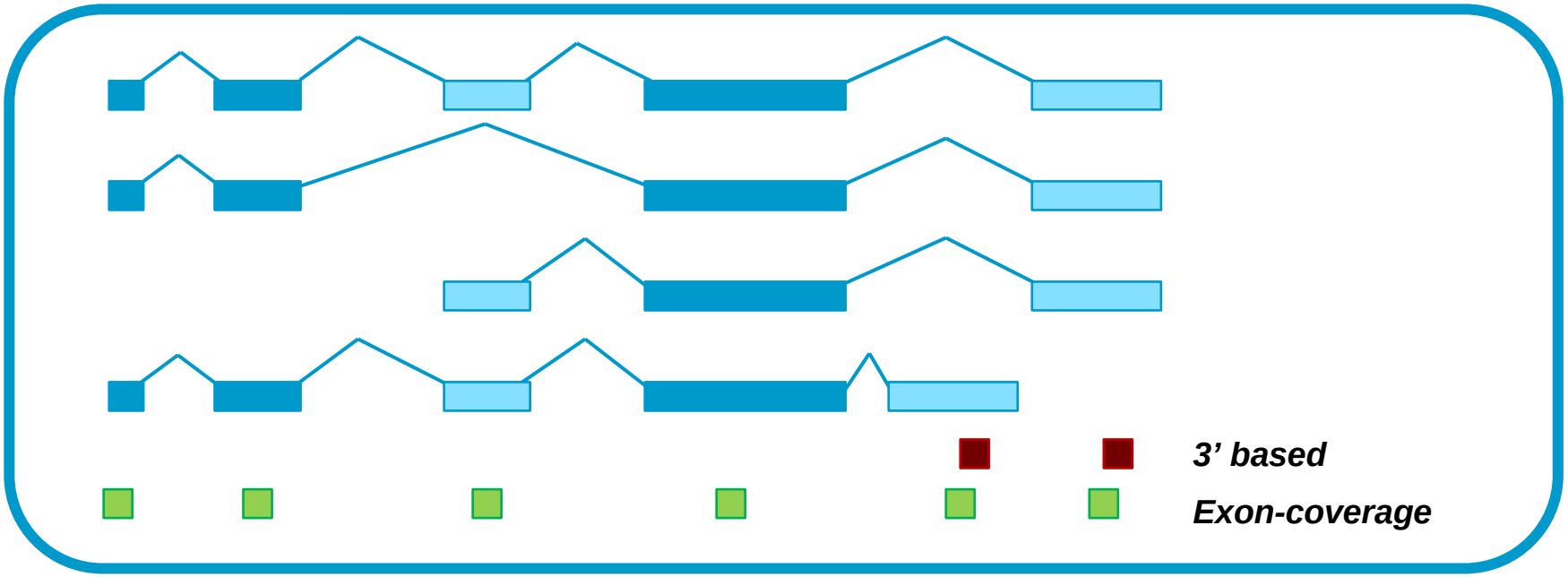


From Wikimedia Commons: http://commons.wikimedia.org/wiki/File:DNA_alternative_splicing.gif

•Gene-level analysis provides a simplified view of expressed genes

•Exon arrays are required to understand the complexity of the transcriptome

Gene-level versus Exon-level Detection



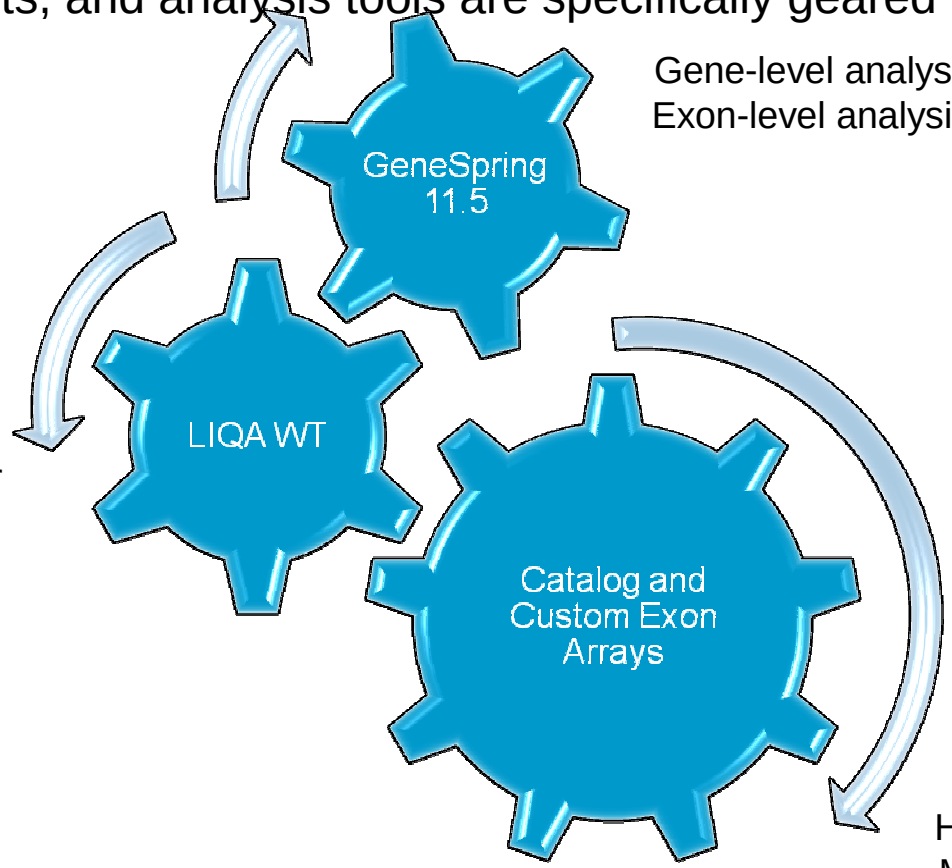
Considerations for an exon array:

•Most assays use oligo-dT primed labeling

•Exon arrays require whole-transcript assays (random primers)

Agilent Exon Analysis Comes in a Complete Package

•Arrays, reagents, and analysis tools are specifically geared for exon analysis



Gene-level analysis
Exon-level analysis

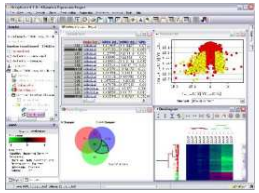
One-color
Two-color
No dye
Cy5 only

Human: 2x400K, 4x180K
Mouse: 2x400K, 4x180K
Rat: 2x400K, 4x180K
Custom: 1x1M, 2x400K, 4x180K, 8x60K

Agilent Gene Expression Exon Workflow

Exon-specific products fit into the global Agilent Gene Expression Workflow

Sample Source	Sample Prep & Isolation	Amplification and Labeling	Microarray Processing	Microarray Scanning	Microarray Analysis	Validation
Universal Reference RNAs	Absolutely RNA Purification Kits	LIQA WT	Exon Catalog Microarrays	High Resolution Microarray C Scanner	Feature Extraction Software	Mx3005P QPCR System
MVP RNA Products	RNA Isolation Kit / RNA Lysis Buffer	One-color RNA Spike-in Kit	Exon Custom Microarrays		FE QC Reports	Brilliant III QPCR Reagents
	RNAse Block	Two-color RNA Spike-In Kit	Gene Expression Hybridization Kit		GeneSpring 11.5	Brilliant III QRT-PCR Reagents
	Total RNA Isolation Mini Kit		Gene Expression Wash Buffer Kit			QPCR Reference RNA
	Plant RNA Isolation Mini Kit		Microarray Hybridization Chamber			2100 Bioanalyzer
	2100 Bioanalyzer		Gasket Slides			DNA Series II Bioanalzyer Kits
	RNA 6000 Series II Kits		SureHyb DNA Microarray Hybridization Oven			
			Ozone Barrier Slide Covers			
			Stabilization and Drying Solution			



Low Input Quick Amp WT Labeling Kits

•For the use of amplifying and labeling whole transcripts prior to hybridization to exon microarrays

•These kits are identical to the LIQA kits for standard gene expression, except with the addition of the WT Primer Mix

Part Number	Product Description	Reactions per Kit	USD List Price
5190-2942	Low Input Quick Amp WT, No dye	24	$635
5190-2943	Low Input Quick Amp WT, One-color	48	$1280
5190-2944	Low Input Quick Amp WT, Two-color	24	$2560
5190-3386	Low Input Quick Amp WT, Cy5	24	$1280

Kit Components: LIQA and LIQA WT

LIQA WT kits are identical to LIQA kits, except with the addition of the WT Primer Mix

Kit and Part Number	LIQA Enzyme Module	Cy3 Dye	Cy5 Dye	WT Primer Mix *NEW*
5190-2305: LIQA, One-color For standard gene expression	✓	✓		
5190-2306: LIQA, Two-color For standard gene expression	✓	✓	✓	
5190-2307: LIQA, Cy5 For standard gene expression	✓		✓	
5190-2308: LIQA, No dye For standard gene expression	✓			
5190-2942: LIQA WT, No dye For exon analysis	✓			✓
5190-2943: LIQA WT, One-color For exon analysis	✓	✓		✓
5190-2944: LIQA WT, Two-color For exon analysis	✓	✓	✓	✓
5190-3386: LIQA WT, Cy5 For exon analysis	✓		✓	✓

Kit Components: LIQA and LIQA WT

Kit Components	Location	LIQA Protocol	LIQA WT Protocol
T7 Primer	Enzyme Module	✓	
5x First Strand Buffer	Enzyme Module	✓	✓
10 mM dNTP Mix	Enzyme Module	✓	✓
AffinityScript RNase Block Mix	Enzyme Module	✓	✓
5x Transcription Buffe	Enzyme Module	✓	✓
NTP Mix	Enzyme Module	✓	✓
T7 RNA Polymerase Blend	Enzyme Module	✓	✓
Nuclease-Free Water	Enzyme Module	✓	✓
WT Primer Mix	WT Primer Mix		✓

- The LIQA WT kits include T7 Primer, not part of the LIQA WT protocol

Low Input Quick Amp WT Labeling Kit for Exon

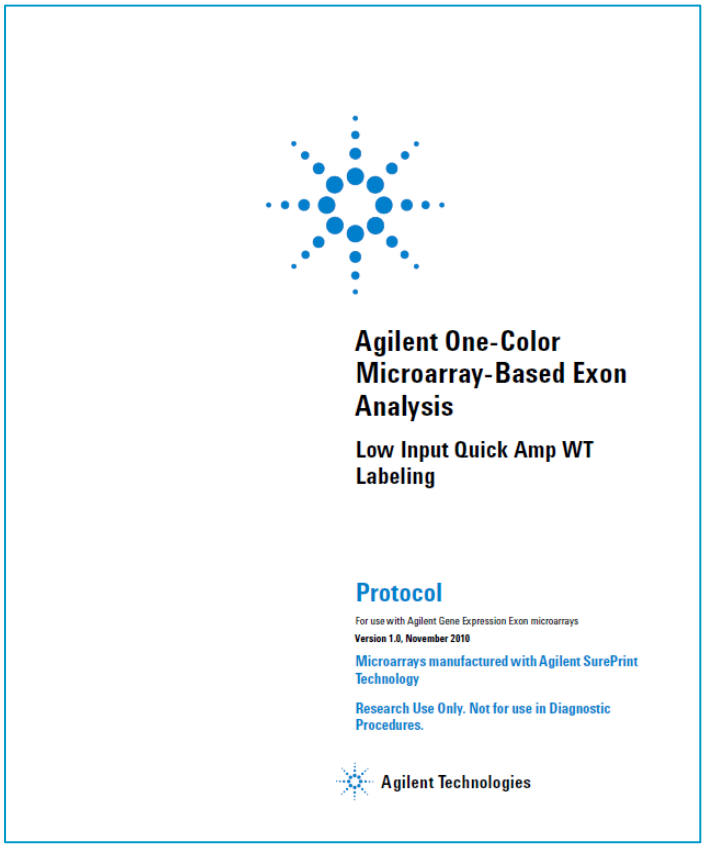
New Manuals, Version 1.0 relevant for all SurePrint G3 formats

What's different in the WT Manual?

- Compatible with Exon Microarrays for whole transcript labeling
- Based on the use of LIQA WT labeling kits, rather than LIQA labeling kits
- The WT Primer Mix replaces the T7-oligo-dT primers
- Input requirements and recommendations for Exon analysis are as follows:

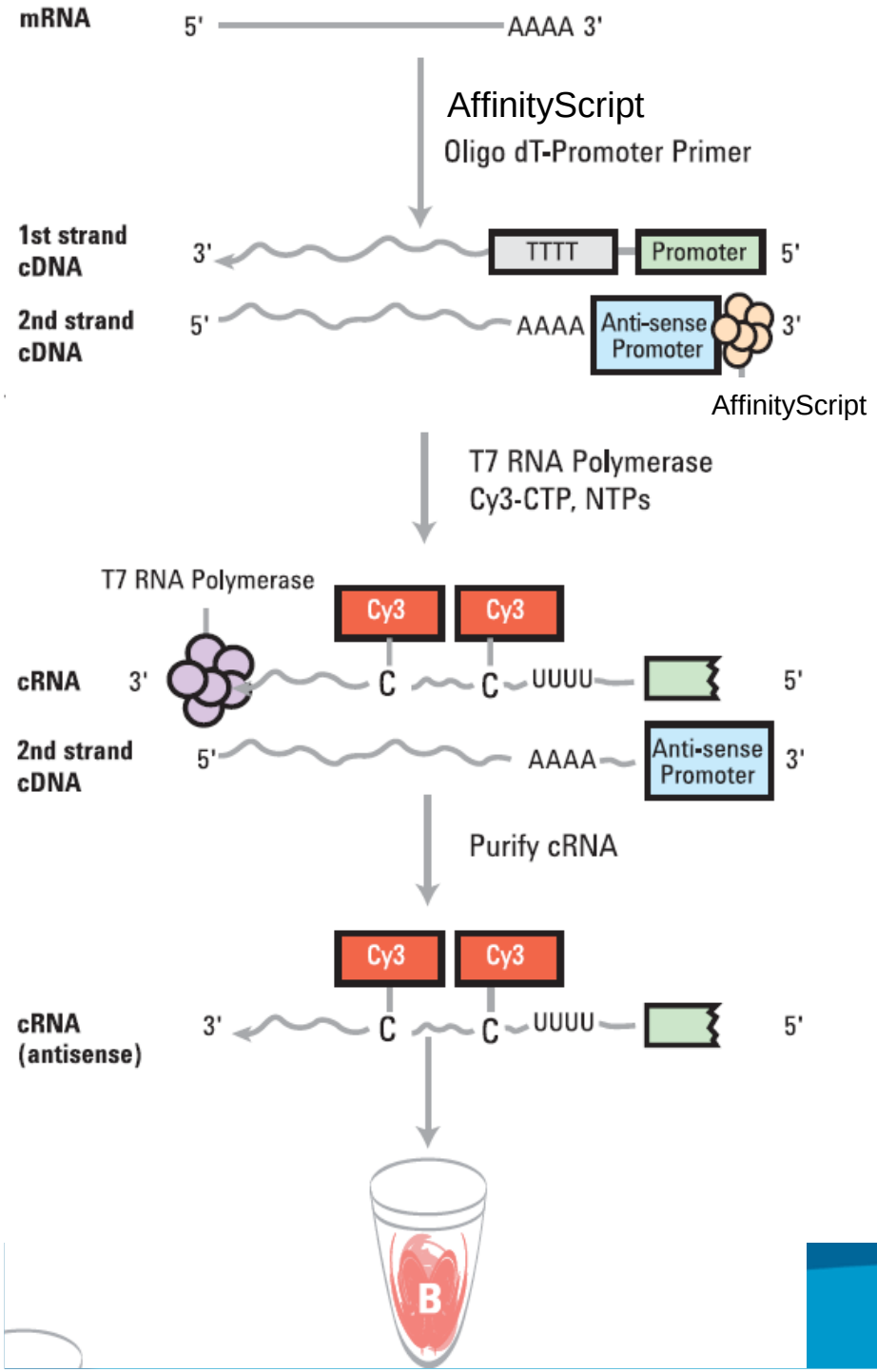
Format	Required	Recommended
8x	25 ng	50 ng
2x	25 ng	50 ng
4x	25 ng	50 ng
1x	100 ng	100 ng

- The recommended Specific Activity hybridization is 15 pmol Cy per µg cRNA
- GeneSpring 11.5 or later is required
- The C Scanner is required, and instructions are only for SurePrint G3 formats



Manual Part Numbers:

One-color	G4140-90042
Two-color	G4140-90052



Overview of Protocol

Workflow diagram for the LIQA WT Labeling Kit:

- Start with 50 ng total RNA
- Create dsDNA containing T7 promoter:
  - AffinityScript, RT with high efficiency
  - Oligo-dT and random primed
- Create labeled cRNA (anti-sense)
  - T7 RNA polymerase
  - Cy3-CTP (Cy5-CTP)
  - Linear amplification IVT
- Purify cRNA → labeled product ready for hyb

✓ Linear amplification to avoid introducing unnecessary biases

✓ Single-tube reaction, no cDNA clean-up step for fast and easy processing

LIQA and LIQA WT Protocols Remain Largely the Same

Available in both O… …pt…

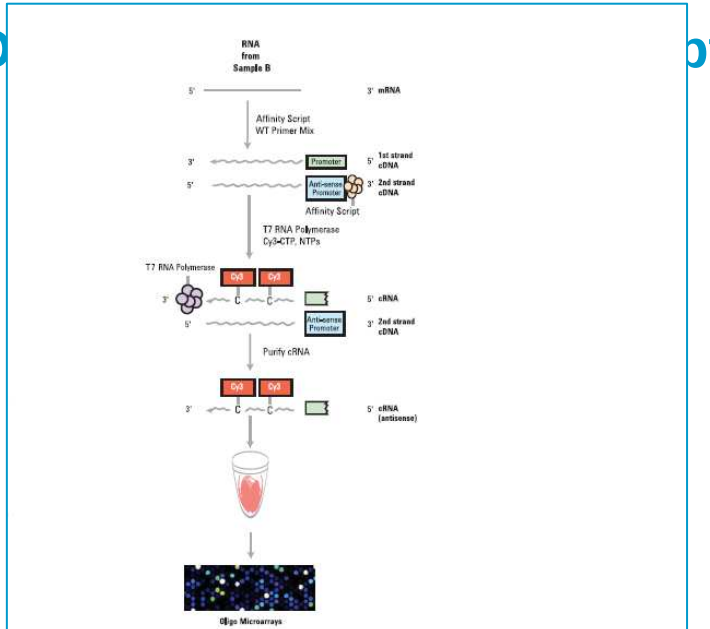


Figure 2 Schematic of amplified cRNA procedure.

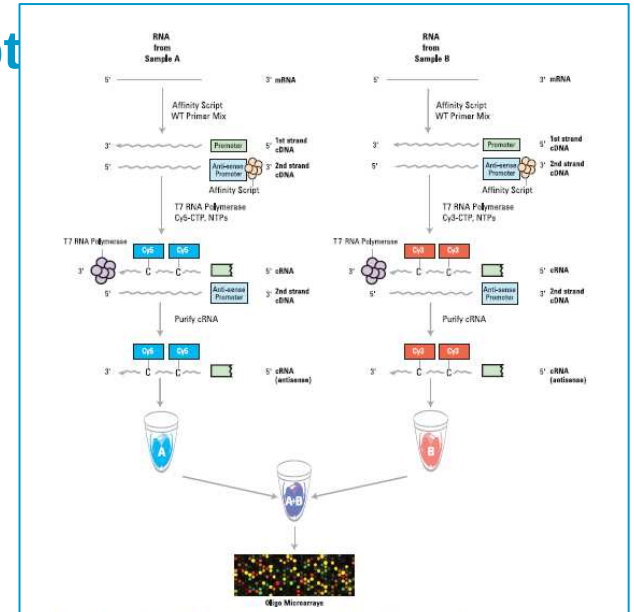


Figure 2 Schematic of amplified cRNA procedure. Generation of cRNA for a two-color microarray experiment is shown.

WT Primer Mix:

- a T7 promoter primer (oligo dT-based primer) and
- a random primer with a T7 promoter

mRNA is primed at the 3’ end and along the transcript

SurePrint G3 Exon Microarrays: Catalog Designs

Part Number	Product Description	# Slides (# Arrays)	USD List Price
G4832A	*SurePrint G3 Human Exon 4x180K Kit*	3 (12)	$3150
G4833A	*SurePrint G3 Mouse Exon 4x180K Kit*	3 (12)	$3150
G4834A	*SurePrint G3 Rat Exon 4x180K Kit*	3 (12)	$3150
G4848A	*SurePrint G3 Human Exon 2x400K Kit*	3 (6)	$2685
G4849A	*SurePrint G3 Mouse Exon 2x400K Kit*	3 (6)	$2685
G4850A	*SurePrint G3 Rat Exon 2x400K Kit*	3 (6)	$2685

PN	AMADID	Product Description	# Slides	USD List Price
G4857A	028679	*SurePrint G3 Human Exon 4x180K Microarray*	1	$1050
G4857A	028726	*SurePrint G3 Mouse Exon 4x180K Microarray*	1	$1050
G4857A	028744	*SurePrint G3 Rat Exon 4x180K Microarray*	1	$1050
G4856A	028680	*SurePrint G3 Human Exon 2x400K Microarray*	1	$895
G4856A	028727	*SurePrint G3 Mouse Exon 2x400K Microarray*	1	$895
G4856A	028728	*SurePrint G3 Rat Exon 2x400K Microarray*	1	$895

SurePrint G3 Exon Microarrays: Custom Options

•Custom options for human, mouse, and rat available for all SurePrint G3 formats

Part Number	Product Description	# Slides (# Arrays)	USD List Price
G4863A	*SurePrint G3 Custom Exon 8x60K*	1 (8)	$1614
G4864A	*SurePrint G3 Custom Exon 4x180K*	1 (4)	$1050
G4865A	*SurePrint G3 Custom Exon 2x400K*	1 (2)	$895
G4866A	*SurePrint G3 Custom Exon 1x1M*	1 (1)	$695

•Custom exon arrays can include any catalog exon probe

•Custom exon arrays can combine exon and standard probes

- Designs including ≥ one exon probe are designated “Exon arrays”, compatible with LIQA WT kits

Agilent Exon Catalog Array Content

Species	Array Format	Genes Targeted	Exons Probes	Probes from 8x60K	Databases Used for Design
Human	4x180K	20,411	174,458	19,128 (56%)	Refseq Build 36.3, RSNM only
	2x400K	27,696	233,164	26,873 (78%)	Refseq Build 36.3 Ensembl Release 52 Unigene Build 216 (Apr 2009) GenBank mRNA (Apr 2009)
Mouse	4x180K	23,215	165,984	19,203 (49%)	Refseq Build 37, RSNM only
	2x400K	33,795	235,714	31,608 (80%)	Refseq Build 37 Ensembl Release 55 Unigene Build 176 (Apr 2009) GenBank mRNA (Apr 2009) RIKEN 3
Rat	4x180K	20,483	160,141	23,444 (50%)	Refseq Build 36.2, RSNM only
	2x400K	26,276	214,270	31,948 (72%)	Refseq Build 36.2 Ensembl Release 55 Unigene Build 177 (Oct 2008) GenBank mRNA (Jan 2009)

Additional features specific to the 2x400K catalog versions (as compared to the 4x180K versions) include:

- Contains probes targeting exons from 35 to 60 bp
- Targets 5’ and 3’ UTRs in addition to gene exons

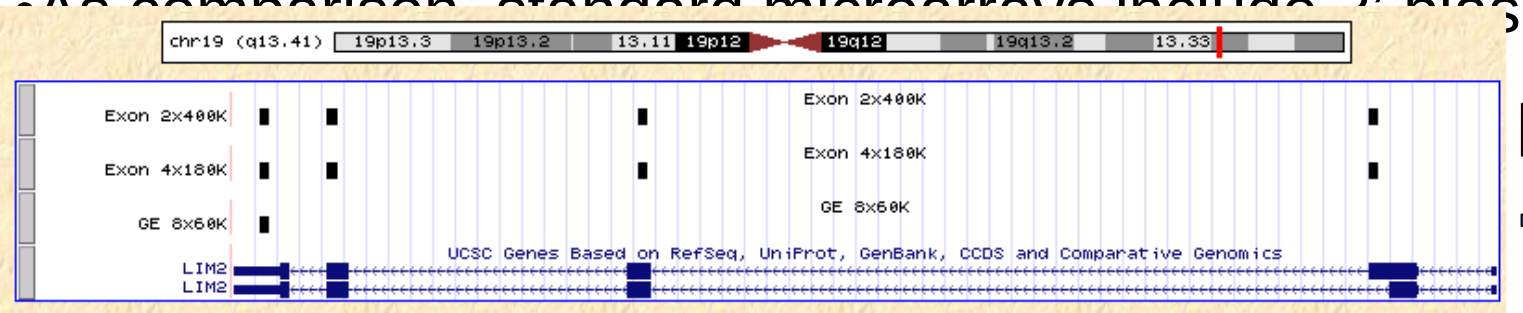
Agilent Gene Expression Catalog Array Content

Species	Array Format	Entrez Gene Targets	Exon Probes	lincRNA Targets
Human	GE 8x60K	27,958	Ø	7,419
	Exon 4x180K	20,411	174,458	Ø
	Exon 2x400K	27,696	233,164	Ø
Mouse	GE 8x60K	34,017	Ø	4,623
	Exon 4x180K	23,215	165,984	Ø
	Exon 2x400K	33,795	235,714	Ø
Rat	GE 8x60K	26,930	Ø	Ø
	Exon 4x180K	20,483	160,141	Ø
	Exon 2x400K	26,276	214,270	Ø

Comprehensive Coverage Taken to a New Level

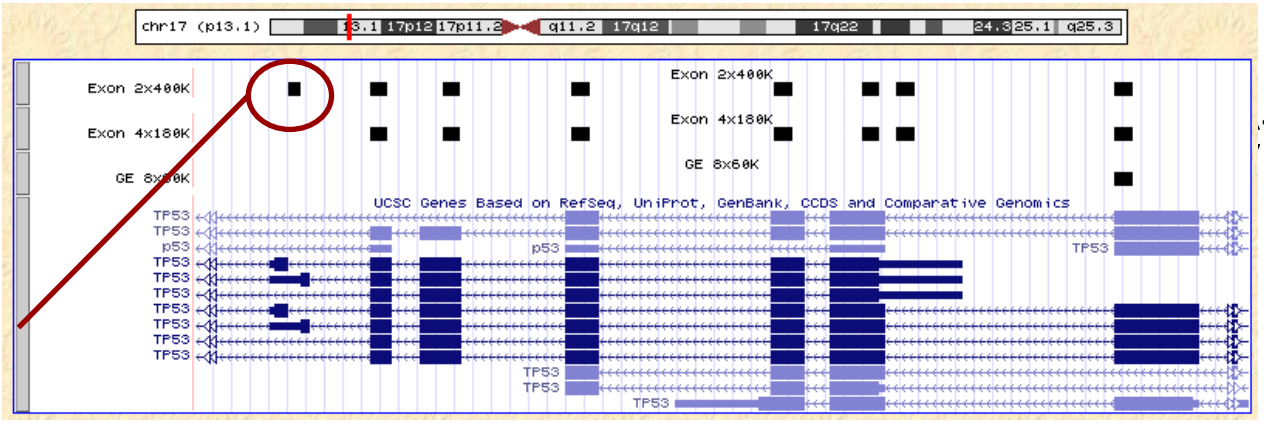
•Agilent SurePrint G3 Exon Arrays include a probe for each exon

•As comparison, standard microarrays include 3' biased probes



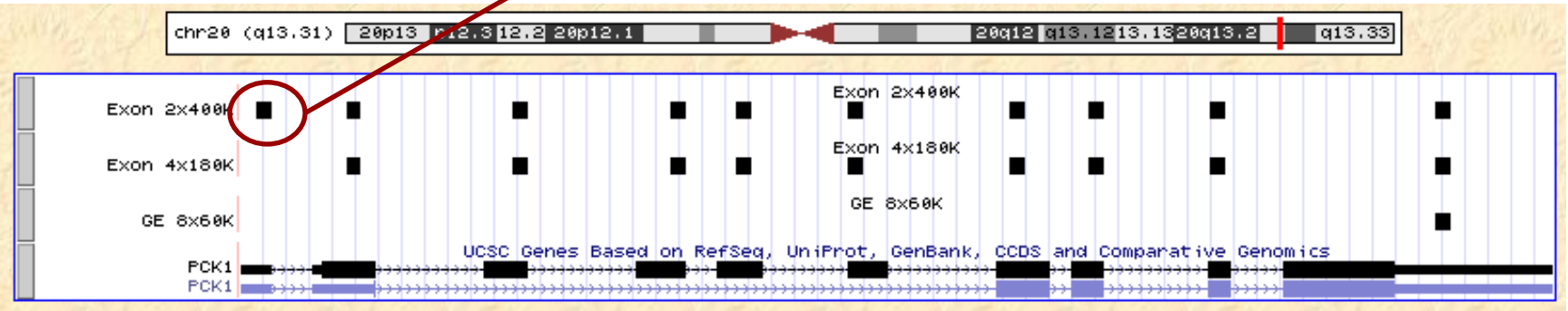
Exon Arrays Offer Choice in Coverage and Cost

- SurePrint G3 Exon 2x400K Microarrays are more comprehensive
- SurePrint G3 E…tive



Probe is 40 bp, unique to 2x400K design

Probe is located in a 5' UTR, unique to 2x400K design

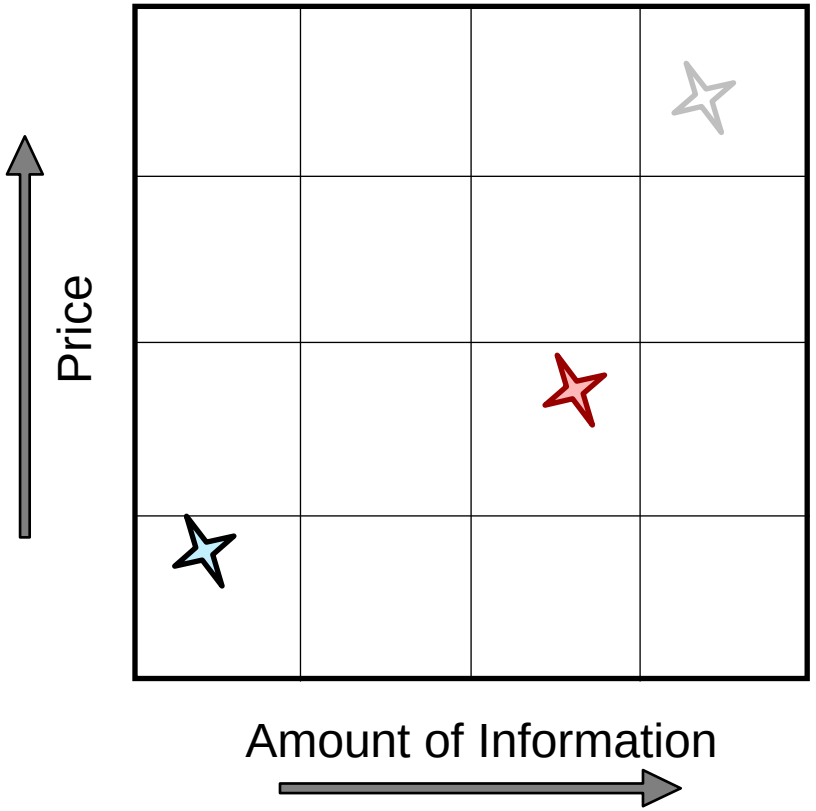


Exon Arrays Provide Affordable Value

Array Type	Offering	Product	Price per Sample
Standard	Gene-level analysis	GE, 8x60K	$202/array $255/sample
Exon	Gene-level analysis Exon-level analysis	Exon, 4x180K	$262/array $315/sample
Exon	Gene-level analysis Exon-level analysis Comprehensive	Exon, 2x400K	$447/array $500/sample

Exon Arrays Provide Affordable Value

Exon arrays provide a cost-effective route to a better understanding of relevant biology



Standard Arrays:
Gene-level analysis
Known genes

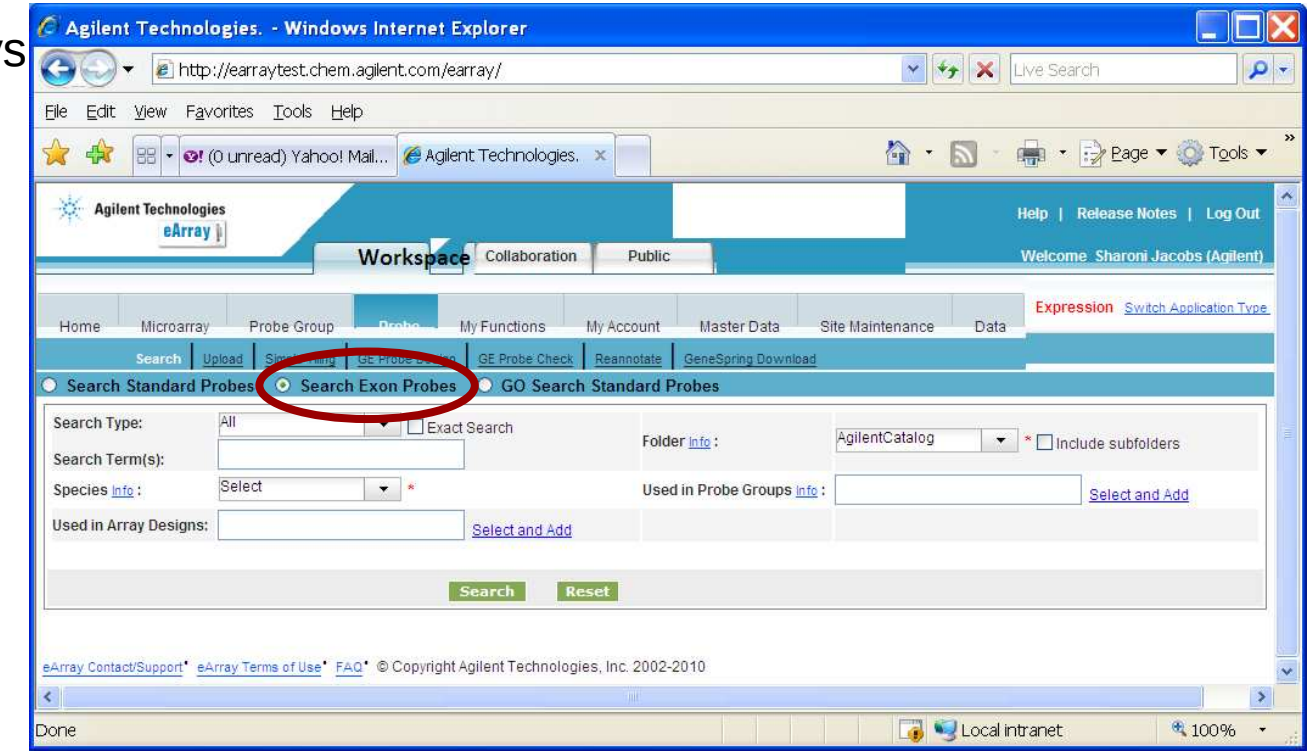
Exon Arrays:
Gene-level analysis
Exon-level analysis
Known genes

RNA-Seq:
Gene-level analysis
Exon-level analysis
Splicing analysis
Known genes
Novel genes
Sequences

eArray Upgraded to Accommodate Exon Custom Design

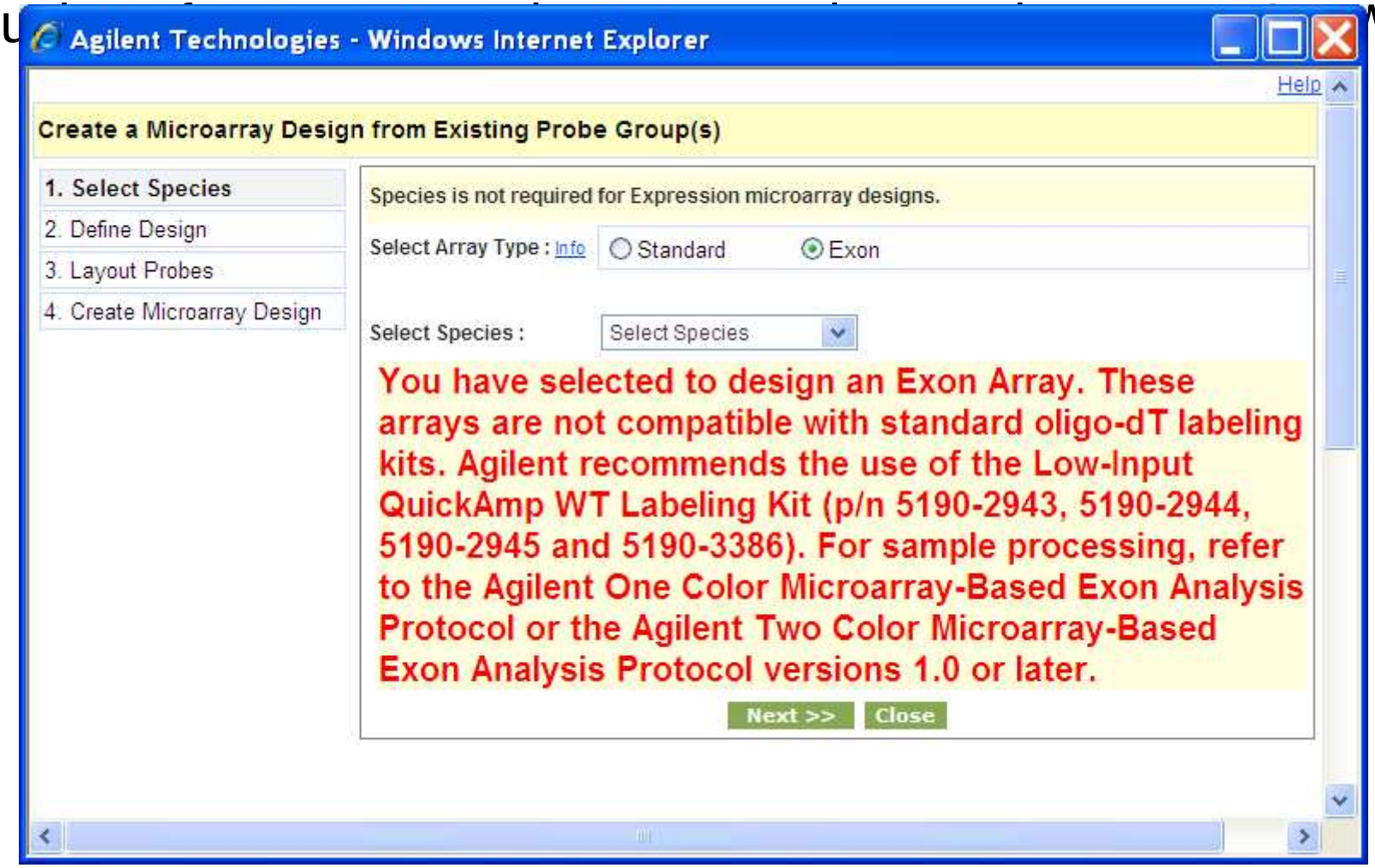
Search for either standard or exon probes

- Exon probes include all catalog exon probes for human, mouse, rat
- Custom designs are designated ‘exon’ if they include one or more exon probes
- Exon arrays



eArray Upgraded to Accommodate Exon Custom Design

Inclu WT

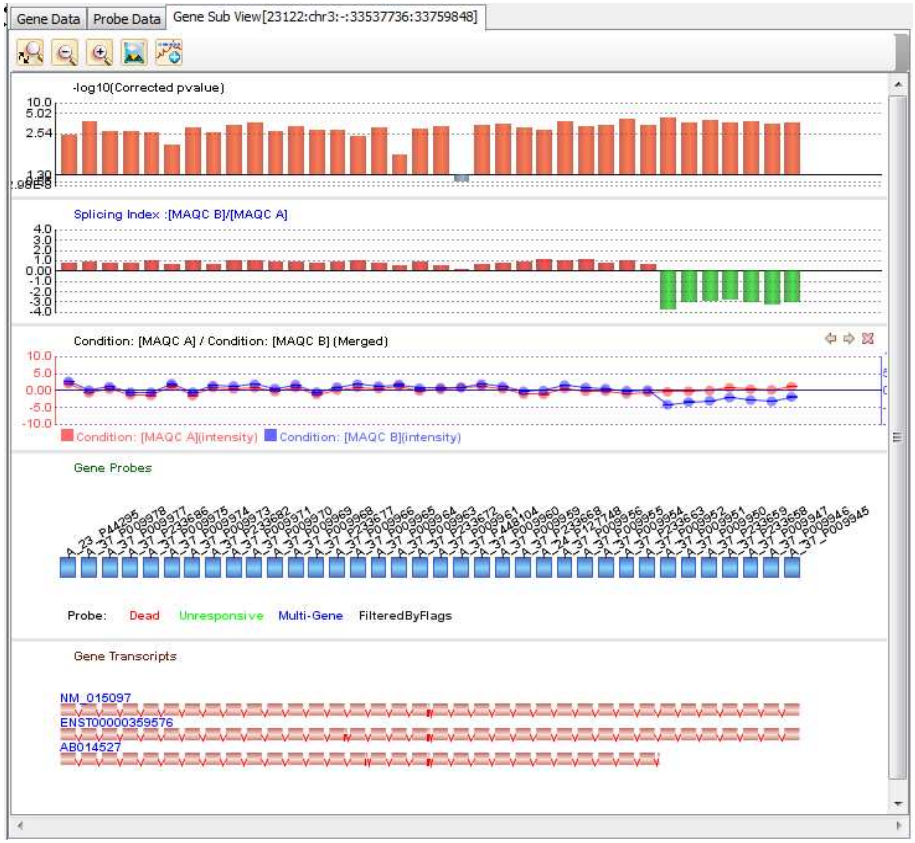


GeneSpring 11.5 Identifies Differentially-expressed Genes and Exons

Identify differentially expressed genes

Identify differentially expressed exons

Calculate corrected p-values for each exon



Screenshot from GeneSpring 11.5

Features & Benefits: SurePrint G3 Exon

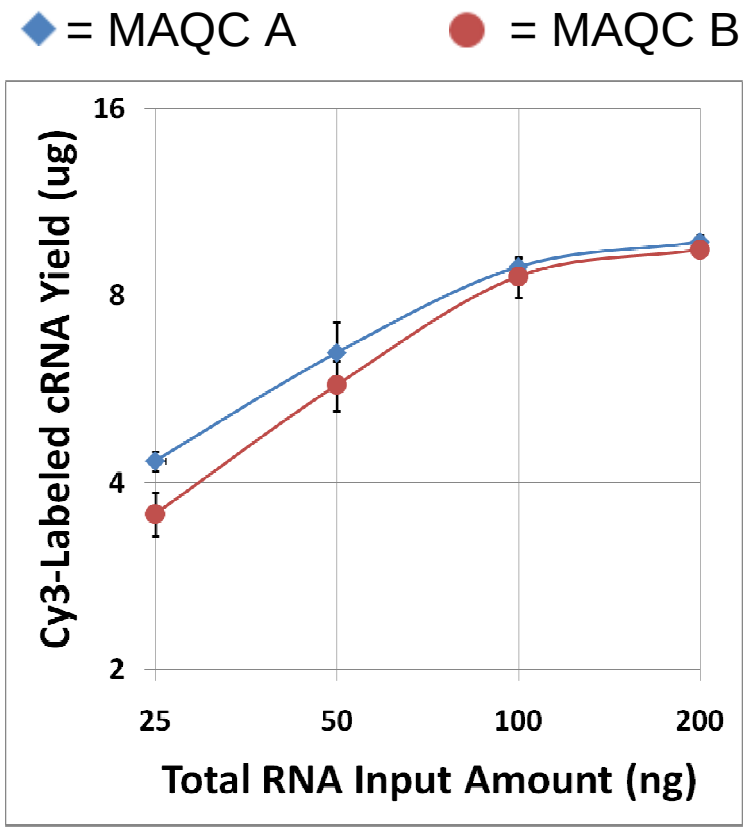
Feature	Benefit
• Two experiments in one: identify both gene-level and exon-level expression changes	• Don't miss out on subtle but important biological changes • Correlate gene- and exon-level changes without unnecessary noise in the system • Efficiency in sample use, processing time and reagents, and costs
• Comprehensive coverage	• Sensitivity to known genes according to recent databases • Study transcripts, not genes to identify subtle but very relevant expression patterns
• Flexibility for catalog and custom	• Perform the experiment that best fits your needs
• Quality data o Wide dynamic range o High reproducibility, accuracy	• Detect low expressors and high expressors • More accurately represent range of expression levels • Feel confident in the results
• Annotations updated regularly	• Annotations stay current with evolving databases
• Compatible with LIQA WT o Low RNA input, down to 25 ng (50 ng recommended) o Single-tube chemistry	• Preserve precious material • Work with small samples • Easy, simple assay

SurePrint G3 Exon Microarrays

The Data

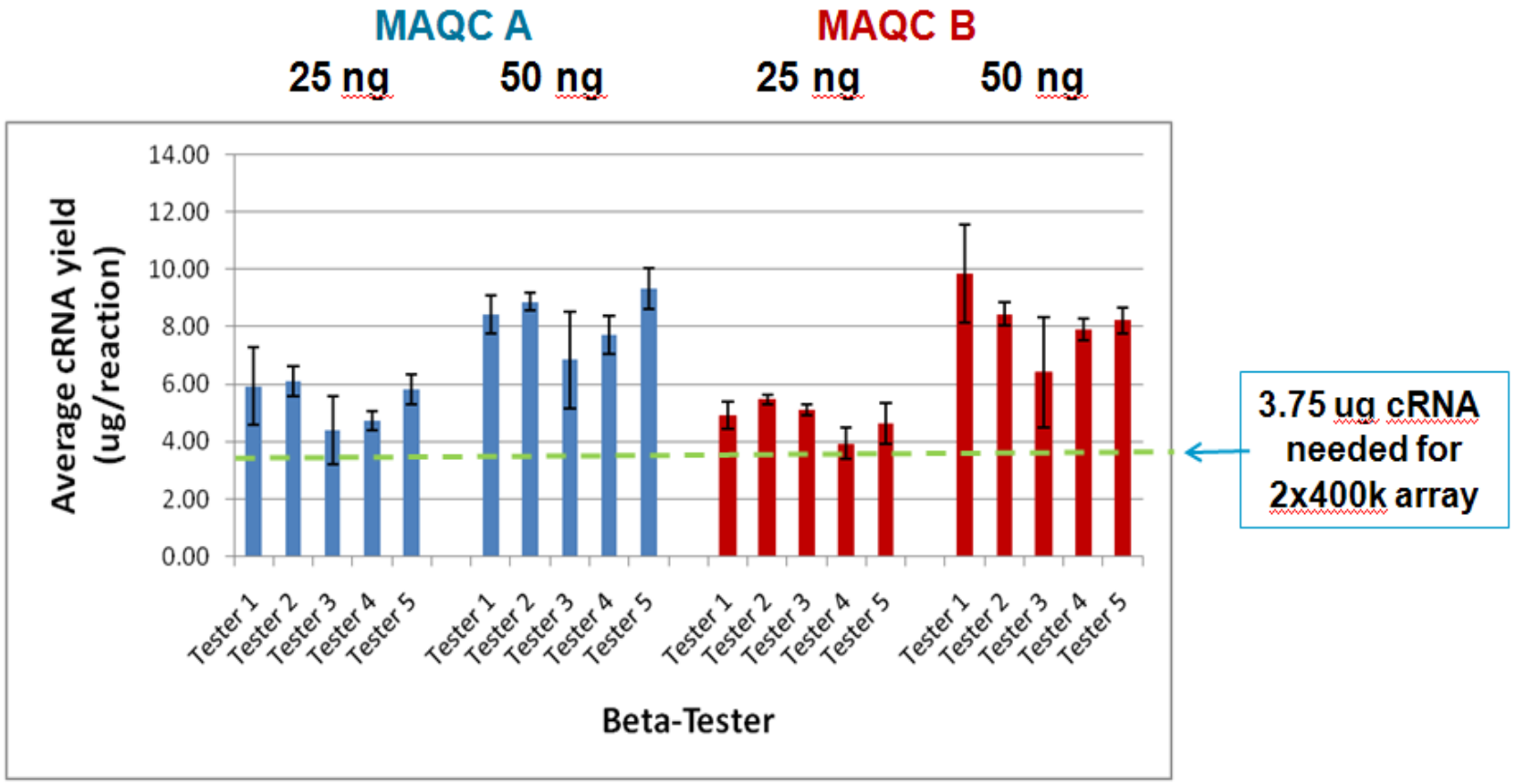
LIQA WT Enables Low Total RNA Input for Exon Analysis

•RNA input requirement as low as 25 ng (50 ng recommended)



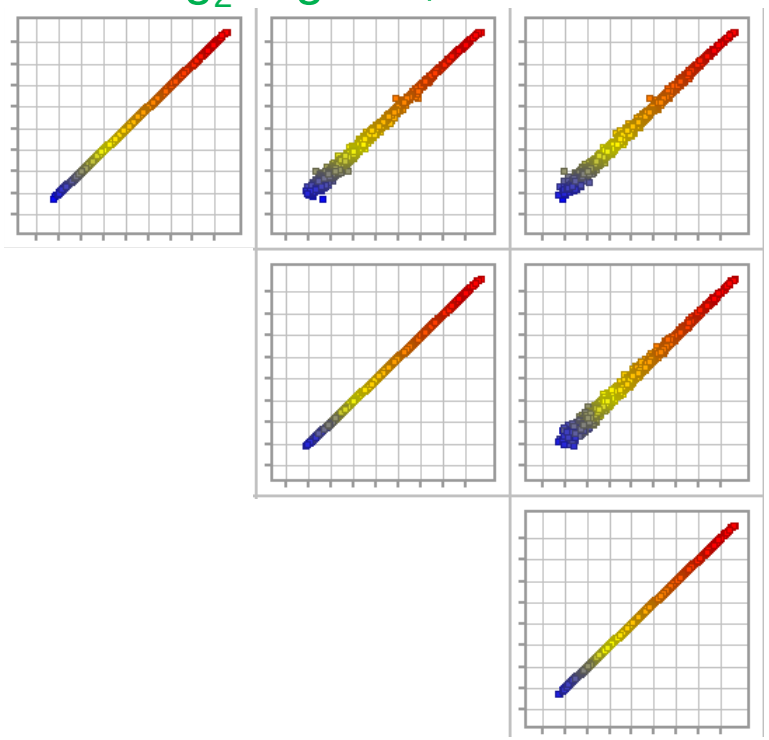
		cRNA Hybridization Requirements	
Array Format	**Recommended RNA Input**	**1-color**	**2-color**
1x formats	100 ng	5.00 µg	2.500 µg
2x formats	50 ng	3.75 µg	1.875 µg
4x formats	50 ng	1.65 µg	825 ng
8x formats	50 ng	600 ng	300 ng

Customers Consistently Obtain Sufficient cRNA Yields Using Low RNA Inputs

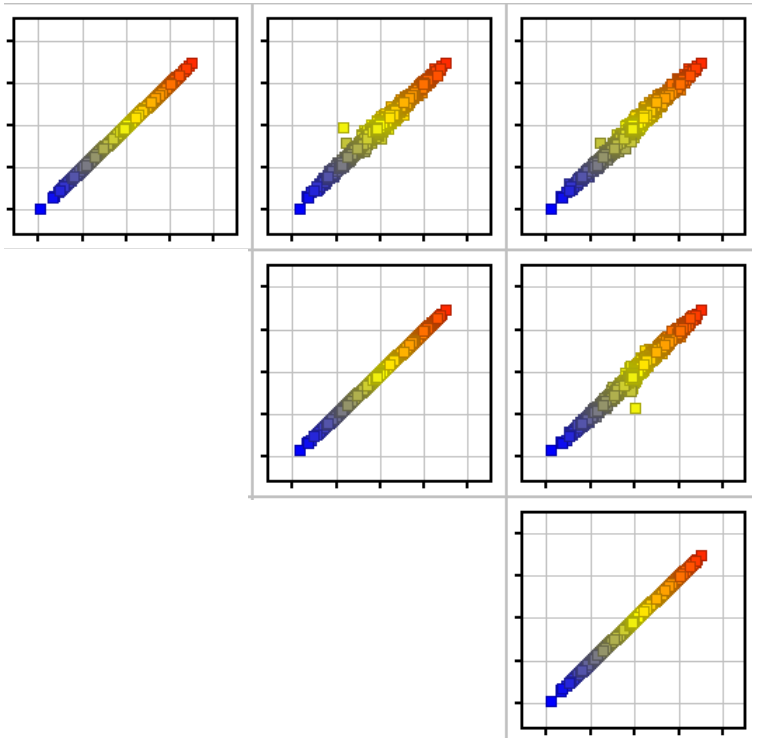


SurePrint G3 Microarrays for High Reproducibility

SurePrint G3 2x400K
50 ng input
Log_2 Signals, 1-color



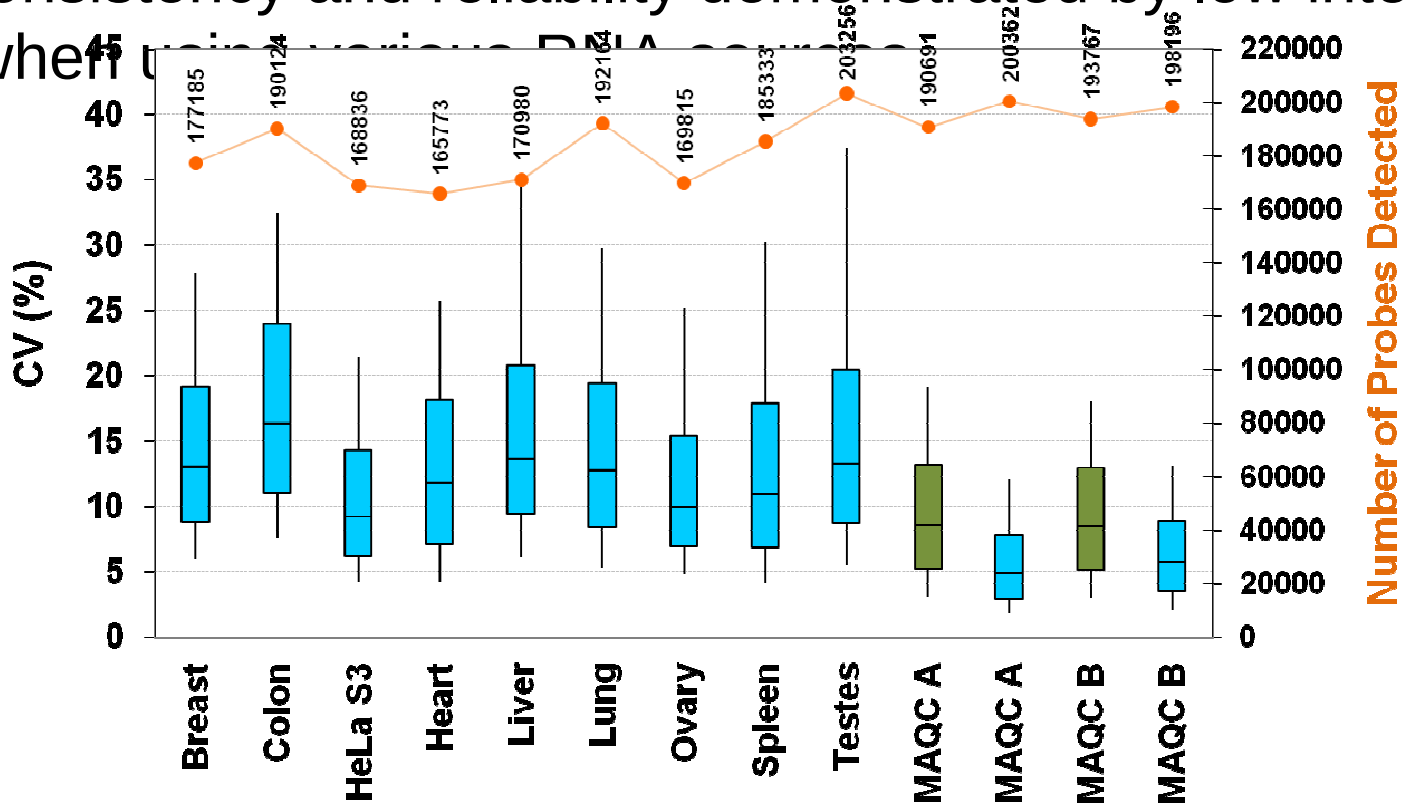
SurePrint G3 2x400K
50 ng input
Log_2 Ratios, 2-color



•Signals and log ratios are highly correlative across technical replicates for both 1-color and 2-color experiments

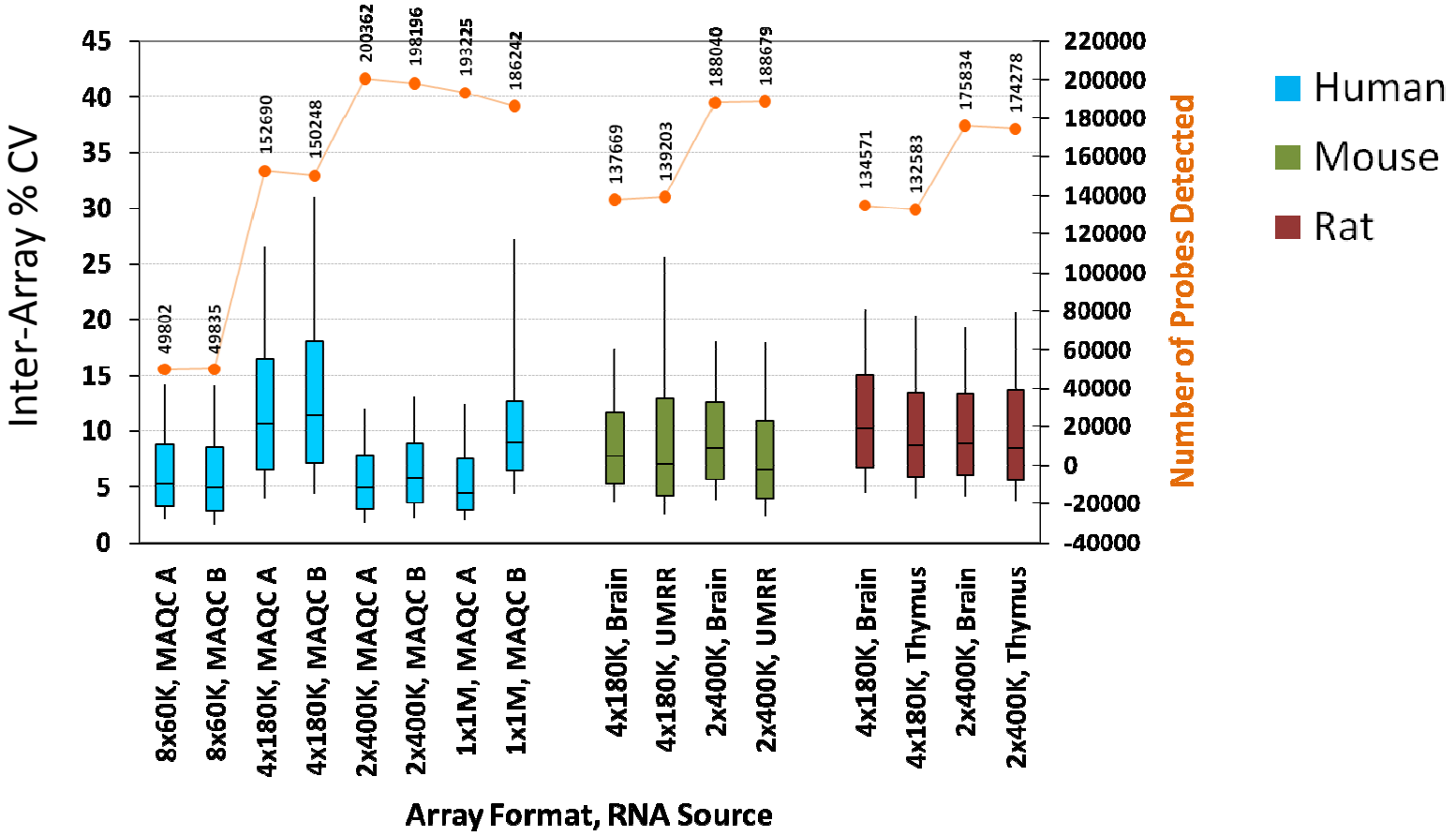
Reproducibility Maintained with Range of Sample Types

High consistency and reliability demonstrated by low inter-array %CV when t



blue bars: 50 ng input RNA
green bars: 25 ng input RNA

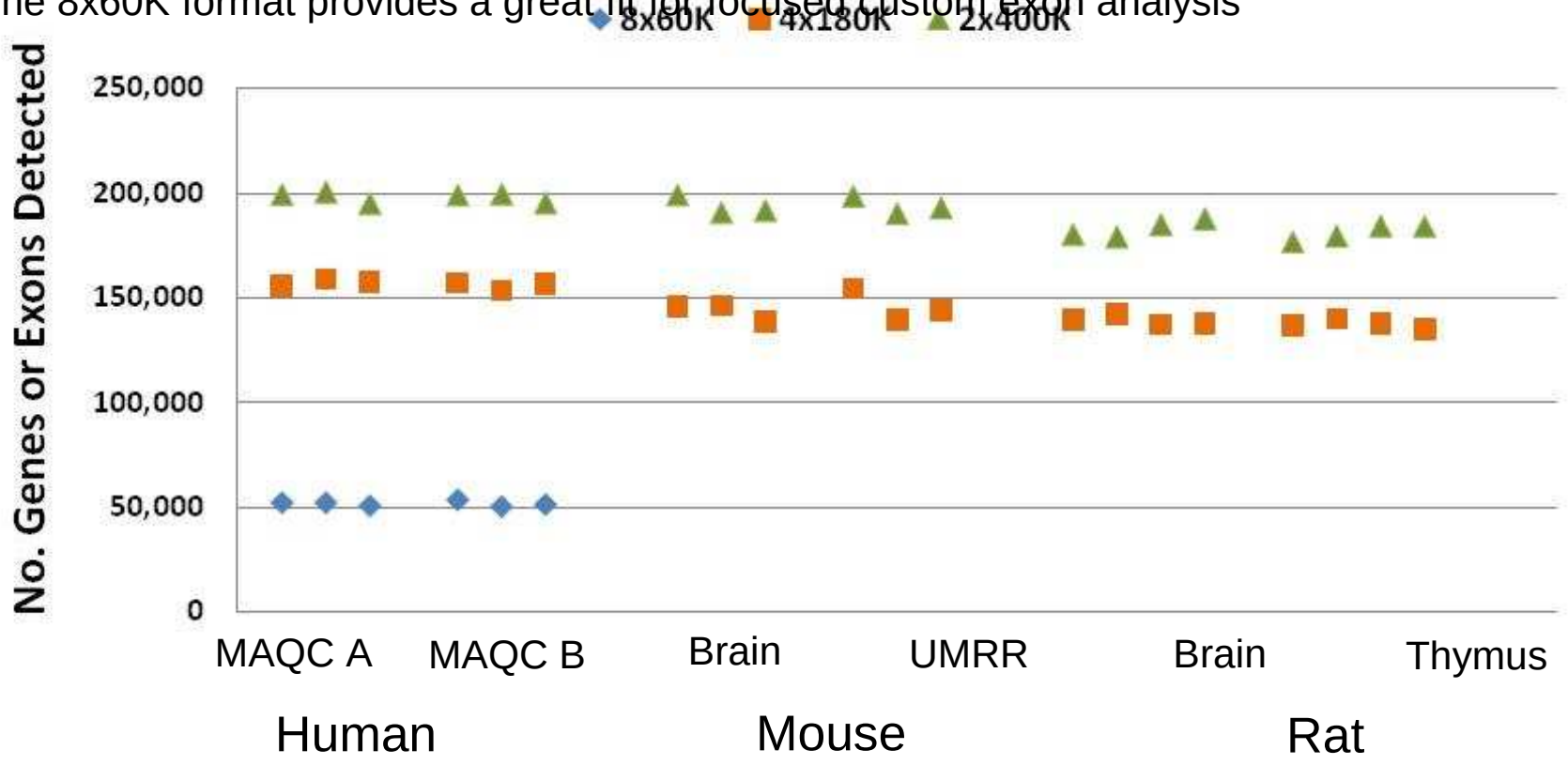
Reproducibility Maintained with All Array Formats



•Formats provide varying amounts of data but with consistent reproducibility across replicate arrays

Exon Microarrays Provide More Coverage

- SurePrint G3 formats provide varying amounts of data to study relevant expression changes
- The 8x60K format provides a great fit for focused custom exon analysis

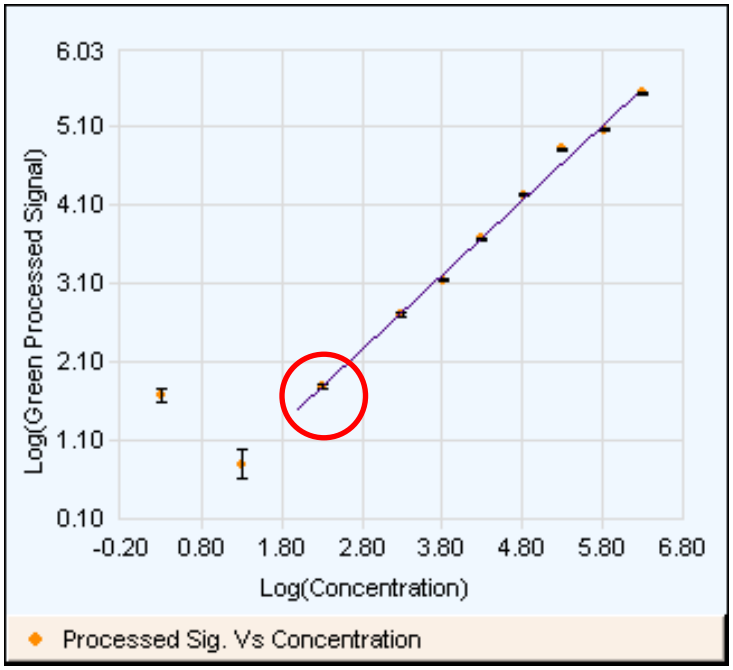


Example based on catalog products

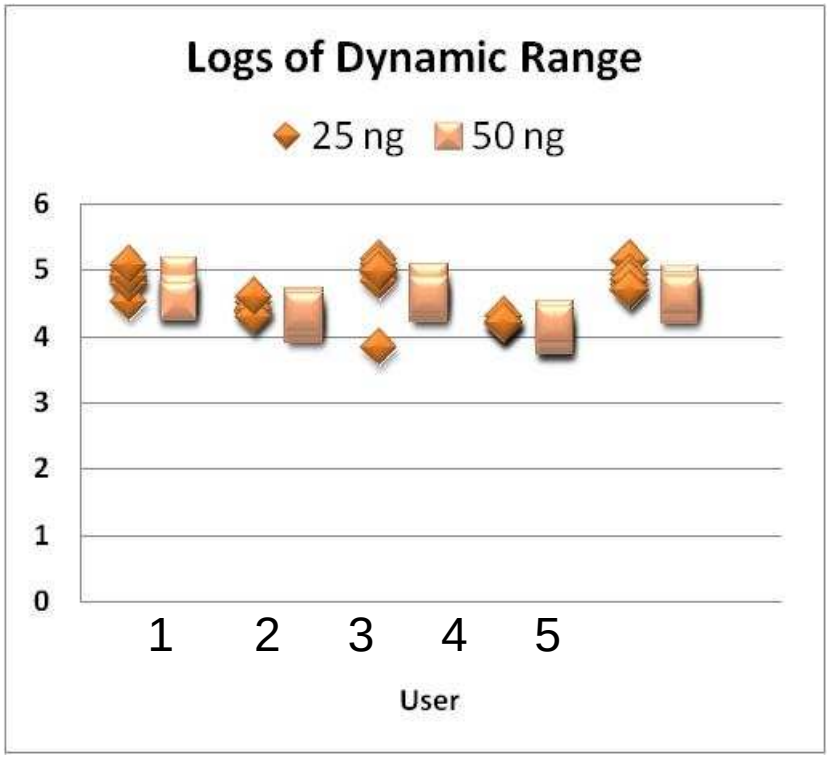
Sensitive Microarray-based Exon Detection

•Agilent Exon Arrays maintain high sensitivity to low-abundance transcripts, as observed through biological and control probes

•Spike-in controls indicate labeling and hybridization success, and sensitive detection



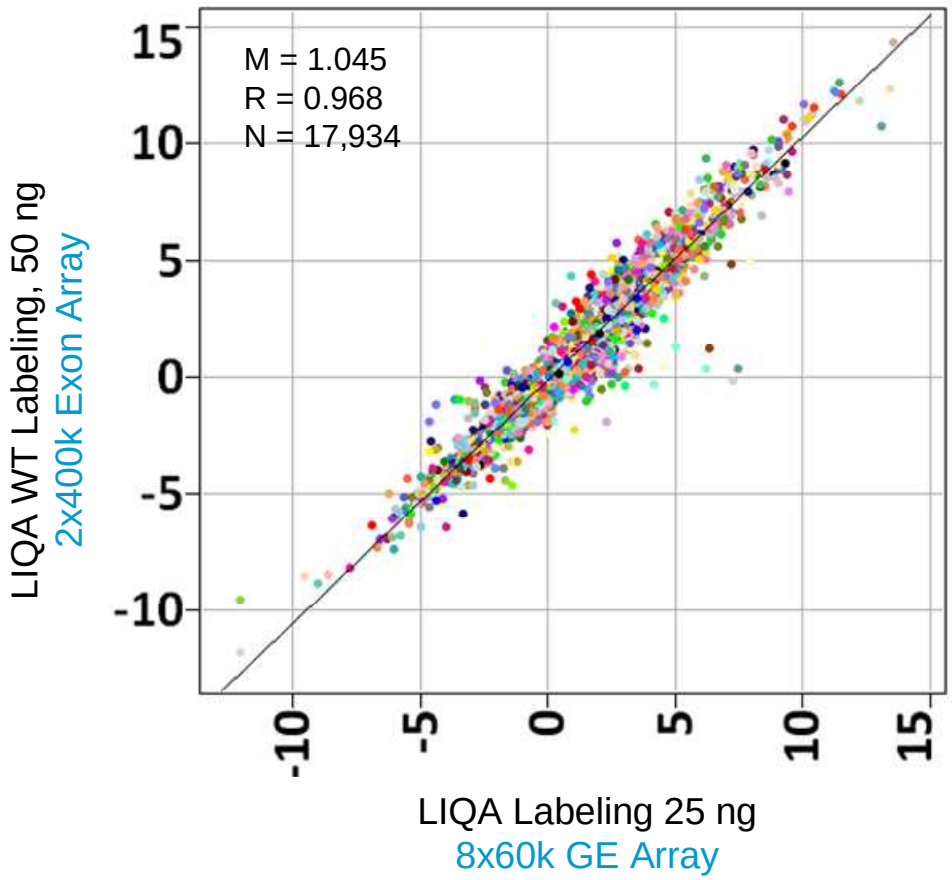
>4 logs



Comparable Results for Exon and Gene Microarrays

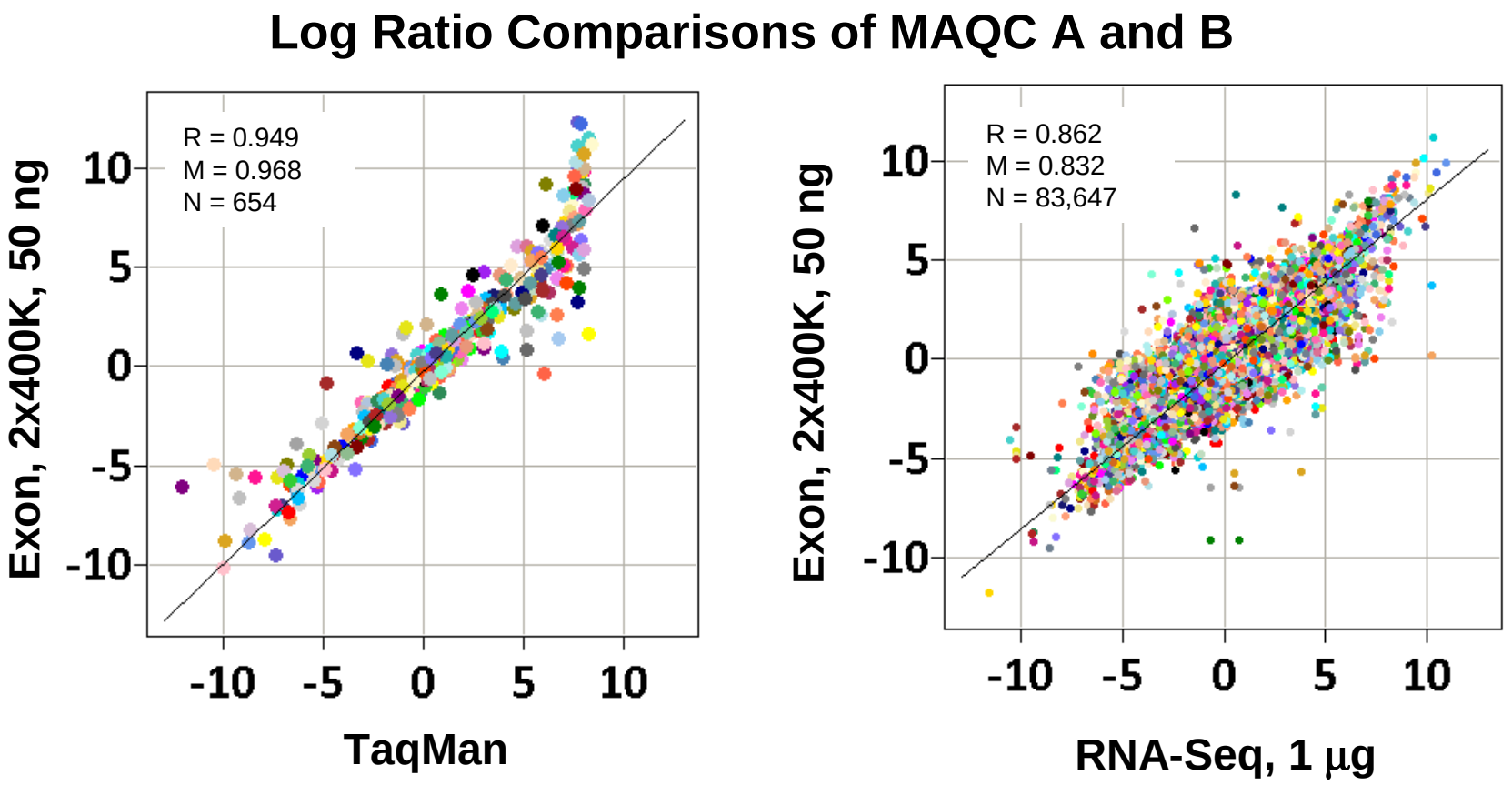
- Exon microarrays provide similar results to standard gene expression arrays
- Both approaches can be used for gene-level analysis

Log Ratio Comparisons of MCF10A/MCF7, 1-color

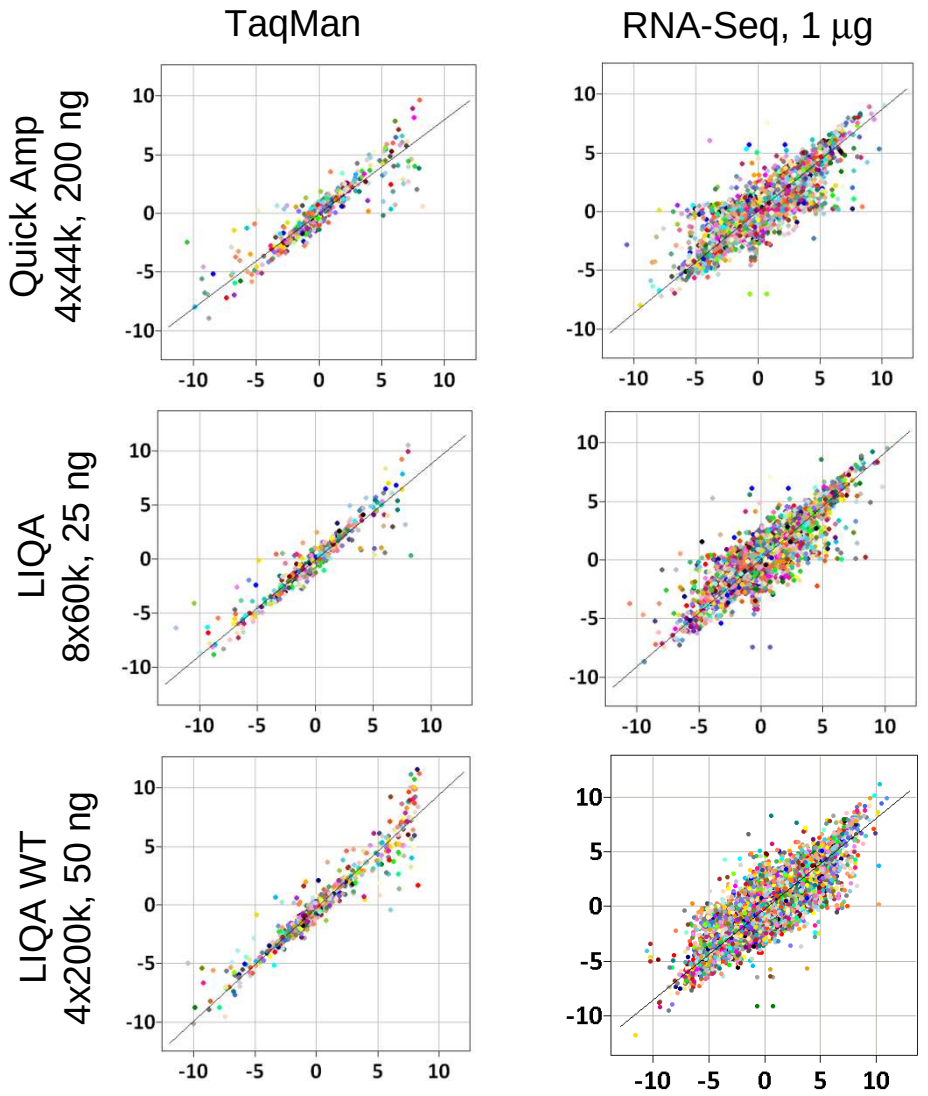


Exon Arrays are Concordant with TaqMan and RNA-Seq

- Probe signals provide an accurate representation of expression levels
- Consistent with multiple alternate technologies



Accurate and Consistent: Comparison to TaqMan qRT-PCR Platform and RNA-Seq

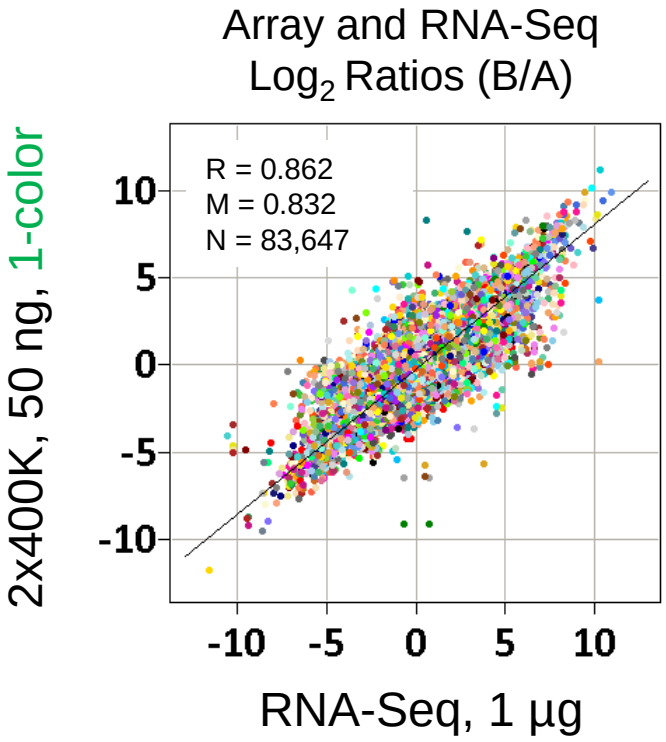


TaqMan	RNA-Seq
N = 578 M = 0.807 R = 0.903	N = 15,622 M = 0.867 R = 0.869
N = 607 M = 0.896 R = 0.935	N = 16,177 M = 0.911 R = 0.891
N = 654 M = 0.968 R = 0.949	N = 83,647 M = 0.832 R = 0.862

SurePrint G3 Microarrays and RNA-Seq: a Comparison

•Strong correlation of log ratios are observed between microarray and RNA-Seq for samples MAQC A and B

•Microarrays are faster, easier, more affordable, and require little RNA input



	Agilent G3 +LIQA WT	RNA-Seq
Starting Material	50 ng	1 µg
Total Sample Prep	6.5 hr	11.5 hr
Hands-on Sample Prep	1.5 hr	3.75 hr
Sample to Data	1.5 days	~ 1 week
Analysis	Simple	Challenging
Price per sample	$$	$$$

Beta-Test Microarrays and TaqMan Comparison

Demonstrates Consistency Across Users