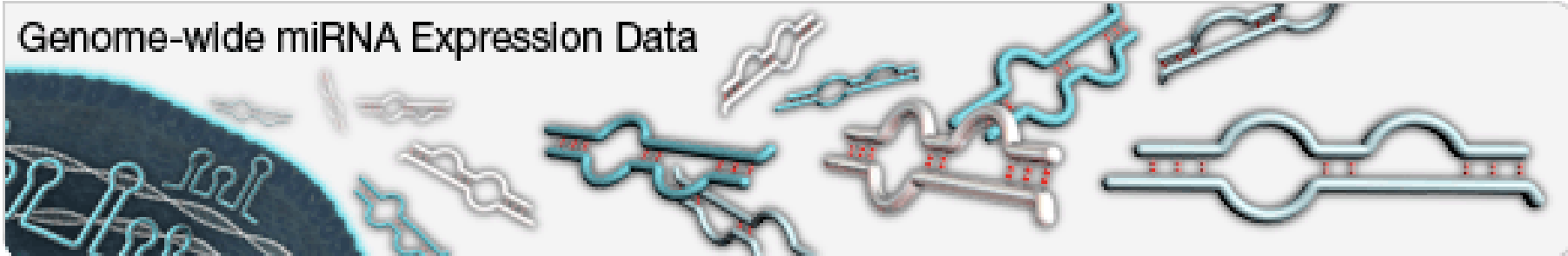


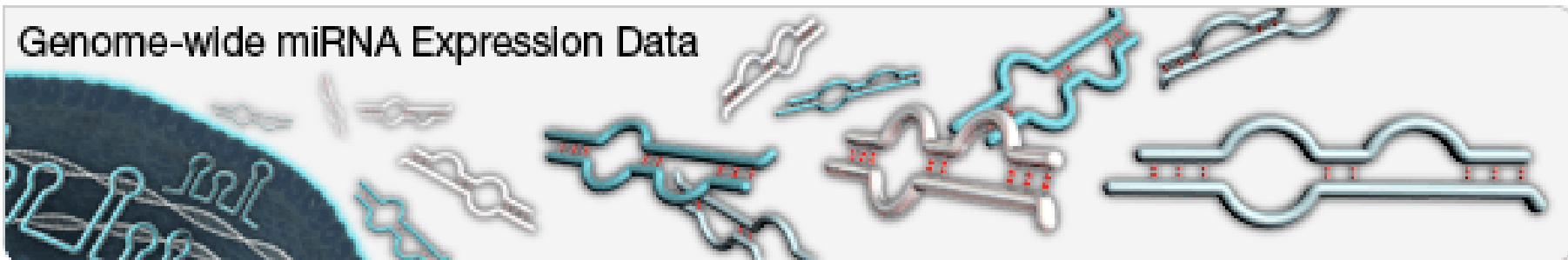
Genome-Wide & Pathway-Focused Analysis With an Advanced qPCR Technology

Genome-wide miRNA Expression Data



Samuel J. Rulli, Jr., Ph.D.
qPCR Applications Scientist
Samuel.Rulli@QIAGEN.com

Genome-Wide & Pathway-Focused Analysis With an Advanced qPCR Technology



Questions? Comments? or Suggestions?
Ask now or contact Technical Support

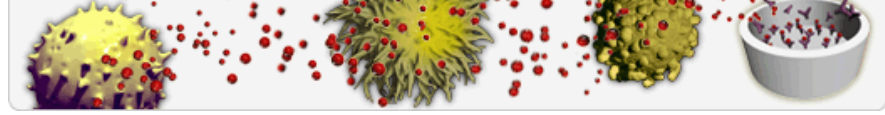
Telephone: 888-503-3187; Email: support@SABiosciences.com

Welcome to SABiosciences: Systems Biology with a Pathway Focused Approach

Accurate and Simple Pathway Expression Analysis



Measure Cytokine Expression



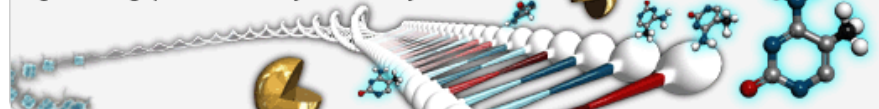
Genome-wide miRNA Expression Data



Successful ChIP in One Day



High-Throughput DNA Methylation Analysis



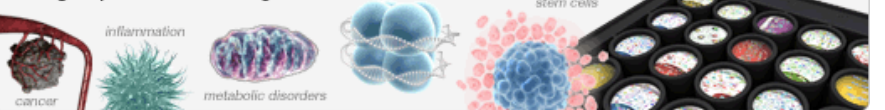
Cell-Based Assays for Signaling



High-potency Genome-wide siRNA and shRNA



Linking Expression and Regulation



- SABiosciences is now a  Company

Introduction to miRNA

miScript miRNA PCR Array Overview

- How It Works
- Portfolio
- Benefit

miScript miRNA PCR Array Applications

- Cancer
- Development & Differentiation
- Genome-Wide Discovery

Endogenously expressed small functional RNAs (19-25 nt)

Regulate mRNA expression post-transcriptionally

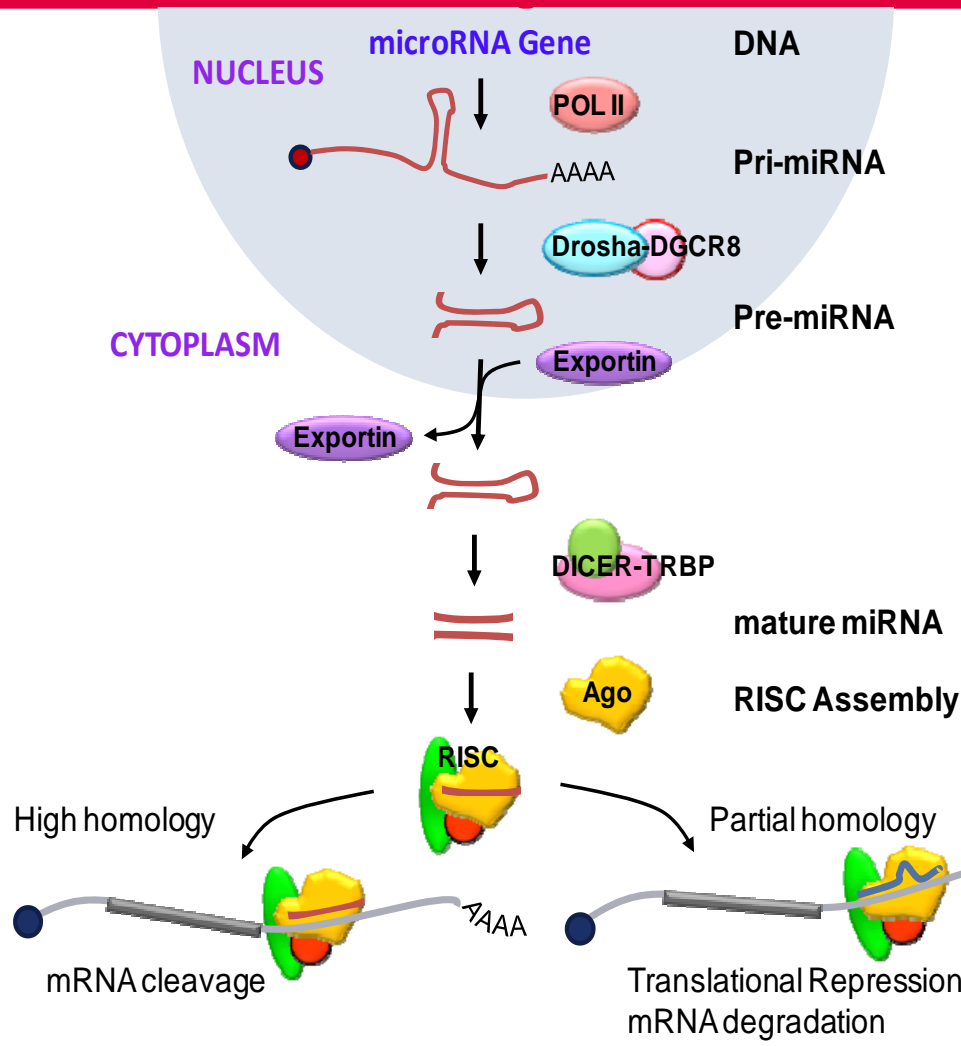
- mRNA degradation, but also translation inhibition

Only partial sequence complementarity required

- One miRNA can potentially regulate hundreds of mRNAs
- One mRNA can be regulated by multiple miRNAs

Another layer of complexity for regulating gene expression

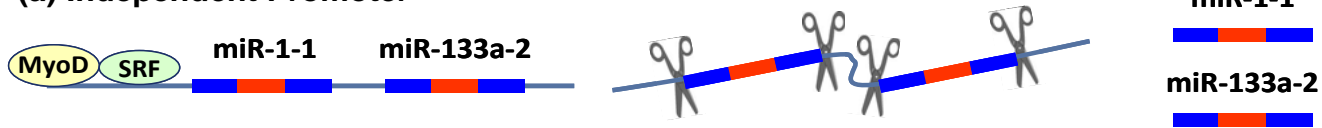
Canonical pathway of microRNA biogenesis



- Transcribed by RNA Polymerase II as a long primary transcript (pri-miRNAs), which may contain more than one miRNA.
- In the nucleus, Pri-miRNAs are processed to hairpin-like pre-miRNAs by RNase III-like enzyme Drosha
- Pre-miRNAs are then exported to the Cytosol by Exportin 5
- In the cytosol RNase III-like Dicer processes these precursors to mature miRNAs
- These miRNAs are incorporated in RISC
- miRNAs with high homology to the target mRNA lead to mRNA cleavage
- miRNAs with imperfect base pairing to the target mRNA lead to translational repression and/or mRNA degradation

Krol, J et.al., (2010) Nature Rev Genetics, 11, 597; Winter, J. et.al., (2009) Nature Cell Biology, 11, 228

(a) Independent Promoter



(b) Intronic



(c) Exonic



- **Intergenic miRNA genes:** either monocistronic or polycistronic with a common promoter
- **Intronic miRNA genes:** present in the introns of protein coding or noncoding genes, can also be in clusters, transcribed by the host gene promoter
- **Exonic miRNAs genes:** rare and often overlap an exon and an intron of a noncoding gene
- miRNAs can be transcribed from the negative strand within or near a protein coding gene



Multiple loci can generate the same mature miRNA

But are under different regulatory control

Stem Loop	CHR	Overlapping transcripts		CHR: Coordinates (GRCh37)
1302-1	12	intergenic		12: 113132839-113132981 [-]
1302-3	2	intergenic		2: 114340536-114340673 [-]
1302-7	8	intergenic		8: 142867603-142867674 [-]
1302-10	15	intergenic		15: 102500662-102500799 [-]
1302-11	19	intergenic		19: 71973-72110 [+]
1302-2	1	intronic	Non protein coding	1: 30366-30503 [+] sense
1302-4	2	intronic	Non protein coding	2: 208133999-208134148 [-]
1302-9	9		Non protein coding	9: 30144-30281 [+]; Sense
1302-5	20	intronic	Protein coding/FAM65C; intron4	20: 49231173-49231322 [-]; Sense
1302-6	7	intronic	Protein coding/HDAC9; intron 1	7: 18166843-18166932 [-] ; Antisense
1302-8	9	intronic	Protein coding/ch9orf174	9: 100125836-100125963 [-]; Antisense

Symbol	HGNC:MIR1302-1	
Description	Homo sapiens miR-1302-1 stem-loop	
Stem-loop	<pre> -- aaag c a ag a u gug - a a uac cag cc agua auuugaauuca uaa caa gaauaau ua uguaag au ucca a a guc gg ucaau uaaacuuaaagu auu guu cuuauua au guauuc ua agggg u au cuua a g cu c c -aa c a c uua </pre>	
	Get sequence	
Deep sequencing	2 reads, 1 experiments	
Genome context	<i>Coordinates (GRCh37)</i> 12: 113132839-113132981 [-]	<i>Overlapping transcripts</i> intergenic

Mature-miR-1302: UUGGGACAUACUUAUGC UAAA

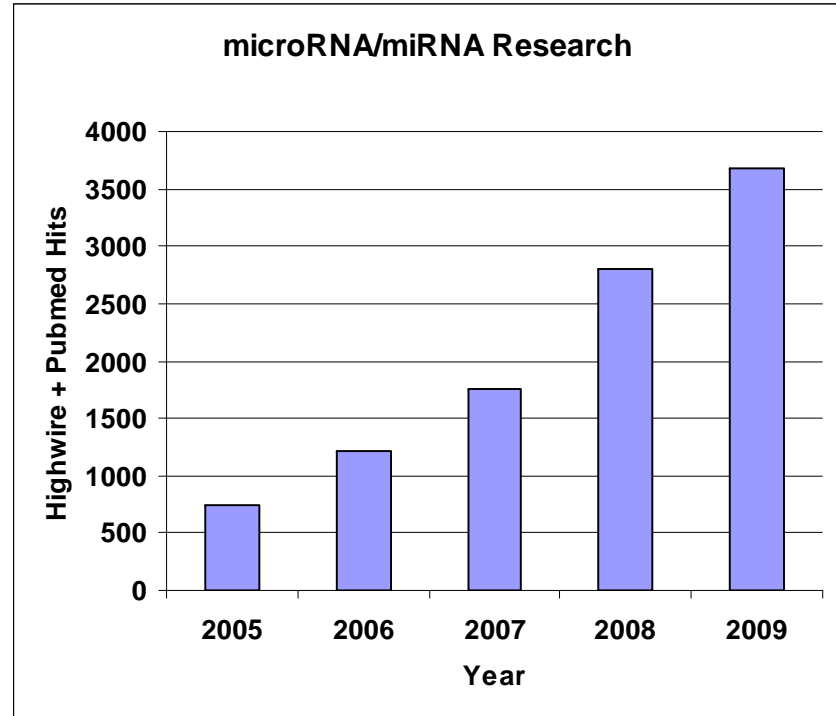
miRNA database: www.mirbase.org

New miRNAs continually discovered (release 16.0)

- Human: 1066
- Mouse: 940
- Rat: 653

Nomenclature

- Pri-miRNA (>100nt)
- Pre-miRNA (~70nt)
- Mature miRNA (~20nt)



Potential for genome-wide coverage, even though >1000 miRNAs identified

miRNA expression profiles correlate with:

- Protein & Gene Expression Levels, Biological Phenotypes

miRNA regulated genes are involved in a variety of biological processes and diseases:

- Cancer
- Development
- Immunology
- Aging
- Heart Diseases
- Neurological Diseases

Northern Blot

- Very low throughput
- Very time consuming and complex

Microarrays

- Profile More Sequences
- Poor Sensitivity & Dynamic Range, Complicated Protocol

Real-Time RT-PCR

- Profiles Fewer Sequences (Up to 384)
- Simpler Protocol
- Better Sensitivity, Specificity & Reproducibility

Introduction to miRNA

miScript miRNA PCR Array Overview

- How It Works
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miScript miRNA PCR Array Applications

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- Genome-Wide Discovery

miRNeasy miRNA Isolation Kit

miScript miRNA 1st Strand cDNA Synthesis Kit

- Preferentially reverse transcribe mature miRNA
- Built-in external RNA control
- One-step reaction

miScript miRNA PCR Arrays and qPCR Primers

- Human, mouse, rat: Search miRNA Primers:

-or- http://www.sabiosciences.com/mirna_pcr_assay.php

- PCR Arrays: Pathways and Genomes

- Cancer
- Cell Differentiation & Development
- Immunopathology
- Inflammation
- miFinder
- Whole Genome (miRNome) – Sanger miRBase V14.0
- Serum (or plasma)
- Brain Cancer miRNA
- Neurological Development & Disease

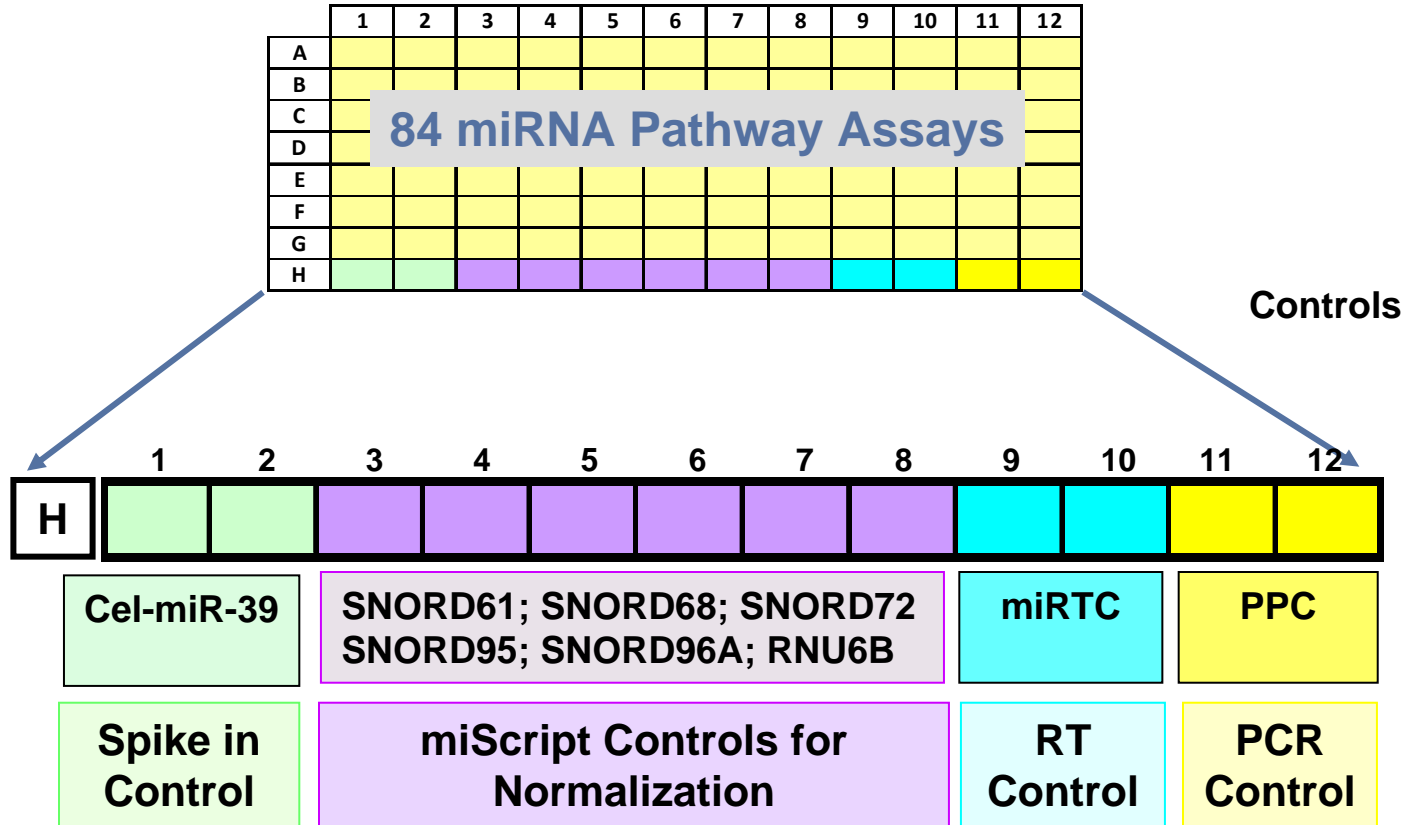
Optimized miScript SYBR[®] Green Master Mix

miScript miRNA Data Analysis Excel Template & Web Portal





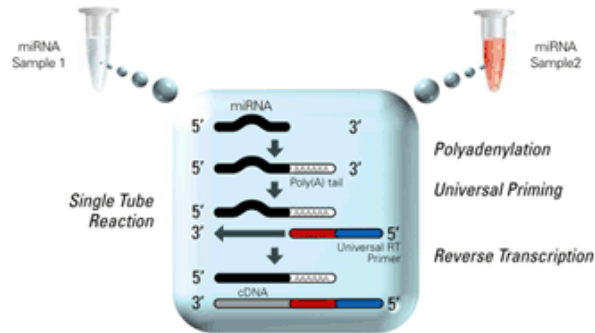
miScript miRNA Array format: Standardized controls (96 well plate)



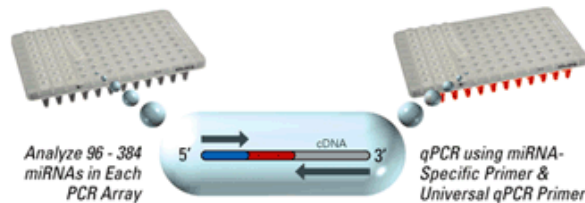
miScript controls: Common for Human, Mouse, Rat and Dog Arrays

miScript miRNA PCR Arrays

1. Convert miRNA to cDNA via Universal Tailing & Reverse Transcription.



2. Add cDNA to qPCR Master Mix. Aliquot Mixture Across PCR Array.



3. Run in Your Real-Time PCR Instrument.



4. Data Analysis.



cDNA Synthesis (miScript II RT KIT)

- 1 hours

Load Plates (Preferably with 8-Channel Pipettors)

- 2 minutes

Run 40 cycle qPCR Program

- 2 hours

Upload and Analyze Data

- 15 minutes

Complete miRNA genome (miRNome)

- Human miScript miRNA PCR Array
- Mouse miScript miRNA PCR Array
- Rat miScript miRNA PCR Array
- Dog miScript miRNA PCR Array

Species	Number of assays (miRBase V16)	Total Number of Plates	
		96 well format	384 well format
Human	1068	12	3
Mouse	947	12	3
Rat	653	8	2
Dog	277	4	1

miRNA Pathway Arrays

- Human, Mouse, Rat:
 - Brain Cancers
 - Cancer
 - Cell Differentiation & Development
 - Immunopathology
 - Inflammation
 - miFinder
 - Neurological Development & Disease
 - Serum and Plasma **(NEW!)**
 - Breast Cancer
 - Ovarian Cancer

All miRNA designs based on mirBase 16



Compatible Instrumentation: 96- & 384-Well Formats



96-Well Blocks: 7000, 7300, 7500, 7700, 7900HT, **ViiA 7**
FAST 96-Well Blocks: **7500, 7900HT**, Step One Plus, **ViiA 7**
FAST 384-Well Block: 7900HT, **ViiA 7**



iCycler, MyiQ, MyiQ2, iQ5, CFX96, CFX384
Opticon, **Opticon 2**, Chromo 4



Mastercycler ep realplex 2/2S/4/4S



LightCycler 480



Mx3000p, Mx3005p, Mx4000p



Rotor-Gene Q, Rotor-Gene 6000

☆ SABiosciences miRNA PCR Array Service Core

Short sequence (21-23nt)

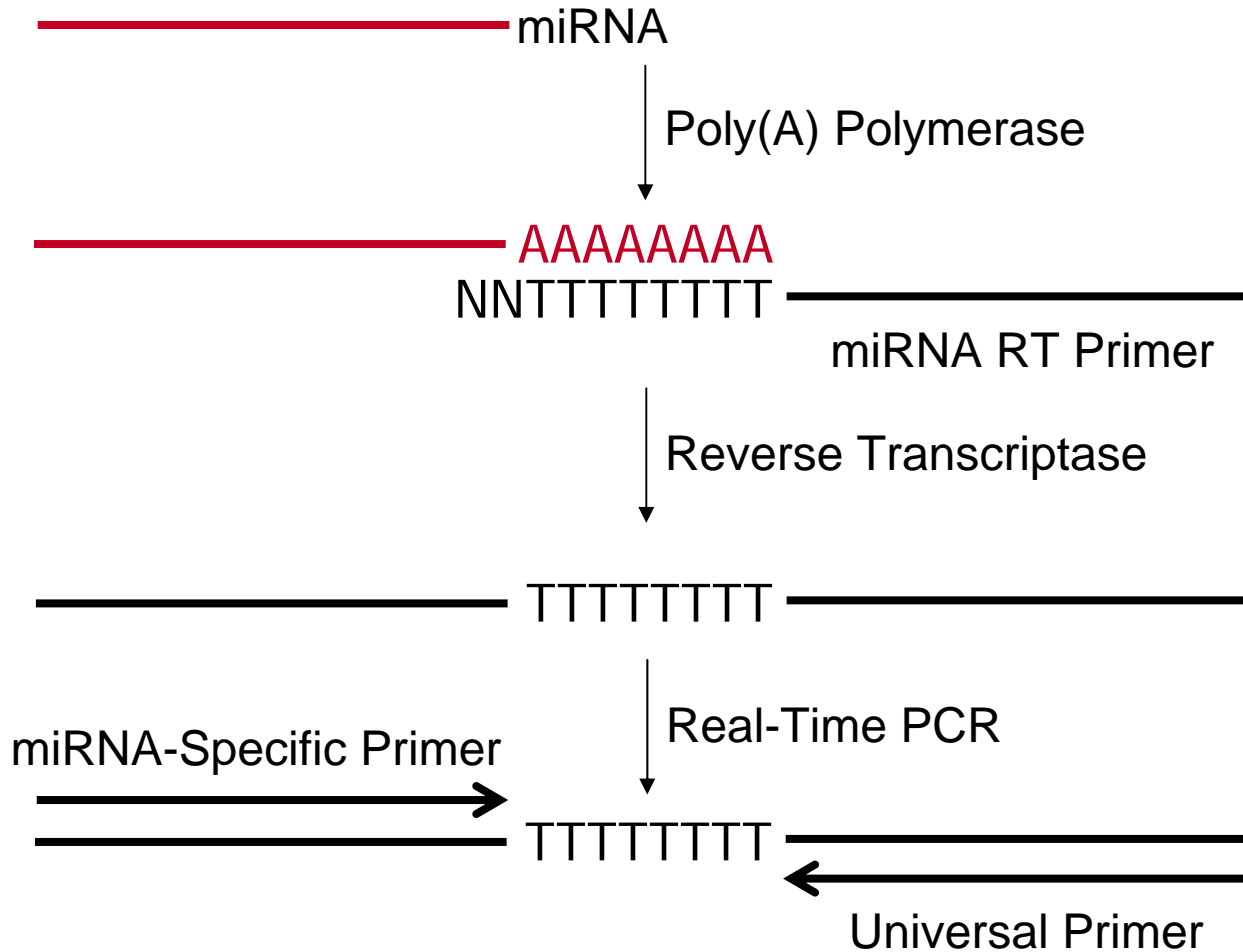
Background from contaminating small RNAs (tRNA, rRNA, snRNA)

Highly homologous

- Many miRNAs have a single nucleotide difference

SABio: Universal miRNA Reverse Transcription

Same cDNA preparation can assay ANY miRNA



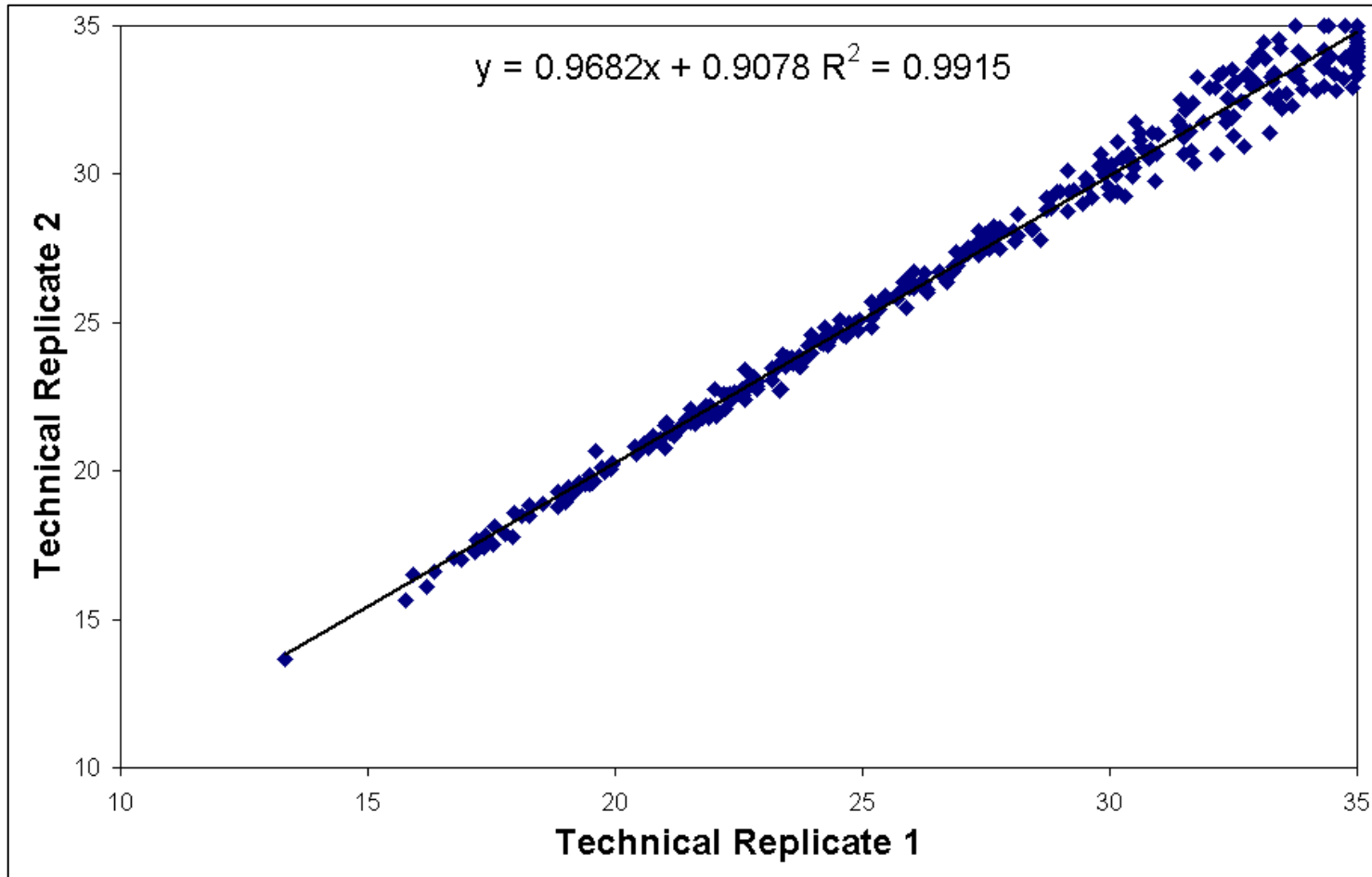
Ease of Universal Reverse Transcription Reaction

- Simpler setup, without primer interaction issues
- Less sample necessary for analyses
- Equal RT reaction for each miRNA, to ensure reproducible expression analysis

Comprehensive miRNA coverage

- cDNA can be saved for new analyses when additional miRNA sequences are discovered

Similar potential specificity, & additional flexibility!



The miscript miRNA PCR Assays are highly reproducible, ensuring run-to-run, plate-to-plate and sample-to-sample reliability.

Technical

- Reproducibility of the PCR Arrays is very high
- Results demonstrate that what you are seeing is a result of biology, not technique.
- RTC & PPC show technical reproducibility on each plate, and comparable across plates.

Biological

- Needed to verify the results are a result of biology
- Need multiple samples
 - At least 3 replicates per sample for statistical analysis
 - p values
 - 95% Confidence Intervals

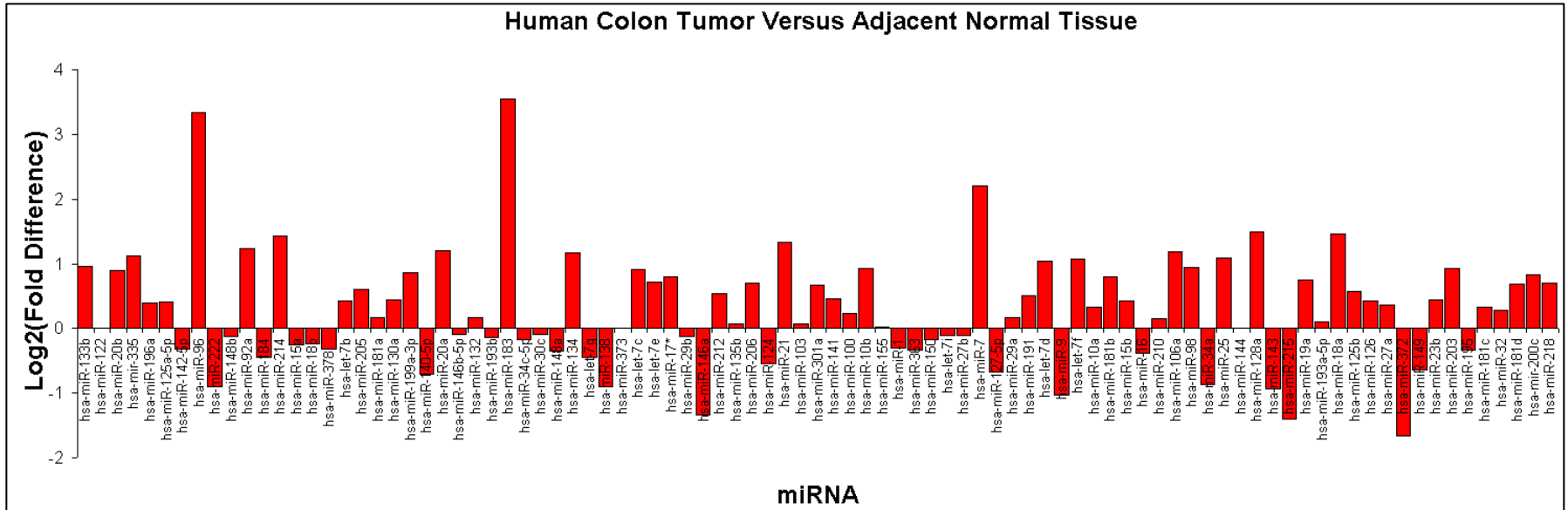
Introduction to miRNA

miScript miRNA PCR Array Overview

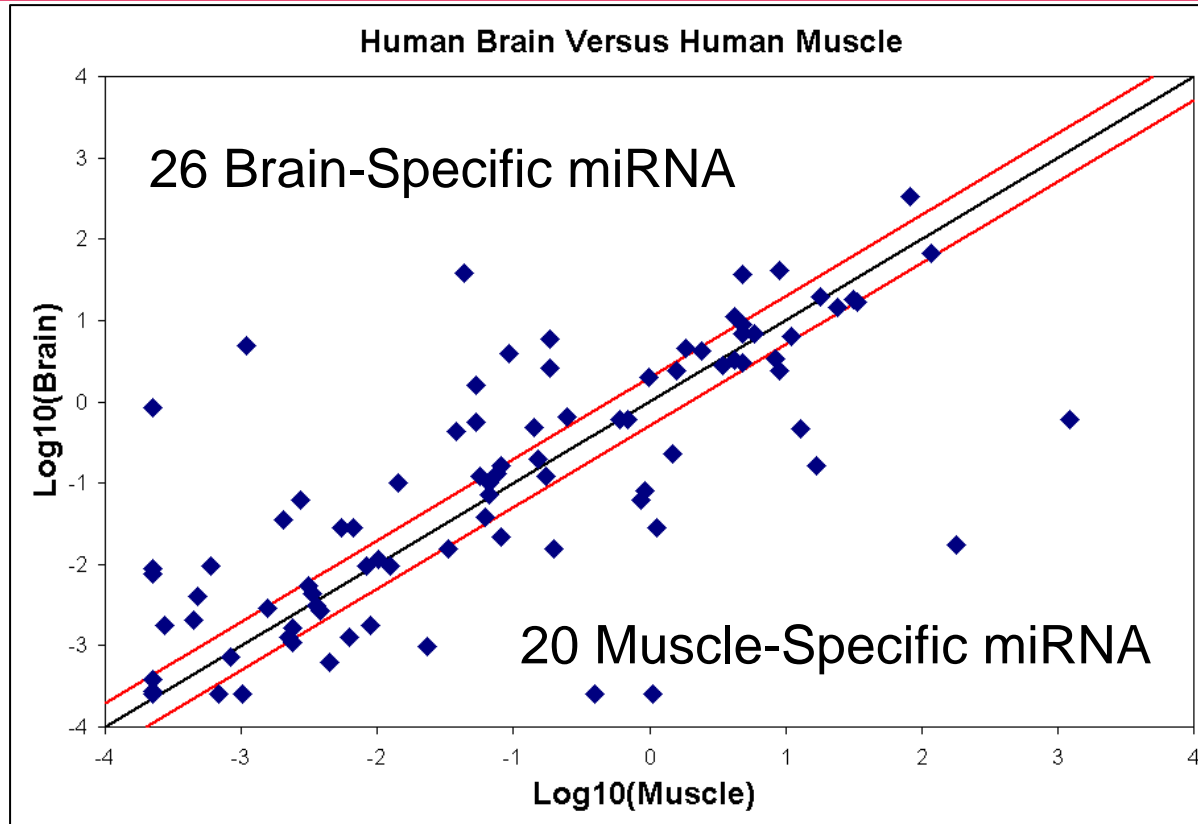
- How It Works
- Portfolio
- Benefit

miScript miRNA PCR Array Applications

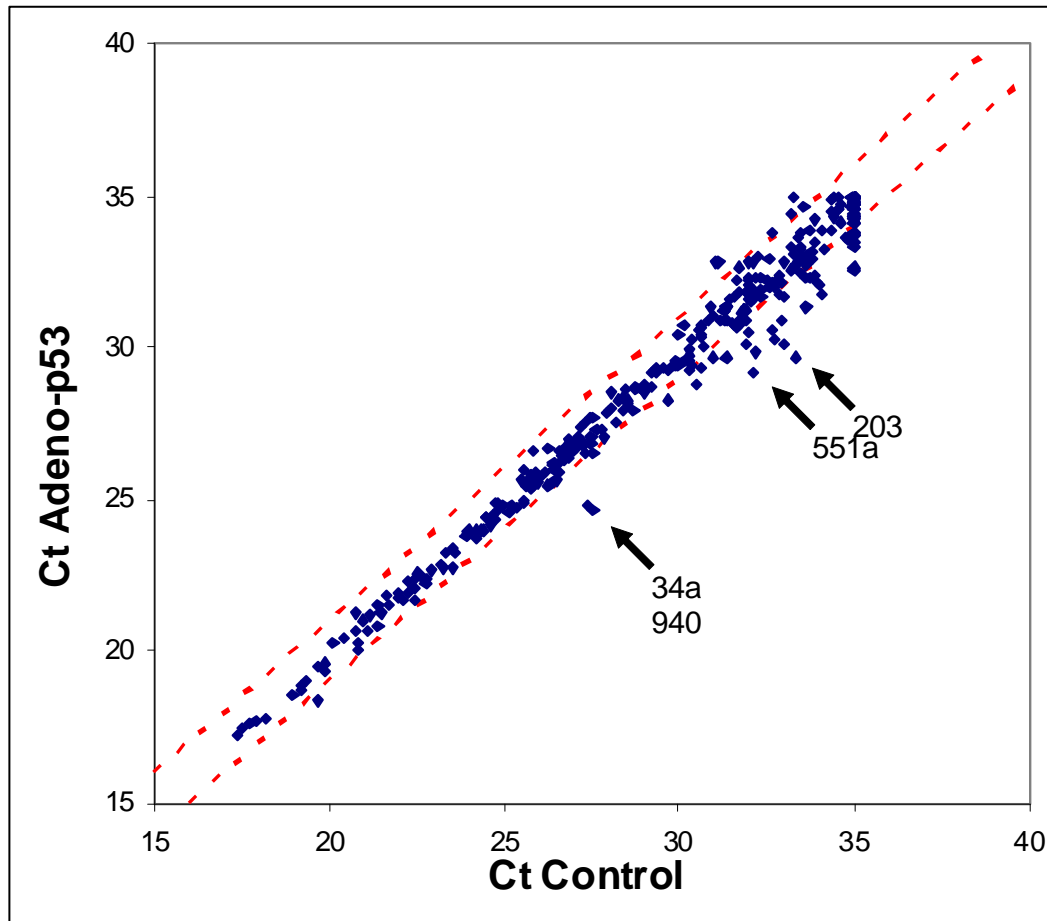
- Cancer
- Development & Differentiation
- Genome-Wide Discovery



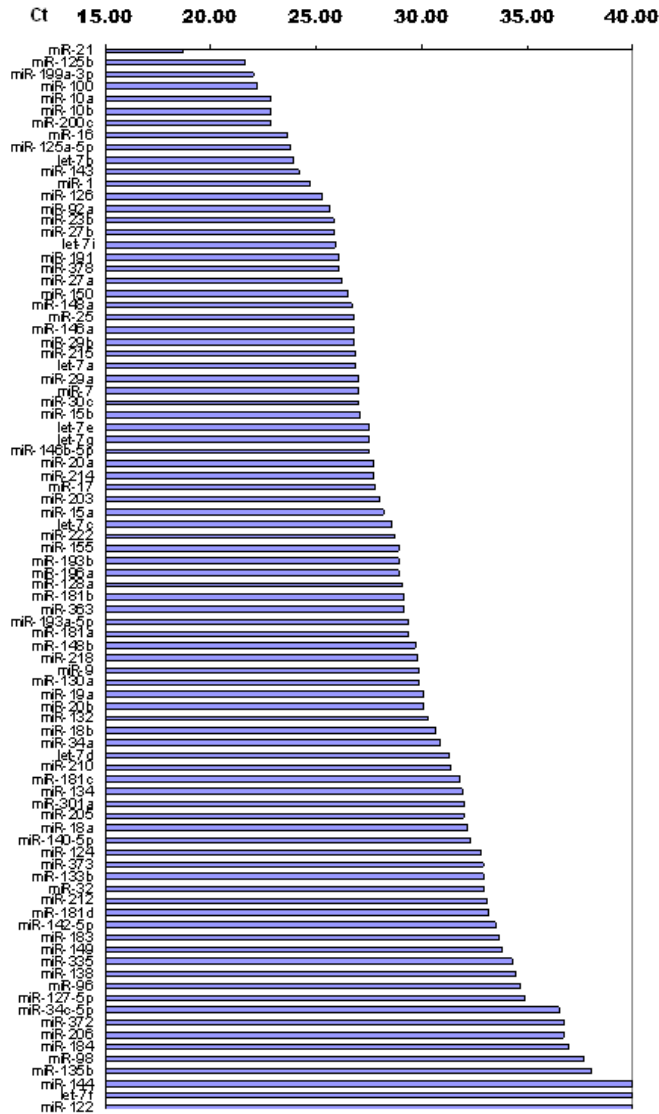
**Human Cancer miscript miRNA PCR Array:
Many Cancer-Specific miRNA Are Up-regulated in a
Colon Tumor.**



Human Cell Differentiation & Development microarray miRNA PCR Array: Identifies 26 Brain- and 20 Muscle-Specific miRNA Potentially Regulating Tissue-Specific Gene Expression.



miscript Human Genome miRNA PCR Array: Identifies Known and Novel miRNA Targets of the p53 Signaling Pathway



Expression rank order of 88 cancer related miRNAs in RNA extracted from one 20µm section of a 2-year old normal human colon FFPE block using the miscript FFPE RNA Extraction Kit.

miRNA Function

- Regulates gene expression post-transcriptionally

miRNA Performance

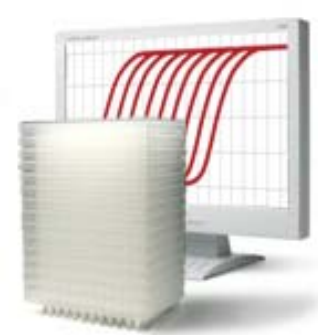
- RT-PCR vs. Microarrays
- Universal vs. Sequence-Specific RT
- Specificity, Sensitivity & Reproducibility

miRNA Applications

- Screen cancer- or development-focused miRNA panels
- Discover novel roles for miRNA sequences

How can YOU analyze miRNA in YOUR research?

... With miscript miRNA PCR Arrays & Assays!



**Experience miRNA PCR Array Performance -
Try them today!**

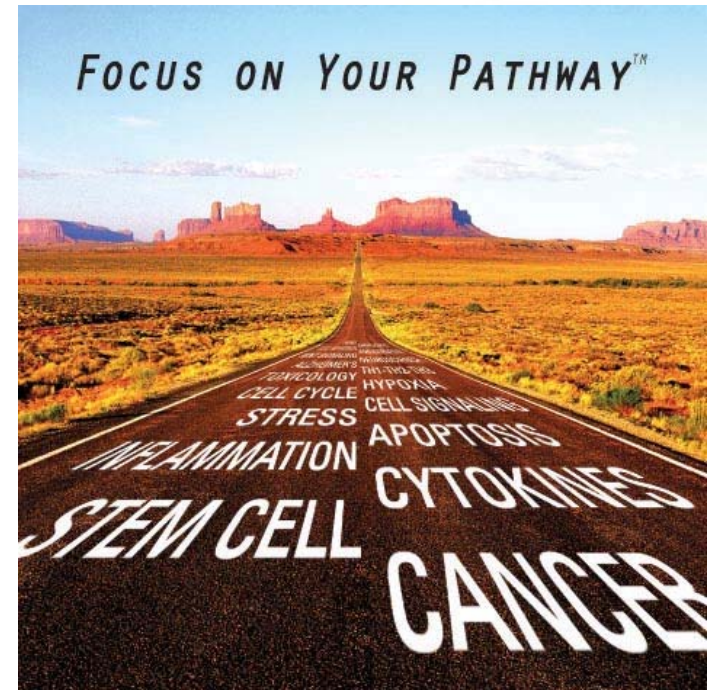
Promo Code: FDK-MIS1

Starter Pack for miscript miRNA PCR Arrays

- 2 96-well or 1 384-well (4x96) PCR Arrays (**Free**)
 - **Any pathway**
- With purchase of:
- mIscript SYBR PCR Kit (200 reaction)
- miscript miRNA 1st Strand cDNA Synthesis Kit

Please call to take advantage of this offer.

Valid for US & Canadian customers



Questions?

Contact Technical Support 9 AM – 6 PM Eastern M – F

Contact: 1-888-503-3187 OR support@SABiosciences.com

Many scientists have discovered the power of miRNA PCR Arrays. Join them on the road to success!

<http://www.sabiosciences.com/promotion/miscriptdemo.php>



What's Next – After PCR Arrays

Validate results with more sample and focused set of genes

- Custom PCR Arrays

Identify Transcription Factors Regulating your Gene

- Biology-on-Array

Assess Biological Impact

- Signal™ Reporters

Knockdown Analysis

- SureSilencing™ shRNA Plasmids
- Flexitube/Flexiplate siRNAs

Analyze interaction between DNA & nuclear proteins

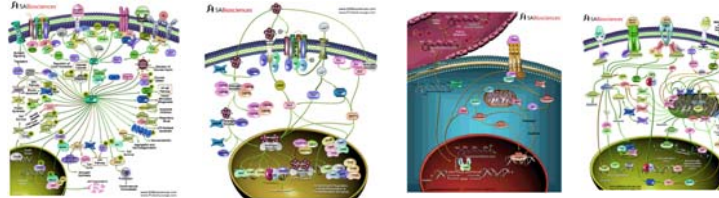
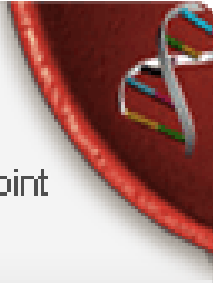
- ChampionChIP™ Chromatin Immunoprecipitation PCR Arrays

Quantify secreted proteins in blood plasma sera

- ELISArrays

Pathway Maps (>100)

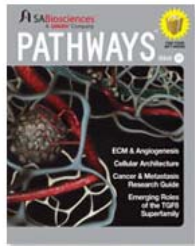
Download PowerPoint Slides [+](#)



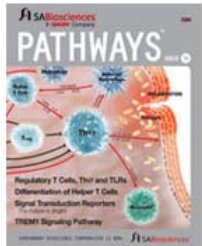
Pathways™ Magazine

[Archives and Newsletters](#) —

Handbooks



Issue 11 / ECM & Angiogenesis



Issue 10 / Inflammation



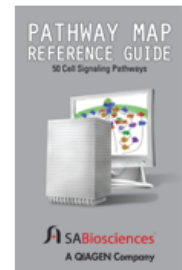
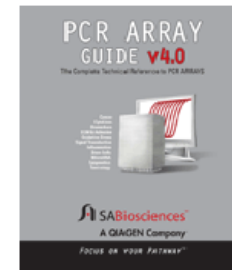
Issue 9 / Stem Cells



Issue 8 / Epigenetics



Issue 7 / TLR



Movie Tutorials

Learn More About PCR Arrays

