

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

Genome-wide miRNA Expression Data

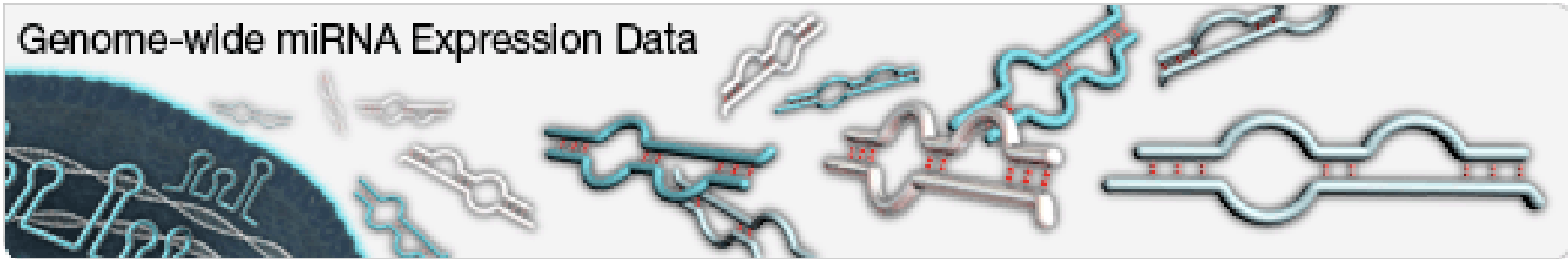


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Genome-wide miRNA Expression Data



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

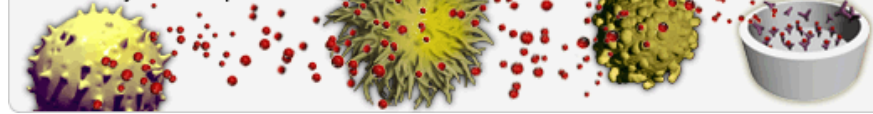
Telephone: 800-988-0325; Email: TechService-AP@qiagen.com

Welcome to SABiosciences: Systems Biology with a Pathway Focused Approach From QIAGEN

Accurate and Simple Pathway Expression Analysis



Measure Cytokine Expression



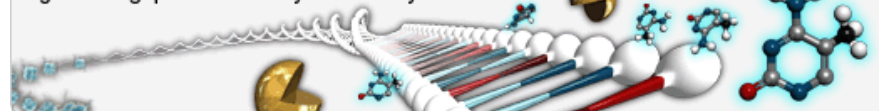
Genome-wide miRNA Expression Data



Successful ChIP in One Day



High-Throughput DNA Methylation Analysis



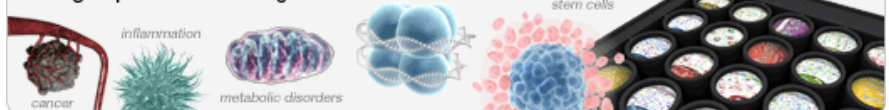
Cell-Based Assays for Signaling



High-potency Genome-wide siRNA and shRNA



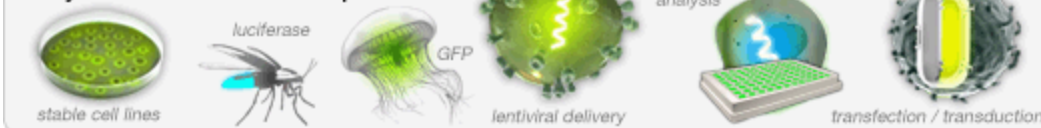
Linking Expression and Regulation



- **SABiosciences (Super Array Biosciences) is now a Company**

Select Upcoming Webinars

Analysis with Cell-Based Reporters



Cell-Based Assays for Pathway and Transcription Factor Activity

Accurate and Simple Pathway Expression Analysis



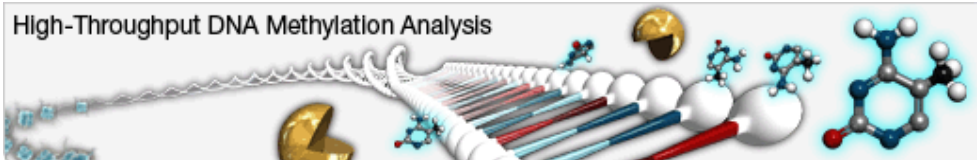
Pathway-Powered Gene Expression Profiling with PCR Arrays



March 12th! (11:30am Beijing Time)

PCR Array DATA Analysis Webinar

High-Throughput DNA Methylation Analysis



Profile the Methylation Status of Multiple Genes Simultaneously Without Bisulfite

Sign up at: <https://www2.gotomeeting.com/register/346489106>

Introduction to miRNA— Tiny molecule, Huge impact

miScript miRNA PCR Array Overview

- Ease-to-use workflow
- Complete solution portfolio
- Superb performance

miScript miRNA PCR Array Applications

- Cancer biomarkers
- Development & differentiation
- Genome-wide discovery

Endogenously expressed small functional RNAs (18-25 nt)

Mature-miR-1302: UUGGGACAUACUUAUGCUGAAA

Regulate mRNA expression post-transcriptionally

- mRNA degradation
- translation inhibition

Only partial sequence complementarity required

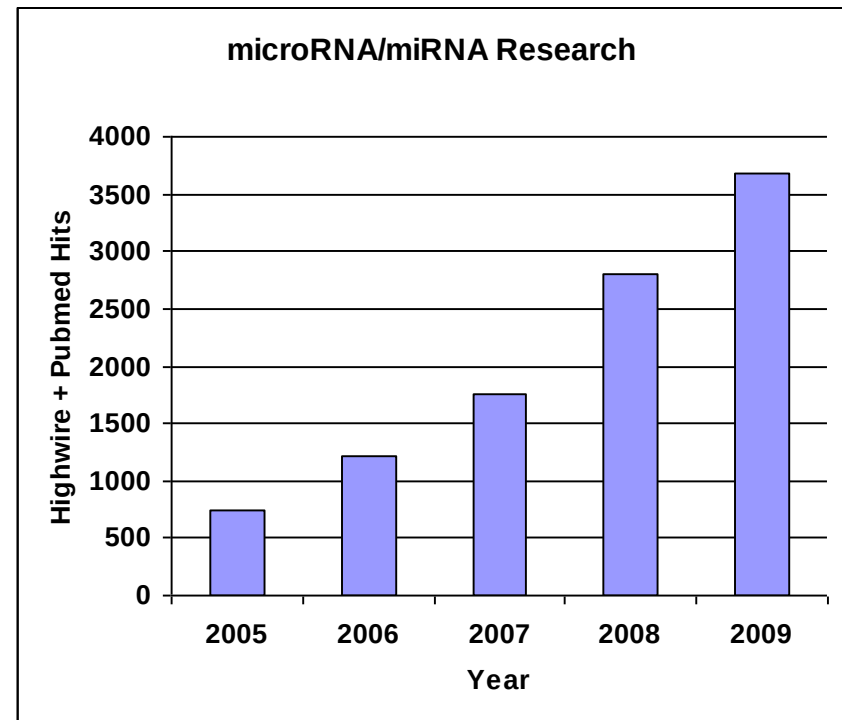
- One miRNA can potentially regulate hundreds of mRNAs
- One mRNA can be regulated by multiple miRNAs

Another layer of complexity for regulating gene expression

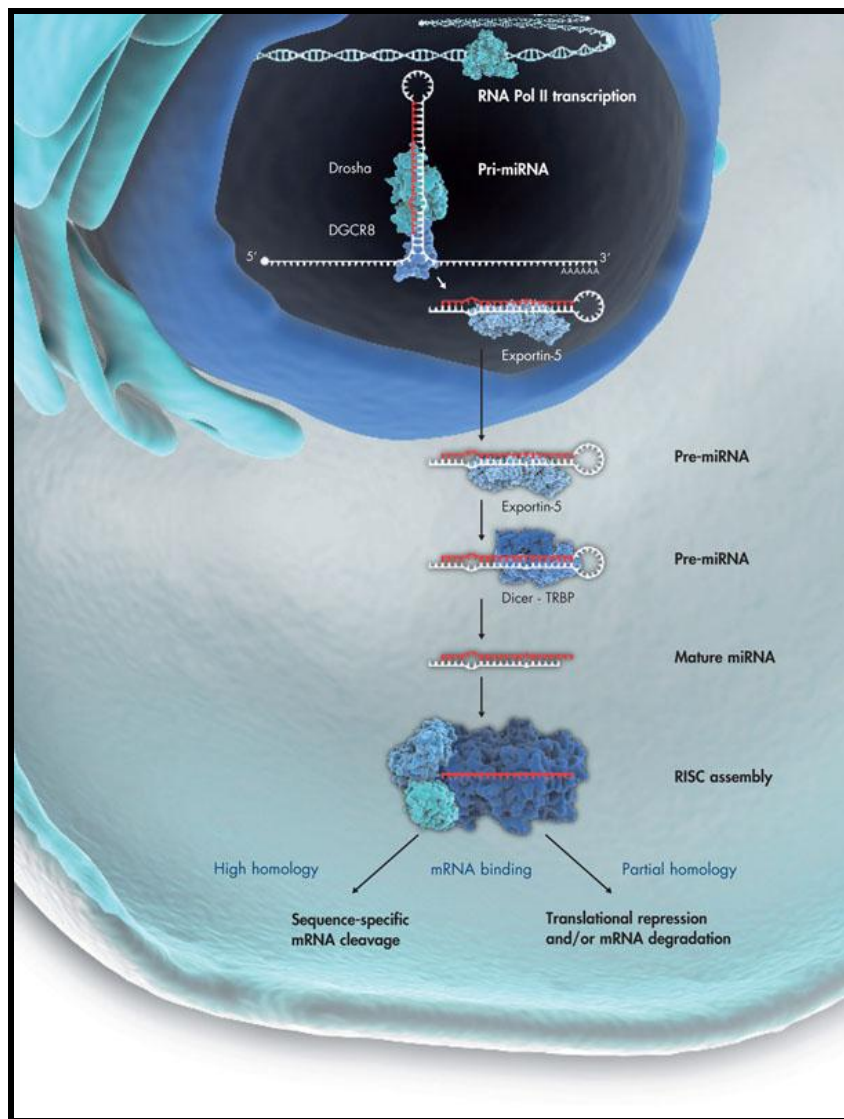
miRNA database: www.mirbase.org

New miRNAs are continually discovered (release 16.0)

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



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

miRNA Genomic Structure

(a) Independent Promoter



(b) Intronic



(c) Exonic



- **Intergenic miRNA genes:** within 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	
1302-1	12	intergenic	
1302-3	2	intergenic	
1302-7	8	intergenic	
1302-10	15	intergenic	
1302-11	19	intergenic	
1302-2	1	intronic	Non protein coding
1302-4	2	intronic	Non protein coding
1302-9	9	intronic	Non protein coding
1302-5	20	intronic	Protein coding/FAM65C; intron4
1302-6	7	intronic	Protein coding/HDAC9; intron 1
1302-8	9	intronic	Protein coding/ch9orf174

Mature-miR-1302: UUGGGACAUACUUAUGC UAAA

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 aguaa auuugaauuuca uaa caa gaauaaa ua uguaag au uccca a a guc gg ucaa uaaacuuaaagu auu guu cuuaauaa au guaaac ua agggg u au cuua a g cu c c -aa c a c uu</pre> Get sequence	
Deep sequencing	2 reads, 1 experiments	
Genome context	Coordinates (<i>GRCh37</i>) 12: 113132839-113132981 [-]	Overlapping transcripts intergenic

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 and differentiation
- Immunology
- Aging
- Heart Diseases
- Neurological Diseases

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

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miRNeasy RNA Extraction Kit

miScript II RT Kit

- Built-in external RNA control
- One-step reaction

miScript miRNA PCR Arrays

miRNome

Pathways/Disease-focused

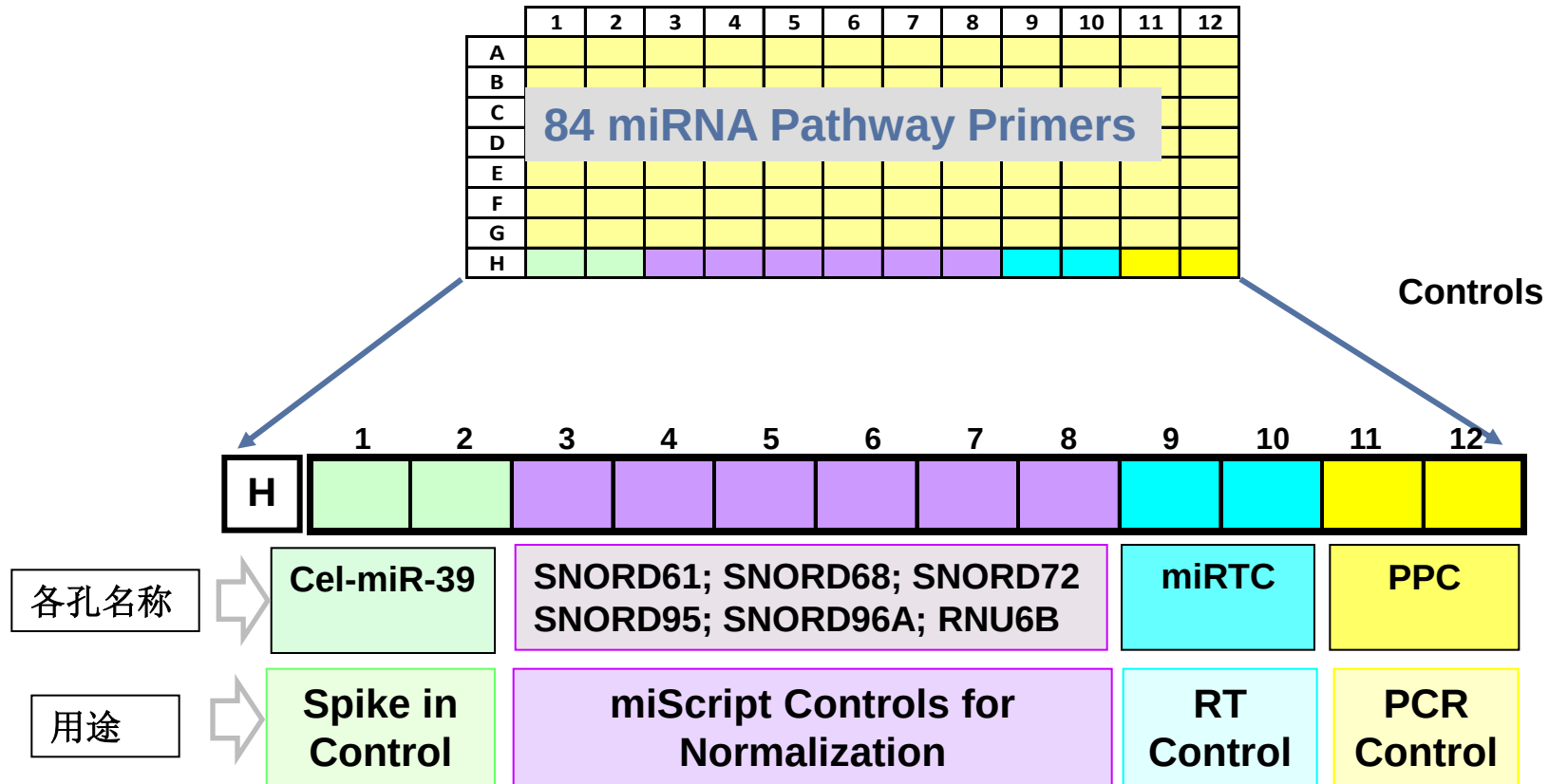
miScript SYBR® Green Master Mix

Optimized and ready-to-use

Data Analysis Web Tool



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



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

Complete miRNA genome (miRNome)

- Human miRNome
- Mouse miRNome
- Rat miRNome
- Dog miRNome

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 Cancer
 - ☐ Cancer
 - ☐ Cell Differentiation & Development
 - ☐ Immunopathology
 - ☐ Inflammation
 - ☐ Cancer PathwayFinder
 - ☐ Neurological Development & Disease
 - ☐ Serum and Plasma **(NEW!)**
 - ☐ Breast Cancer
 - ☐ Ovarian Cancer

All miRNA designs based on mirBase 16



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



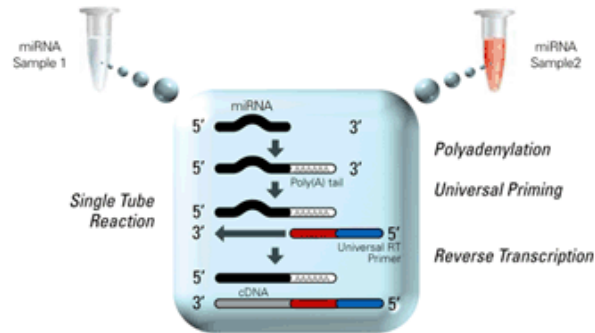
Mx3000p, Mx3005p, Mx4000p



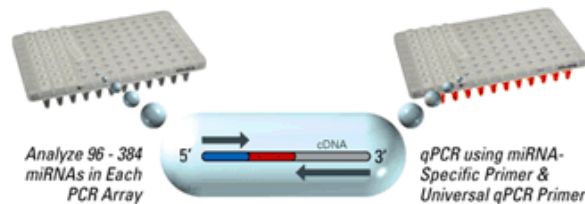
Rotor-Gene Q, Rotor-Gene 6000

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

■ 2 minutes

Run qPCR Program

■ 2 hours

Analyze Data

■ 15 minutes

Short sequence (18-25nt)

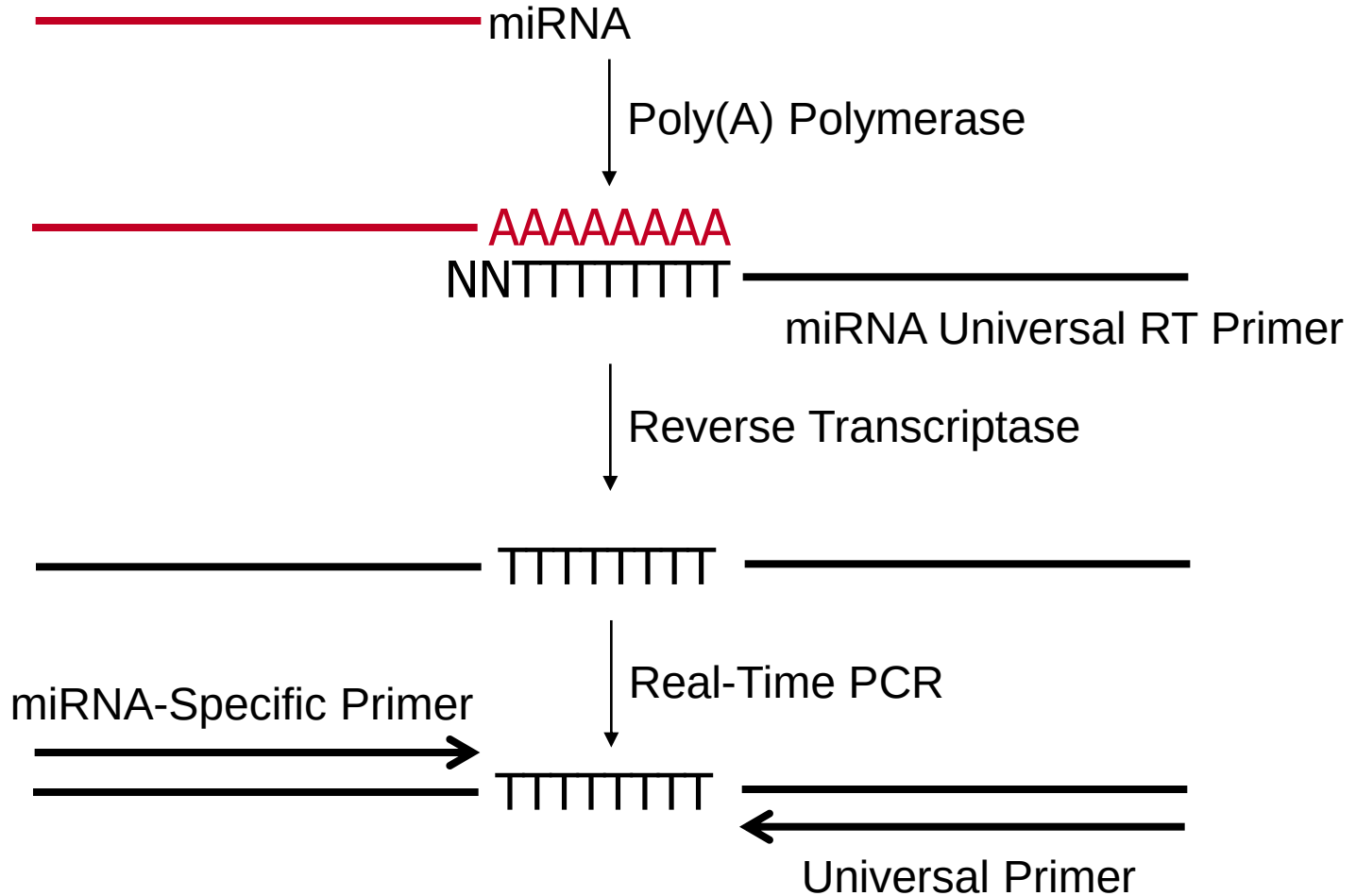
Background from contaminating small RNAs (tRNA, rRNA, snRNA)

Highly homologous

- Many miRNAs have a single nucleotide difference

miScript: Universal miRNA Reverse Transcription

Same cDNA preparation can convert ANY miRNA



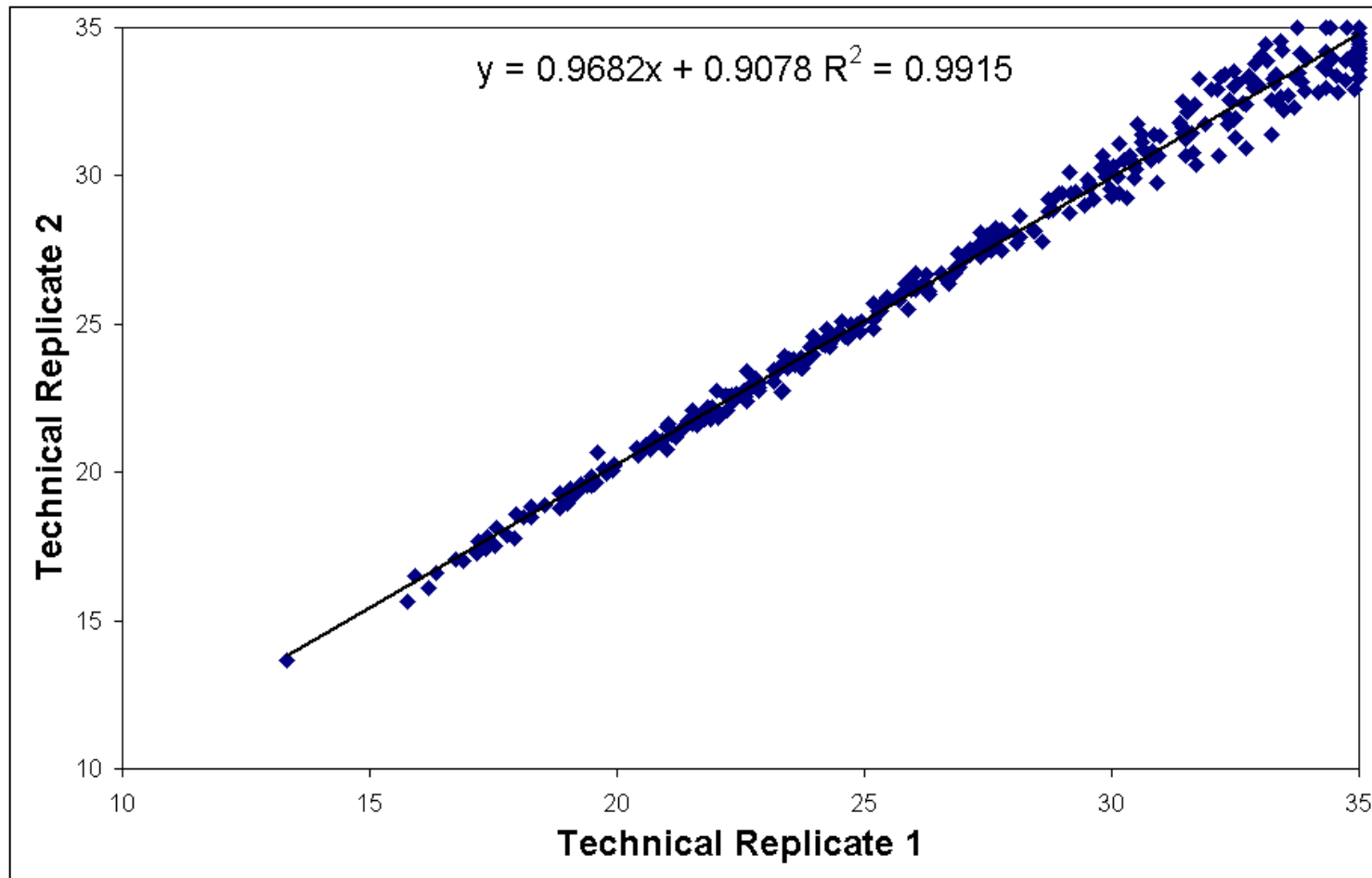
Ease of use

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

One-for-all: 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 Arrays 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

- Need 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

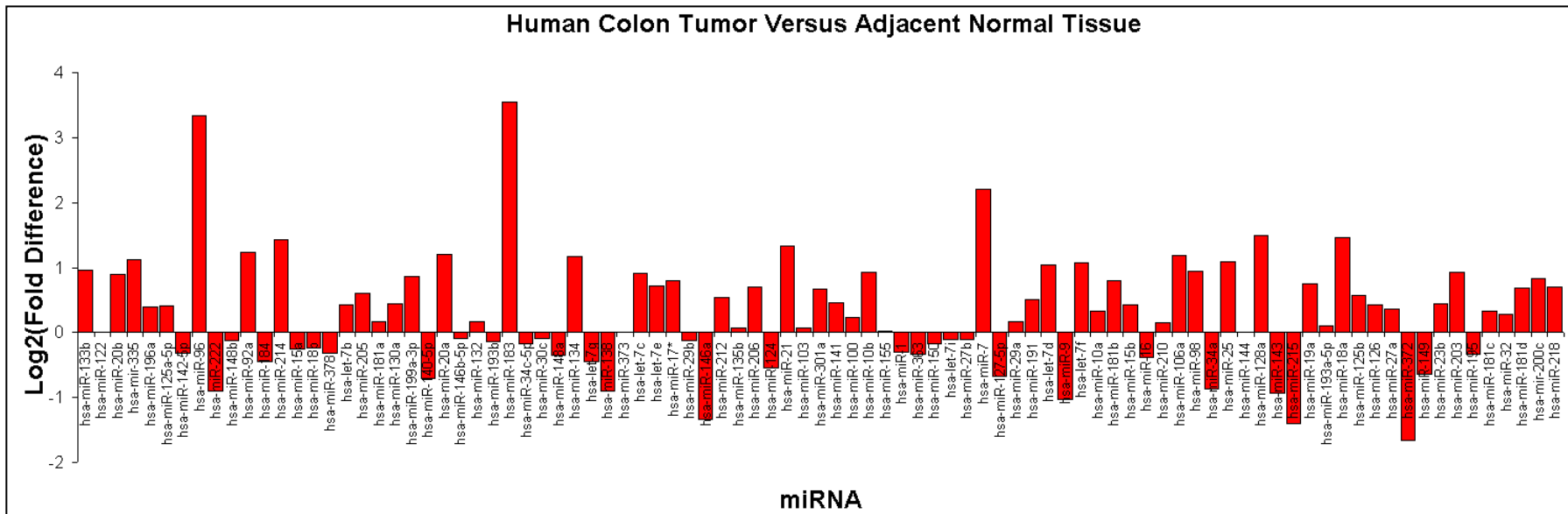
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miScript miRNA PCR Array Overview

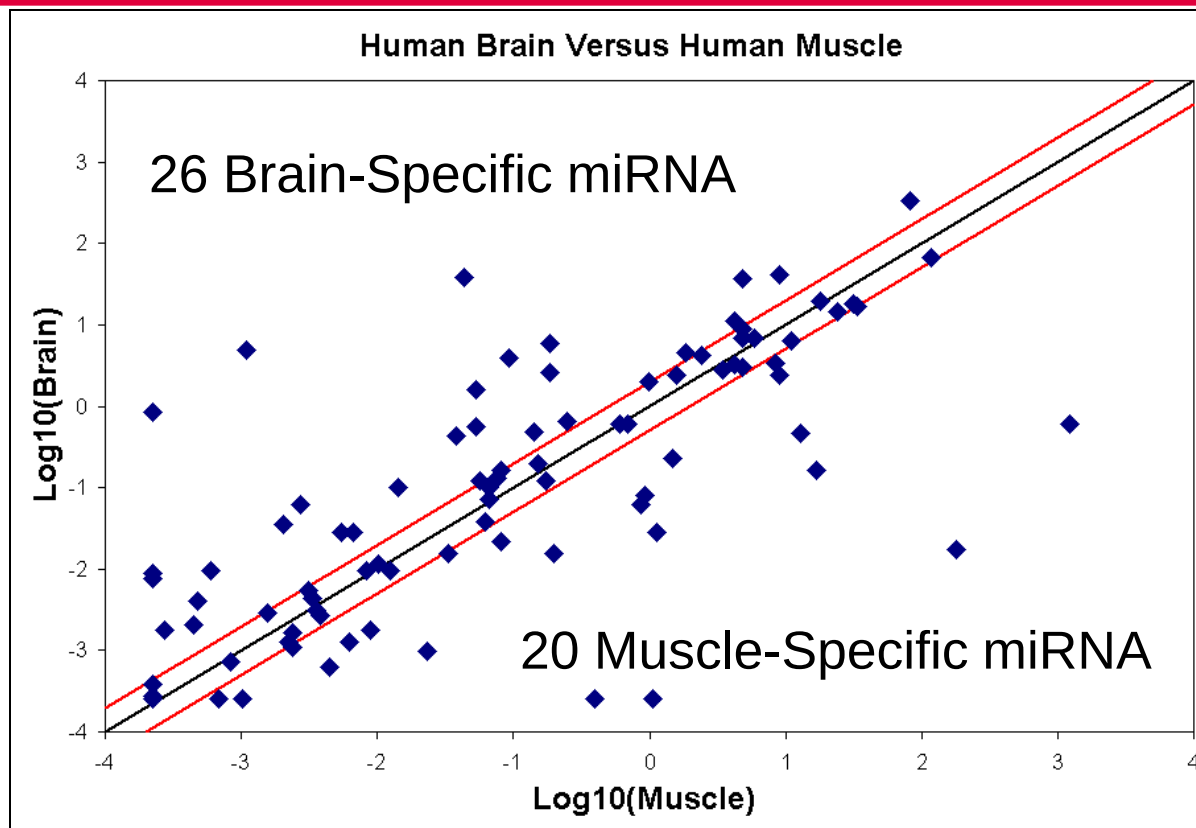
- Ease-to-use workflow
- Complete solution portfolio
- Superb performance

miScript miRNA PCR Array Applications

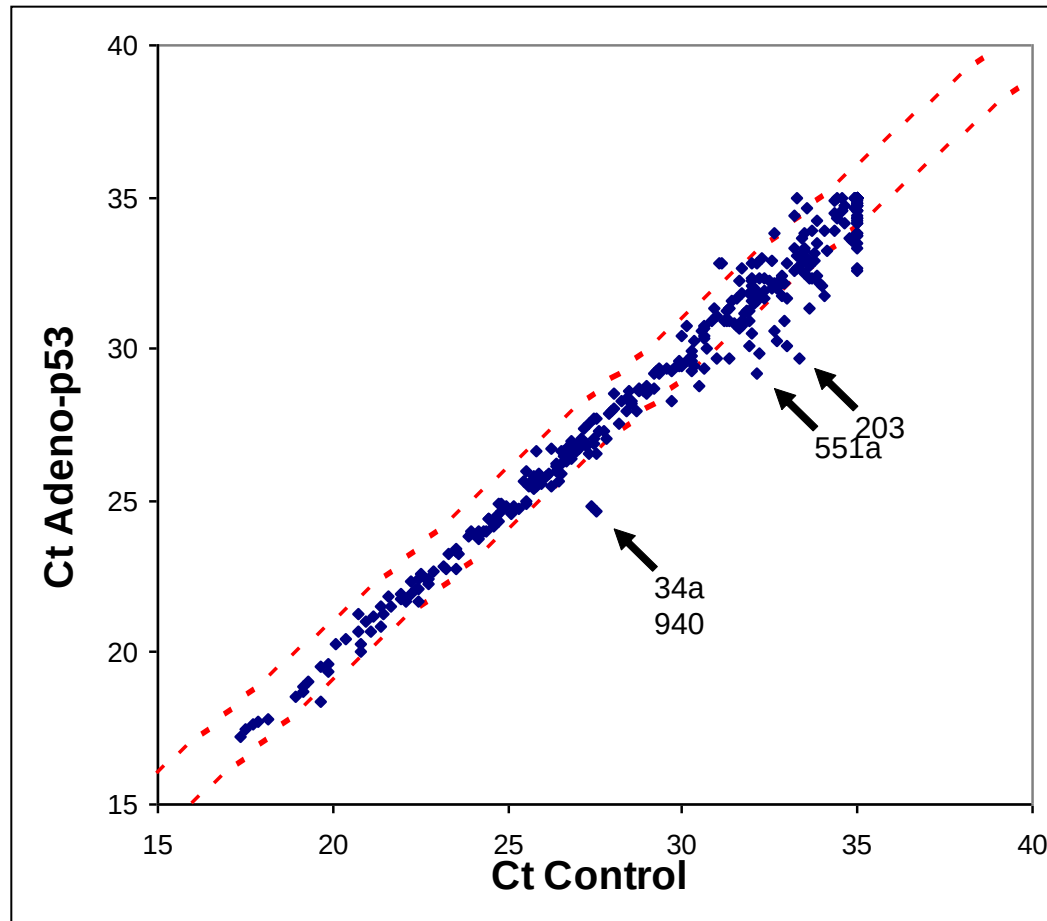
- Cancer biomarkers
- Development & differentiation
- Genome-wide discovery



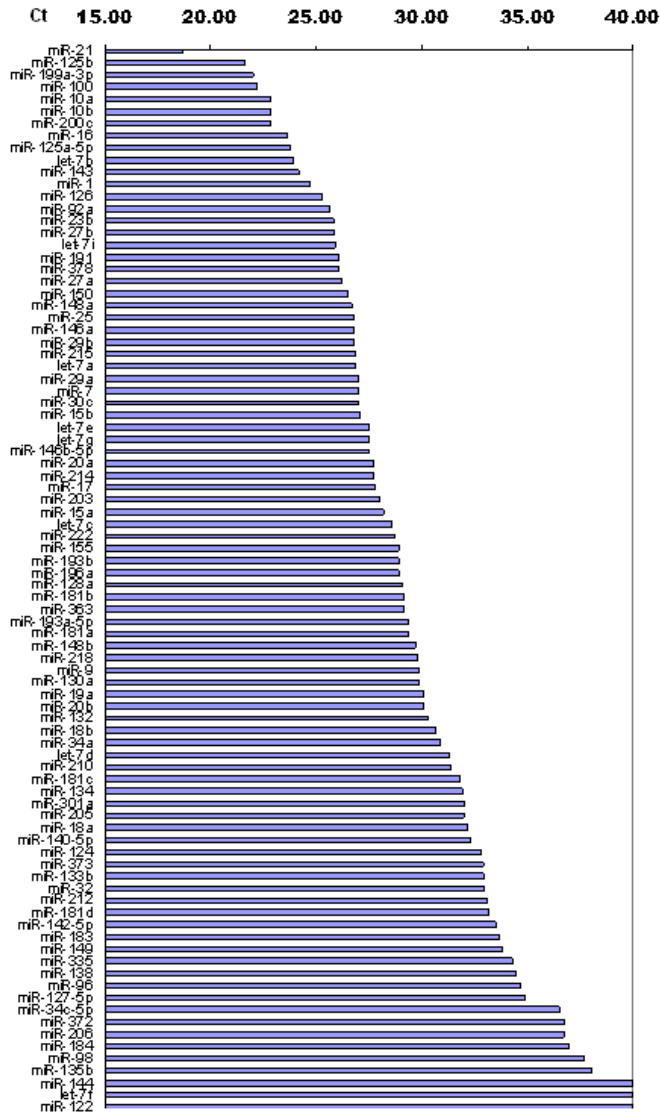
**Human Cancer miScript miRNA PCR Array:
Many Cancer-Specific miRNA Are Up-regulated in a
Colon Tumor.**



Differentiation & Development miScript miRNA PCR Array: Identifies 26 Brain- and 20 Muscle-Specific miRNAs potentially regulating tissue-specific gene expression.



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



Expression of 88 cancer related miRNAs
FFPE blocks.

Sample: 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

- 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!**

Validate results with more samples and focused set of genes

- Custom miRNA PCR Arrays

Assess gene expression and biological impact

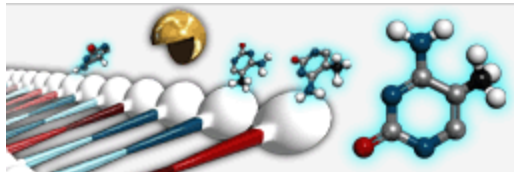
- Signal™ Reporters



- Pathway focused PCR Arrays (>150 pathway)

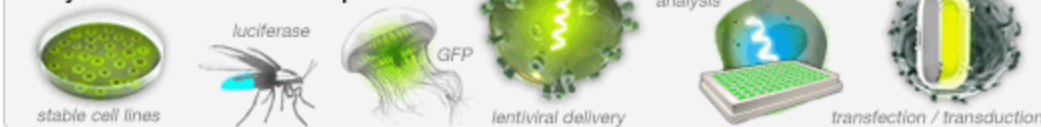
Analyze regulation of miRNA expression

- Human Cancer miRNA DNA Methylation PCR Array



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Analysis with Cell-Based Reporters



**Cell-Based Assays for
Pathway and Transcription
Factor Activity**

Accurate and Simple Pathway Expression Analysis



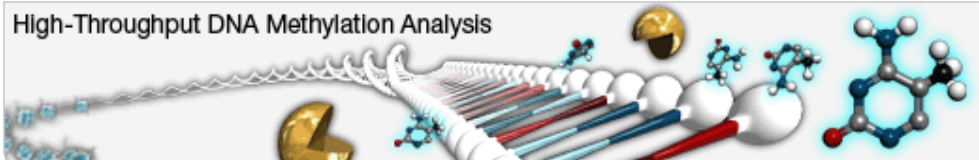
**Pathway-Powered Gene
Expression Profiling with
PCR Arrays**



March 12th! (11:30am Beijing Time)

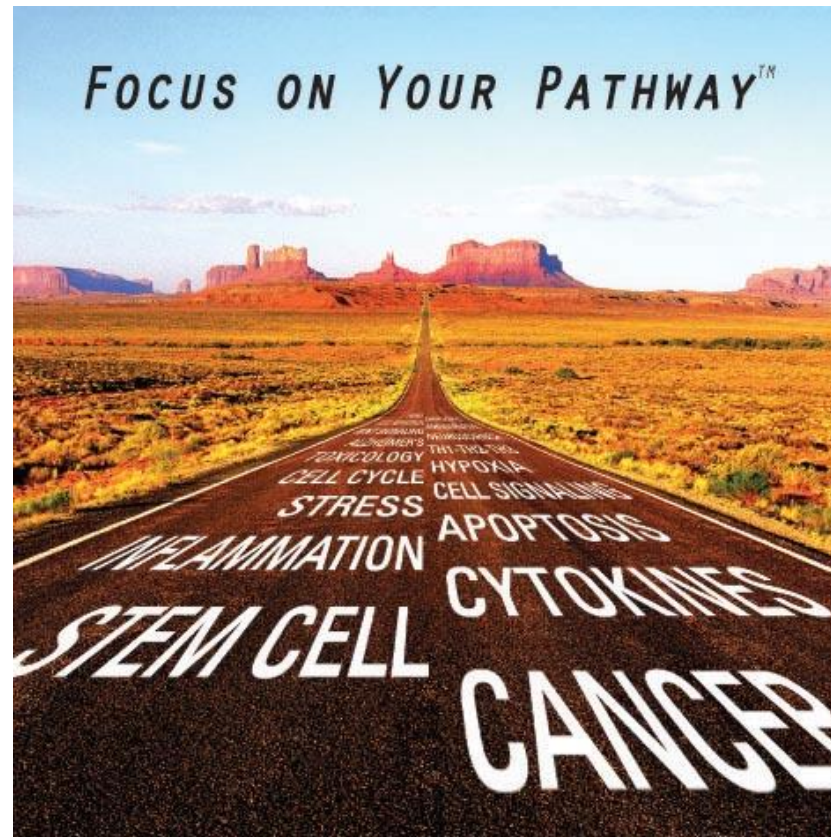
**PCR Array DATA
Analysis Webinar**

High-Throughput DNA Methylation Analysis



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Multiple Genes Simultaneously
Without Bisulfite**

Sign up at: <https://www2.gotomeeting.com/register/346489106>



Contact Local Sales Representatives

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

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miScript SYBR Green PCR kit

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