PCR ARRAY
HANDBOOK v3.0
The Complete Technical Guide to PCR ARRAYS

Cancer
Cytokines
Biomarkers
ECM & Adhesion
Oxidative Stress
Signal Transduction
Inflammation
Stem Cells
MicroRNA
Epigenetics
Toxicology

NEW 100 Selected Peer-reviewed Publications

SA Biosciences

Focus on your Pathway™
PCR ARRAY HANDBOOK VERSION 3.0

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What Are PCR ARRAYS?

RT² Profiler™ PCR Arrays are the most reliable and sensitive gene expression profiling technology for analyzing focused panels of genes in signal transduction, biological process, or disease-related pathways using real-time PCR.

How Are PCR ARRAYS Utilized?

RT² Profiler PCR Arrays are exceptionally valuable tools for studying cancer, immunology, stem cells, toxicology, biomarker discovery/validation, and phenotypic analysis of cell and tissue samples (fresh, frozen, and fixed).

Why Use RT² Profiler™ PCR ARRAYS?

- **Simplicity:**
  The simplicity of RT² Profiler PCR Arrays makes routine expression profiling practical in any research laboratory with a real-time instrument.

- **Performance:**
  RT² Profiler PCR Arrays have the sensitivity, reproducibility, specificity, and reliability to accurately profile multiple genes simultaneously in 96- or 384-well formats.

- **Relevance:**
  RT² Profiler PCR Arrays focus on profiling the genes relevant to the pathways or disease states important to your research.

Anatomy of a 96-well RT² Profiler PCR ARRAY

**HUMAN INFLAMMATORY CYTOKINES AND RECEPTORS PCR ARRAY**

**SYBR® Green Versus TaqMan® Chemistries**

Figure 1: Each Well in a PCR Array Measures the Expression of a Gene Related to a Pathway or Disease State. Each cataloged PCR Array contains a list of the pathway-focused genes as well as five housekeeping (reference) genes on the array. Wells H6 through H12 contain a panel of proprietary controls to monitor genomic DNA contamination (HSDC) as well as the first strand synthesis (RTG) and real-time PCR efficiency (PPC).

How PCR ARRAYS Work

1. **Convert Total RNA to cDNA.**

2. **Add cDNA to RT² SYBR® Green Master Mix. Aliquot Mixture Across PCR Array.**

3. **Run in Your Real-Time PCR Instrument.**

4. **Data Analysis.**

**Figure 2: Comparable Biological Results.** Gene expression analysis was compared between RT² Profiler PCR Arrays (SYBR Green-based) and the TaqMan platform. Regression analysis of fold differences, with data normalized against POL2, demonstrate that both platforms yield similar biological results.

**Figure 3: Sensitivity with RT² SYBR Green Versus TaqMan Chemistry.** PCR amplicons detected using the same primer pair with or without TaqMan probes in either SYBR Green or TaqMan chemistry. SYBR green chemistry yields earlier Ct's for each dilution, demonstrating better sensitivity than TaqMan chemistry.

**Application: Angiogenesis**

*Figure 4: Relative Fold Change Between Disorganized and Organized Colonies Using the RT² Profiler Angiogenesis PCR Array.* RNA isolated from unorganized T4-2 cells treated with a control antibody (IgG) or reverted to an organized colony by blocking EGF signaling (mAB252) was reverse transcribed and relative gene expression data was obtained using the Human Angiogenesis PCR Arrays. The expression profile of 84 genes relevant to Angiogenesis as well as 5 housekeeping genes was assayed. Fold change calculations were done using SABiosciences’ data analysis software which automatically calculates the fold change in gene expression between the treated and control groups.

**Application: Immune Response**

*Figure 5: Common Cytokine PCR Array Identified 23 Up-Regulated and 6 Down-Regulated Genes Following PBMC Stimulation.* Triplicate total RNA samples from human peripheral blood mononuclear cells (either untreated or stimulated with 50 ng/mL PMA and 1 μg/mL ionomycin for 6 hours) were characterized with the Human Common Cytokine PCR Array. Twenty-three cytokine genes are up-regulated (p-value < 0.0005) including interleukins, colony stimulating factors, and TNF ligands after 6 hours of stimulation. Six interleukin and TNF ligand genes are down-regulated under the same conditions.

**Application: Determining Drug Toxicity with PCR ARRAYS**

*Figure 6: Stress and Toxicity PathwayFinder™ PCR Array Uncovered Distinct Gene Expression Profiles Associated with Liver Toxicity Caused by 3 PPARγ Agonists.* RNA from HepG2 cells treated with three different glutaric acid PPARγ agonists for type 2 diabetes mellitus was characterized, and the results were compared to that of a vehicle (DMSO) control. The drug withdrawn due to idiosyncratic liver toxicity (Rosiglitazone), induces very different changes in the expression of stress-related genes than two safer drugs still on the market (Avandia and Actos).

**Application: ECM PCR ARRAYS for Cancer Biomarker Discovery**

*Figure 7: ECM and Cell Adhesion PCR Arrays Revealed Up- and Down-Regulated Genes in Breast Cancer.* Total RNA from a normal human breast and a human breast tumor were characterized in technical triplicates, and the relative expression levels for each gene in the two samples are plotted against each other in the Scatter Plot. Genes encoding the matrix metalloproteinases (MMP3 and MMP9) and their inhibitors (TIMP3) are up-regulated, while genes encoding integrins (ITGB3 and ITGB4) are down-regulated, by at least three-fold (outside the silver field) in breast tumors relative to normal tissue.

**Popular Pathway-Focused PCR ARRAYS**

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<thead>
<tr>
<th>Angiogenesis</th>
<th>Inflammatory Cytokines &amp; Receptors</th>
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<tr>
<td>Apoptosis</td>
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<td>Toll-Like Receptor Signaling Pathway</td>
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<tr>
<td>Extracellular Matrix and Adhesion</td>
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**Custom PCR Arrays**

(Detailed Information on Page 8)

**Gene Expression Analysis Services**

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**Complete the RT² Profiler PCR ARRAY System**

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<th>Plate Format</th>
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Add up to 4 Genes to any PCR Array

**PCR ARRAY Accessories**

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**FREE** PCR Array Data Analysis Software
How The PCR ARRAY System Works

SABiosciences RT² Profiler PCR Arrays are a complete system for Pathway-Focused Gene Expression Analysis. From Sample Preparation to Data Analysis, the PCR Array system includes four components that GUARANTEE high-quality, reproducible, and reliable gene expression data.

RT² Profiler PCR ARRAYS
Each pathway-focused PCR Array includes 89-wet bench validated qPCR Primers Assays (including 5 housekeeping genes) and a proprietary control panel.

RT² SYBR Green qPCR Master Mixes
A unique formulation of buffers (GE) that co-evolved with the primer design algorithm provides high amplification efficiencies. Available with reference dyes (ROX, Fluorescein or without).

RT² First Strand cDNA Synthesis Kit
An external RNA Control detected by the PCR Array tests the quality of input RNA. It also features a proprietary genomic DNA elimination buffer essential for eliminating residual gDNA, ensuring specific detection of mRNA.

GE Effectively Removes Genomic DNA

Figure 1: Elimination of Genomic DNA Contamination. RNA from HEK 293T cells, mouse spinal tissue, mouse brain tissue, or rat brain tissue was characterized on SYBR Green PCR Arrays before (blue bars) and after (red bars) treatment with gDNA Elimination Buffer from the RT² First Strand Kit.

RT² SYBR GREEN Master Mix

Figure 2: Monitoring Inhibition in Reverse Transcription. Human universal RNA was added with magnesium salt to simulate RNA degradation or added with TRizol® reagent to simulate contamination that inhibits enzyme activity. RT First Strand Kit was used for cDNA synthesis.

FREE Data Analysis Software
The power of the PCR Array to assess the expression of a pathway-focused set of genes over a wide range of detection yields an abundance of data. With our FREE PCR Array Data Analysis tool, go from raw C_v values to fold change results displayed in a variety of formats (Scatter Plots, Volcano Plots, Clustergram) in a MATTER OF MINUTES.
RT² Profiler PCR Arrays are used and trusted by thousands of research scientists for pathway-focused gene expression analysis. Several factors, including the RT² Primer Assay design algorithm, the proprietary control panel, and the strict manufacturing and quality control procedures, ensure the outstanding performance and reliability of our PCR Arrays. Each PCR Array and every qPCR Primer Assay is wet-bench validated to guarantee their performance, with results demonstrating several performance parameters demonstrated here.

**Distinct Specificity**

The complete PCR Array System, with high quality input RNA, is guaranteed to yield single bands without primer dimers or other secondary products. The proprietary primer design algorithm incorporates more than ten thermodynamic and sequence alignment criteria, and our wet-bench validation provides confidence that every real-time qPCR Assay accurately represents the expression of the queried gene. Over 20,000 gene-specific RT² PCR Primer Assays have been designed and shipped to satisfied customers.

![Graph showing signal intensity vs. Tm](image1.jpg)

**Figure 1: PCR Arrays Amplify A Single Gene-Specific Product in Every Reaction.** Universal total RNA was characterized for four chemokine and chemokine receptors using RT² Primer Assays, followed by a dissociation (melting) curve analysis. PCR Arrays specifically detect individual genes despite the expression of related gene family members in the same RNA sample.

**High Sensitivity and Wide Dynamic Range**

A key benefit of using pathway-focused PCR Arrays for gene expression analysis is that genes that are over expressed can be measured as reliably as those that are under expressed. The complete PCR Array System yields > 85% positive call with 25 ng - 5 μg RNA or >90% with as a little as 1 ng PreAMP RNA. The 8-log wide dynamic range provided by real-time PCR is unparalleled when comparing a pathway-focused gene panel of varying expression levels across a variety of samples.

![Graph showing fluorescence vs. cycle number](image2.jpg)

**Figure 3: PCR Arrays Detect RNA Across a Wide Dynamic Range.** Ten-fold serial dilutions of Human ORRNAs were characterized with the respective RT² qPCR Primer Assays.

**Uniform PCR Amplification Efficiency**

One prerequisite for PCR Array technology is that the amount of template product doubles with every cycle. The more the assays deviate from this ideal, the error in the fold change calculation (ΔCt) increases exponentially. Only with consistently high amplification efficiencies can PCR Arrays yield meaningful comparison of gene expression levels of all genes simultaneously. The unique combination of SA Biosciences' proprietary primer design algorithm and rigorous testing of every primer assay by hand guarantees the high performance of every primer assay on the PCR Arrays.

**Superb Reproducibility**

Regardless of user or instrument used, the complete PCR Array System demonstrates strong correlations across technical replicates, lots, and instruments with average correlation coefficients > 0.99 insuring reliable detection of differences in expression between biological samples.

![Graph showing Ct from user A vs. B](image3.jpg)

**Figure 4: PCR Arrays Yield Highly Reproducible Results.** Four replicate sets of raw threshold data (A-D) obtained by two different scientists (A & B) at two different times on Human Drug Metabolism RT² Profiler PCR Arrays are directly compared. The results demonstrate a high degree of correlation (R² > 0.990).

**RT² Profiler PCR Arrays: A Trusted & Reliable System**

PCR Arrays have been used by thousands of researchers who have successfully submitted and published their PCR Array results in very high impact journals, including Science, PNAS, Cancer Research, the Nature & Cell family of journals, and others (See Pages 18-23).
What Is the RT² Nano PreAmp™ cDNA Synthesis Kit?

RT² Nano PreAmp cDNA Synthesis Kit and Primer Mixes are a breakthrough technology enabling expression analysis starting from as little as 1 ng of total RNA. It employs a proprietary preamplification process to faithfully increase the amount of targeted cDNA for PCR Array analysis. This technology empowers RT² Profiler PCR Arrays to accurately analyze nanogram levels of total RNA.

Samples that can NOW be characterized with real-time PCR Arrays include:

- **Laser Captured Microdissection Samples (LCM)**
- **Fine Needle Aspiration Biopsies (FNAB)**
- **Stem Cell Clusters or Embryoid Bodies**
- **Flow Cytometry / Fluorescent-Activated Cell Sorting (FACS)**

Combined with PCR Arrays, the RT² Nano PreAmp cDNA Synthesis Kit and Primer Mixes extends the PCR Array System to accurately analyze a pathway-focused set of genes with as little as 1 ng of total RNA.

- **RT² Nano PreAmp cDNA Synthesis Kit**: Proprietary kits include optimized reagents for first strand cDNA synthesis and preamplification from only 1 ng of total RNA.
- **RT² Nano PreAmp cDNA Synthesis Primer Mixes**: Ready-to-use primer mixes for amplifying pathway-specific cDNA templates on corresponding RT² Profiler PCR Arrays.

Benefits of RT² Nano PreAmp cDNA Synthesis Technology

- **Robust Performance on Small Samples**: Analyze up to 4 different PCR Arrays starting with as little as 1 ng of Total RNA.
- **Easy Workflow and Designed for Routine Use**: Simple and quick procedures with minimal hands-on time to preamplify target templates in under 2 hrs.
- **Superior Sensitivity**: Maximally enhances the sensitivity of RT² Profiler PCR Arrays to analyze limited amounts of RNA.

![Image](image_url)

**Figure 1. RT² Nano PreAmp Further Increases the Sensitivity of PCR Arrays**

Different amounts of human universal RNA were converted to cDNA with (red) or without (blue) RT² Nano preamplification. The unamplified and preamplified samples were then analyzed on the Human Inflammatory Cytokines and Receptors PCR Array (PAHS-011), which contains 84 pathway-specific assays, plus controls, including 5 assays for housekeeping genes. Threshold cycle values (Ct) were obtained and any genes with a C< 35 were considered to be present. Results indicate that with Nano preamplification, a 33.7% increase in positive call rate is observed in samples with as little as 1 ng RNA.

![Image](image_url)

**Figure 2. Unbiased Amplification Process - Highly Comparable ΔCt Values Between Preamplified and Unamplified cDNA from Human Liver Tumor RNA.** First strand cDNA was synthesized from 5 ng of human liver tumor RNA. One-quarter of each RT product was used for preamplification with the RT² Nano PreAmp cDNA Master Mix Kit plus the Human Cancer PathwayFinder™ Nano PreAmp Primer Mix. Unamplified cDNA synthesized from 500 ng of the same liver tumor RNA sample was used as the control. Preamplified and unamplified cDNA samples were then analyzed on the Human Cancer PathwayFinder™ PCR Array, and the threshold cycle values (Ct) were obtained. The ΔCt value for each gene was calculated by subtracting the average C of the five reference genes (B2M, HPRT1, RPL13A, GAPDH, and ACTB) on the PCR Array from the C of each gene of interest. The concordance of the ΔCt values between preamplified and unamplified samples was evaluated by regression analysis. Data points with a C>35 were considered to be absent genes and were excluded from the analysis. The dashed line represents the ideal slope of 1.0. The solid line shows a linear regression fit with the R² and slope indicated. The high correlations between preamplified and unamplified cDNA were also obtained from universal RNA samples (data not shown).

### RT² Nano PreAmp cDNA Synthesis Products

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<tr>
<td>RT² Nano PreAmp cDNA Synthesis Kit</td>
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<td>RT² Nano PreAmp cDNA Synthesis Primer Mixes*</td>
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<td>Human Cancer PathwayFinder™</td>
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* PreAmp Primer Mixes for Custom PCR Arrays available
Gene Expression Analysis from FFPE Samples

An innovative solution enabling the accurate qRT-PCR analysis of Formalin-fixed Paraffin-embedded (FFPE) samples. The RT² FFPE PreAmp technology utilizes multiplex tandem PCR to preamplify gene-specific cDNA with minimal bias. This kit is intended for preamplification of first-strand cDNA from fragmented total RNA from FFPE samples for gene expression analysis with RT² Profiler PCR Arrays.

The combination of a simplified Xylene-Free RNA extraction and a high-fidelity amplification process maximizes recovery of RNA and microRNA (miRNA). RT² Profiler PCR Arrays facilitate easy and reliable expression analysis of genes associated with a biological pathway or a diseased state from FFPE samples.

Benefits of RT² FFPE PreAMP PCR ARRAY System

- **Quick and Efficient**: High quality and high-yield total RNA and miRNA isolation from FFPE samples in 70 minutes
- **Superior Sensitivity**: PreAMP protocol significantly enhances qRT-PCR detection sensitivity for FFPE samples
- **Easy Workflow**: Simple Xylene-Free procedure and robust performance

RT² FFPE PreAmp Increases Detection of Genes Previously Classified as “Absent”

Figure 1. Highly Comparable Gene Expression Fold Change Results between FFPE Preamplified and Unamplified Samples. RNA extracted from FFPE spleen and intestine samples were extracted using the RT² FFPE RNA Extraction Kit and converted to cDNA with and without preamplification. All four cDNAs were analyzed on the Human Cancer PathwayFinder™ PCR Array. The ∆∆Ct comparison and genes with raw Ct values lower than 33 in both unamplified spleen and intestine samples are presented.

RT² FFPE PreAMP Performance

- **Increased positive call rate from FFPE samples**
- **Increased detection of genes previously classified as “Absent”**
- **Unbiased amplification of preamplified genes**
- **Faithful conservation of biological changes**

Figure 2. Genes extracted from FFPE samples previously classified as “Absent” are now detectable after RT² FFPE Preamplification. RNA was extracted from FFPE spleen sample human with the RT² FFPE RNA Extraction Kit and reverse transcribed to cDNA using RT FFPE preamplification (red bars) and without Preamp (blue bars). Results of the Human Cancer PathwayFinder PCR Array showed 55% of unamplified genes were virtually undetectable with no genes in the 10-20 Ctg range. Preamplified genes with Ctg values > 30, shift into the reliably quantitative range (Ctg = 10–20).

RT² FFPE PreAmp & RNA Extraction Products

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<td>Custom RT² FFPE PreAmp Primer Mixes for PCR ARRAYS</td>
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<tr>
<td>Instrument-Specific SYBR® Green qPCR Master Mixes</td>
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What Are Custom PCR ARRAYS?

Custom RT™ Profiler™ PCR Arrays are a high-throughput approach for profiling the expression of your genes of interest. Choose from any gene in the human, mouse, rat, Rhesus Macaque or Drosophila genomes (up to 384 different genes). Whether your interests are in biomarker discovery, microarray followup, drug development, disease characterization, or signal transduction mechanisms, Custom RT™ Profiler PCR Arrays enable focused expression analysis on your genes of interest.

Why Custom PCR ARRAYS from SABiosciences?

- **Performance:** Each assay within a Custom RT™ Profiler PCR Array is designed and wet bench-validated using a set of rigorous parameters to insure the genes in your sample across a wide dynamic range are reproducibly recognized and quantified.

- **Flexibility:** Custom RT™ Profiler PCR Arrays are available in a number of easy-to-use formats for quick sample loading and data analysis.

- **Turnaround Time:** Submit your 96- or 384-gene list and receive your Custom PCR Arrays in 2 weeks.

Gene Layouts

### 96-well Custom PCR ARRAYS

- 12 Genes, 8 Samples / Plate
- 24 Genes, 4 Samples / Plate
- 32 Genes, 3 Samples / Plate
- 48 Genes, 2 Samples / Plate
- 96 Genes, 1 Sample / Plate
- All Formats

### 384-well Custom PCR ARRAYS

- 16 Genes, 24 Samples / Plate
- 32 Genes, 12 Samples / Plate
- 48 Genes, 8 Samples / Plate
- 96 Genes, 4 Samples / Plate
- Up to 384 Genes, 1 Sample / Plate
- All Formats

---

**Customer Data:** Validate Microarray Expression Analyses

**MECP2 Responsive Genes**

![Graph showing gene expression changes](image)

**MECP2 Tg**

**MECP2 null**

**Activated**

**Repressed**

Figure 1: Gene Expression Changes in Hypothalamus of MECP2 Mouse Models. Validation of expression changes for 66 genes by qPCR analysis. Gene expression levels from microarray analyses were validated in four MECP2-Tg males and four MECP2-null males. Data is plotted as relative up-regulation (red) or down-regulation (blue) over wild-type (P < 0.05, t test). Each column represents a single gene, and represents data from four samples for each genotype.


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**PCR ARRAY Accessories**

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<td>SYBR® Green Master Mixes (see page 16)</td>
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**FREE PCR Array Data Analysis Software**

Data shown is used with permission of Science and respective authors.
The ChampionChip™ System provides the first complete platform for the analysis of in vivo protein-DNA interactions using Chromatin Immunoprecipitation (ChIP) and real-time PCR (qPCR) detection. With validated high-quality ChIP-grade antibodies and PCR primers for any genes' promoter region, a simple and robust one-day preparation assay quickly delivers reliable and biologically relevant results. ChIP qPCR is a powerful and versatile method for the analysis of chromatin DNA bound by transcription factors, co-regulators, modified histones, chromatin remodeling proteins, or other nuclear factors from live cells. However, the tedious process and variable results have limited many researchers' ability to adopt this technique to study dynamic protein-DNA interactions in native chromatin environments. The ChampionChip™ System yields the most reliable ChIP assay results with qPCR precision in just a single day.

How the ChampionChip™ System Works

The ChampionChip One-Day Kit simplifies the usual two- to five-day ChIP protocol down to a manageable six to eight hours. Its crosslink reversal step is much faster and less tedious than conventional methods, and its DNA purification step yields a larger quantity and higher quality ChIP DNA than other one-day kits.

A. Chromatin Immunoprecipitation (ChIP)

1. ChIP-Ready Chromatin Preparation
   - Fix and Harvest Cells: 30 min
   - Sonicate Chromatin: 30 min
   - Optional: Quickly Evaluate Fragmentation Size: 40 min

2. Immunoprecipitation
   - Pre-Clear: 50 min
   - Anti-TF or -Histone Antibody & Control IgG: 1-2 hr
   - Protein A Beads: 1 hr
   - Wash: 30 min

3. ChIP and Input Fraction DNA Isolation
   - Reverse Cross-Linking and Elution: 30 min
   - DNA Spin-Column Purification: 10 min

B. Real-Time PCR

C. Data Analysis (ChIP PCR Array Analysis Software) 15 min

Figure 1: The Entire ChampionChip System Protocol Can Be Completed in a Single Day. The ChampionChip System includes a simplified high-performance One-Day ChIP Kit, ChIP-Grade Antibodies, real-time PCR primers, and a FREE ChIP PCR Array Data Analysis Suite.

High Specificity

Figure 2: The ChampionChip System Readily and Correctly Identifies Different Euchromatin and Heterochromatin Loci. ChampionChip antibodies against modified histones (H3K4me2, H3K27me3, H3K9me3) or control IgG were used for precipitating chromatin from HeLa cells. Each ChIP DNA fraction was analyzed by real-time PCR using primers specific for the ALDOA, MYO-D, and SAT2 loci to calculate percentages of co-precipitating DNA relative to input.

Figure 3: Differential Histone Modification Tiling Across the Sequence of Any Gene. ChampionChip antibodies for modified histones (H3Ac, H3K4me2, H3K27me3) or control IgG were used for precipitating chromatin from one million HeLa cells. Each ChIP DNA fraction was analyzed with a ChampionChip Tiling Array representing 30 one-kb tile intervals across the genomic sequence of the CDKN1A gene. The results obtained from three independent experiments are consistent with active transcription of the CDKN1A gene.

Figure 4: Treatment with 5-Fluorouracil Increases CDKN1A Gene Expression and p53 Binding in Cell Lines Expressing Wild-Type But Not Mutant p53. Replicate samples from A549, HepG2, and PC3 cells were treated with 5-FU (300 μM, 6 hr) and either subjected to ChIP with an anti-p53 antibody followed by qPCR analysis of the CDKN1A-2kb p53 binding site, or harvested for RNA to analyze CDKN1A expression by real-time RT-PCR. The results of both assays are expressed as the fold increase upon 5-FU treatment.

ChampionChip qPCR System

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FREE ChIP PCR Array Data Analysis Software
What are miRNAs?

MicroRNAs are endogenous single-stranded RNA molecules 19-25 nucleotides in length, synthesized in a regulated manner from larger RNA molecules. Many miRNA sequences have been found in a variety of species [for example, ~700 miRNAs in humans, and ~800 in mice].

Why Use SABiosciences miRNA PCR ARRAYS?

Detecting every miRNA across the entire genome in a specific and sensitive way is a very technologically challenging task. Many miRNA family members and otherwise distinct miRNA species have very similar sequences. Moreover, other RNA species such as snRNA, tRNA, miRNA, and rRNA can cause non-specific amplification, making the specific analysis of mature miRNA even more problematic. SABiosciences’ proprietary miRNA detection technology enables uniformly high PCR amplification efficiencies, allowing simultaneous detection of miRNA under uniform cycling conditions.

The RT® miRNA PCR Array accurately analyzes the expression of up to 96 or 384 microRNA sequences simultaneously on any real-time PCR instrument. SABiosciences’ patent-pending miRNA technology integrates a universal-tailing and reverse transcription reaction specific for miRNA with accurate expression level measurement of distinct miRNA sequences that may differ by a single nucleotide base. RT® miRNA PCR Arrays are the most specific and sensitive technology for analyzing genome-wide miRNA expression.

- **Sensitivity:** As little as 0.5 µg total RNA needed
- **Multi-Sequence Flexibility:** Analyze up to 384 sequences simultaneously
- **Simplicity:** As easy as a real-time PCR Array experiment

Why Study miRNA?

MicroRNA represents a new layer of regulation in endogenous gene transcription and translation. Since there are ~700 miRNAs in humans, with each miRNA potentially having hundreds of targets, the majority of genes may be subject to regulation by one or more miRNAs. miRNAs are already being considered as cancer biomarkers, and their importance is being realized in a variety of other research areas, such as differentiation, neurobiology and immunology. There are three major ways to start studying miRNA:

1. **Expression Analysis:**
   The best technology for determining the expression of miRNA is the SABiosciences miRNA PCR Array System.

2. **Bioinformatic Prediction:**
   Identification of miRNAs that potentially regulate your genes of interest is possible with our powerful yet simple bioinformatic algorithm at:
   

3. **Functional Studies:**
   The function of individual miRNAs can be identified via miRNA over expression or suppression of miRNA function. [Over 500 miRNAs in their endogenous genomic context are available as expression constructs].

How miRNA PCR ARRAYS Work

*As Easy as a Real-time PCR Experiment*

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

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


4. Data Analysis.

**Specificity**

![Single Nucleotide Mismatch Specificity](image)

**A**

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**B**

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<tr>
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<tr>
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<td>AACCUGAUAGAGCAUCUGUG</td>
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**Figure 1:** miRNA qPCR Assays Distinguish Single Nucleotide Mismatches. RT® miRNA PCR Assays and a competitor’s assays for miR-99a and miR-100 were used to detect both corresponding synthetic templates, whose sequences differ by only one nucleotide (A). Relative detection of the off-target template is calculated as a percentage of the correct template detection (A). RT® miRNA PCR Assays’ proprietary primer design specifically discriminates closely related sequences better than competing assays.
Sensitivity and Dynamic Range

1000X MORE SENSITIVE THAN COMPETITORS

\[ y = -3.3683x + 41.596 \]
\[ R^2 = 0.9982 \]

Figure 2: 1000-Fold Higher Sensitivity Than Our Competitors. Samples containing serially diluted synthetic miR-658 template spiked into a constant amount of small RNA that lacks miR-658, were analyzed with miR-658-specific RT\(^\text{\textsuperscript{\textregistered}}\) miRNA qPCR Assays and other commercial assays. The resulting threshold cycle values are plotted versus the amount of synthetic template used. RT\(^\text{\textsuperscript{\textregistered}}\) miRNA PCR Assays’ proprietary reaction formulation detects 1000-fold lower concentrations of miR-658 with a wider linear dynamic range than competing assays.

Accuracy

UNIFORM HIGH AMPLIFICATION EFFICIENCIES

\[ E = 92.5 \]
\[ E = 91.7 \]

Figure 3: miRNA PCR Assays Yield the Most Accurate Results. Threshold cycle values from miR-16 and miR-21 specific RT\(^\text{\textsuperscript{\textregistered}}\) miRNA qPCR Assays are plotted versus the amount of HEK293 smallRNA in a serial dilution series. Serial dilutions of pooled synthetic cDNA templates were similarly used to calculate the amplification efficiencies of 468 assays (average efficiency of 95.37% ± 6.19%). Consistently high amplification efficiencies and sensitivity enable miRNA PCR Arrays to accurately analyze multiple sequences simultaneously using the ΔΔC\(_T\) method.

Reproducibility

HIGH TECHNICAL REPRODUCIBILITY

\[ y = 0.9682x + 0.9078 \]
\[ R^2 = 0.9915 \]

Figure 4: miRNA PCR Assays Yield Highly Reproducible Results. Duplicate samples of human brain smallRNA were characterized with the Human Genome RT\(^\text{\textsuperscript{\textregistered}}\) miRNA PCR Array, and the raw threshold cycle values from each array were plotted against each other. The linear fit of the data with a strong correlation factor (RT\(^\text{\textsuperscript{\textregistered}}\) > 0.99) indicates that miRNA PCR Array results can be reliably compared between plates, runs, biological replicates, and samples.

Application: Cancer Research - Colon Cancer Biomarkers

HUMAN COLON TUMOR VS. ADJACENT NORMAL TISSUE

Figure 5: RT\(^\text{\textsuperscript{\textregistered}}\) Human Cancer miRNA PCR Array Identifies Potential Colon Cancer Biomarkers. Small RNA isolated from human colon tumor and matched adjacent normal tissue (Biochain) were characterized with PCR Arrays containing assays specific for 88 cancer-related human miRNA sequences. Fold-differences are calculated from raw C\(_T\) values normalized to a panel of housekeeping small nuclear RNA. Several miRNA biomarkers are up-regulated in colon cancer.

Application: Stem Cell Research - Osteogenic miRNA Markers

Figure 6: Cell Differentiation and Development miRNA PCR Arrays Identify Sequences Potentially Regulating Osteogenesis. Human adipose tissue-derived mesenchymal stem cells (hMSC) grown in normal or osteogenic differentiation medium for 16 days were stained with Alizarin Red (A) and isolated for relative miRNA expression profiling. The results are shown in the scatter plot (B). Many miRNA sequences exhibit unique time-dependent changes in expression compared to undifferentiated controls (C).

### RT\(^\text{\textsuperscript{\textregistered}}\) miRNA PCR ARRAYS

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### miRNA PCR ARRAY Accessories

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FREE miRNA PCR Array Data Analysis Software
RT² PROFILER™ PCR ARRAYS
- Technology Overview (page 2)
- Over 100 Pathways Available (page 24)
- Start With as Little as 1 ng of RNA (page 6)
- Analyze FFPE Samples with PCR Arrays (page 7)
- Over 600 Peer-reviewed Publications
- Custom PCR Arrays Available for Human, Mouse, Rat, Rhesus Macaque and Drosophila
- To Learn More, Please Visit the PCR Product Web Page:
  www.SABiosciences.com/RTPCR.php

RT² MicroRNA PCR ARRAYS
- Regulation of Gene Transcription and Translation (page 10)
  Cancer
  Cell Differentiation and Development
  miFinder™ Whole Genome

Methyl-Profiler™ PCR ARRAYS
- Accurate Detection of DNA Methylation at CpG Islands Without Bisulfite (page 12)
  Breast Cancer
  Gastric Cancer
  Liver Cancer
  Lung Cancer
  Prostate Cancer

* NEW PCR Array
### CYTOKINES / INFLAMMATION
- Chemokines & Receptors
- Common Cytokine
- Inflammatory Cytokines and Receptors
- Inflammatory Response and Autoimmunity
- Interferon and Receptor
- Interferon-α, β Response
- JAK / STAT Signaling Pathway
- NFκB Signaling Pathway
- T Cell Energy & Immune Tolerance
- T-cell and B-cell Activation
- TGFβ BMP Signaling Pathway
- Th1 / Th2 / Th17
- TLR-Like Receptor
- Signaling Pathway
- TNF Ligand and Receptor
- Unfolded Protein Response

### ECM / ADHESION
- Angiogenesis
- Angiogenic Growth Factors & Angiogenesis Inhibitors
- Atherosclerosis
- Chemokines and Receptors
- Common Cytokine
- Endothelial Cell Biology
- Extracellular Matrix and Adhesion Molecules
- Growth Factors
- Inflammatory Cytokines and Receptors
- MAP Kinase Signaling Pathway
- Mesenchymal Stem Cell
- NFκB Signaling Pathway
- Osteogenesis
- TGFβ BMP Signaling Pathway
- Tumor Metastasis
- TNF Ligand and Receptor

### NEUROSCIENCE
- Alzheimer's Disease
- Apoptosis
- cAMP / Ca2+ Signaling Pathway
- Drug Transporters
- Embryonic Stem Cells
- GPCR Signaling Pathway
- Heat Shock Proteins
- Hedgehog Signaling Pathway
- Hypoxia Signaling Pathway
- Mesenchymal Stem Cell
- Neurogenesis and Neural Stem Cell
- Neuroscience Ion Channels and Transporters
- Neurotransmitter Receptors and Regulators
- Neurotrophin and Receptors
- Nitric Oxide Signaling Pathway
- Notch Signaling Pathway
- Oxidative Stress and Antioxidant Defense
- Stem Cell

### SIGNAL TRANSDUCTION
- cAMP / Ca2+ Signaling PathwayFinder
- EGFR / PDGF Signaling Pathway
- GPCR Signaling PathwayFinder
- Hedgehog Signaling Pathway
- Insulin Signaling Pathway
- JAK / STAT Signaling Pathway
- Lipoxygenase Signaling and Cholesterol Metabolism
- MAP Kinase Signaling Pathway
- NFκB Signaling Pathway
- Notch Signaling Pathway
- Nuclear Receptors and Coregulators
- PI3K-AKT Signaling Pathway
- Sign Transduction Pathway
- TGFβ BMP Signaling Pathway
- Toll-Like Receptor Signaling Pathway
- Transcription Factors
- Ubiquitination Pathway
- Wnt Signaling Pathway

### STEM CELL / DEVELOPMENT
- Cell Cycle
- Dendritic and Antigen Presenting Cell
- Embryonic Stem Cells
- Extracellular Matrix and Adhesion Molecules
- GPCR Signaling PathwayFinder
- Hedgehog Signaling Pathway
- Hematopoietic Stem Cells and Hematopoiesis
- Homeobox (HOX) Genes
- Lipoprotein Signaling and Cholesterol Metabolism
- Mesenchymal Stem Cell
- Neurogenesis and Neural Stem Cell
- Neurotrophin & Receptors
- Notch Signaling Pathway
- Osteogenesis
- Stem Cell
- T-cell and B-cell Activation
- TGFβ BMP Signaling Pathway
- Toll-Like Receptor Signaling Pathway
- Transcription Factors
- Wnt Signaling Pathway

### TOXICOLOGY / DRUG ADME
- Apoptosis
- Autophagy
- Cancer Drug Resistance and Metabolism
- Cancer PathwayFinder
- Cell Cycle
- DNA Damage Signaling Pathway
- Drug Metabolism
- Drug Metabolism: Phase I Enzymes
- Drug Metabolism: Phase II Enzymes
- Drug Transporters
- GPCR Signaling PathwayFinder
- Lipoprotein Signaling & Cholesterol Metabolism
- Mitochondria
- Oxidative Stress and Antioxidant Defense
- p53 Signaling Pathway
- PI3K-AKT Signaling Pathway
- Stress and Toxicity PathwayFinder

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<td>Prostate Cancer</td>
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The Methyl-Profiler™ DNA Methylation PCR Array System is an innovative technology enabling fast and accurate detection of DNA methylation status at CpG islands. This technology replaces the tedious and inefficient bisulfite-based methods with simple and simultaneous selective restriction digests of either methylated or unmethylated DNA, and takes advantage of the quantitative power of real-time PCR. The PCR Array format reveals the DNA methylation status of gene panels related to diseases or pathways. The individual primer pairs allow analysis of the DNA methylation status of any human or mouse gene. The reliability and simplicity of the procedure makes this technology ideal for profiling DNA methylation and biomarkers of stem cell growth and differentiation, cancer, and other human diseases.

**How DNA Methylation PCR ARRAYS Work**

1. **DNA Digestion.**
   - **Mix DNA + Digestion Buffer**
   - **Split into 4 Fractions**

2. **Real-Time PCR.**
   - **37°C (6 hr - overnight)**
   - **RT SYBR® Green qPCR Master Mix**

3. **Data Analysis.**

The human genome contains many long hypermethylated stretches of CpG dinucleotide-rich sequences. In this sea of CpG methylation, unmethylated CpG-rich sequences, known as “CpG islands”, are found in the promoters of most transcriptionally active genes. These normal patterns of DNA methylation are perturbed in cancer cells, where specific tumor suppressor genes (TSG) become hypermethylated, causing their expression to be silenced. Since every tumor type has a unique “methylation profile”, or panel of hypermethylated genes, the analysis of TSG hypermethylation has become very important for basic cancer research, clinical diagnostics, and therapeutic applications.

The Methyl-Profiler PCR Array System fulfills the need to rapidly and simultaneously determine the methylation status of more genes in more samples in a higher-throughput fashion. The current time- and labor-intensive methodologies require bisulfite conversion of unmethylated cytosines to uracil followed by either sequence analysis or PCR using primers sensitive to the resulting base conversion. Bisulfite conversion is not only tedious but is also inefficient and damages DNA. The resulting low yields of DNA make bisulfite-based methods unsuitable for the analysis of small samples.

**Why Use the Methyl-Profiler™ PCR ARRAY System?**

- **Simple, Fast and Reliable:**
  - No bisulfite conversion and ready-to-use.

- **Disease- or Pathway-Focused Gene Sets:**
  - Simultaneously detect DNA methylation of 24 or 96 genes.

- **Genome-wide Coverage:**
  - Primers to detect methylation of your favorite genes.

The Methyl-Profiler PCR Array System relies on the differential cleavage of target sequences by two different restriction endonucleases whose activities require either the presence or absence of methylated cytosines in their respective recognition sequences. As real-time PCR quantifies the relative amount of intact DNA remaining after each enzyme digestion, the methylation status of individual genes and the methylation profile across a gene panel are reliably and easily calculated. The high yield of DNA from the restriction digests and PCR amplification allow the analysis of smaller, more heterogenous samples.

**RESULTS COMPAREABLE TO BISULFITE SEQUENCING**

![Graph comparing bisulfite sequencing to PCR array results.](image)

**Figure 1:** Methyl-Profiler PCR Assays Yield Results Consistent with Bisulfite Sequencing.

The methylation status of the cadherin 1 gene (CDH1) was determined using bisulfite sequencing and Methyl-Profiler PCR Assays in three breast cancer cell lines known to have very different CDH1 methylation patterns.

To validate the accuracy of the Methyl-Profiler PCR Array System, its results were compared with those generated by bisulfite sequencing, the gold standard for DNA methylation analysis. The methylation status for both the CDH1 (Figure 1) and CDH13 (data not shown) genes observed in three different breast cancer cell lines by the two methods match very closely. Real-time PCR characterization of methylation-dependent and methylation-sensitive restriction enzyme digestions directly quantifies unmethylated and hypermethylated genomic DNA, respectively. The results indicate that the sensitivity and specificity of the Methyl-Profiler PCR Array System rivals bisulfite sequencing, suggesting that it can readily replace more tedious bisulfite PCR validation methods.

Make your DNA Methylation analysis as quick and painless as possible with the Methyl-Profiler DNA Methylation PCR System and EXCEL-based data analysis.

Download our FREE EXCEL Data Analysis Software:

Primary tumors are typically very heterogeneous, containing a mixture of both cancerous and noncancerous cells. Therefore, reliable tumor characterization requires detecting smaller amounts of hypermethylated DNA diluted in an unmethylated background. Methyl-Profiler PCR Assays have the sensitivity required to detect hypermethylated DNA from breast cancer cells even when they represent only five percent of the total cell population (Figure 2).

**Applications**

**Methyl-Profiler™ DNA Methylation PCR Arrays**

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* Methyl-Profiler PCR Arrays are available in Signature Panels (24 genes) & Comprehensive Panels (96 genes).

**PCR ARRAY Accessories**

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<tr>
<td>SYBR Green Only Master Mix</td>
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**Figure 2:** Methyl-Profiler PCR Assays Detect Hypermethylation in Heterogeneous Samples Containing As Little As Five Percent Tumor DNA. SKBR3 breast cancer cell line and normal blood genomic DNA (encoding hypermethylated and unmethylated H21, respectively) were mixed in different ratios. Using Human H21 Methyl-Profiler qPCR Primers, the percentage of hypermethylated H21 relative to total promoter DNA in each mixture was detectable down to five percent.

**Figure 3:** Methyl-Profiler PCR Arrays Validate Breast Cancer Gene Methylation Status in Breast Cancer Cell Lines. Heat map comparison of the hypermethylation status of 24 genes in the genomic DNA of three breast cancer cell lines and blood genomic DNA as determined by Human Breast Cancer Signature Panel DNA Methylation PCR Arrays.

To demonstrate that Methyl-Profiler PCR Arrays can validate methylation biomarkers, we first scanned published results to design a cataloged PCR Array representing a signature panel of the 24 most frequently methylated genes in human breast tumors. We then analyzed the methylation profile of this gene panel in three different breast cancer cell lines (Figure 3). The results further strengthen the correlation of these biomarkers with breast cancer.

**Figure 4:** Methyl-Profiler PCR Arrays Discover New Candidate Breast Cancer DNA Methylation Biomarkers. Heat map comparison of the hypermethylation status of a panel of 79 transcription factor genes in six breast cancer cell lines and a normal epithelial cell line as determined with Custom DNA Methylation PCR Arrays.

To demonstrate that Methyl-Profiler PCR Arrays can also discover new biomarkers, we arranged a custom array containing a panel of candidate transcription factor genes, whose methylation status had not been previously associated with breast cancer (Figure 4). We found that breast cancer cell lines also hypermethylate this gene panel, potentially providing a new discovery source for cancer biomarkers.

The Methyl-Profiler DNA Methylation PCR Array System is ideally suited to genomic DNA hypermethylation analysis for both basic research applications and clinical biomarker development. The simple two-step procedure is considerably faster and easier than current bisulfite sequencing and bisulfite PCR methods, and yields closely matching results with equivalent sensitivity.
SYBR Green Detection is a popular approach used in quantifying gene expression analysis with RT-PCR. It relies on the preferential binding of the SYBR green dye to double-stranded DNA, resulting in strong fluorescence emission signals, with the signal intensity proportional to the amount of double-stranded DNA present.

High quality PCR reaction components are essential for achieving superior amplification specificity and efficiency. SABiosciences offers a complete solution for using SYBR Green PCR Arrays with the RT SYBR Green qPCR Master Mixes. Each mix includes a Hot Start Taq DNA polymerase, which provides tighter control over activity, and other proprietary chemical components that significantly minimize primer dimer formation, thereby enhancing amplification efficiencies for even the most difficult-to-amplify genes. The higher SYBR Green signal from our formulations provides greater sensitivity and ensures clean results without sacrificing specificity or amplification efficiency.

Brighter SYBR Green Signal

**Figure 1: RT SYBR Green qPCR Master Mixes Provide Greater Sensitivity with a Brighter SYBR Green Signal.** Four commercial master mixes were used to detect the expression of human ACTB from the same universal reference RNA. The amplification (A) and the dissociation curves (B) for the master mix from SABiosciences demonstrate a sharper amplification curve and a brighter SYBR Green signal than observed with three competing master mixes.

**Figure 2: RT SYBR Green qPCR Master Mixes Provide Greater Sensitivity Without Sacrificing Specificity.** RT SYBR Green qPCR Master Mixes and Competitor I Master Mixes were used in qPCR assays to detect the human MMP13 and MMP15 mRNA in reference RNA. RT SYBR Green qPCR Master Mixes provide detection of the genes at an earlier threshold cycle value (Ct). The real-time dissociation curves and agarose gel electrophoresis characterization reveal the presence of a non-specific secondary product generated with the competitor's master mix which is not amplified by the RT SYBR Green qPCR Master Mix.

**RT SYBR Green qPCR Master Mix from SABiosciences**
- Proven in >20,000 Genes & in >600 Peer-Reviewed Publications

**Compatible PCR Instruments**
- Applied Biosystems (ABI): 5700, 7000, 7300, 7500, 7500 FAST, 7700, 7900HT,
  - StepOnePlus (96- and 384-well blocks)
- Bio-Rad: CFX96, CFX384, iCycler, IQ5, MyIQ, Chromo4, Opticon, Opticon 2
- Stratagene: Mx3000P, Mx3005P, Mx4000
- Roche: LightCycler 480 (96- and 384-well blocks)
- Eppendorf: Mastercycler ep realplex
- Takara: TP800
- RblId: BioMark

**RT SYBR Green qPCR Master Mixes**

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<th>Product</th>
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<td></td>
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* Plate format is 384-well.
FREE Web-Based PCR ARRAY Data Analysis Software

This integrated web-based software package for the PCR Array System automatically performs all ΔΔCt-based fold-change calculations from your uploaded raw threshold cycle data. Simply providing the array catalog number annotates the results to the correct gene list. The web portal delivers results not only in a tabular format but also in scatter, volcano, clustergram, and multi-group plots. Perform any pair-wise comparison between groups of experimental replicates by defining your own fold-change and statistical significance thresholds, or compare all of the groups side-by-side. The web portal also helps you correctly interpret the genomic DNA, reverse transcription efficiency, and positive PCR control well data. Make your pathway-focused gene expression analysis quick and painless with the PCR Array System and the PCR Array Data Analysis Suite.

- **Simple**: Just upload your data and define your parameters*
- **Convenient**: No downloading or installation required
- **Publication-Ready Output**: Export all results as FREE EXCEL files or PNG image files

* EXCEL-based data analysis templates are available from our website.

**INSTRUCTIONS**
1. Upload your data in a simple EXCEL file format.
2. Define your housekeeping genes and experimental groups.
3. Choose an automatically generated data analysis result

Take a test run with pre-loaded sample data set today:  
http://www.SABiosciences.com/pcarraydataanalysis.php

OR

Join our next live webinar entitled: “PCR Array Data Analysis Tutorial” at:  
http://www.SABiosciences.com/seminarlist.php

---

**Figure 1:** The Scatter Plot Compares Gene Expression Levels Between Two Experimental Conditions. The graph plots the log2 of normalized gene expression levels in a control condition (x-axis) versus an experimental condition (y-axis). Symbols outside the gray area indicate fold-differences larger than a threshold that you can define. The red symbols in the upper-left corner readily identify up-regulated genes, and the green symbols in the lower-right corner readily identify down-regulated genes.

**Figure 2:** The Volcano Plot Indicates the Statistical Significance of Gene Expression Changes. The x-axis plots the log2 of the fold-differences, while the y-axis plots p-values based on student's t-test of your replicate raw C, data. The red and green symbols outside the gray area conveniently have the same meaning as the Scatter Plot. Symbols in the Volcano Plot above the blue line readily identify fold-differences at least as statistically significant as a threshold that you can define.

---

**Service Core for PCR ARRAY Gene Expression Analysis**

SABiosciences provides comprehensive PCR Array Gene Expression Analysis Services for all of your real-time PCR-based needs. SABiosciences’ Service Team has years of experience in gene expression profiling with quantitative SYBR® Green real-time PCR, as well as RNA isolation of the quantity and quality required by real-time PCR experiments.

**Why Use Our Gene Expression Analysis Services?**
- No access to a real-time PCR instrument
- Lack the time or manpower to perform the microarray or PCR experiments needed to complete your project
- Prefer to run an optimized pilot project before bringing the technology in-house for a large scale project

Let our in-house experts serve as your “external core facility” to save you time, money, and effort in performing your expression analysis projects. The services are flexible and can be tailored to exactly meet your specific needs. Simply submit experimental samples and receive gene expression profiling results in a matter of days.

**Features of the PCR ARRAY Service**
- Free consultation on your experimental design to help you take advantage of our expertise
- Confirmation of RNA sample concentration and quality
- Unbiased reverse transcription of RNA into cDNA template, including elimination of genomic DNA contamination
- Detailed and flexible data analysis according to your needs
- Quick results
- Competitive and affordable prices

**RNA Isolation Services**

- **Standard**: Preparation from Cells, Tissue, and Blood
- **Specialized**: Preparation from FFPE, LCM, FNAB and Other Samples
References Listed by Pathway-Focused PCR Arrays

ANGIOGENESIS


APOPTOSIS


ATHEROSCLEROSIS


BREAST CANCER & ESTROGEN RECEPTOR SIGNALING


APPLICATION: CANCER BIOLOGY

PIK3CA EXPRESSION IN LUNG CANCER CELLS

---

Figure: PIK3CA mRNA Expression in Multiple Lung Cancer Cell Lines. PIK3CA mRNA expression was compared among cell lines having different features, such as PIK3CA alterations or mutations of other genes involved in the EGFR signaling pathway. PIK3CA mRNA expression levels were expressed relative to the mean levels in six HBEc cell lines. PIK3CA mRNA expression in PIK3CA gain or EGFR mutant cell lines were significantly increased compared with that of wild-type cell lines. However, PIK3CA mutant lines do not express increased mRNA levels. Horizontal bars indicate mean values. The Kruskal-Wallis test with Dunn’s multiple comparison test was used to determine significance.

CANCER PATHWAYFINDER™


CELL CYCLE


CHEMOKINES & RECEPTORS


Cheung KP, Yang E, Godbath AW. Memory-like CD8+ T cells generated during homeostatic proliferation differ to J Immunol. 2009 Sep 1;183(5):3364-72.


COMMON CYTOKINES


CUSTOM PCR ARRAYS


DNA DAMAGE


DRUG METABOLISM: PHASE I ENZYMES


EXTRACELLULAR MATRIX & ADHESION MOLECULES


ENDOTHELIAL CELL BIOLOGY


GROWTH FACTORS


HYPOXIA SIGNALING


APPLICATION: INFLAMMATORY CYTOKINES & RECEPTORS

Figure: Lineage-dependent Differences in Expression of Treg Functional Genes. Quantitative PCR analysis of selected genes related to Treg function in CD4+CD25+ cells from Fossi, Fulani, and European donors infected with Plasmodium falciparum (malaria). CD4+CD25+ cells were isolated from 12 Fossi (red bars) and 12 Fulani (blue bars) donors included in the study and 10 European donors (gray bars), and lysed to obtain total RNA. Equal amounts of RNA (50 ng) from each donor were reverse-transcribed and amplified in duplicate in RT® Custom PCR Arrays to simultaneously examine the mRNA levels of nine selected genes related to Treg activity using PPIA, GAPDH, and ACTB as housekeeping genes (HKG). Data was normalized to the mean values of the HKG, and a relative amount of RNA was calculated using the 2^-ΔΔCT method.


Chittur S, Parr B, Marcovici G. Inhibition of Inflammatory Gene Expression in Keratinocytes Using a Composition Evid Based Complement Alternat Med. 2009 Aug 19.


Montgomery CP, Daum RS. Transcription of inflammatory genes in the lung after infection with Influenza J Immunol. 2009 May 7;75(9):2519-47.


**INFLAMMATORY RESPONSE & AUTOIMMUNITY**


**INSULIN SIGNALLING**


**INTERFERON α, β RESPONSE**


**INTERFERON (IFN) & RECEPTOR**


**JAK / STAT SIGNALING**


**LIPOPROTEIN SIGNALING & CHOLESTEROL**

Zhou C, King N, Chen KY, Breslow JL. Activation of pregnane X receptor induces hypercholesterolemia in wild-type and J Lipid Res. 2009 May 12.

**MAP KINASE SIGNALING**


**APPLICATION: NEUROSCIENCE**

![MECP2 Responsive Genes](image_url)

**Figure**: Gene Expression Changes in Hypothalamus of MECP2 Mouse Models. Validation of expression changes by qPCR analysis. Gene expression levels from microarray analyses were validated in four MECP2-Tg males and four MECP2-null males. Data is plotted as relative up-regulation (red) or down-regulation (blue) over WT (P < 0.05, t-test). Each row represents a single gene, and each column represents data for four samples from each genotype.


**NFκB SIGNALING**


OXIDATIVE STRESS & ANTIOXIDANT DEFENSE


OSTEONECROSIS


p53 SIGNALING


SIGNAL TRANSDUCTION PATHWAYFINDER


T CELL ANERGY & IMMUNE TOLERANCE


TGFβ BMP SIGNALING


Th1, Th2, Th3


Th17 FOR AUTOIMMUNITY & INFLAMMATION


TOLL-LIKE RECEPTOR SIGNALING


TRANSCRIPTION FACTORS


TOXICOLOGY


TUMOR METASTASIS


WNT SIGNALING


Data is used with permission from Science, PNAS, Cancer Research, & respective authors.

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Patent Pending.
## RT² Profiler PCR Arrays

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<td>Toll-Like Receptor Signaling Pathway</td>
<td>PAHS-018</td>
<td>PAMM-018</td>
<td>PARN-018</td>
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<tr>
<td>Transcription Factors</td>
<td>PAHS-075</td>
<td>PAMM-075</td>
<td>PARN-075</td>
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<tr>
<td>Tumor Metastasis</td>
<td>PAHS-028</td>
<td>PAMM-028</td>
<td>PARN-028</td>
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<tr>
<td>Ubiquitination (Ubiquitylation)</td>
<td>PAHS-079</td>
<td>PAMM-079</td>
<td>PARN-079</td>
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<tr>
<td>Wnt Signaling Pathway</td>
<td>PAHS-043</td>
<td>PAMM-043</td>
<td>PARN-043</td>
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## PCR Arrays

<table>
<thead>
<tr>
<th>Size</th>
<th>96-well</th>
<th>96-well</th>
<th>384-well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>2 Arrays</td>
<td>12 Arrays</td>
<td>24 Arrays</td>
</tr>
</tbody>
</table>

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**Compatible Instrument**

- ** ABI 7500**
- ** ABI 7500HT**
- ** ABI 7500HT FAST 96-well Block**
- ** ABI 7500HT FAST 384-well Block**
- ** ABI StepOnePlus**

**Plate**

- **A**
- **B**
- **C**
- **D**
- **E**
- **F**
- **G**

---

**Master Mix**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>RT SYBR® Green w/ ROX®</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA-012</td>
<td>2 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-012-12</td>
<td>12 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-012-24</td>
<td>24 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-012-8</td>
<td>4 Arrays (384-well)</td>
</tr>
<tr>
<td>PA-112</td>
<td>25 ml</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>RT SYBR® Green w/ Fluorescin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA-011</td>
<td>2 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-011-12</td>
<td>12 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-011-24</td>
<td>24 Arrays (96-well)</td>
</tr>
<tr>
<td>PA-011-8</td>
<td>4 Arrays (384-well)</td>
</tr>
<tr>
<td>PA-110</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

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**PCR Accessories**

- **First Strand Kit**
- **RNA Isolation Kit**
- **NanoPreAMP Kit**
- **NanoPreAMP Primer Mixes**
- **FPERA Extraction Kit**
- **FP-E PreAMP Kit**
- **FP-P ReAMP Primer Mixes**
- **PhoReal Assays**

---

**Support@SABiosciences.com**

USA TEL: 888.503.3187 FAX: 888.465.9859
### RT<sup>™</sup> miRNA PCR Arrays

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Mouse</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Genome (96-well)</td>
<td>MAH-100</td>
<td>MAM-100</td>
<td>8 Plates</td>
</tr>
<tr>
<td>Whole Genome (384-well)</td>
<td>MAH-3100</td>
<td>MAM-3100</td>
<td>2, 12, 24 Plates</td>
</tr>
<tr>
<td>Cancer (96-well)</td>
<td>MAH-102</td>
<td>MAM-102</td>
<td>2, 12, 24 Plates</td>
</tr>
<tr>
<td>Cancer (384-well)</td>
<td>MAH-102</td>
<td>MAM-102</td>
<td>4 Plates</td>
</tr>
<tr>
<td>Cell Differentiation/Development (96-well)</td>
<td>MAH-103</td>
<td>MAM-103</td>
<td>2, 12, 24 Plates</td>
</tr>
<tr>
<td>Cell Differentiation/Development (384-well)</td>
<td>MAH-103</td>
<td>MAM-103</td>
<td>4 Plates</td>
</tr>
<tr>
<td>miFinder&lt;sup&gt;™&lt;/sup&gt; (96-well)</td>
<td>MAH-001</td>
<td>MAM-001</td>
<td>2, 12, 24 Plates</td>
</tr>
<tr>
<td>miFinder&lt;sup&gt;™&lt;/sup&gt; (384-well)</td>
<td>MAH-001</td>
<td>MAM-001</td>
<td>4 Plates</td>
</tr>
<tr>
<td>RT&lt;sup&gt;™&lt;/sup&gt; qPCR-Graded&lt;sup&gt;™&lt;/sup&gt; miRNA Isolation Kit</td>
<td>MA-03</td>
<td>MA-03</td>
<td>12 Samples</td>
</tr>
<tr>
<td>RT&lt;sup&gt;™&lt;/sup&gt; qRNA&lt;sup&gt;™&lt;/sup&gt; First Strand Kit</td>
<td>Various</td>
<td>Various</td>
<td>200 Reactions</td>
</tr>
<tr>
<td>RT&lt;sup&gt;™&lt;/sup&gt; qRNA&lt;sup&gt;™&lt;/sup&gt; qPCR Assays</td>
<td>Various</td>
<td>Various</td>
<td>200 Reactions</td>
</tr>
</tbody>
</table>

### Methyl-Profiler<sup>™</sup> DNA Methylation PCR Arrays

<table>
<thead>
<tr>
<th></th>
<th>Signature Panel*</th>
<th>Complete Panel*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Breast Cancer</td>
<td>MeAH-011</td>
<td>MeAH-8010</td>
</tr>
<tr>
<td>Human Gastric Cancer</td>
<td>MeAH-021</td>
<td>MeAH-8020</td>
</tr>
<tr>
<td>Human Liver Cancer</td>
<td>MeAH-031</td>
<td>MeAH-8030</td>
</tr>
<tr>
<td>Human Lung Cancer</td>
<td>MeAH-041</td>
<td>MeAH-8040</td>
</tr>
<tr>
<td>Human Prostate Cancer</td>
<td>MeAH-051</td>
<td>MeAH-8050</td>
</tr>
<tr>
<td>Human Colon Cancer</td>
<td>MeAH-061</td>
<td>MeAH-8060</td>
</tr>
<tr>
<td>Human Stem Cell Transcription Factor</td>
<td>MeAH-511</td>
<td>MeAH-512</td>
</tr>
<tr>
<td>Human Inflammatory Response</td>
<td>MeAH-521</td>
<td>MeAH-521</td>
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<tr>
<td>Human T Cell Activation</td>
<td>MeAH-531</td>
<td>MeAH-531</td>
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<tr>
<td>Human Cytokine Production</td>
<td>MeAH-541</td>
<td>MeAH-541</td>
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<tr>
<td>Methyl-Profiler DNA Methylation Enzyme Kit (12 samples)</td>
<td>MeA-03</td>
<td>MeA-03</td>
</tr>
<tr>
<td>Human qPCR Primers for Promoter CpG Islands (200 reactions)</td>
<td>Inquire</td>
<td>Inquire</td>
</tr>
<tr>
<td>Custom Methyl-Profiler PCR Array</td>
<td>Inquire</td>
<td>Inquire</td>
</tr>
</tbody>
</table>

* Methyl-Profiler Signature Panels profile the promoter methylation status of 24 genes and Complete Panels profile 96 genes.

### ChampionChip<sup>™</sup> PCR Arrays

<table>
<thead>
<tr>
<th></th>
<th>Size</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChampionChip PCR Arrays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Stem Cell Transcription Factors</td>
<td>4, 12, 24 Plates</td>
<td>GH-501</td>
</tr>
<tr>
<td>Human Oncogene &amp; Tumor Suppressor Genes</td>
<td>4, 12, 24 Plates</td>
<td>GH-502</td>
</tr>
<tr>
<td>Human T Helper Cell Differentiation</td>
<td>4, 12, 24 Plates</td>
<td>GH-503</td>
</tr>
<tr>
<td>ChampionChip One-Day Kit</td>
<td>12 samples</td>
<td>GA-101</td>
</tr>
<tr>
<td>ChampionChip Antibody Kits (antibody and qPCR controls)</td>
<td>12 Samples</td>
<td>GA-111</td>
</tr>
<tr>
<td>Human RNA Polymerase II</td>
<td>12 Samples</td>
<td>GA-112</td>
</tr>
<tr>
<td>Human p53</td>
<td>12 Samples</td>
<td>GA-112</td>
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<tr>
<td>Human Histone H3Ac</td>
<td>12 Samples</td>
<td>GAH-7201</td>
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<tr>
<td>Human Histone H4Ac</td>
<td>12 Samples</td>
<td>GAH-7201</td>
</tr>
<tr>
<td>Human Histone H3K4me1, H3K4me2, H3K4me3</td>
<td>12 Samples</td>
<td>GAH-3203</td>
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<tr>
<td>Human Histone H3K9me1, H3K9me2, H3K9me3</td>
<td>12 Samples</td>
<td>GAH-3203</td>
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<tr>
<td>Human Histone H3K27me3</td>
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<td>ChampionChip qPCR Primers - Whole Genome</td>
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<td>Various</td>
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<tr>
<td>Custom ChampionChip qPCR Arrays</td>
<td>Inquire</td>
<td>Inquire</td>
</tr>
</tbody>
</table>

### FREE Data Analysis Software


### Service Core for Gene Expression Analysis

- RT<sup>™</sup> Profiler PCR Array - Focus on Pathways or Diseases: [www.SABiosciences.com/RTtmProfilerService.php](http://www.SABiosciences.com/RTtmProfilerService.php)
- RT<sup>™</sup> Real-Time PCR - Analyze Individual Genes and Verify Array Data: [www.SABiosciences.com/RTtmRealTimePCR.php](http://www.SABiosciences.com/RTtmRealTimePCR.php)