

## RT<sup>2</sup> Profiler™ PCR Arrays: Pathway-Focused Gene Expression Profiling with qRT-PCR

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**Abstract:** This paper evaluates the performance of the newest technique for monitoring the expression of a panel of pathway- or disease-specific genes: the RT<sup>2</sup> Profiler PCR Array System from SA Biosciences. The PCR Array System combines the quantitative performance of SYBR® Green-based real-time PCR with the multiple gene profiling capabilities of a microarray. The PCR Array is a 96- or 384-well plate containing RT<sup>2</sup> qPCR Primer Assays for a set of 84 related genes, plus five housekeeping genes, and three controls. A complete system includes an instrument-specific master mix and an optimized first strand synthesis kit. This paper presents scientific data showing that PCR Arrays have the sensitivity, reproducibility, and specificity expected from real-time PCR techniques. As a result, this technology brings focused gene expression profiling to any biological laboratory setting with a real-time PCR instrument.

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### Introduction

The RT<sup>2</sup> Profiler PCR Array System is the most reliable and accurate tool for analyzing the expression of a focused panel of genes using SYBR Green-based real-time PCR. It brings together the quantitative performance of real-time PCR and the multiple gene profiling capability of microarrays. Each PCR Array profiles the expression of 84 genes relevant to a specific pathway or disease state. Expression levels are measured by gene-specific RT<sup>2</sup> qPCR Primer Assays optimized for simultaneous use in the PCR Array System.

RT<sup>2</sup> qPCR Primer Assays are key components in the PCR Array System. Each qPCR assay on the array is uniquely designed for use in SYBR Green real-time PCR analysis. The assay design criteria ensure that each qPCR reaction will generate single, gene-specific amplicons and prevent the co-amplification of non-specific products. The qPCR Assays used in PCR Arrays are optimized to work under standard conditions enabling a large number of genes to be assayed simultaneously. Their specificity is guaranteed by SABiosciences when RT<sup>2</sup> SYBR Green qPCR Master Mixes are used as part of the complete PCR Array System protocol.

The RT<sup>2</sup> Profiler PCR Array System is specifically designed to meet the unique challenges of profiling pathway-focused sets of genes using real-time PCR. Simultaneous gene expression analyses require similar qPCR efficiencies for accurate comparison among genes. RT<sup>2</sup> qPCR Primer Assays are designed with an amplicon size ranging from 100 to 250 bp and with PCR efficiencies uniformly greater than

90%. Overall, more than 10 thermodynamic criteria are included in the design of each RT<sup>2</sup> qPCR Primer Assay to ensure the most reliable and accurate results for pathway-based gene expression analysis in the PCR Array System.

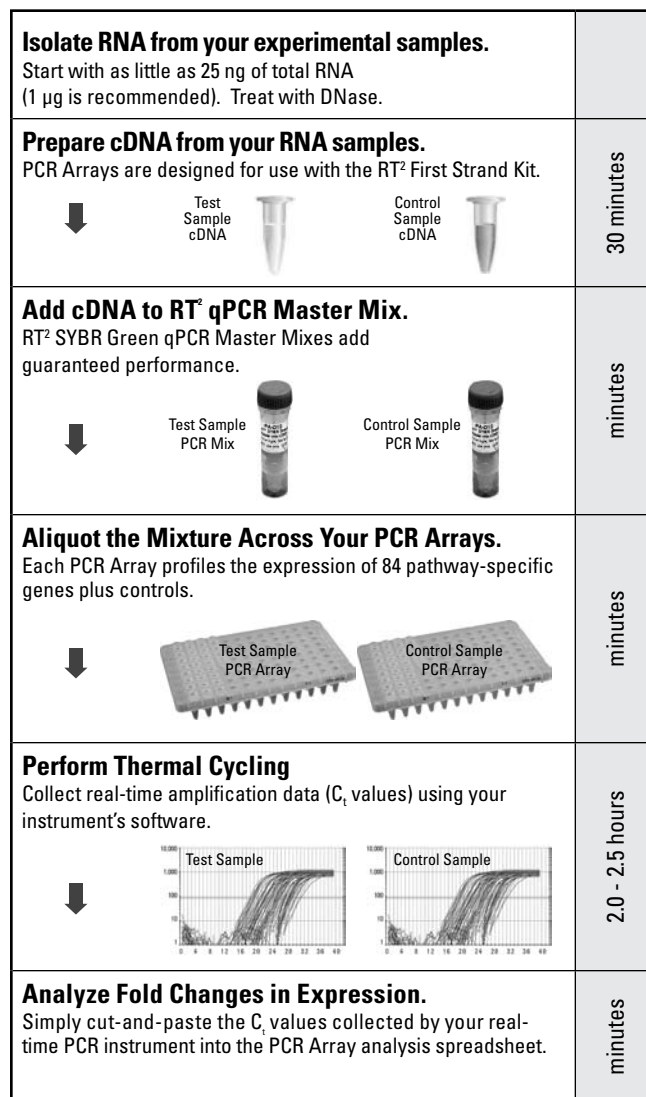
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## Experimental Protocol

Figure 1 depicts an overview of the PCR Array procedure. The protocol takes only two hours to perform (per sample) from start to finish. Start by converting the experimental RNA samples into PCR template with the RT<sup>2</sup> First Strand Kit. Then, combine the template with an instrument-specific and ready-to-use RT<sup>2</sup> SYBR Green qPCR Master Mix. Add equal aliquots of this mixture (25  $\mu$ l for 96-well or 10  $\mu$ l for 384-well plates) to each well of the same PCR Array plate containing the pre-dispensed gene-specific primer sets, and perform PCR. Use your instrument's software to calculate the threshold cycle ( $C_t$ ) values for all the genes on each PCR Array. Finally, calculate fold-changes in gene expression for pair-wise comparison using the  $\Delta\Delta C_t$  method. A simple examination of  $C_t$  value consistency for the housekeeping genes quickly indicates the proper normalization method. A similarly quick evaluation of the built-in RNA quality controls elements provides the relative levels of genomic DNA contamination and inhibitors of either the reverse transcription or the PCR itself.

## How It Works



**Figure 1: The Complete PCR Array Procedure is Easy-to-use and Requires Minimal Hands-on Time.**

## PCR Array Design and Gene Content

Each RT<sup>2</sup> Profiler PCR Array contains gene-specific qPCR assays for a thoroughly researched set of 84 genes relevant to a pathway or disease state and three RNA quality control elements (See Figure 2 for the layout of a typical PCR Array). Researchers are able to focus on genes related to their biological pathway or disease state with our pre-designed pathway- or application-specific gene panels. By limiting the range to less than one hundred genes (instead of thousands at one time), analysis can be achieved much faster and with greater precision due to the highly specific, yet smaller amount of data to analyze. As a result, more meaningful data can be obtained in less time. This process also streamlines the preparation stages of the experiment because the relevant genes are already grouped into one ready-to-use assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7	Gene 8	Gene 9	Gene 10	Gene 11	Gene 12
B	Gene 13	Gene 14	Gene 15	Gene 16	Gene 17	Gene 18	Gene 19	Gene 20	Gene 21	Gene 22	Gene 23	Gene 24
C	Gene 25	Gene 26	Gene 27	Gene 28	Gene 29	Gene 30	Gene 31	Gene 32	Gene 33	Gene 34	Gene 35	Gene 36
D	Gene 37	Gene 38	Gene 39	Gene 40	Gene 41	Gene 42	Gene 43	Gene 44	Gene 45	Gene 46	Gene 47	Gene 48
E	Gene 49	Gene 50	Gene 51	Gene 52	Gene 53	Gene 54	Gene 55	Gene 56	Gene 57	Gene 58	Gene 59	Gene 60
F	Gene 61	Gene 62	Gene 63	Gene 64	Gene 65	Gene 66	Gene 67	Gene 68	Gene 69	Gene 70	Gene 71	Gene 72
G	Gene 73	Gene 74	Gene 75	Gene 76	Gene 77	Gene 78	Gene 79	Gene 80	Gene 81	Gene 82	Gene 83	Gene 84
H	HK1	HK2	HK3	HK4	HK5	GDC	RTC	RTC	RTC	PPC	PPC	PPC

**Figure 2: Layout of the Cataloged PCR Arrays**

Wells A1 through G12 contain individual qPCR assays for 84 genes relevant to a biological pathway or disease state. Wells H1 through H5 contain a panel of housekeeping genes (HK1-HK5) used for normalizing the PCR Array data. Well H6 contains a Genomic DNA Control (GDC) primer set that specifically detects non-transcribed, repetitive genomic DNA with a high level of sensitivity. Wells H7 through H9 contain replicate Reverse Transcription Controls (RTC). These elements verify the efficiency of the RT reaction with a qPCR assay that specifically detects template synthesized from the first strand synthesis kit's built-in external RNA control. The replicate Positive PCR Controls (PPC) in wells H10 through H12 report on the efficiency of the polymerase chain reaction itself. These elements use a pre-dispensed artificial DNA sequence and the primer set that detects it. The two sets of replicate control wells (RTC and PPC) also test for inter-well and intra-plate consistency.

## PCR Array Benefits

- Pathway-Focused:** Efficiently profiles the expression of a large panel of genes relevant to a pathway or disease state
- Simple and Accurate:** Easy-to-use qRT-PCR based procedure provides high performance levels
- Easy Access:** Brings the power of expression profiling to any lab with real-time PCR capabilities

## Pathway-Focused PCR Arrays

The 96- or 384-well format of the RT<sup>2</sup> Profiler PCR Arrays is uniquely suited to SABiosciences' pathway-focused design concept. This product line combines the current understanding of important biological pathways with real-time PCR technology to generate application-specific research tools. To compile each product's comprehensive list of genes and to continually expand the breadth of available products, a systematic process comprised of literature surveys, database searches, expert review, and user feedback is utilized. SABiosciences now has the largest collection of pathway and application specific human, mouse, and rat PCR Arrays available on the market. (For examples, see Table 1.) This knowledge-based design merges the benefits of hypothesis-driven and discovery-based research, allowing researchers to answer highly specific questions in a systematic fashion. These pre-designed application-specific PCR Arrays accelerate, simplify, and improve life science research by saving time, effort, and resources. Currently, PCR Arrays are available for many pathways including apoptosis, inflammation, signal transduction, cancer and other diseases. Visit the SABiosciences web site ([www.SABiosciences.com](http://www.SABiosciences.com)) for a complete list.

**Table 1: Examples of Cataloged Pathway-Focused PCR Arrays**

Research Application	PCR Array Example	Gene Content Selected for the PCR Array Example
Biological Process	Human Apoptosis	TNF Ligands and their Receptors BCL2 Family Members Caspases Death and Effector Domains ATM and p53 Pathways
Functionally or Structurally Related Genes	Mouse Common Cytokines	Interferons and Interleukins Bone Morphogenetic Proteins Tumor Necrosis Factors Other Various Growth Factors
Signal Transduction Pathways	Human NF- $\kappa$ B Signaling Pathway	Extracellular Ligands and Receptors NF- $\kappa$ B and I $\kappa$ B Family Members Kinases Transcription Factors Responsive Genes
Disease	Human Cancer PathwayFinder™	Cell Cycle Control and DNA Damage Repair Apoptosis and Cell Senescence Cell Adhesion Angiogenesis Invasion and Tumor Metastasis

## Customized PCR Arrays

For researchers who have special gene expression profiling needs, SABiosciences offers a streamlined custom design and array production service. SABiosciences' Custom PCR Arrays provide researchers the flexibility to 1) validate a focused panel of genes identified by a high-density, genome-wide microarray, 2) modify the gene content of an existing PCR Array to better fit their research project, or 3) characterize a pathway or otherwise focused gene panel not covered by one of the cataloged PCR Arrays. The content of a PCR Array may also be subdivided into multiple sets of a smaller number of gene targets. This format allows for the characterization of multiple biological or technical replicates on the same array and during the same run. Like the cataloged products, Custom PCR Arrays are also available in either 96- or 384-well plate formats.

## The Complete PCR Array System

The complete PCR Array System includes the RT<sup>2</sup> Profiler PCR Arrays, the RT<sup>2</sup> SYBR Green qPCR Master Mixes and the RT<sup>2</sup> First Strand Kit. These system components are optimized for SYBR Green real-time PCR detection. The primer design and the optimized master mix formulation work together to insure the specificity of each assay in the array. The instrument-specific PCR Array plate formats and the master mixes containing the appropriate reference dyes also provide the PCR Arrays with the flexibility to match most real-time PCR platforms. The RT<sup>2</sup> First Strand Kit provides superior sensitivity and an External RNA Control detected by the PCR Array that helps test the quality of the input RNA material.

### Why the PCR Array System Works: Component 1: RT<sup>2</sup> qPCR Primer Assays

The greatest challenge for the PCR Array system is the amplification of every relevant, pathway- or disease-focused gene during the same run. The same uniform PCR conditions must be used while still achieving the high level of sensitivity, specificity, and reproducibility expected of real-time PCR. SABiosciences has designed the best possible qPCR assays and optimized the PCR master mix formulation for SYBR Green detection by experimentally testing thousands of qPCR assays under many reaction conditions.

#### RT<sup>2</sup> qPCR Primer Assays: Key Primer Design Criteria

Three of the most important primer design criteria in our experimentally verified computer algorithm:

- 1. Specificity:** Using BLAST and other algorithms, the specificity of each primer set is measured against the entire human, mouse, or rat genome to prevent the amplification of sequence-related, non-specific secondary products. The primer specificity is also checked against the *E. coli* genome to assure that the primers do not amplify bacterial genomic DNA, a common but minor contaminant of many Taq DNA polymerases.
- 2. Uniformity:** To use the same annealing temperature for every well in each PCR Array, only primer pairs with similar GC contents, melting temperature ( $T_m$ ), and other chemical and physical properties are used.
- 3. Efficiency:** Short amplicons (~ 100 to 200 bp) have been chosen for our primer pairs so that the enzyme replicates the entire sequence in the time allotted by the cycling program. Several filters are also used to strengthen the 3-prime anchoring of the primers, eliminating the amplification of dimers and other non-specific annealing events.

## Why the PCR Array System Works: Component 2: RT<sup>2</sup> qPCR Master Mixes

PCR master mix quality also plays an important role in the performance of SYBR Green-based real-time PCR. A tightly controlled hot-start Taq DNA polymerase is a critical component for success. The RT<sup>2</sup> qPCR Master Mixes from SABiosciences utilize a unique and proprietary chemically-modified HotStart Taq polymerase which only gains full activity after its heat activation step. Under these conditions, non-specific priming events occurring at low temperatures are not extended. Other master mixes often amplify the resulting templates into non-specific products which can cause false positive results. In addition, the RT<sup>2</sup> qPCR Master Mixes include proprietary chemical components that further minimize primer dimer formation and ensure high amplification efficiencies for even the most difficult to amplify genes. The combination of the RT<sup>2</sup> qPCR Primer Assay design and the high performance of the RT<sup>2</sup> SYBR Green qPCR Master Mix formulation is the foundation for the guaranteed specificity of the assays on the PCR Array.

## Why the PCR Array System Works: Component 3: RT<sup>2</sup> First Strand Kit

The RT<sup>2</sup> First Strand Kit contains all of the reagents needed not only to convert RNA into first strand cDNA, but also for the removal of genomic DNA from the RNA in the same simple two-step 30-minute reaction. A proprietary genomic DNA elimination buffer completely removes any residual genomic DNA from your RNA sample. Then, the optimized formulation also allows you to directly use the RNA preparation for reverse transcription and finally real-time PCR without affecting reaction performance. By eliminating genomic DNA contamination, real-time PCR signal intensities accurately reflect the relative level of gene-specific mRNA transcript.

The kit also includes a built-in External RNA Control, an *in vitro* transcript with an artificial sequence designed to help test for inhibitors of reverse transcription. The Reverse Transcription Control (RTC) in the PCR Array specifically detects cDNA template generated by the kit from the external RNA control. A reproducible threshold cycle value from this control indicates a consistent and high level of RNA quality and transcription efficiency. Such a result provides a greater degree of confidence in the final results.

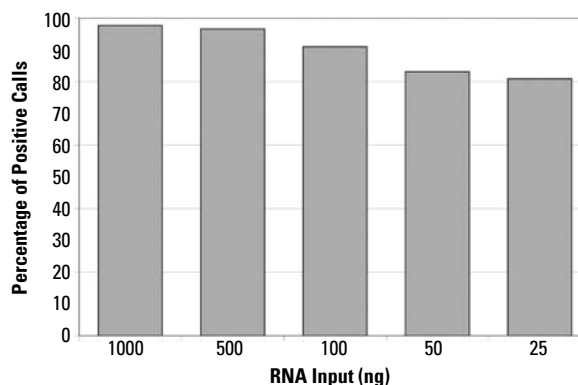
The RT<sup>2</sup> First Strand Kit is optimized for use with the RT<sup>2</sup> SYBR Green qPCR Master Mixes and subsequent gene expression analysis with the RT<sup>2</sup> Profiler PCR Arrays as part of the complete PCR Array System. Random hexamers and oligo-dT prime reverse transcription in an unbiased manner and capture more difficult-to-detect genes. The reverse transcriptase, optimized magnesium concentration, and other buffer components maximize cDNA product yield and length. The RT<sup>2</sup> First Strand Kit contains a complete set of reagents for the conversion of RNA into PCR template and provides greater control over RNA quality than other available kits or enzyme sources. Table 2 summarizes the features of the RT<sup>2</sup> Profiler PCR Array System.

**Table 2: Features of Complete RT<sup>2</sup> Profiler PCR Array System**

<b>Array Design</b>	84 pathway-focused genes
	5 housekeeping genes
	1 Genomic DNA control
	3 Reverse Transcription Controls (RTC)
	3 Positive PCR controls (PPC)
<b>Primer Design</b>	Specificity: Sequence alignment filter
	Uniformity: Consistent melting and annealing temperatures
	Efficiency: Short amplicon sequence
<b>Master Mix</b>	Instrument-specific SYBR Green formulations
	Supports all ABI, Bio-Rad, MJ Research, and Stratagene platforms
	Hot Start Enzyme: No extension of non-specific priming events No amplification of secondary products like primer dimers
<b>First Strand Synthesis</b>	Optimized gDNA elimination buffer prevents false positive signals
	Built-in External RNA Control to test for inhibitors of RT

## PCR Array Performance: Sensitivity

Researchers continually attempt to detect genes at ever lower levels of expression and in ever smaller amounts of total RNA. To meet these needs, the PCR Array System must pass a very stringent test of sensitivity. A wide variety of universal RNA amounts were characterized with the PCR Array System and an array representing inflammatory cytokine and receptor genes that are known to be expressed at very low levels. Figure 3 plots the percent positive call (the percentage of genes with  $C_t < 35$ ) versus the amount of input RNA. The results indicate that the PCR Array System achieves greater than 80 percent positive calls with input total RNA amounts as low as 25.0 ng and as high as 1.0  $\mu$ g (or even 5.0  $\mu$ g) per array plate. For other pathways or gene panels expressed at higher levels, the sensitivity of the system may be further improved, potentially yielding high positive call rates with even lower amounts of input total RNA. However, the recommended amount of input RNA for first-time users is 0.5 to 1.0  $\mu$ g to assure a maximum number of positive calls. The minimum recommended RNA amount is 25.0 ng, because the percent positive call drops significantly with less material.



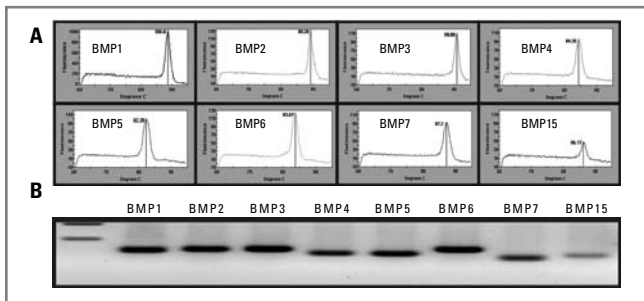
**Figure 3: High Positive Call Rates with as little as 25 ng of Total RNA**

The RT<sup>2</sup> Profiler PCR Array System yields high positive call rates with as little as 25 ng of total RNA. Different amounts of XpressRef™ Human Universal RNA (25, 50, 100, 500, and 1000 ng) were characterized with the Human Inflammatory Cytokines and Receptors PCR Array, the RT<sup>2</sup> First Strand kit and the RT<sup>2</sup> SYBR Green/Fluorescein qPCR Master Mix on the Bio-Rad iCycler instrument. The percent positive call rate (the percentage of genes with  $C_t < 35$ ) is plotted versus the input amount of total RNA.

## PCR Array Performance: Specificity

The PCR Array System has been designed and optimized for the SYBR Green based detection method used by most real-time systems, making the PCR Array System very flexible and widely applicable. Concerns have been raised over the specificity of SYBR Green-based detection and its ability to amplify only one gene-specific amplicon product, because it detects double-stranded DNA non-specifically. SABiosciences' experimentally verified primer design algorithm, used for the PCR Arrays, guarantees the generation of single, gene-specific amplicons without the co-amplification of primer dimers or other non-specific secondary products.

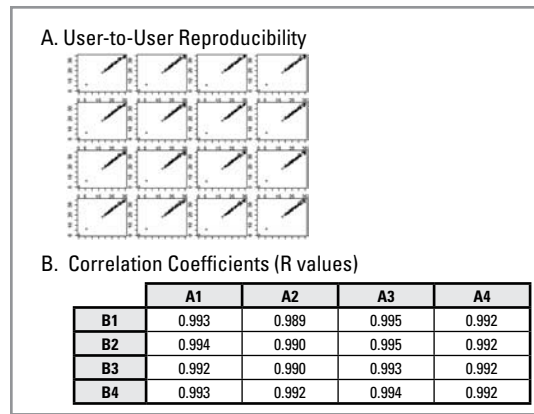
For an example of a stringent test of PCR Array specificity, we characterized the real-time PCR dissociation curves of each gene on a PCR Array representing highly homologous members of the TGFβ and Bone Morphogenetic Protein (BMP) gene families. Products were also characterized by agarose gel electrophoresis. Figure 4 displays the representative dissociation curves and the agarose gel results for the BMP gene family. Each dissociation curve contains only one peak, and each agarose gel lane contains only one band of the predicted size. The results indicate that the PCR Array amplifies gene-specific products despite the expression of highly homologous members of the same gene family in the same RNA sample. The optimized PCR Array System now brings a level of specificity to SYBR Green-based detection that most thought could be achieved only by more expensive probe-based methods.



**Figure 4: High Specificity with the PCR Array System**  
The RT<sup>2</sup> Profiler PCR Arrays demonstrate a high degree of specificity for their target genes. XpressRef Human Universal Total RNA (5 µg) was characterized on the Human TGFβ / BMP Signaling Pathway PCR Array using the RT<sup>2</sup> SYBR Green/Fluorescein qPCR Master Mix on the Bio-Rad iCycler instrument. After a standard melting curve program, dissociation curves were obtained (Panel A), and the products were characterized by agarose gel electrophoresis (Panel B).

## PCR Array Performance: Reproducibility

The quantitative nature of real-time PCR should impart a high degree of reproducibility onto the PCR Array System. To test this notion, two different end-users characterized, in technical replicates (n = 4), the same universal total RNA sample, each with two separate manufacturing lots of a cataloged PCR Array on two separate days. The raw threshold cycle values for the entire array's gene panel were then compared between each user's replicates and all four of the other user's replicates. Figure 5 displays the resulting scatter plots and correlation coefficients. Each comparison yields the predicted ideals of straight lines with slopes of 1.0 and correlation coefficients of 0.99 or greater. The results demonstrate the high degree of plate-to-plate, run-to-run, and replicate-to-replicate reproducibility inherent in the PCR Array System technology, even at the level of raw data.

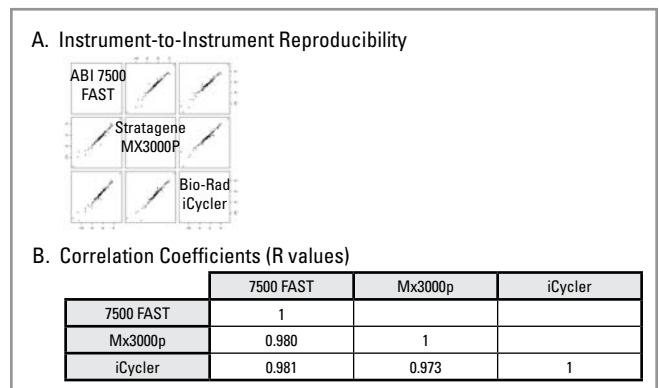


**Figure 5: High User-to-User Reproducibility**  
The PCR Array System demonstrates a high degree of user-to-user reproducibility. Two different end-users characterized template cDNA prepared from Human XpressRef Universal Total RNA (5.0 µg) in technical quadruplicates using the Human Drug Metabolism PCR Array and the RT<sup>2</sup> SYBR Green / Fluorescein qPCR Master Mix on the Bio-Rad iCycler. Panel A compares the raw threshold cycle values of the array's gene panel as determined by each of the first end-user's replicates versus each of the second end-user's replicates. Panel B lists the correlation coefficient of the linear curve fit for each scatter plot comparison.

To directly demonstrate that the results from the PCR Array System are indeed reproducible, the fold-differences in the expression of drug metabolism genes between two different RNA samples were compared across three different real-time PCR instrument platforms. In each gene expression profile comparison shown in Figure 6, the curve fit to a straight line with a slope of one (1) has a correlation coefficient of 0.97 or higher. Assuming good RNA sample preparation and proper execution of the PCR Array protocol, any differences observed in gene expression levels are attributable to the biological conditions under study and not experimental variation associated with this level of reproducibility in the technology itself. Table 3 summarizes the typical performance of the RT<sup>2</sup> Profiler PCR Array.

**Table 3: Typical Performance of the RT<sup>2</sup> Profiler PCR Array**

<b>Sensitivity</b>	80 % Positive Call with as little as 25 ng
<b>Dynamic Range</b>	At least five (5) orders of magnitude
<b>Specificity</b>	Primers amplify single, target-specific PCR products
<b>Reproducibility</b>	Correlation coefficients (R) □ 0.99 for intra-lab raw C <sub>t</sub> values Correlation coefficients (R) □ 0.97 for inter-lab fold-change values Average standard deviation of 0.25 threshold cycles



**Figure 6: High Instrument-to-Instrument Reproducibility**  
PCR Arrays demonstrate a high degree of instrument-to-instrument reproducibility. Two different MAQC RNA samples were characterized using the Human Drug Metabolism PCR Array and either the RT<sup>2</sup> SYBR Green / Fluorescein qPCR Master Mix on the Bio-Rad iCycler or the RT<sup>2</sup> SYBR Green / ROX qPCR Master Mix on either the Stratagene Mx3000p or the ABI 7500 FAST instrumentation. The fold-difference in the expression of the entire array's gene panel between the two RNA samples determined by each instrument was calculated and compared with both of the other two instruments in scatter plots (Panel A) and the correlation coefficients of the linear curve fits (Panel B).

## PCR Array Application Example I: Identifying and Monitoring Oncogenic Pathways

**Materials and Methods:** Template cDNAs prepared from normal human breast and human breast tumor #1 total RNA (BioChain Institute, Inc., 5.0 µg) were characterized in technical triplicates using the Human Cancer PathwayFinder PCR Array and the RT<sup>2</sup> SYBR Green/Fluorescein qPCR Master Mix on the iCycler PCR System.

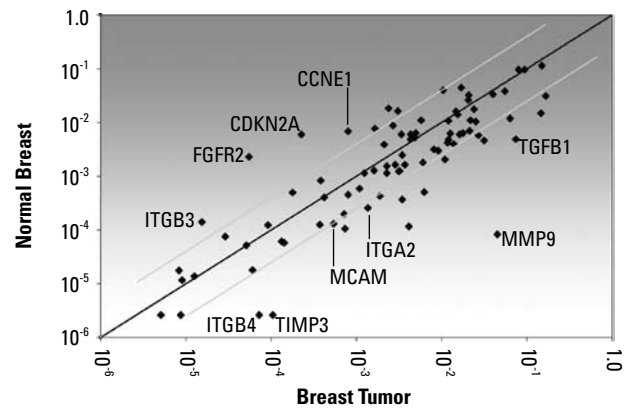
Triplicate total RNA samples prepared from normal human breast and human breast tumor #2 total RNA (BioChain Institute, Inc., 1.0 µg) were converted into template cDNA and then characterized using the Human Extracellular Matrix and Adhesion Molecules PCR Array and the RT<sup>2</sup> SYBR Green/Fluorescein qPCR Master Mix on the iCycler<sup>®</sup> PCR System.

**Results:** Gene expression profiling is important for discovering and validating tumor biomarkers and therapeutic targets. Using the Cancer PathwayFinder PCR Array and the Human Extracellular Matrix and Adhesion Molecules PCR Array, we examined the gene expression profiles exhibited by two different human breast tumors relative to normal tissues. The study compared the relative expression of both tumorigenesis- and adhesion-related genes between each tumor sample and a normal breast tissue sample. This study provides an example of the identification of a pathway affected by the transformation of a particular tumor type.

Total RNA samples from normal breast tissue and the first of two unmatched breast tumor were analyzed using the Cancer PathwayFinder PCR Array. This PCR Array includes representative genes from the following biological pathways involved in tumorigenesis: adhesion, angiogenesis, apoptosis, cell cycle control, cell senescence, DNA damage repair, invasion, metastasis, signal transduction molecules, and transcription factors.

Figure 7 displays a scatter plot report of the results from the Cancer PathwayFinder PCR Array experiment, indicating the positions of several noteworthy genes based on their large fold-differences in expression between the normal breast and the breast tumor samples. Of the 84 cancer pathway-focused genes in this array, 24 genes demonstrated at least a 3-fold difference in gene expression between normal breast tissue and the breast tumor. Up-regulation was observed in 17 genes, while 7 genes appeared to be down-regulated in the tumor samples, for a total of 24 differentially regulated genes (Table 4).

A subset of six of the 24 genes (ITGA2, ITGA4, ITGB3, MCAM, MMP9, and TIMP3) represents adhesion and extracellular matrix molecules. ITGB3 was down-regulated, while the other five genes were up-regulated. The results suggest that changes in the expression of genes involved in cellular interactions played an important role in the transformation of this and perhaps other breast tumors. To further test this hypothesis and to analyze the expression of other adhesion-related genes, a second breast tumor sample was characterized using a cellular adhesion-focused PCR Array.



**Figure 7: Relative expression comparison for 84 cancer-related genes between normal human breast and human breast tumor #1.**

The figure depicts a log transformation plot of the relative expression level of each gene ( $2^{\Delta\Delta C_t}$ ) between breast tumor (x-axis) and normal breast (y-axis). The gray lines indicate a four-fold change in gene expression threshold.

**Table 4: Changes in expression for cancer-related genes between normal human breast and human breast tumor #1.**

Genes from the experiment in Figure 7 that exhibit a three-fold or greater change in expression between normal and tumor breast tissue are listed.

Gene	Fold change Tumor/Normal	t-Test p value	Average Raw $C_t$	
			Tumor	Normal
MMP9	542.45	0.0000	21.8	30.0
TIMP3	39.85	0.0000	30.5	35.0
TNF	35.51	0.0000	25.2	29.5
ITGA4	27.54	0.0001	31.1	35.0
TGFB1	15.10	0.0000	21.1	24.1
BCL2	12.27	0.0012	24.6	27.4
FOS	9.74	0.0003	20.1	22.5
GZMA	9.30	0.0003	25.5	27.9
TEK	6.88	0.0003	27.7	29.7
JUN	6.88	0.0008	22.3	24.2
APAF1	5.34	0.0018	23.8	25.4
ATM	5.34	0.0001	19.9	21.5
ITGA2	5.34	0.0042	26.8	28.4
PIK3R1	5.34	0.0001	21.3	22.9
SYK	4.65	0.0003	22.5	23.9
PLAUR	4.44	0.0007	26.4	27.7
MCAM	4.14	0.0000	28.2	29.4
PLAU	3.61	0.0132	27.8	28.8
ETS2	3.44	0.0015	23.5	24.4
ANGPT1	3.36	0.0028	31.3	32.2
FAS	3.36	0.0031	24.7	25.6
TERT	3.29	0.0314	34.1	35.0
NFKB1	3.07	0.0068	22.9	23.6
NME4	3.07	0.0019	24.1	24.9
ERBB2	-3.29	0.0000	25.9	23.3
ITGA3	-3.78	0.0000	23.9	21.1
UCC1	-4.65	0.0003	26.6	23.5
MYC	-5.34	0.0004	25.7	22.4
SNCG	-7.73	0.0000	26.0	22.2
CCNE1	-8.48	0.0000	27.6	23.7
ITGB3	-9.08	0.0026	33.3	29.3
CDKN2A	-26.91	0.0000	29.4	23.8
FGFR2	-41.74	0.0007	31.5	25.2

Total RNA samples from normal breast tissue and the second of the two unmatched breast tumors were characterized on the Extracellular Matrix and Adhesion Molecules PCR Array. Genes that displayed at least a 3-fold difference in expression between the samples are listed in Table 5. On this array, a larger number of genes exhibited differential expression in the second tumor than was observed for the first tumor on the Cancer PathwayFinder PCR Array. A total of 38 genes had a different level of expression in the breast tumor than in the normal breast tissue, with 27 genes showing up-regulation and 11 genes showing down-regulation.

**Table 5: Changes in relative expression for genes encoding ECM and adhesion molecules between normal human breast and human breast tumor #2.**

The table lists genes that exhibit at least a three-fold difference in expression in the breast tumor sample when compared to the normal breast tissue. The raw threshold cycle (C<sub>t</sub>) values seen in the two samples are also listed for comparison.

Gene	Fold change	t-Test p value	Average Raw C <sub>t</sub>	
	Tumor/Normal		Tumor	Normal
CTNND2	229.39	0.0000	23.8	31.6
TIMP3	104.57	0.0000	28.4	35.0
SELE	43.46	0.0000	26.3	31.7
MMP1	36.97	0.0000	27.9	33.0
MMP3	34.50	0.0000	29.9	35.0
KAL1	31.45	0.0000	23.1	28.0
MMP13	21.73	0.0000	26.9	31.2
MMP10	16.47	0.0000	31.0	35.0
MMP16	16.09	0.0000	25.3	29.2
FN1	11.92	0.0512	29.9	33.4
CD44	11.92	0.0046	23.5	27.0
TNC	10.87	0.0000	22.9	26.2
MMP9	10.62	0.0001	27.1	30.4
SELP	9.46	0.0001	26.1	29.2
MMP11	7.51	0.0000	25.0	27.9
COL7A1	7.00	0.0057	30.9	33.7
CSPG2	6.39	0.0000	24.0	26.6
COL4A2	5.56	0.0009	23.9	26.3
TNA	5.43	0.0001	26.9	29.3
COL11A1	5.31	0.0017	30.7	33.0
THBS1	4.84	0.0185	24.1	26.3
SELL	4.21	0.0002	24.7	26.7
HAS1	3.93	0.0010	27.5	29.4
CTNND1	3.84	0.0007	30.4	32.2
ITGA4	3.34	0.0000	25.4	27.1
ITGA7	3.34	0.0003	27.6	29.3
THBS2	3.19	0.0058	26.1	27.7
SPP1	-3.08	0.0000	23.6	21.9
ITGB5	-3.31	0.0000	23.2	21.4
CTNNB1	-3.31	0.0003	21.2	19.4
ITGAV	-4.57	0.0072	26.5	24.2
CNTN1	-5.25	0.0001	28.8	26.3
MMP7	-5.37	0.0000	25.7	23.2
ITGB3	-7.25	0.0094	32.1	29.2
ADAMTS1	-9.35	0.0003	25.5	22.2
LAMA3	-10.26	0.0000	24.7	21.2
NCAM1	-23.02	0.0000	30.9	26.3
ITGB4	-30.38	0.0000	26.6	21.6

The first and second breast tumor sample displayed concordant results for four genes (MMP9, TIMP3, ITGA4, and ITGB3) that changed expression in the same direction on the Cancer PathwayFinder PCR Array and the Extracellular Matrix and Adhesion Molecules PCR Array. These results not only further verify that cellular adhesion genes changed their expression in these two particular breast cancer tumors, but also suggest a more general role for these genes in breast tissue transformation.

These types of studies provide a new and convenient way to investigate the mechanisms underlying oncogenesis of specific tumors on a pathway-focused basis. The data shown here is consistent with known principles, that changes in the expression of genes related to cellular adhesion play a role in the transformation of breast tissue<sup>1-2</sup>. Alterations in the expression of these genes enhance or inhibit metastasis of the tumor from its original location and may aid tumor invasion into a new tissue or organ. A PCR Array focusing on Human Tumor Metastasis is available and could be used to continue this study.

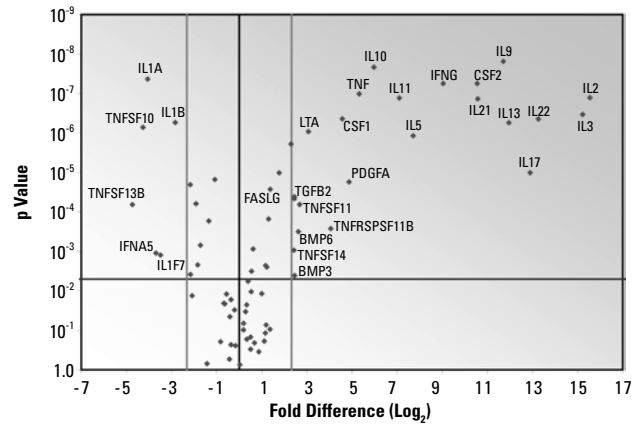
## PCR Array Application Example II: Monitoring Cytokine Expression Levels

**Materials and Methods:** Peripheral Blood Mononuclear Cells (PBMC) were treated with or without 50 ng/ml PMA + 1 µg/ml ionomycin for 6 or 24 hours. After each incubation period, total RNA was isolated from each preparation, and first strand cDNAs were prepared from 500 ng total RNA of each sample using the RT<sup>2</sup> First Strand Kit. Template cDNAs were characterized in technical triplicates using the Human Common Cytokine PCR Array with the RT<sup>2</sup> SYBR Green/ROX qPCR Master Mix on the 7500 FAST<sup>®</sup> Real-Time PCR System (Applied Biosystems). Fold changes in gene expression between the stimulated and resting PBMC RNA were calculated using the  $\Delta\Delta C_t$  method in the PCR Array Data Analysis template.

To validate the results obtained from the PCR Array, the protein level of eight selected cytokines secreted by the PBMC (IL-2, 4, 5, 10, 12, 13, and IFN- $\gamma$  and TNF- $\alpha$ ) was measured. Cell supernatants were collected at different time points (0, 6, 24, and 48 hours) and the cytokines were measured by enzyme-linked immunosorbent assay (ELISA) using the Human Th1 / Th2 Cytokines Multi-Analyte Profiler ELISArray<sup>™</sup> Kit. Optical Density (OD) readings for each protein analyte from the samples were compared to a standard curve for quantification of the amount of protein in the original samples.

**Results:** Cytokine quantification is an important element in studies of inflammation and immune responses. Quantitative RT-PCR, a rapid and sensitive assay, is the preferred method to quantify cytokine mRNA levels because they are often expressed at low levels. The PCR Array System offers a simple, reliable and sensitive tool for multiple cytokine profiling. Using the Human Cytokine PCR Array, we have monitored the mRNA levels of 84 different cytokines in stimulated versus and untreated human peripheral blood mononuclear cells (PBMC).

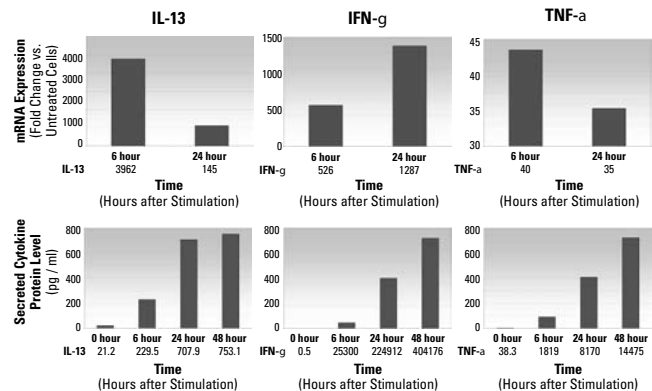
The gene expression results identify 23 up-regulated and 6 down-regulated genes (with >5 fold-change and  $p < 0.005$ ) upon 6 hours of stimulation. At 24 hours, the effects of PMA-ionomycin on genes such as BMP's, CSF's, IFN $\gamma$ , IL1 $\beta$ , IL6, IL11, TGF $\beta$  and TNF are continuously observed, while the effect on other genes such as interleukin 2, 3, 5, 9, 10, 13, 17 and 22 diminishes twenty-four hours after stimulation (Figure 8 and Table 6). To validate these results, the protein levels of 8 selected cytokines secreted by the PBMC was measured using a multiplex ELISA array (Figure 9). The effects of these mRNA expression changes were observed in the changes in cytokine production induced by PMA ionomycin at 6 hours after stimulation. The induction in cytokine production by PMA-ionomycin was sustained up to 48 hours after stimulation, despite the observation of the subdued mRNA expression for some cytokines at 24 hours after stimulation.



**Figure 8: RNA isolated from resting PBMC or PBMC stimulated with PMA ionomycin for 6 or 24 hours were characterized on the Human Common Cytokine PCR Array.**

Log fold-changes in gene expression between PBMC stimulated with PMA ionomycin and resting PBMC are plotted against t-test p-values to produce a "volcano plot". The higher the position, the more significant the gene's fold-change. Genes plotted farther from the central axis have larger changes in gene expression. Thresholds for fold-change (vertical lines, 5-fold) and significant difference (horizontal line,  $p < 0.005$ ) were used in this display.

Using the Common Cytokine PCR Array, we identified 29 genes that exhibited at least a five-fold change in gene expression between resting and PMA ionomycin stimulated peripheral blood mononuclear cells at 6 hours after stimulation. Our data show that changes in cytokine mRNA levels detected by PCR Arrays accurately predict changes in protein levels measured by ELISA. Hence, the PCR Array offers a simple, reliable and sensitive tool for multiple cytokine profiling.



**Figure 9. The effects of PMA-ionomycin on the secretion of the eight selected cytokines were assessed by multiplex cytokine ELISA.**

As shown in the above graphs, in parallel with the PCR Array results (upper panel), a marked increase in cytokine release (lower panel) was seen for IL-13, and IFN-g and TNF-a. The induction in cytokine secretion by PMA-ionomycin were sustained up to 48 hours of stimulation, despite the observation of the subdued mRNA expression for some cytokines such as IL-13 and TNF-a after 24 hours of stimulation.



**Table 6. List of cytokines induced or down regulated in Phorbol Myristate Acetate Ionomycin-stimulated Peripheral Blood Mononuclear Cells (PBMC) versus resting PBMC.**

The significance of the change in gene expression between the two samples was evaluated by unpaired Student t-test for each gene. The level of statistical significance is set at <0.005. Genes that show at least a five-fold difference in expression between the two samples are listed in the table. After six hours of stimulation, a total of 29 genes have at least a 5-fold change in expression between the stimulated and resting PBMC, with 23 genes having increased expression and six genes having decreased expression in stimulated PBMC.

Gene	6 HOURS AFTER STIMULATION				24 HOURS AFTER STIMULATION			
	Average Raw C <sub>t</sub> Value		Stimulated / Resting		Average Raw C <sub>t</sub> Value		Stimulated / Resting	
	Stimulated	Resting	Fold Change	t-test p-value	Stimulated	Resting	Fold Change	t-test p-value
IL2	14.64	29.99	<b>47820.23</b>	0.0000	13.54	26.91	<b>11190.60</b>	0.0000
IL3	19.53	34.56	<b>38218.94</b>	0.0000	18.46	30.35	<b>4020.99</b>	0.0000
IL22	21.08	34.14	<b>9823.35</b>	0.0000	24.26	30.62	<b>87.02</b>	0.0000
IL17	21.51	34.21	<b>7601.14</b>	0.0000	20.63	32.26	<b>3365.64</b>	0.0000
IL13	21.05	32.80	<b>3961.96</b>	0.0000	23.65	30.74	<b>144.67</b>	0.0000
IL9	23.49	35.00	<b>3339.31</b>	0.0000	22.22	31.15	<b>516.75</b>	0.0000
IL21	19.76	30.13	<b>1522.26</b>	0.0000	20.00	30.09	<b>1152.06</b>	0.0000
CSF2	16.80	27.15	<b>1494.38</b>	0.0000	15.53	26.86	<b>2714.87</b>	0.0000
IFNG	13.57	22.41	<b>525.91</b>	0.0000	13.94	24.19	<b>1287.18</b>	0.0000
IL5	21.89	29.40	<b>208.71</b>	0.0000	25.77	29.35	<b>12.70</b>	0.0000
IL11	24.22	31.12	<b>136.74</b>	0.0000	25.35	34.35	<b>542.45</b>	0.0000
IL10	21.43	27.21	<b>62.77</b>	0.0000	26.37	24.33	<b>-3.87</b>	0.0015
TNF	17.91	23.04	<b>40.00</b>	0.0000	18.69	23.72	<b>34.54</b>	0.0000
PDGFA	24.17	28.84	<b>29.22</b>	0.0000	23.27	28.05	<b>29.11</b>	0.0000
CSF1	21.27	25.64	<b>23.73</b>	0.0000	20.64	23.85	<b>9.78</b>	0.0000
TNFRSF11B	30.39	34.25	<b>16.63</b>	0.0003	30.63	32.16	<b>3.06</b>	<b>0.0060</b>
LTA	22.19	25.06	<b>8.39</b>	0.0000	20.26	24.76	<b>23.92</b>	0.0000
TNFSF11	26.61	29.10	<b>6.40</b>	0.0001	27.28	29.61	<b>5.30</b>	0.0001
BMP6	26.37	28.79	<b>6.14</b>	0.0003	26.40	29.28	<b>7.84</b>	0.0000
BMP3	31.45	33.71	<b>5.50</b>	0.0041	35.00	34.71	<b>-1.16</b>	<b>0.1996</b>
FASLG	20.90	23.16	<b>5.46</b>	0.0000	21.54	24.16	<b>6.48</b>	0.0000
TGFB2	28.98	31.23	<b>5.43</b>	0.0000	30.88	33.36	<b>5.91</b>	0.0029
TNFSF14	32.77	35.00	<b>5.37</b>	0.0009	33.51	35.00	<b>2.98</b>	0.0003
TNFSF8	20.16	22.27	<b>4.92</b>	0.0000	19.94	24.17	<b>19.88</b>	0.0000
TNFSF13	29.20	30.38	<b>2.60</b>	0.0000	31.80	26.02	<b>-52.10</b>	0.0000
BMP4	32.11	33.29	<b>2.58</b>	0.0935	28.99	32.54	<b>12.38</b>	0.0003
IL6	18.77	19.88	<b>2.47</b>	0.0002	19.92	22.49	<b>6.29</b>	0.0000
GDF10	33.11	34.08	<b>2.23</b>	0.1166	32.95	29.13	<b>-13.30</b>	0.0006
IL20	31.75	32.56	<b>2.00</b>	0.0117	32.27	35.00	<b>7.03</b>	0.0001
IL4	32.00	32.31	<b>1.42</b>	0.3010	33.36	32.22	<b>-2.08</b>	0.0025
TNFSF12	26.05	26.25	<b>1.32</b>	0.0057	29.28	23.84	<b>-41.16</b>	0.0000
IL12A	27.19	27.19	<b>1.14</b>	0.0971	27.18	27.18	<b>1.06</b>	<b>0.3060</b>
IL1F6	30.28	29.72	<b>-1.29</b>	0.2311	33.34	30.17	<b>-8.48</b>	0.0046
IL18	29.14	28.53	<b>-1.33</b>	0.0449	33.32	28.83	<b>-21.26</b>	0.0000
LTB	22.22	21.47	<b>-1.48</b>	0.0120	27.18	20.42	<b>-102.54</b>	0.0000
IL17C	28.78	27.95	<b>-1.55</b>	0.0213	31.86	27.66	<b>-17.31</b>	0.0001
IFNK	29.27	28.40	<b>-1.60</b>	0.0206	29.73	27.14	<b>-5.71</b>	0.0011
IL16	23.52	22.25	<b>-2.11</b>	0.0000	24.75	20.97	<b>-12.91</b>	0.0000
TNFSF4	28.43	26.89	<b>-2.54</b>	0.0002	27.96	25.45	<b>-5.38</b>	0.0000
IL1F9	29.69	28.07	<b>-2.68</b>	0.6977	26.92	22.81	<b>-16.34</b>	0.0000
IL15	29.46	27.55	<b>-3.28</b>	0.0007	28.79	26.32	<b>-5.23</b>	0.0000
IFNB1	31.11	29.07	<b>-3.58</b>	0.0022	34.37	30.03	<b>-19.03</b>	0.0015
BMP8B	29.36	27.25	<b>-3.76</b>	0.0001	31.35	28.51	<b>-6.74</b>	0.0018
IL12B	35.00	32.72	<b>-4.25</b>	0.0132	31.24	29.86	<b>-2.46</b>	0.0049
TGFA	29.29	26.92	<b>-4.49</b>	0.0000	27.96	24.06	<b>-14.06</b>	0.0000
IL1B	18.66	15.64	<b>-7.12</b>	0.0000	20.12	16.46	<b>-11.93</b>	0.0000
IL1F7	34.52	30.84	<b>-11.19</b>	0.0012	35.00	30.85	<b>-16.76</b>	0.0000
IFNA5	33.53	29.65	<b>-12.89</b>	0.0011	31.19	29.13	<b>-3.93</b>	0.0002
IL1A	24.27	20.02	<b>-16.62</b>	0.0000	25.48	23.24	<b>-4.46</b>	0.0000
TNFSF10	26.16	21.70	<b>-19.22</b>	0.0000	25.41	20.73	<b>-24.20</b>	0.0000
TNFSF13B	29.68	24.75	<b>-26.62</b>	0.0001	31.27	22.50	<b>-411.10</b>	0.0001

## Summary:

The RT<sup>2</sup> Profiler PCR Array System is the ideal tool for analyzing the expression of a focused panel of genes. The flexibility, simplicity, and convenience of standard SYBR Green PCR detection methodology make the PCR Array System accessible for routine use in any research laboratory. The correct combination of instrument-specific plate format and master mix matches the PCR Array System with the most popular real-time instrument platforms. The arrays feature a pathway-focused or a customizable gene content design, while demonstrating the sensitivity, specificity, and reproducibility performance expected of real-time PCR. The focused design of this system decreases the amount of time necessary to complete the experiment and facilitates easier and more straightforward data analysis. Using this system, results can be generated with as little as 25 ng or as much as 5 µg of total RNA starting material. The specificity of the system guarantees the amplification of only one gene-specific product in each reaction meaning that the expression level result confidently reflects only the gene of interest. The reproducibility of the system (with intra-lab and inter-lab correlations greater than 0.99 and 0.97, respectively) demonstrates that the same results are obtainable by multiple end-users. As a result, the RT<sup>2</sup> Profiler PCR Array System is indeed ideally suited to allow every laboratory to combine the performance of real-time PCR with the profiling capabilities of a microarray.

## References:

1. Ross JS, Linette GP, Stec J, Clark E, Ayers M, Leschly N, Symmans WF, Hortobagyi GN, Puzstai L. Breast cancer biomarkers and molecular medicine: part II. *Expert Rev Mol Diagn* 2004; 4(2):169-88
2. Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, Pergamenschikov A, Williams CF, Zhu SX, Lee JC, Lashkari D, Shalon D, Brown PO, Botstein D. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci U S A*. 1999; 96(16):9212-7.

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# PCR Array Buyer's Guide

Step 1: Find your pathway in the list below. For complete PCR Array gene lists, see our web site at: [www.SABiosciences.com/ArrayList.php](http://www.SABiosciences.com/ArrayList.php)

Step 2: Determine which PCR Array format fits the instrument in your lab using the Real-Time PCR Systems table.

Step 3: Select your pack sizes and reagents. Place your order by phone, fax, or e-mail:

Phone: 888.503.3187 Fax: 888.465.9859 E-mail: [order@SABiosciences.com](mailto:order@SABiosciences.com)

Pathway / Topic Focus	PCR Array Catalog Number
Angiogenesis	PAXX-024Y
Angiogenic Growth Factors & Angiogenesis Inhibitors	PAXX-072Y
Apoptosis	PAXX-012Y
Atherosclerosis	PAXX-038Y
Breast Cancer and Estrogen Receptor Signaling	PAXX-005Y
cAMP and Calcium Signaling Pathway	PAXX-066Y
Cancer Drug Resistance and Metabolism	PAXX-004Y
Cancer PathwayFinder™	PAXX-033Y
Cell Cycle	PAXX-020Y
Chemokines and Receptors	PAXX-022Y
Common Cytokines	PAXX-021Y
Diabetes	PAXX-023Y
DNA Damage Signaling Pathway	PAXX-029Y
Drug Metabolism	PAXX-002Y
Drug Metabolism: Phase I Enzymes	PAXX-068Y
Drug Transporters	PAXX-070Y
Endothelial Cell Biology	PAXX-015Y
Extracellular Matrix and Adhesion Molecules	PAXX-013Y
Growth Factors	PAXX-041Y
Hypoxia Signaling Pathway	PAXX-032Y
Inflammatory Cytokines and Receptors	PAXX-011Y
Insulin Signaling Pathway	PAXX-030Y
Interferons (IFN) and Receptors	PAXX-064Y
JAK / STAT Signaling Pathway	PAXX-039Y
MAP Kinase Signaling Pathway	PAXX-061Y
Neuroscience Ion Channels and Transporters	PAXX-036Y
Neurotransmitter Receptors and Regulators	PAXX-060Y
Neurotrophins and Receptors	PAXX-031Y
NF- $\kappa$ B Signaling Pathway	PAXX-025Y
Nitric Oxide Signaling Pathway	PAXX-062Y
Notch Signaling Pathway	PAXX-059Y
Obesity	PAXX-017Y
Osteogenesis	PAXX-026Y
Oxidative Stress and Antioxidant Defense	PAXX-065Y
p53 Signaling Pathway	PAXX-027Y
Signal Transduction PathwayFinder™	PAXX-014Y
Stem Cell	PAXX-405Y
Stress and Toxicity PathwayFinder™	PAXX-003Y
TGF $\beta$ / BMP Signaling Pathway	PAXX-035Y
Th17 for Autoimmunity and Inflammation	PAXX-073Y
Th1-Th2-Th3	PAXX-034Y
Toll-Like Receptor Signaling Pathway	PAXX-018Y
Tumor Metastasis	PAXX-028Y
Tumor Necrosis Factor (TNF) Ligands and Receptors	PAXX-063Y
Wnt Signaling Pathway	PAXX-043Y
Housekeeping Genes	PAXX-000Y
RT <sup>2</sup> RNA QC PCR Array - quality control plates	PAXX-999Y
Custom Options -	Inquire

"XX" = HS, MM, RN (Human, Mouse, Rat) see web site for availability

## Real-Time PCR Systems

Determine the plate type and master mix that fits your real-time PCR system.

	Instrument Make and Model	PCR Array Plate Format (Cat. No. Y = )	Required Master Mix
Applied Biosystems	ABI 7000	A	PA-012
	ABI 7300	A	
	ABI 7500 Standard Block	A	
	ABI 7500 FAST Block	C	
	ABI 7900HT Standard 96 Block	A	
	ABI 7900HT FAST 96 Block	C	
	ABI 7900HT 384-well Block	E	
	ABI 5700 (Perkin Elmer)	A	
ABI 7700 (Perkin Elmer)	A		
Bio-Rad	iCycler, iQ5	A	PA-011
	iQ5	A	
	MyiQ	A	
	PA-010	Chromo 4 (MJ Research)	A
		Opticon (2) (MJ Research)	D
Stratagene	Mx3005p	A	PA-012
	Mx3000p	A	
	Mx4000	D	
Roche	LightCycler 480 96 Block	F	PA-010
	LightCycler 480 384 Block	G	
Eppendorf	Mastercycler ep realplex	A	Inquire

## Pack Sizes and Required Reagents

Volume discounts are built into the PCR Array 12-pack and 24-pack sizes.

PCR Array Pack Sizes
Two (2) 96-well PCR Arrays
Twelve (12) 96-well PCR Arrays
Twenty-Four (24) 96-well PCR Arrays
Four (4) 384-well PCR Arrays (Format "E")

RT<sup>2</sup> SYBR Green qPCR Master Mixes are required for use with PCR Arrays.

Master Mix Pack Sizes (Number of 96-well PCR Arrays Accommodated)	Catalog Number
Two-Pack (2)	PA-01#
Twelve-Pack (12)	PA-01#-12
Twenty-Four Pack (24)	PA-01#-24

The RT<sup>2</sup> First Strand Kit is required for use with PCR Arrays.

For best results, we also recommend the RT<sup>2</sup> qPCR-Grade RNA Isolation Kit.

<b>RT<sup>2</sup> First Strand Kit</b> (Cat. No. C-03, enough for 12 reactions)
<b>RT<sup>2</sup> qPCR-Grade RNA Isolation Kit</b> (Cat. No. PA-001, enough for 12 RNA isolations)