# Gene and miRNA qPCR Arrays



## Gene qPCR Arrays

Cancer Gene qPCR Arrays Pathway Gene qPCR Arrays Custom Gene qPCR Arrays Supporting Reagents

### miRNA qPCR Arrays

Cancer miRNA qPCR Arrays
Focus-Group miRNA qPCR Arrays
miRNome qPCR Arrays
Custom miRNA qPCR Arrays
Supporting Reagents

Custom qPCR Arrays and Services

Data Analysis Tool



# Introduction

## **QPCR Array System**

GeneCopoeia qPCR arrays are a reliable yet easy-to-use tool to study gene expression profiling using SYBR® Green-based real-time PCR technology. The 96- or 384-well arrays are designed for profiling a panel of genes or miRNAs related to a specific disease or pathway in various tissues or cells. The resulting differential expressions help researchers to identify those that are biologically significant and relevant to their research.

qPCR arrays can also be used for your microarray and NGS data validation, biomarker discovery, and gene function study verification.

### Why GeneCopoeia qPCR Array System?

#### **Validated Primers**

Each primer is designed using a proprietary algorithm and has been experimentally validated

#### **Robust Performance**

Stringent QC ensures high quality, specificity, and sensitivity (detects as low as 4 copies of RNA)

#### **Research Focused**

Disease or pathway-focused, or custom-made with your own genes or miRNAs of interest

Category	Product	Description
Gene qPCR Arrays	ExProfile™ cancer gene qPCR arrays	Cancer related 21 different cancer types available
	ExProfile™ pathway-focused gene qPCR arrays	Disease- or other focus-group related
	Supporting reagents	RNAzol <sup>®</sup> RT RNA isolation kit All-in-One <sup>™</sup> first-strand cDNA synthesis kit All-in-One <sup>™</sup> qPCR mix All-in-One <sup>™</sup> qPCR validated primers
miRNA qPCR Arrays	miProfile™ miRNome qPCR arrays	Human: Covering 1,700+ miRNAs based on miRBase v18 Mouse: Covering 800+ miRNAs based on miRBase v18
	miProfile™ cancer miRNA qPCR arrays	Cancer related Human: 15 cancer types available Mouse: 11 cancer types available
	miProfile™ disease and focusgroup miRNA qPCR arrays	Disease- or other focus-group related
	Supporting reagents	RNAzol® RT RNA isolation kit  All-in-One™ qPCR mix  All-in-One™ miRNA qRT-PCR detection kit  All-in-One™ miRNA first-Strand cDNA synthesis kit  All-in-One™ miRNA qPCR validated primers
Custom PCR Arrays & Services	Gene or miRNA custom qPCR arrays and services	96-well format (6 layouts) and 384-well format (8 layouts) Capable of in-house array processing and data analysis
Data Analysis Tool	Online data analysis tool	Free and easy-to-use

## **■■** ExProfile Mene qPCR Arrays

The qPCR array system allows researchers to amplify 96- or 384-well gene-specific products under a uniform cycling condition. In each 96-well plate, there are up to 84 pairs of qPCR primers and 12 wells of controls which are used to monitor the efficiency of the entire experimental process – from reverse transcription to qPCR reaction.

### Gene qPCR Array Layout (96-Well)

	1	2	3	4	5	6	7	8	9	10	11	12
Α	1	2	3	4	5	6	7	8	9	10	11	12
В	13	14	15	16	17	18	19	20	21	22	23	24
С	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
Е	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
н	GDC	GDC	HK1	HK2	нкз	HK4	HK5	HK6	RT	RT	PCR	PCR

- Wells 1-84: Pathway or disease related genes
- HK1-6: Housekeeping genes as endogenous positive controls as well as for array normalization
- **GDC**: Genomic DNA controls to detect genomic DNA contamination
- RT: Spike-in reverse transcription controls to monitor the efficiency of the RT reaction
- PCR: Positive PCR controls to verify the PCR efficiency

#### **How qPCR Array Works**

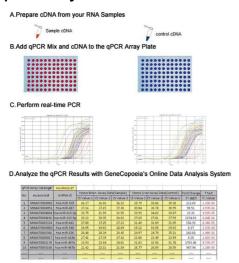


Figure 1. qPCR array experimental work flow

- A. Convert total RNA into cDNA with All-in-One™ First-Strand cDNA Synthesis Kit
- B. Mix cDNA template and All-in-One™ qPCR Mix and aliquot the mixture across the PCR Array
- C. Run the array plate in real-time PCR instrument (Bio-Rad, or Applied Biosystem, or Roche)
- D. Analyze qPCR array data using GeneCopoeia's free online data analysis tool

#### **Performance Data**

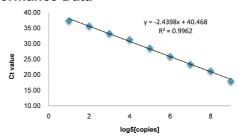


Figure 2. Broad linear range and high sensitivity

Mouse total RNA with serially diluted spike-in control RNA were reverse-transcribed using All-in-One™ First Strand cDNA Synthesis Kit. The reverse-transcribed cDNA samples were detected using All-in-One™ qPCR Mix and spike-in control specific primers deposited in a 96-well plate. The resulting Ct values were plotted against the log5 of the amount of spike-in control RNA. The data demonstrated a broad linear dynamic range from 4 to 1.6\*10<sup>6</sup> copies of input RNA as well as high sensitivity.

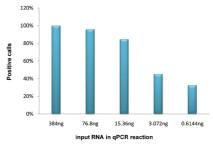


Figure 3. High positive calls with as little as 15.36 ng of total RNA Different amounts of MCF\_7 total RNA (1000, 200, 40, 8, 1.6ng) were analyzed with the Human Breast Cancer Gene qPCR Arrays (PAG-HGBE96-01). The percentage of positive calls (Ct < 35) is plotted against the input amount of total RNA in each qPCR reaction.

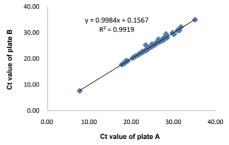


Figure 4. High inter-array reproducibility

Two ExProfile™ qPCR gene array replicates (plate A and B) were analyzed using human total RNA (10-tissue mix) on the Bio-Rad iQ5. The Ct values of the replicate plates were plotted against each other.  $R^2 > 0.99$  was observed for high inter-array reproducibility.  $R^2 > 0.99$  was also observed for intra-array reproducibility (data not shown).

# Gene qPCR Arrays

## **■■** ExProfile Cancer Gene qPCR Arrays

ExProfile™ Cancer Gene qPCR Arrays profile the expression of cancer-related genes, which are carefully chosen for their close cancer correlation based on a thorough literature search of peer-reviewed publications. Twenty-one different cancer types are available for human.

Catalog #	Product Name	# of Genes	# of Plates
PAG-HCAD96	Exprofile™ Human Adenocarcinoma Gene qPCR Array	168	2
PAG-HCBA96	Exprofile™ Human Brain Cancer Gene qPCR Array	252	3
PAG-HCBE96	Exprofile™ Human Breast Cancer Gene qPCR Array	504	6
PAG-HCBL96	Exprofile™ Human Bladder Cancer Gene qPCR Array	420	5
PAG-HCCR96	Exprofile™ Human Colorectal Cancer Gene qPCR Arrays	336	4
PAG-HCCV96	Exprofile™ Human Cervical Cancer Gene qPCR Arrays	84	1
PAG-HCED96	Exprofile™ Human Endometrial Cancer Gene qPCR Arrays	82	1
PAG-HCHN96	Exprofile™ Human Head and Neck Cancer Gene qPCR Arrays	504	6
PAG-HCKD96	Exprofile™ Human Kidney Cancer Gene qPCR Arrays	84	1
PAG-HCLK96	Exprofile™ Human Leukemia Gene qPCR Arrays	504	6
PAG-HCLU96	Exprofile™ Human Lung Cancer Gene qPCR Arrays	504	6
PAG-HCLV96	Exprofile™ Human Liver Cancer Gene qPCR Arrays	168	2
PAG-HCLY96	Exprofile™ Human Lymphoma Gene qPCR Arrays	420	5
PAG-HCML96	Exprofile™ Human Myeloma Gene qPCR Arrays	84	1
PAG-HCOV96	Exprofile™ Human Ovarian Cancer Gene qPCR Arrays	336	4
PAG-HCPC96	Exprofile™ Human Pancreatic Cancer Gene qPCR Arrays	168	2
PAG-HCPS96	Exprofile™ Human Prostate Cancer Gene qPCR Arrays	412	5
PAG-HCSK96	Exprofile™ Human Skin Cancer Gene qPCR Arrays	252	3
PAG-HCSM96	Exprofile™ Human Stomach Cancer Gene qPCR Arrays	168	2
PAG-HCTR96	Exprofile™ Human Thyroid Cancer Gene qPCR Arrays	84	1
PAG-HCTT96	Exprofile™ Human Testicular Cancer Gene qPCR Arrays	68	1

## **■■** ExProfile **■** Pathway Gene qPCR Arrays

GeneCopoeia offers a comprehensive list of pathway and disease-focused gene qPCR arrays for your research of interest. While the arrays are in the development stage, you are welcome to call us and tell us what pathways you are interested in, so we can develop the most suitable products for you.

# **III** ExProfile™ Custom Gene qPCR Arrays

GeneCopoeia can also make custom gene qPCR arrays for your own gene list. Simply provide us with a list of gene accession numbers and instrument model, and we will help you to build custom qPCR arrays for your specific instrument type.

# **Gene qPCR Supporting Reagents**

## **Gene qPCR Array Reagents and Validated Primers**

### RNAzol® RT RNA Isolation Reagent

RNAzol® RT RNA Isolation Kit is the most effective reagent for isolation of total RNA and small RNA from human, animal, plant, bacterial and viral origin. (Catalog#: E01010A)

#### All-in-One™ First-Strand cDNA Synthesis Kit

The All-in-One™ First-Strand cDNA Synthesis Kit includes a reverse transcriptase and a specialized set of reagents designed to yield cDNA that is optimal for gene cloning, cDNA library creation and quantitative PCR amplification.

Catalog #	Product
AORT-0020	All-in-One™ First-Strand cDNA Synthesis Kit (20 reactions)
AORT-0060	All-in-One™ First-Strand cDNA Synthesis Kit (60 reactions)

#### All-in-One™ qPCR Mix

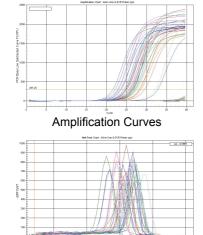
The All-in-One™ qPCR Mix uses high-fidelity hot-start polymerase, an optimized reaction buffer and high-quality dNTPs to enable specific and sensitive amplification from even low-copy RNA (cDNA) or DNA samples.

Catalog#	Product
AOPR-0200	All-in-One™ qPCR Mix (200 qPCR reactions)
AOPR-0600	All-in-One™ qPCR Mix (600 qPCR reactions)
AOPR-1000	All-in-One™ qPCR Mix (1000 qPCR reactions)
AOPR-4000	All-in-One™ qPCR Mix (4000 qPCR reactions)

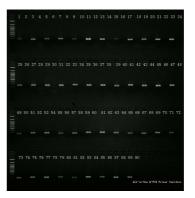
#### All-in-One™ qPCR Validated Primers

Experimentally validated gene-specific primers for human, mouse and rat

Catalog#	Product
Variable	All-in-One™ qPCR Primer(20 µl x 500 reactions)



Melting Curves



**Figure 5.** Forty-five pairs of gene-specific All-in-One™ qPCR primers were experimentally validated to yield a single dissociation curve peak and to generate a single amplification of the correct size for the targeted genes. A cDNA pool containing reverse transcribed products of total RNA from 10 different human tissues (lung, liver, testicle, ovary, spleen, brain, placenta, pancreas, heart and mammary glands), was used as the qPCR validation template.

# miRNA qPCR Arrays

## miProfile™ miRNA qPCR Array System

The miProfile™ miRNA qPCR Arrays are designed for profiling the expression of pre-defined or customized sets of miRNAs in various tissues or cells of interest to discover the miRNAs that are specifically important to your research. Each 96-well plate contains up to 84 pairs of PCR primers (forward: miRNA-specific primer; reverse: universal primer), which are pre-deposited in each well.

### Available miRNA qPCR Array Products

- miRNome qPCR Arrays
- Cancer miRNA qPCR Arrays
- Disease and Focus-Group miRNA qPCR Arrays
- Custom miRNA gPCR Arrays

#### **Required Reagents**

- RNAzol® RT RNA Isolation Reagent
- All-in-One™ miRNA First-Strand cDNA Synthesis Kit
- All-in-One™ miRNA qRT-PCR Detection Kit
- All-in-One™ qPCR Mix

### **Advantages**

#### Genome-wide or focused coverage

- · Largest genome-wide miRNA coverage
- Cancer-related groups
- Customized miRNA arrays for focused study

#### Validated miRNA primers

 Each miRNA primer is designed using a proprietary algorithm and experimentally validated

#### Robust performance

- Sensitive Detect miRNA from as little as 10 pg of small RNA or 20 pg of total RNA
- Specific Be able to distinguish miRNAs with single nucleotide mismatches.
- Broad linearity

   Allow miRNAs at a variety of expression levels to be detected simultaneously
- Reproducible High reproducibility (R<sup>2</sup>>0.99) for interarray and intra-array replicates

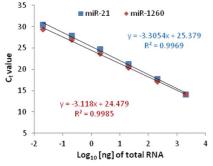


Figure 6. Broad linear range and high sensitivity Starting with a serially diluted amount of human colon cancer total RNA, miR-21 and miR-1260 were detected using All-in-One™ miRNA qRT-PCR Detection Kit. The resulting Ct values were plotted against the log10 of the amount of input total RNA. The data demonstrated a broad linear dynamic range from 20 pg to 2 µg of input total RNA as well as high sensitivity. This allows a variety of expression levels of miRNAs, including low abundant ones, to be detected simultaneously.

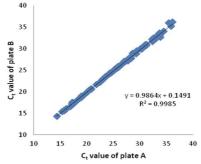


Figure 7. High inter-array reproducibility Two miProfile™ qPCR array replicates (plate A and B) were analyzed using human total RNA (10-tissue mix) on the Bio-Rad iQ5. The Ct values of the replicate plates were plotted against each other. R² > 0.99 were observed for high interarray reproducibility. R² > 0.99 is also observed for intra-array reproducibility (data not shown)



Figure 8. Specificity of miRNA detection miRNA miR-29a and miR-29c with one single nucleotide mismatch (B) can be distinguished. Relative detection, defined as a percentage of the perfect match (100% x 2 - $\triangle$ Ct), was calculated using the Ct values of on-target and off-target assays, which were performed to detect miRNA plasmid DNA templates using All-in-One miRNA qRT-PCR Detection Kits (A).

# miRNA qPCR Arrays

# **I** miProfile™ Cancer miRNA qPCR Arrays

miRNAs are known for their extensive involvement in cancer development and progression, and their altered expression patterns that may affect the cell cycle and survival program. The miProfile™ Cancer miRNA qPCR Arrays allow researchers to profile the differential expression of cancer-related miRNAs in order to study the role of miRNA in cancer pathogenesis, and identify markers for cancer classification, diagnosis, and prognosis.

Catalog#	Product Name	# of miRNAs	# of Plates
PAM-HC96	Human cancer miRNA qPCR arrays	420	5
PAM-HCN96	Human brain cancer miRNA qPCR arrays	84	1
PAM-HCB96	Human breast cancer miRNA qPCR arrays	168	2
PAM-HCX96	Human leukemia miRNA qPCR arrays	168	2
PAM-HCL96	Human lung cancer miRNA qPCR arrays	168	2
PAM-HCO96	Human ovarian cancer miRNA qPCR arrays	168	2
PAM-HCY96	Human bladder cancer miRNA qPCR arrays	79	1
PAM-HCC96	Human colorectal cancer miRNA qPCR arrays	84	1
PAM-HCE96	Human endometrial cancer miRNA qPCR arrays	84	1
PAM-HCG96	Human gastric cancer miRNA qPCR arrays	80	1
PAM-HCH96	Human hepatocellular carcinoma miRNA qPCR arrays	168	2
PAM-HCZ96	Human lymphoma miRNA qPCR arrays	84	1
PAM-HCM96	Human melanoma miRNA qPCR arrays	84	1
PAM-HCT96	Human head and neck cancer miRNA qPCR arrays	84	1
PAM-HCP96	Human pancreatic cancer miRNA qPCR arrays	84	1
PAM-HCQ96	Human prostate cancer miRNA qPCR arrays	84	1
PAM-MCB96	Mouse breast cancer miRNA qPCR arrays	84	1
PAM-MCN96	Mouse brain cancer miRNA qPCR arrays	84	1
PAM-MCO96	Mouse ovarian cancer miRNA qPCR arrays	84	1
PAM-MCQ96	Mouse prostate cancer miRNA qPCR arrays	84	1
PAM-MCC96	Mouse colorectal cancer miRNA qPCR arrays	84	1
PAM-MCH96	Mouse hepatocellular carcinoma miRNA qPCR arrays	84	1
PAM-MCL96	Mouse lung cancer miRNA qPCR arrays	84	1
PAM-MCM96	Mouse melanoma miRNA qPCR arrays	84	1
PAM-MCP96	Mouse pancreatic cancer miRNA qPCR arrays	84	1
PAM-MCT96	Mouse head and neck cancer miRNA qPCR arrays	84	1
PAM-MCX96	Mouse leukemia cancer miRNA qPCR arrays	84	1

# miRNA qPCR Arrays

## **III** miProfile™ Disease and Focus-Group miRNA qPCR Arrays

These arrays profile the expression of disease-related or other focus-group miRNAs, which are carefully chosen for their close disease, pathway, or process correlation, allowing researchers to study the differential expression of these miRNAs to gain understanding of the role of miRNA in disease pathogenesis or cellular pathways as well as to identify or validate key markers.

Catalog#	Product Name	Species	# of Genes	# of Plates
PAM-HF96	Human inflammatory miRNA qPCR arrays	Human	84	1
PAM-HH96	Human heart disease miRNA qPCR arrays	Human	84	1
PAM-HI96	Human immunopathology miRNA qPCR arrays	Human	84	1
PAM-HK96	Human iPS cells miRNA qPCR arrays	Human	168	2
PAM-HM96	Human muscle disease miRNA qPCR arrays	Human	84	1
PAM-HT96	Human toxicology related miRNA qPCR arrays	Human	84	1
PAM-HX96	Human serum and plasma miRNA qPCR arrays	Human	168	2
PAM-MF96	Mouse inflammatory miRNA qPCR arrays	Mouse	84	1
PAM-MH96	Mouse heart disease miRNA qPCR arrays	Mouse	84	1
PAM-MI96	Mouse immunopathology miRNA qPCR arrays	Mouse	84	1
PAM-MX96	Mouse serum and plasma miRNA qPCR arrays	Mouse	84	1

# **III** miProfile™ miRNome qPCR Arrays

The miProfile™ Human miRNome qPCR Arrays are a set of twenty-one 96-well plates covering 1,700 of the best characterized and annotated miRNAs based on miRBase v18. Arrays are also available for mouse covering 834 annotated miRNAs.

The miProfile™ Human Single-Nucleotide Mismatch miRNA qPCR Arrays are available as stand-alone products for users who want to study these miRNAs using specific PCR conditions.

Catalog#	Product Name	# of miRNAs	# of Plates
PAM-HG96	Human miRNome miRNA qPCR arrays	1,700 (miRBase v18)	21 x 96-well
PAM-HS96	Human single-nucleotide mismatch miRNA qPCR arrays	61	1 x 96-well
PAM-HG384	Human miRNome miRNA qPCR arrays	1,700 (miRBase v18)	5 x 384-well
PAM-MG96	Mouse miRNome miRNA qPCR arrays	834 (miRBase v18)	10 x 96-well
PAM-MG384	Mouse miRNome miRNA qPCR arrays	834 (miRBase v18)	3 x 384-well

# miProfile™ miRNA qPCR Array Custom Services

GeneCopoeia can also make custom miRNA qPCR arrays for the miRNAs of your interest. Simply provide us with a list of miRNAs and your instrument model, and we will help you to build custom qPCR arrays for your specific instrument type.

# miRNA qPCR Supporting Reagents

## miRNA qRT-PCR Reagent Kits and Validated Primers

### RNAzol® RT RNA Isolation Reagent

RNAzol<sup>®</sup> RT RNA Isolation Kit is the most effective reagent for isolation of total RNA and small RNA from human, animal, plant, bacterial and viral origin. (Catalog#: E01010A)

#### All-in-One™ qPCR Mix

The All-in-One™ qPCR Mix uses high-fidelity hot-start polymerase, an optimized reaction buffer and high-quality dNTPs to enable specific and sensitive amplification from even low-copy RNA (cDNA) or DNA samples.

(Catalog#: AOPR-0200, AOPR-0600, AOPR-1000, AOPR-4000)

#### All-in-One™ miRNA First-Strand cDNA Synthesis Kit

The All-in-One™ First-Strand cDNA Synthesis Kit is optimized to work with miProfile™ miRNA qPCR arrays to generate reliable end results.

(Catalog#: AMRT-0020, AMRT-0060)

#### All-in-One™ miRNA gRT-PCR Detection Kit

The All-in-One™ RT-PCR Detection Kit includes both RT and PCR reagents. It combines PCR technology and SYBR® Green-based detection to make fast and accurate quantification of mature miRNAs from as little as 10 pg of small RNA or 20 pg of total RNA samples. The kit is designed for All-in-One™ miRNA qPCR validated primers.

(Catalog#: AOMD-Q020, AOMD-Q060)

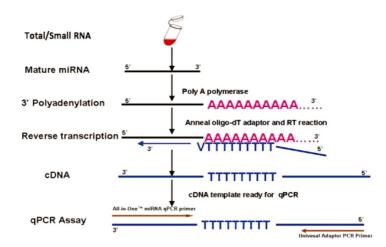


Figure 9. Overview of steps involved in the All-in-One™ miRNA qRT-PCR Detection Kit. After 3' polyadenylation, M-MLV RTase in conjuction with a unique oligo dT adaptor primer reverse transcribes the poly A miRNAs. The qRT-PCR mix containing SYBR® Green specifically detects the reverse transcribed miRNAs.

#### All-in-One™ miRNA qPCR Validated Primers

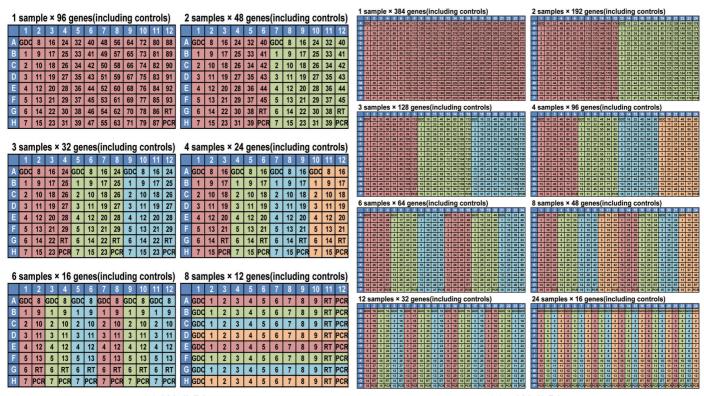
The All-in-One<sup>™</sup> qPCR miRNA-specific primers are designed using a proprietary algorithm and experimentally validated. When used in combination with the All-in-One<sup>™</sup> miRNA qRT-PCR Reagent Kits, the All-in-One<sup>™</sup> miRNA primers generate reliable and reproducible high performance in quantitative PCR assays.

# **Custom qPCR Array Services**

## Custom Gene and miRNA qPCR Array Services

GeneCopoeia can also make custom gene qPCR arrays or custom miRNA qPCR arrays based on your own choice of genes or miRNAs. 96-well and 384-well formats are available for your specific instrument type. Different layouts are available for each format based on the number of genes or miRNAs you are interested in. Positive PCR controls and spike-in reverse transcription controls are required in custom arrays for QC purpose to ensure the RT and PCR reaction efficiency.

#### **Custom Array Layouts**



96-Well Plate 384-Well Plate

#### **How to Order Custom qPCR Arrays**

- 1. Provide your own gene list (gene symbol and accession number) or miRNA list
- 2. Choose array layout, housekeeping genes, and control genes
- 3. Determine the number and type of plates
- 4. Summit your custom array request form and contact GeneCopoeia technical support at 866-360-9531 or inquiry@genecopoeia.com for a quote

#### **Custom Array Processing and Data Analysis**

Do not have a real-time PCR instrument?

- 1. Send your samples to GeneCopoeia (RNA, cell, tissue)
- 2. GeneCopoeia's experienced scientists will process the samples and run the qPCR array experiments
- 3. Thorough data analysis results will be emailed to you

## **QPCR Array Data Analysis Tool**

The qPCR array data analysis tool performs  $\Delta\Delta$ Ct based fold change calculations from your uploaded raw threshold cycle data to the excel data analysis file. Results are automatically analyzed in multiple formats and annotated to your gene list.

$\begin{array}{c} \text{AVG} \ \Delta C_t \\ (\text{Ct}(\text{GOI}) \text{ - Ave Ct} \\ (\text{HKG})) \end{array}$		2^-ΔC <sub>t</sub>		Fold Difference	T-TEST	Fold Up- or Down-Regulation	Comments
Test Sample	Control Sample	Test Sample	Control Sample	Test Sample /Control Sample	p value	Test Sample /Control Sample	
1.12	1.35	4.6E-01	3.9E-01	1.18	0.184963	1.18	OKAY
8.65	9.34	2.5E-03	1.5E-03	1.62	0.035272	1.62	OKAY
4.41	6.77	4.7E-02	9.2E-03	5.14	0.000004	5.14	Type 1
8.20	8.02	3.4E-03	3.9E-03	0.88	0.394106	-1.14	Type 2
0.70	2.01	6.2E-01	2.5E-01	2.48	0.000000	2.48	OKAY
4.74	5.74	3.7E-02	1.9E-02	2.00	0.000600	2.00	Type 1
4.31	3.97	5.0E-02	6.4E-02	0.79	0.146488	-1.26	OKAY
5.77	9.35	1.8E-02	1.5E-03	12.03	0.000059	12.03	Type 1
4.82	7.54	3.5E-02	5.4E-03	6.58	0.000040	6.58	Type 1
2.62	7.55	1.6E-01	5.3E-03	30.45	0.000011	30.45	Type 1
9.95	9.77	1.0E-03	1.1E-03	0.88	0.358349	-1.13	Type 2
0.94	2.05	5.2E-01	2.4E-01	2.16	0.000056	2.16	OKAY
3.77	6.05	7.4E-02	1.5E-02	4.87	0.000253	4.87	Type 1
5.31	1.32	2.5E-02	4.0E-01	0.06	0.052092	-15.91	OKAY

#### **Fold Change**

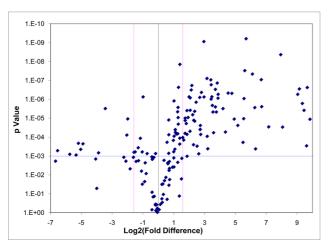
The fold change is calculated using the normalized gene expression  $(2^{-4} - \Delta Ct)$  of the test sample divided by the normalized gene expression  $(2^{-4} - \Delta Ct)$  of the control sample.

#### Fold Up- or Down-Regulation

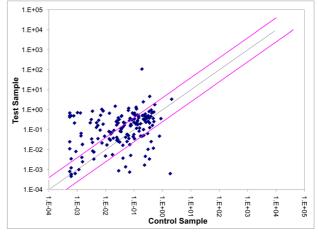
If the fold change = 1, it means there is no up- or down-regulation.

If the fold change > 1, it indicates upregulation.

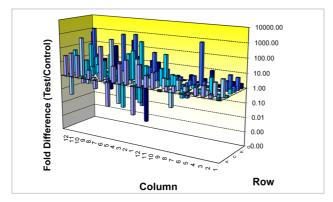
If the fold change < 1, it indicates down-regulation.



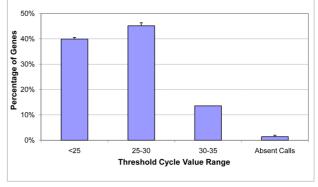
**Figure 10.** Volcano Plot -the black line indicates a fold change in gene expression of 1. The pink lines indicate the desired fold change in gene expression threshold as defined by the user.



**Figure 11.** Scatter Plot -the black line indicates fold changes  $((2 \land (-\Delta Ct)))$  of 1. The pink lines indicate the desired fold change in gene expression threshold defined by the user.



**Figure 12.** A 3-D profile showing up- and down-regulation results.



**Figure 13.** The percentage of genes that generate a certain Ct value. If Ct > 35, the gene is considered absent.



## GeneCopoeia Headquarters

9620 Medical Center Drive, Suite 101 Rockville, MD 20850, USA

Email inquiry@genecopoeia.com

Tel +1 (301) 762-0888 Toll free +1 (866) 360-9531 Fax +1 (301) 762-3888 Website www.genecopoeia.com

## Fulengen Co., Ltd.

8F, Building D

Guangzhou International Business Incubator Guangzhou Science Park Guangzhou, China 510663

Email sales@fulengen.com Tel +86 (20) 3205-2376 Fax +86 (20) 3205-2877 Website www.fulengen.com

### **Distributors**

Australia United Bioresearch Products Pty Ltd www.unitedbioresearch.com.au

Brazil Ambriex, S/A Japan Cosmo Bio Co., Ltd.

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