QIAGEN

Technical Guide to QIAGEN PCR Arrays

RT² Profiler PCR Arrays
RT² lncRNA PCR Arrays
miScript PCR Arrays

Sample to Insight

Total RNA discovery with RT² and miScript PCR Arrays

Explore the RNA universe

Whatever your destination within the RNA universe, QIAGEN will help you get there. The miRNeasy kits deliver pure, high-quality total RNA from a broad range of samples. The RT² and miScript PCR arrays are a complete solution both for focused analysis of gene and microRNA expression and for validation of microarray and RNA sequencing experiments. Together with the powerful analytics tools of GeneGlobe® and QIAGEN Ingenuity® Pathway Analysis, these products give you a smooth path from your sample to high-quality results.

From one sample – the whole range of RNA results

With our workflow (Figure 1), you can take any single sample and discover the secrets of the messenger RNA (mRNA), long non-coding RNA (lncRNA) and microRNA (miRNA). Whether you're studying serum or plasma samples, FFPE tissues, cultured cell lines or exosomes, the kits are optimized to provide excellent results – even with limited amounts of sample.

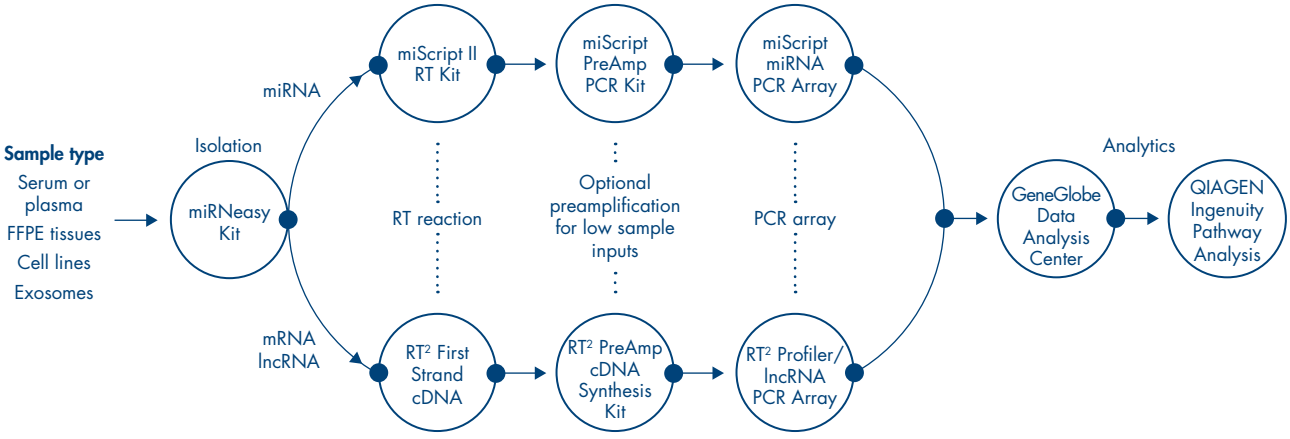


Figure 1. From any sample to pathway insights – the total RNA workflow from QIAGEN.

Laboratory-validated real-time PCR-based RNA quantification and verification

Real-time PCR provides flexibility and speed for time-critical assays. It is universally recognized as the best means of quantifying and verifying the contents of both annotated and novel mRNA, miRNA and lncRNA. Its success relies on having the right technology to give:

- Sensitivity – assays designed to efficiently detect and quantify even the rarest target
- Specificity – on-target amplification from each single assay to the full array
- Dynamic range – reliable with large or small input volumes, robust or rare targets, one assay or thousands

QIAGEN is a world-renowned supplier of sensitive, specific and dynamic, laboratory-verified PCR-based solutions that address all the requirements of RNA profiling work.

Start the easy way – with miRNeasy and exoRNeasy

Get total RNA from every sample

Our miRNeasy Kits isolate pure, high-quality total RNA, including mRNA, miRNA and lncRNA, from cells, easy- and difficult-to-lyse tissues, FFPE samples and all biofluids including serum, plasma, CSF, urine and cell culture media.

These kits enable efficient enrichment of RNA down to approximately 18 nt in size, even when low amounts of starting material are used. This versatility and quality is achieved thanks to phenol/guanidine-based lysis of samples followed by silica membrane-based purification (Figure 2).

The power of miRNeasy lysis

The QIAzol® Lysis Reagent included in the kit is a monophasic solution of phenol and guanidine thiocyanate. It is designed to facilitate tissue lysis, inhibit RNases and remove most of the cellular DNA and proteins from the lysate by organic extraction.

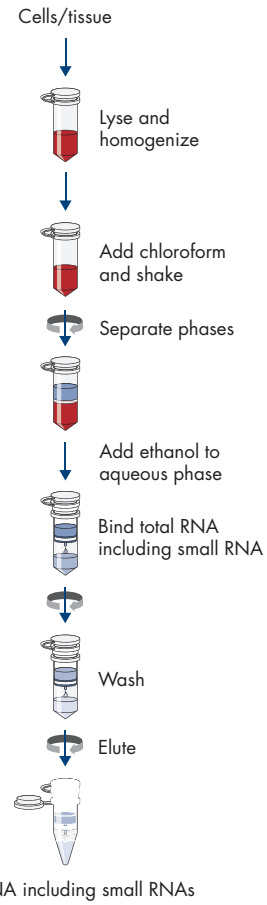


Figure 2. The miRNeasy workflow.

High-purity RNA from FFPE tissues

The crosslinking and fragmentation that occurs during the FFPE process can make purification of nucleic acids challenging. The miRNeasy FFPE Kit provides special lysis and incubation conditions to reverse formalin crosslinking of RNA, giving efficient purification without further degradation. To remove even trace amounts of DNA, which can impair RT-PCR, the RNA is treated with both DNase and DNase Booster Buffer.

Circulating miRNA purification from serum or plasma

With their potential to serve as biomarkers for the detection of cancers and other diseases, circulating miRNA are desirable assay targets. The miRNeasy Serum/Plasma Kit enables total RNA purification from even very small sample volumes. The miRNeasy Serum/Plasma Spike-In Control supports normalization when working with such samples.

What about exosomes?

Exosomes also have considerable potential in the study of cancers and other diseases. Using similar principles of purification of total RNA, we developed the exoRNeasy Serum/Plasma Maxi Kit, which provides microvesicle isolation in just 20 minutes (Figure 3). A subsequent 35-minute isolation procedure yields total RNA from these extracellular vesicles. Just one hour from sample to microvesicle RNA means you can spend less time getting the RNA and more time deciphering what it means.

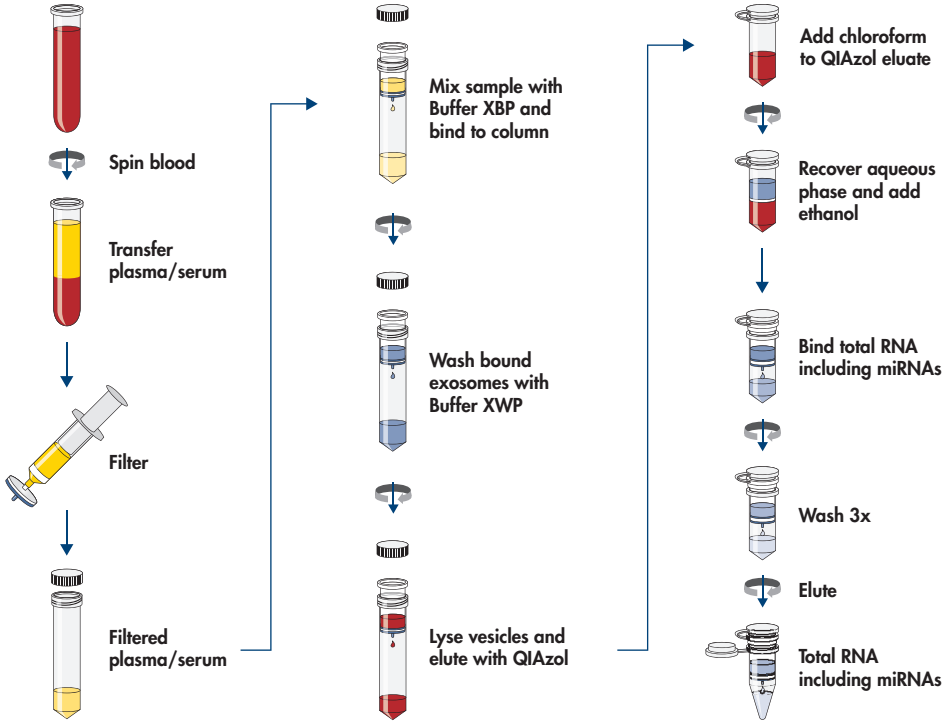


Figure 3. The exoRNeasy Serum/Plasma Maxi Kit workflow.

Profiling coding and noncoding RNAs by pathway & disease

Unlock the secrets of the transcriptome

RT² Profiler and RT² lncRNA PCR Arrays are highly reliable and sensitive technologies for analyzing focused panels of genes to discover their roles in signal transduction, biological processes or disease pathways. They use a straightforward workflow based on RT-PCR (Figure 4) and can identify basal and up- or downregulated expression of genes (Figure 5).

Each PCR Array contains a list of pathway-focused genes along with 5 housekeeping (reference) genes. In addition, they contain a panel of patented controls to monitor genomic DNA contamination, strand cDNA synthesis and real-time PCR efficiency.

Why use RT² Profiler and lncRNA PCR Arrays?

- **Content**: Select from over 180 different pathways, diseases or biological processes and
  perform routine gene expression analysis on any qPCR instrument
- **Control**: The integrated controls allow the comparison of results results from start to finish for consistency
- **Customization**: Easily modify catalog arrays to fit your needs by adding up to 4 genes to make a Modified RT² PCR Array; or use your gene list to make a full Custom RT² PCR Array

From screening canonical pathways to validation of RNA sequencing – there is always the right RT² PCR Array for you!

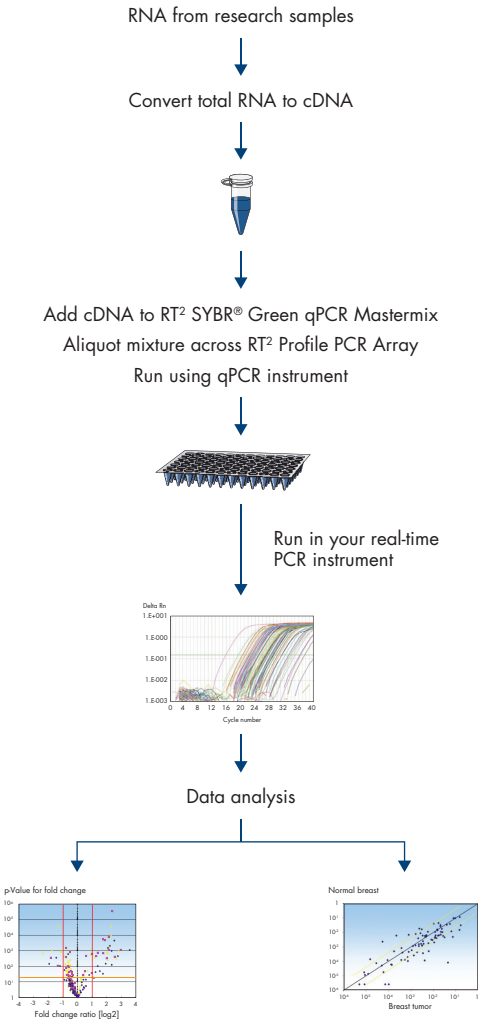


Figure 4. The workflow for the RT² PCR Arrays.

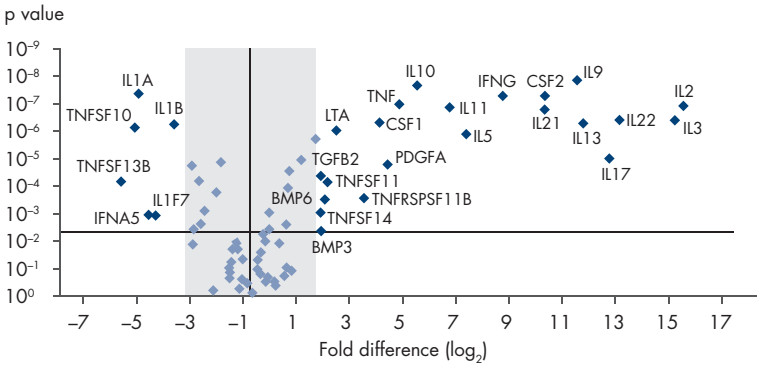


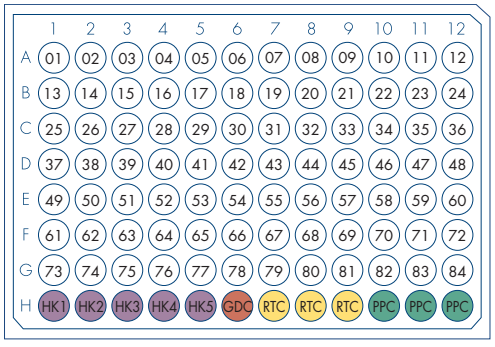
Figure 5. The Common Cytokine RT² Profiler PCR Array identifies 23 upregulated and 6 downregulated genes following PBMC stimulation. Total RNA samples from human peripheral blood mononuclear cells (either untreated or stimulated with 50 ng/ml PMA and 1 mg/ml ionomycin for 6 hours) were characterized in triplicate using the human Common Cytokine RT² Profiler PCR Array. Results show upregulation of 23 genes (>5-fold, p<0.0005) including interleukins, colony-stimulating factors and TNF ligands and downregulation of 6 interleukin and TNF ligand genes.

RT² PCR Array plate layouts and controls

Patented Controls standardize qPCR performance

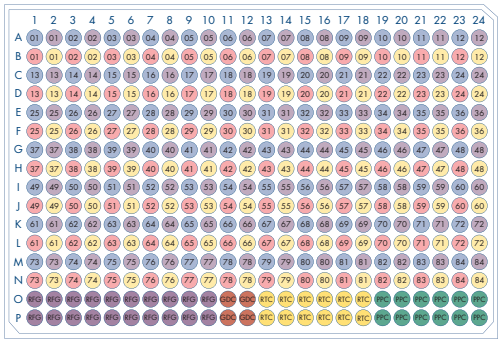
Multiple reference or housekeeping genes (RF or HK) and patented controls (US patent #8,597,938) are a feature of every catalog RT² PCR Array (Figures 6 and 7). The inclusion of multiple reference genes enables researchers to choose the best way to normalize their qPCR experiments.

Figure 6. Key to the controls in the RT² PCR Array plates. The genomic DNA control (GDC) assay is a sensitive assay that detects the presence of genomic DNA. The reverse transcription control (RTC) assay detects an artificial RNA template provided with the RT² First Strand Kit. The positive PCR controls (PPC) monitor for PCR inhibitors. During data analysis, the online software monitors ratios between the PPC and RTC to calculate the reverse transcription efficiency. The software also looks at both variability in a single sample and across samples by monitoring the reproducibility of the RTC and PPC assays.



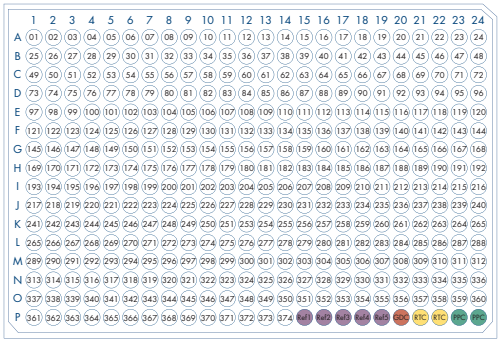
Catalog RT² PCR Array plate layout for 96-well qPCR instruments.

1 sample per plate = 96 qPCR assays per sample



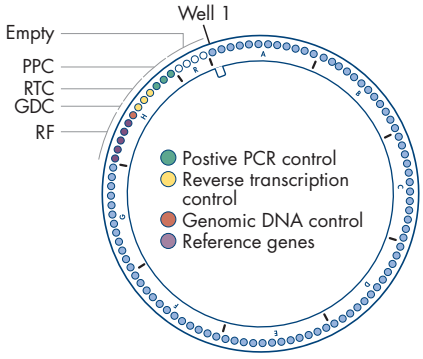
Catalog RT² PCR Array plate layout for 384-well qPCR instruments.

4 samples per plate = 96 qPCR assays per sample



Catalog High-content RT² PCR Array plate layout for 384-well qPCR instruments.

1 sample per plate = 384 qPCR assays per sample



Catalog RT² PCR Array plate layout for 100-well rotary qPCR instruments.

1 sample per plate = 96 qPCR assays per sample

Figure 7. 96- and and 384-well plate and 100-well ring layouts. The key to the controls is in Figure 6.

miRNA expression profiling using miScript miRNA PCR Arrays

miScript miRNA PCR Arrays are mature miRNA-specific forward primers that have been arrayed in miRNome and biologically relevant, pathway-focused panels (Table 1). These PCR arrays are provided in ready-to-use, 96- and 384-well plate and 100-well Rotor-Disc® formats. miScript miRNA PCR Arrays are available for various species, provide guaranteed high performance and are fully customizable. Each array contains controls that allow monitoring of the complete experiment from sample preparation to data analysis, including data normalization controls, reverse transcription controls and PCR controls. Every assay in a miScript miRNA PCR Array has been bench validated to ensure sensitive and specific detection of mature miRNA via real-time PCR.

Table 1. The available miScript miRNA PCR Arrays

Array	Species
Complete miRNome	Human, mouse, rat, dog, rhesus macaque
miFinder	Human, mouse, rat, dog, rhesus macaque
Brain Cancer	Human, mouse, rat
Breast Cancer	Human, mouse, rat
Cancer PathwayFinder	Human, mouse, rat
Cell Differentiation & Development	Human, mouse, rat
Immunopathology	Human, mouse, rat
Inflammatory Response & Autoimmunity	Human, mouse, rat
Neurological Development & Disease	Human, mouse, rat
Ovarian Cancer	Human, mouse, rat
Serum & Plasma	Human, mouse, rat
Custom Array	Human, mouse, rat, dog, rhesus macaque, and other species

A straightforward, rapid workflow

miRNA expression profiling with miScript miRNA PCR Arrays is simple and robust (Figure 8). cDNA preparation with the miScript II RT Kit using miScript HiSpec Bufferis followed by the addition of a premix of cDNA, miScript Universal Primer, QuantiTect® SYBR Green PCR Master Mix and RNase-free water to a miScript miRNA PCR Array. The reaction is run in a real-time PCR cycler and data analysis is performed using the miScript miRNA PCR Array Data Analysis Tool.

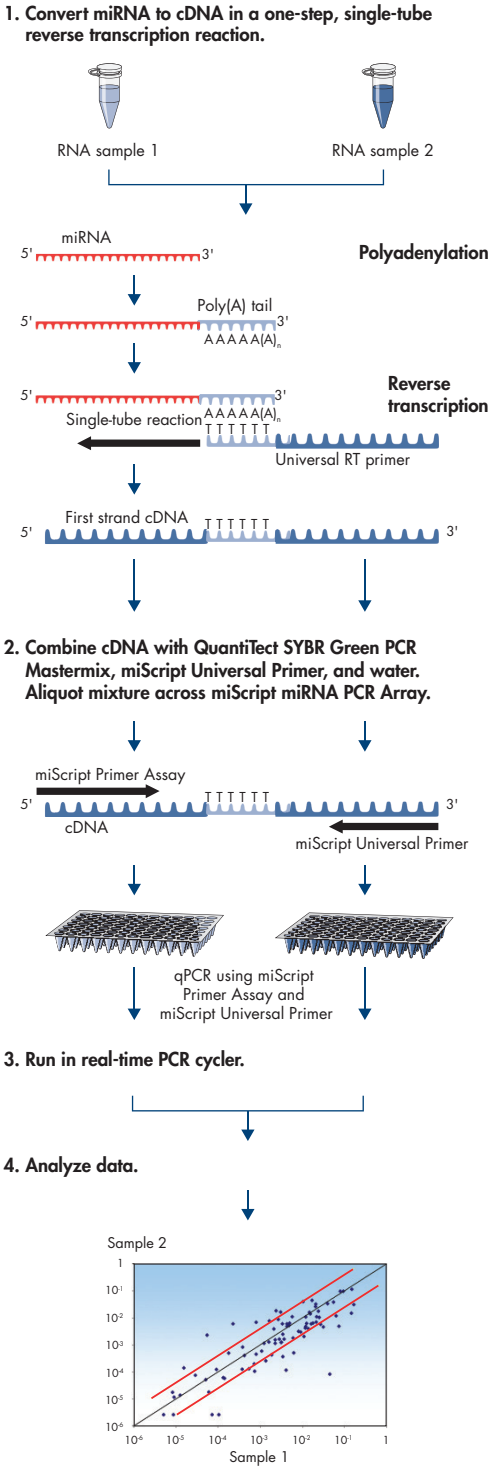


Figure 8. The workflow for the miScript miRNA PCR Arrays.

PCR Arrays by Application

Research area	Apoptosis research	Biomarker research	Cancer research	Cell cycle research
Array type	**Process, pathway or cell type for analysis**			
RT² Profiler PCR Arrays	Apoptosis	Alzheimer's disease	Angiogenesis	Apoptosis
	Autophagy	Angiogenesis	Apoptosis	Autophagy
	Cancer pathways	Breast cancer and estrogen receptor signaling	Breast cancer and estrogen receptor signaling	Cancer pathways
	Cell cycle	Cancer pathways	Cancer drug resistance and metabolism	Cell cycle
	DNA damage signaling pathway	Cell surface markers	Cancer pathways	DNA damage signaling pathway
	DNA repair	Dendritic and antigen presenting cell	Cell cycle	DNA repair
	Endothelial cell biology	Epigenetic chromatin modification enzymes	DNA Damage Signaling Pathway	Epithelial to mesenchymal transition (EMT)
	Heat shock proteins	Epigenetic chromatin remodeling factors	EGF/PDGF signaling pathway	MAP kinase signaling pathway
	NFκB signaling pathway	Epithelial to mesenchymal transition (EMT)	Epithelial to mesenchymal transition (EMT)	mTOR signaling
	Oxidative stress and antioxidant defense	Extracellular matrix and adhesion molecules	MAP kinase signaling pathway	Neurogenesis and neural stem cell
	p53 signaling pathway	Glucose metabolism	p53 Signaling Pathway	NFκB Signaling Pathway
	PI3K-AKT signaling pathway	Hematopoietic stem cells and hematopoiesis	PI3K-AKT signaling pathway	p53 signaling pathway
RT² lncRNA PCR Array	Apoptosis lncRNAs	lncRNA biomarkers	Cancer lncRNAs	Cell cycle regulatory lncRNAs
miScript miRNA PCR Array	Apoptosis	Serum & plasma	Tumor suppressor	Cell cycle regulatory miRNAs
	Common miRNAs	Tumor suppressor	Cancer stem cells	Cell differentiation & development
	Tumor suppressor	Inflammatory response & autoimmunity	Cancer regulatory miRNAs	Tumor suppressor miRNAs
	miRNome miRBase version 21			

Inflammation research	ECM/adhesion research	Neuroscience research	Signal transduction research	Stem cell research	Toxicology/drug ADME research
Process, pathway or cell type for analysis					
Chemokines and receptors	Angiogenic growth factors and angiogenesis inhibitors	Alzheimer's disease	cAMP/Ca^{2+} signaling pathways	Adipogenesis	Cancer drug resistance and metabolism
Common cytokines	Atherosclerosis	Apoptosis	EGF/PDGF Signaling pathway	Dendritic and antigen-presenting cell	Cancer pathways
Inflammasomes	Chemokines and receptors	Autophagy	G protein-coupled receptors	Embryonic stem cells	Cardiotoxicity
Inflammatory cytokines and receptors	Common cytokine	Drug transporters	GPCR signaling pathways	Hedgehog signaling pathway	Cell cycle
Inflammatory response and autoimmunity	Embryonic stem cells	Embryonic stem cells	Heat shock proteins	Hematopoietic stem cells and hematopoiesis	DNA damage signaling pathway
Interferon α, β response	Endothelial cell biology	GPCR signaling pathways	Hedgehog signaling pathway	Homeobox (HOX) genes	Drug metabolism
Interferon and receptor	Extracellular matrix and adhesion molecules	Heat shock proteins	Insulin signaling pathway	Lipoprotein signaling and cholesterol metabolism	Drug metabolism: phase I enzymes
JAK/STAT signaling pathway	Glycosylation	Hedgehog signaling pathway	JAK/STAT signaling pathway	Mesenchymal stem cell	Drug metabolism: phase II enzymes
NFκB signaling pathway	MAP kinase signaling pathway	Huntington's disease	MAP kinase signaling pathway	Neurogenesis and neural stem cell	Drug transporters
T Cell energy and immune tolerance	Mesenchymal stem cell	Hypoxia signaling pathway	mTOR signaling	Neurotrophin and receptors	GPCR signaling pathway
T-cell and B-cell activation	NFκB signaling pathway	Mesenchymal stem cell	NFκB signaling pathway	Notch signaling pathway	Hepatoxicology
TGFβ BMP signaling pathway	Osteogenesis	Neurogenesis and neural stem cell	Nuclear receptors and coregulators	Osteogenesis	Lipoprotein signaling and cholesterol metabolism
Inflammation response lncRNAs	ECM & adhesion lncRNAs	Neuroscience lncRNAs	Signal transduction lncRNAs	Development & differentiation lncRNAs	Common lncRNA biomarkers
T-cell & B-cell activation	Fibrosis	Neurological development & disease	Hypoxia signaling	Stem cells	Serum & plasma
Immunopathology	Cell differentiation & development	Neuropathic & inflammatory pain	Common miRNAs	Cell differentiation & development	Liver miRNAs
Inflammatory response & autoimmunity	Apoptosis	Serum & plasma	Apoptosis	Cancer stem cells	Cardiovascular miRNAs
miRNome miRBase version 21					

Discover biomarkers in serum or plasma

The range of pathway-focused miScript miRNA PCR Arrays is continually expanding to enable new discoveries about the roles of miRNAs in biological processes. Content is selected using our proprietary methodology, which ensures that the arrays are up-to-date and biologically relevant.

One of the most exciting areas of current miRNA research involves the assessment of miRNAs present in serum or plasma samples. The relatively stable, extracellular miRNAs in serum and plasma have great potential as biomarkers for a variety of diseases. Some could find use in liquid biopsies – a minimally invasive way of assessing disease states.

The Serum & Plasma miScript miRNA PCR Array has been developed to enable rapid profiling of the 84 most relevant disease-associated miRNAs in serum and plasma, supporting liquid biopsy research.

miScript miRNA PCR Array layout

Our arrays contain miScript Primer Assays for 84 to 372 miRNAs along with controls for data normalization, reverse transcription and PCR. The formats are shown in Figure 9. The key to the controls is shown in Figure 10 with additional details in Table 2.

Figure 10. Key to the controls for qPCR performance in the miScript miRNA PCR Array plates. Data normalization controls include 6 miScript PCR Controls (SN1–6) and a spike-in control for monitoring microRNA isolation (*C. elegans* miR-39; Ce). The Ce assay detects the Syn-cel-miR-39 miScript miRNA Mimic. The reverse transcription control (miRTC) assay detects an artificial RNA template provided with the arrays. The positive PCR controls (PPC) monitor for PCR inhibitors.

Table 2. The controls for qPCR performance in the miScript miRNA PCR Array plates

Control	Purpose
C. elegans miR-39 miScript Primer Assay (Ce)	Alternative data normalization using exogenously spiked Syn-cel-miR-39 miScript miRNA Mimic
6 snoRNA/snRNA Primer Assays (miScript PCR Controls; SN1–6)	Data normalization using the $\Delta\Delta C_T$ method of relative quantification
miRNA reverse transcription control (miRTC)	Assessment of reverse transcription performance
Positive PCR control (PPC)	Assessment of PCR performance

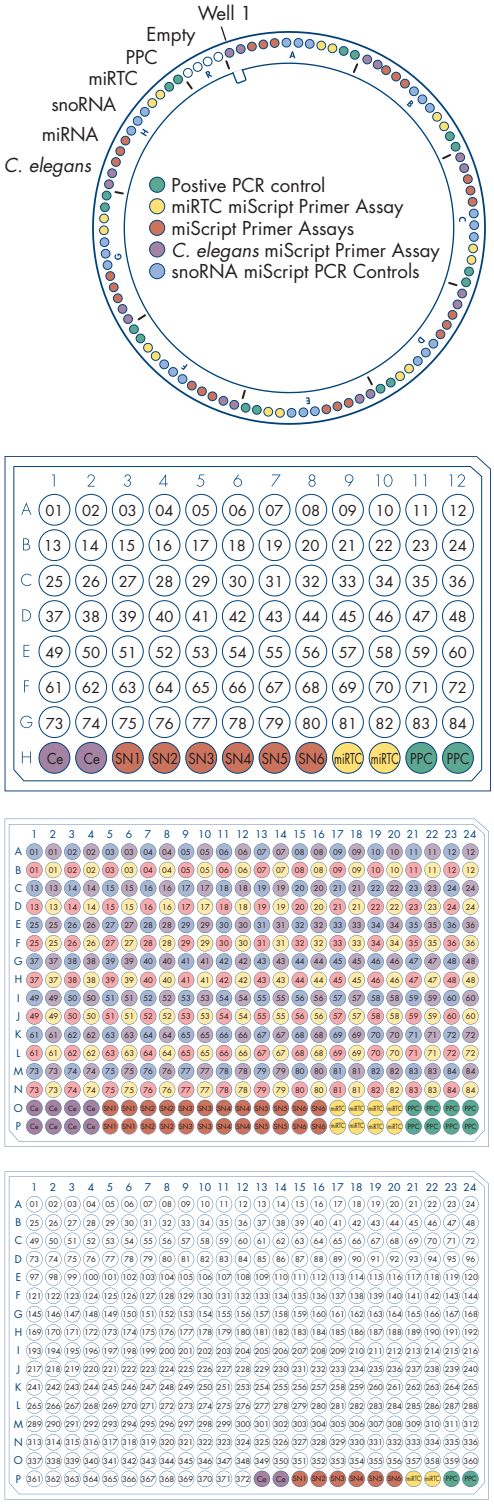
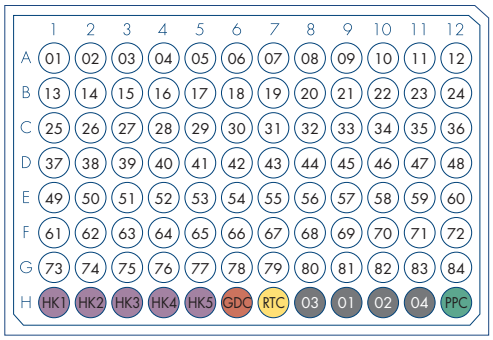


Figure 9. The layouts of the miScript miRNA PCR Array plates: the 100-well ring; 96-well plate, and 384-well plates set up for four samples or 1 sample. Control key in Figure 10.

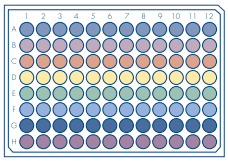
Modified and Custom PCR Arrays

Modified PCR Arrays

Add any 4 genes back to a cataloged array to instantly create an affordable and unique solution for your research. This is an excellent tool for scaling up your single gene experiments to a pathway, or to verify the role of your favorite genes in a biological process. These arrays are available for RT² Profiler and RT² lncRNA PCR Arrays.

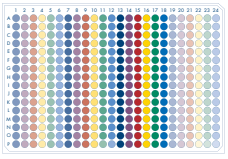


96-well format

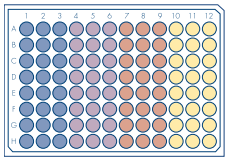


12 genes – 8 samples/plate

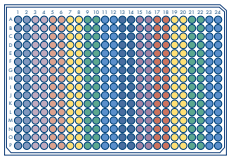
384-well format



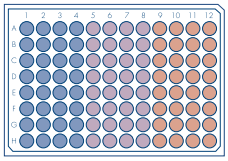
16 genes – 24 samples/plate



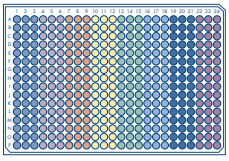
24 genes – 4 samples/plate



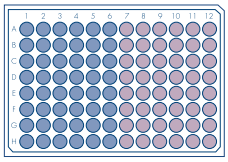
32 genes – 12 samples/plate



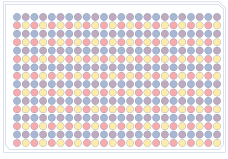
12 genes – 3 samples/plate



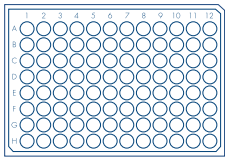
48 genes – 8 samples/plate



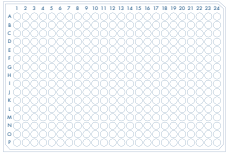
48 genes – 2 samples/plate



96 genes – 4 samples/plate



96 genes – 1 sample/plate



384 genes – 1 sample/plate

Figure 11. The layouts of Custom RT² PCR Array plates.

Custom RT² and miScript PCR Arrays

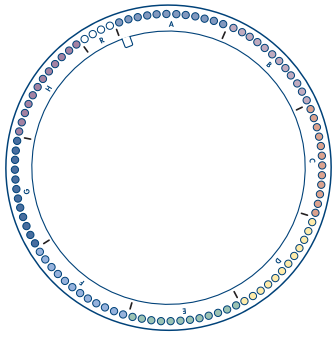
Custom RT² and miScript PCR Arrays employ a high-throughput approach for profiling the expression of genes of interest. Choose any miRNA or combine mRNA and lncRNA genes from the supported species and chose from several different plate layouts (Figures 11 and 12). Whether your interests are in biomarker discovery, drug development, disease characterization, signal transduction or RNA-seq and microarray followup, the Custom RT² and miScript PCR Arrays enable superior qPCR performance without the need for primer validation or optimization.

Each qPCR assay used in a custom array goes through the same laboratory-verification process as our standard catalog arrays.

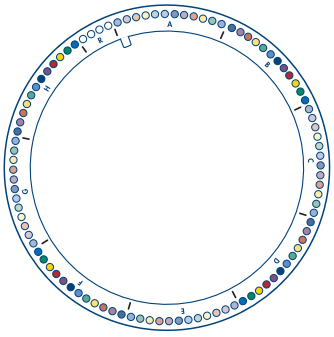
Custom RT² and miScript PCR Array are delivered within 3 weeks of ordering and are available in 96- and 384-well plates and 100-ring disc formats suitable for QIAGEN Rotor-Gene® Q instruments.

Start building your arrays today at **qiagen.com/myPCRarray**.

100-well disc format



12 genes – 8 samples/plate



96 genes – 1 sample/plate

Figure 12. The layouts of the Custom RT² PCR Array Ring.

Full support for data analysis

GeneGlobe RT² and miScript PCR Array Data Analysis Center

This integrated web-based software package for the RT² and miScript PCR Array systems automatically performs all $\Delta\Delta C_T$-based fold-change calculations from your uploaded raw data. Simply providing the array catalog number(s) annotates the results to the correct gene list.

The web portal delivers results not only in a tabular format but also scatter, volcano, cluster-gram and multi-group plots (Figure 13). 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 to correctly interpret genomic DNA, reverse transcription efficiency and positive PCR control well data.

Make your pathway-focused gene expression analysis quick and painless with by using the GeneGlobe Data Analysis Portal:

- **Simple**: Just upload your data and define your parameters
- **Convenient**: No downloading or installation required
- **Publication-ready**: Export all results as free Excel® files or .png image files

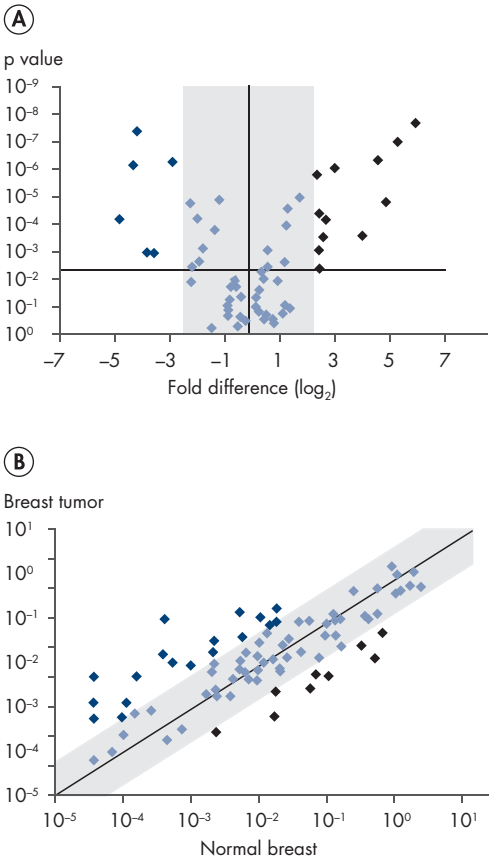


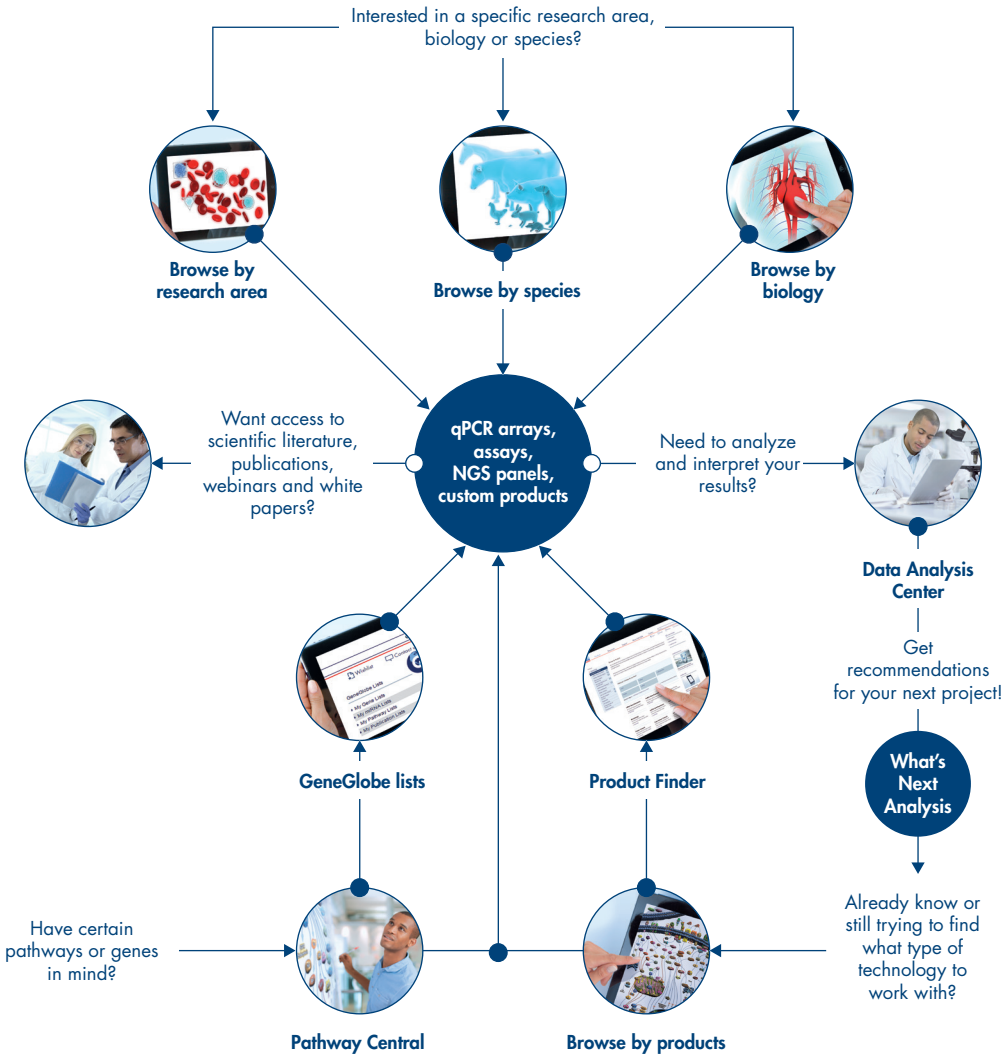
Figure 13. Example output plots from the GeneGlobe Data Analysis Center. A The volcano plot shows the fold change and statistical significance of gene expression changes. Relevent gene expression changes can be ranked by the p-value allowing researchers to follow-up on highly consistent fold change values. This becomes very important when trying to determine if genes with small fold changes are relevent for future experiments. **B** The scatter plot indicated the fold difference of gene expression changes. Two samples can quickly be compared using this plot.

Instructions

1. Upload your data in a simple Excel file format.
2. Define your experimental groups and replicates.
3. Select your normalization genes or have them automatically selected for you.
4. Export your data analysis results and graphs.

Test it with a pre-loaded sample data set at **qiagen.com/geneglobe/dataanalysis**.

GeneGlobe makes your sample to insight research quest easier!



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