QIAGEN

RT^2 Profiler PCR Array Gene Expression Analysis Report

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Sample to Insight

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Introduction

Cataloged arrays

RT^2 Profiler PCR Arrays are highly reliable and sensitive gene expression profiling tools for analyzing focused panels of genes in signal transduction, biological processes or disease research pathways using real-time PCR. Each cataloged RT^2 Profiler PCR Array contains a list of the pathway-focused genes as well as five housekeeping (reference) genes on the array. In addition, each array contains a panel of proprietary controls to monitor genomic DNA contamination (GDC) as well as the first strand synthesis (RTC) and real-time PCR efficiency (PPC). The qPCR Assays used in PCR Arrays are laboratory-verified and optimized to work under standard conditions enabling a large number of genes to be assayed simultaneously. Their specificity is guaranteed when RT^2 SYBR Green qPCR Master Mixes are used as part of the complete PCR Array System protocol.

In this study, 96 genes were profiled on 3 samples with the PAHS-032Z.

Summary and workflow

Cataloged arrays

1. Mature RNA was isolated using an RNA extraction kit according to the manufacturer's instructions.
2. RNA quality was determined using a spectrophotometer and was reverse transcribed using a cDNA conversion kit.
3. The cDNA was used on the real-time RT² Profiler PCR Array (QIAGEN, Cat. no. PAHS-032Z) in combination with RT² SYBR® Green qPCR Mastermix (Cat. no. 330529).

C_T values were exported to an Excel file to create a table of C_T values. This table was then uploaded on to the data analysis web portal at **http://www.qiagen.com/geneglobe**. Samples were assigned to controls and test groups. C_T values were normalized based on a/an Automatic selection from HKG panel of reference genes.

The data analysis web portal calculates fold change/regulation using delta delta C_T method, in which delta C_T is calculated between gene of interest (GOI) and an average of reference genes (HKG), followed by delta-delta C_T calculations (delta C_T (Test Group)-delta C_T (Control Group)). Fold Change is then calculated using 2^ (-delta delta C_T) formula. The data analysis web portal also plots scatter plot, volcano plot, clustergram, and heat map.

This data analysis report was exported from the QIAGEN web portal at GeneGlobe.

Gene table

RT^2 Profiler™ PCR Array Human Hypoxia Signaling Pathway

Position	RefSeq Number	Symbol	Description
A01	NM_001124	ADM	Adrenomedullin
A02	NM_000676	ADORA2B	Adenosine A2b receptor
A03	NM_000034	ALDOA	Aldolase A, fructose-bisphosphate
A04	NM_001039667	ANGPTL4	Angiopoietin-like 4
A05	NM_181726	ANKRD37	Ankyrin repeat domain 37
A06	NM_004039	ANXA2	Annexin A2
A07	NM_080649	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1
A08	NM_001668	ARNT	Aryl hydrocarbon receptor nuclear translocator
A09	NM_001184	ATR	Ataxia telangiectasia and Rad3 related
A10	NM_003670	BHLHE40	Basic helix-loop-helix family, member e40
A11	NM_000057	BLM	Bloom syndrome, RecQ helicase-like
A12	NM_004052	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
B01	NM_004331	BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like
B02	NM_001731	BTG1	B-cell translocation gene 1, anti-proliferative
B03	NM_001216	CA9	Carbonic anhydrase IX
B04	NM_004354	CCNG2	Cyclin G2
B05	NM_006837	COPS5	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)
B06	NM_000308	CTSA	Cathepsin A
B07	NM_019058	DDIT4	DNA-damage-inducible transcript 4
B08	NM_025219	DNAJC5	DnaJ (Hsp40) homolog, subfamily C, member 5
B09	NM_001955	EDN1	Endothelin 1
B10	NM_022051	EGLN1	Egl nine homolog 1 (C. elegans)
B11	NM_053046	EGLN2	Egl nine homolog 2 (C. elegans)
B12	NM_001964	EGR1	Early growth response 1
C01	NM_004095	EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1
C02	NM_001428	ENO1	Enolase 1, (alpha)
C03	NM_000799	EPO	Erythropoietin
C04	NM_014584	ERO1A	ERO1-like (S. cerevisiae)
C05	NM_000504	F10	Coagulation factor X
C06	NM_001993	F3	Coagulation factor III (thromboplastin, tissue factor)
C07	NM_005252	FOS	FBJ murine osteosarcoma viral oncogene homolog
C08	NM_000158	GBE1	Glucan (1,4-alpha-), branching enzyme 1
C09	NM_000175	GPI	Glucose-6-phosphate isomerase
C10	NM_002103	GYS1	Glycogen synthase 1 (muscle)
C11	NM_001530	HIF1A	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
C12	NM_017902	HIF1AN	Hypoxia inducible factor 1, alpha subunit inhibitor
D01	NM_152794	HIF3A	Hypoxia inducible factor 3, alpha subunit
D02	NM_000189	HK2	Hexokinase 2
D03	NM_002133	HMOX1	Heme oxygenase (decycling) 1
D04	NM_178849	HNF4A	Hepatocyte nuclear factor 4, alpha
D05	NM_003897	IER3	Immediate early response 3
D06	NM_000598	IGFBP3	Insulin-like growth factor binding protein 3
D07	NM_015167	JMJD6	Jumonji domain containing 6
D08	NM_005566	LDHA	Lactate dehydrogenase A
D09	NM_002306	LGALS3	Lectin, galactoside-binding, soluble, 3
D10	NM_002317	LOX	Lysyl oxidase

Position	RefSeq Number	Symbol	Description
D11	NM_005921	MAP3K1	Mitogen-activated protein kinase kinase kinase 1
D12	NM_000245	MET	Met proto-oncogene (hepatocyte growth factor receptor)
E01	NM_002415	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
E02	NM_004994	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
E03	NM_005962	MXI1	MAX interactor 1
E04	NM_005746	NAMPT	Nicotinamide phosphoribosyltransferase
E05	NM_003743	NCOA1	Nuclear receptor coactivator 1
E06	NM_006096	NDRG1	N-myc downstream regulated 1
E07	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
E08	NM_000603	NOS3	Nitric oxide synthase 3 (endothelial cell)
E09	NM_002539	ODC1	Ornithine decarboxylase 1
E10	NM_000917	P4HA1	Prolyl 4-hydroxylase, alpha polypeptide I
E11	NM_000918	P4HB	Prolyl 4-hydroxylase, beta polypeptide
E12	NM_002610	PDK1	Pyruvate dehydrogenase kinase, isozyme 1
F01	NM_002616	PER1	Period homolog 1 (Drosophila)
F02	NM_004566	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
F03	NM_004567	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
F04	NM_002626	PFKL	Phosphofructokinase, liver
F05	NM_002627	PFKP	Phosphofructokinase, platelet
F06	NM_002629	PGAM1	Phosphoglycerate mutase 1 (brain)
F07	NM_002632	PGF	Placental growth factor
F08	NM_000291	PGK1	Phosphoglycerate kinase 1
F09	NM_002648	PIM1	Pim-1 oncogene
F10	NM_002654	PKM	Pyruvate kinase, muscle
F11	NM_002658	PLAU	Plasminogen activator, urokinase
F12	NM_005349	RBPJ	Recombination signal binding protein for immunoglobulin kappa J region
G01	NM_006666	RUVBL2	RuvB-like 2 (E. coli)
G02	NM_000602	SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
G03	NM_004207	SLC16A3	Solute carrier family 16, member 3 (monocarboxylic acid transporter 4)
G04	NM_006516	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1
G05	NM_006931	SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3
G06	NM_003234	TFRC	Transferrin receptor (p90, CD71)
G07	NM_000546	TP53	Tumor protein p53
G08	NM_000365	TPI1	Triosephosphate isomerase 1
G09	NM_006472	TXNIP	Thioredoxin interacting protein
G10	NM_003367	USF2	Upstream transcription factor 2, c-fos interacting
G11	NM_003374	VDAC1	Voltage-dependent anion channel 1
G12	NM_003376	VEGFA	Vascular endothelial growth factor A
H01	NM_001101	ACTB	Actin, beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein, large, P0
H06	SA_00105	HGDC	Human Genomic DNA Contamination
H07	SA_00104	RTC	Reverse Transcription Control
H08	SA_00104	RTC	Reverse Transcription Control
H09	SA_00104	RTC	Reverse Transcription Control
H10	SA_00103	PPC	Positive PCR Control
H11	SA_00103	PPC	Positive PCR Control
H12	SA_00103	PPC	Positive PCR Control

Data analysis setup

Sample management

Control Group	**Group 1**	**Group 2**
Sample 1	Sample 2	Sample 3

Pre-amplification

A pre-amplification using the appropriate species- and pathway-specific RT^2 PreAMP Primer Mix was not performed and the appropriate corrections were made during the data analysis procedure.

Lower limit of detection

The C_T cut-off was set to 35

Data quality control (QC)

Quality checks performed and results

Test Performed	Test Result
1. PCR Array Reproducibility	All Samples Passed
2. RT Efficiency	All Samples Passed
3. Genomic DNA Contamination	All Samples Passed

Normalization analysis

Automatic selection from HKG panel

Groups	**Samples**	**GAPDH**	**Geometric Mean**	**Average Geometric Mean**
Control Group	Sample 1	18.93	18.93	18.93
Group 1	Sample 2	18.9	18.90	18.90
Group 2	Sample 3	20.2	20.20	20.20

This method automatically selects an optimal set of internal control / housekeeping / normalization genes for the analysis from the available housekeeping gene panel on the PCR Array. The software measures and identifies the genes with the most stable expression via a non-normalized calculation. The C_T values for these genes are then geometrically averaged and used for the $\Delta\Delta C_T$ calculations.

Results

Fold regulation comparison and p-value

Control Group	Test Group	Fold Regulation cut off	p-Value cut off
Control Group	Group 1	2	0.05

Position	Gene Symbol	Fold Regulation	p-Value	Comments
A04	ANGPTL4	5.10	N/A	A
A05	ANKRD37	2.69	N/A	
A12	BNIP3	3.12	N/A	
B03	CA9	15.67	N/A	A
B07	DDIT4	6.11	N/A	A
B10	EGLN1	2.07	N/A	
C03	EPO	-4.17	N/A	B
C05	F10	-2.08	N/A	B
D02	HK2	7.36	N/A	
D04	HNF4A	-54.57	N/A	A
D05	IER3	-3.43	N/A	A
E06	NDRG1	7.26	N/A	
E10	P4HA1	2.01	N/A	
E12	PDK1	4.06	N/A	
F02	PFKFB3	2.03	N/A	
F03	PFKFB4	5.35	N/A	
G04	SLC2A1	2.19	N/A	
G05	SLC2A3	2.55	N/A	
G06	TFRC	-4.53	N/A	
G09	TXNIP	-3.71	N/A	
G12	VEGFA	4.08	N/A	
H10	PPC	-2.45	N/A	

Control Group	Test Group	Fold Regulation cut off	p-Value cut off
Control Group	Group 2	2	0.05

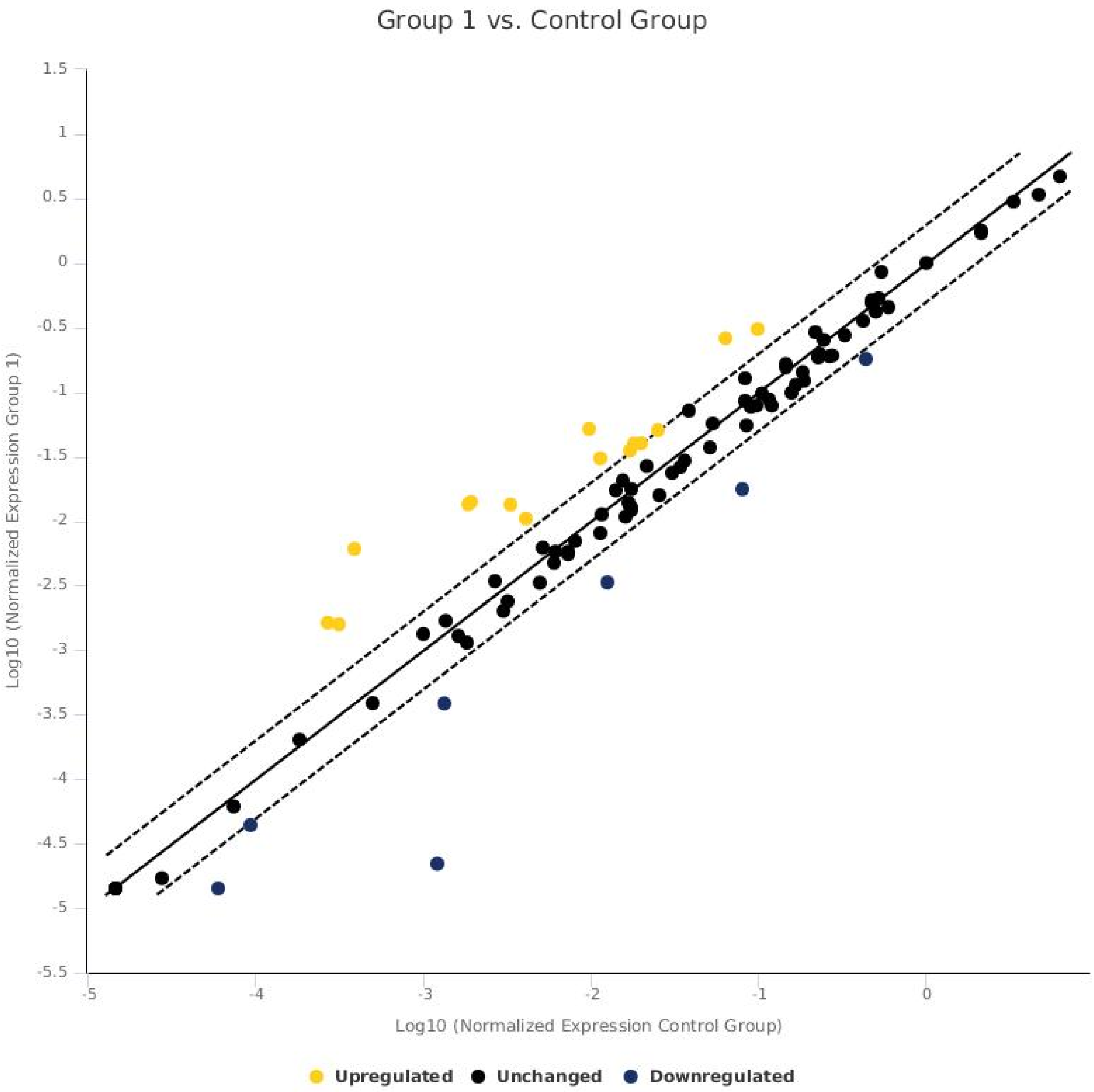
Position	Gene Symbol	Fold Regulation	p-Value	Comments
A01	ADM	2.41	N/A	C
A04	ANGPTL4	3.78	N/A	A
A07	APEX1	-2.60	N/A	
A11	BLM	-2.35	N/A	
A12	BNIP3	4.79	N/A	
B03	CA9	116.97	N/A	A
B05	COPS5	-2.27	N/A	
B07	DDIT4	6.28	N/A	A
B09	EDN1	-2.45	N/A	
B12	EGR1	-2.19	N/A	
C03	EPO	2.19	N/A	B
D01	HIF3A	2.41	N/A	C
D02	HK2	4.63	N/A	
D04	HNF4A	-4.06	N/A	A
D07	JMJD6	-2.01	N/A	
D10	LOX	2.71	N/A	
E06	NDRG1	30.48	N/A	
E09	ODC1	-3.25	N/A	
E10	P4HA1	3.48	N/A	
E12	PDK1	3.68	N/A	
F03	PFKFB4	3.05	N/A	
F07	PGF	3.34	N/A	B
F08	PGK1	2.01	N/A	
G01	RUVBL2	-2.31	N/A	
G03	SLC16A3	2.30	N/A	B
G06	TFRC	-2.13	N/A	
G09	TXNIP	3.71	N/A	
G12	VEGFA	4.23	N/A	
H02	B2M	-2.16	N/A	
H06	HGDC	2.41	N/A	C
H11	PPC	2.79	N/A	
H12	PPC	2.16	N/A	

Fold-Change (2^ (- Delta Delta C_T)) is the normalized gene expression (2^(- Delta C_T)) in the Test Sample divided the normalized gene expression (2^ (- Delta C_T)) in the Control Sample. Fold-Regulation represents fold-change results in a biologically meaningful way. Fold-change values greater than one indicates a positive- or an up-regulation, and the fold-regulation is equal to the fold-change. Fold-change values less than one indicate a negative or down-regulation, and the fold-regulation is the negative inverse of the fold-change.

The p values are calculated based on a Student's t-test of the replicate 2^ (- Delta C_T) values for each gene in the control group and treatment groups.

Scatter Plot

Test Group	Control Group	Fold Regulation Threshold
Group 1	Control Group	2



The scatter plot compares the normalized expression of every gene on the array between the two selected groups by plotting them against one another to quickly visualize large gene expression changes. The central line indicates unchanged gene expression. The dotted lines indicate the selected fold regulation threshold. Data points beyond the dotted lines in the upper left and lower right sections meet the selected fold regulation threshold.

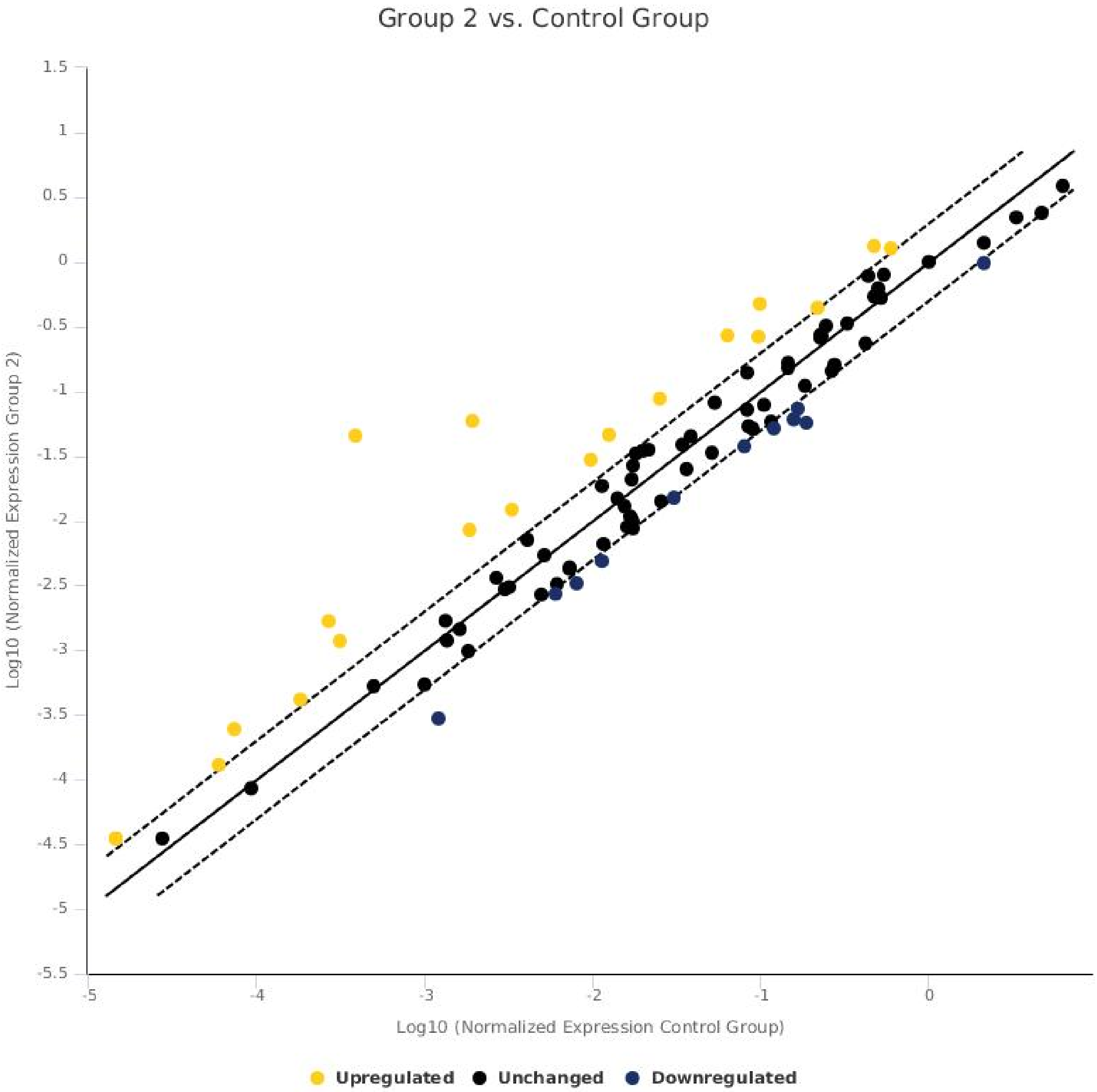
Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
B03	CA9	15.67	A	PPH01751A
D02	HK2	7.36		PPH00983B
E06	NDRG1	7.26		PPH02202B
B07	DDIT4	6.11	A	PPH11172C
F03	PFKFB4	5.35		PPH14224A
A04	ANGPTL4	5.10	A	PPH02234F
G12	VEGFA	4.08		PPH00251C
E12	PDK1	4.06		PPH20207A
A12	BNIP3	3.12		PPH00301C
A05	ANKRD37	2.69		PPH65925A
G05	SLC2A3	2.55		PPH02044B
G04	SLC2A1	2.19		PPH02043C
B10	EGLN1	2.07		PPH12056B
F02	PFKFB3	2.03		PPH00024F
E10	P4HA1	2.01		PPH02052A

Genes Under-Expressed in Group 1 vs. Control Group

Position	**Gene Symbol**	**Fold Regulation**	**Comments**	**RT2 Catalog**
D04	HNF4A	-54.57	A	PPH05540A
G06	TFRC	-4.53		PPH00990H
C03	EPO	-4.17	B	PPH01338C
G09	TXNIP	-3.71		PPH02855A
D05	IER3	-3.43	A	PPH10008F
H10	PPC	-2.45		
C05	F10	-2.08	B	PPH17879A

Test Group	**Control Group**	**Fold Regulation Threshold**
Group 2	Control Group	2



The scatter plot compares the normalized expression of every gene on the array between the two selected groups by plotting them against one another to quickly visualize large gene expression changes. The central line indicates unchanged gene expression. The dotted lines indicate the selected fold regulation threshold. Data points beyond the dotted lines in the upper left and lower right sections meet the selected fold regulation threshold.

Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
B03	CA9	116.97	A	PPH01751A
E06	NDRG1	30.48		PPH02202B
B07	DDIT4	6.28	A	PPH11172C
A12	BNIP3	4.79		PPH00301C
D02	HK2	4.63		PPH00983B
G12	VEGFA	4.23		PPH00251C
A04	ANGPTL4	3.78	A	PPH02234F
G09	TXNIP	3.71		PPH02855A
E12	PDK1	3.68		PPH20207A
E10	P4HA1	3.48		PPH02052A
F07	PGF	3.34	B	PPH01155F
F03	PFKFB4	3.05		PPH14224A
H11	PPC	2.79		
D10	LOX	2.71		PPH01956A
H06	HGDC	2.41	C	
A01	ADM	2.41	C	PPH02039C
D01	HIF3A	2.41	C	PPH02544A
G03	SLC16A3	2.30	B	PPH18809E
C03	EPO	2.19	B	PPH01338C
H12	PPC	2.16		
F08	PGK1	2.01		PPH02049A

Genes Under-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
D04	HNF4A	-4.06	A	PPH05540A
E09	ODC1	-3.25		PPH00987C
A07	APEX1	-2.60		PPH02201A
B09	EDN1	-2.45		PPH00653A
A11	BLM	-2.35		PPH02711B
G01	RUVBL2	-2.31		PPH07411A
B05	COPS5	-2.27		PPH02029A
B12	EGR1	-2.19		PPH00139A
H02	B2M	-2.16		PPH01094E
G06	TFRC	-2.13		PPH00990H
D07	JMJD6	-2.01		PPH09860A

Volcano Plot

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 1	Control Group	2	0.05

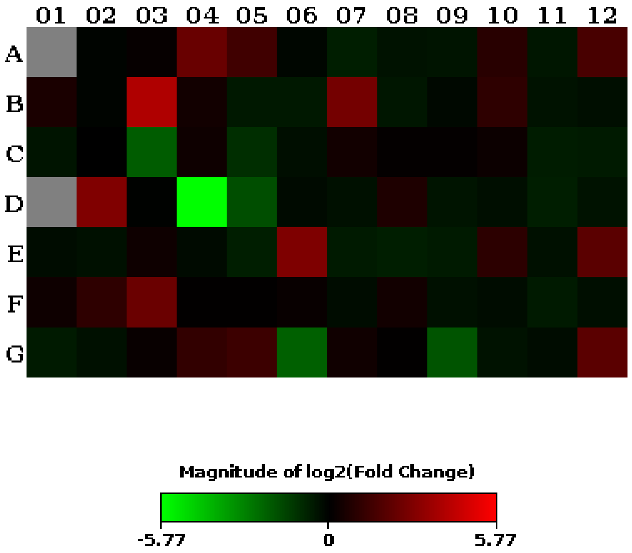
In order to generate a Volcano Plot, each group must contain at least 3 arrays.

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 2	Control Group	2	0.05

In order to generate a Volcano Plot, each group must contain at least 3 arrays.

Heat Map

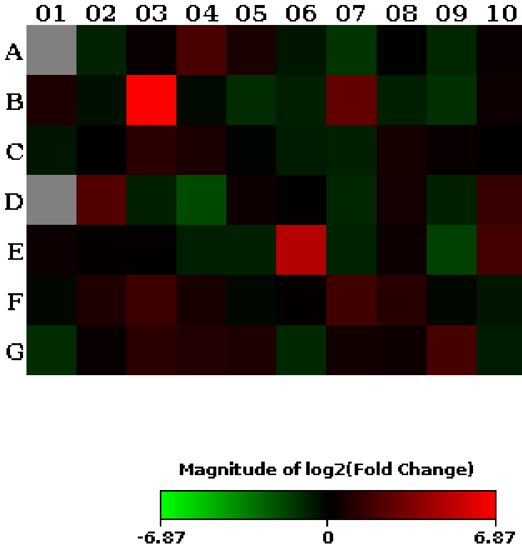
Treated Group	**Control Group**
Group 1	Control Group



Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ADM -1.02 C	ADORA2B -1.06	ALDOA 1.09	ANGPTL4 5.10 A	ANKRD37 2.69	ANXA2 -1.10	APEX1 -1.60	ARNT -1.33	ATR -1.37	BHLHE40 1.88	BLM -1.41	BNIP3 3.12
B	BNIP3L 1.53	BTG1 -1.08	CA9 15.67 A	CCNG2 1.33	COPS5 -1.46	CTSA -1.44	DDIT4 6.11 A	DNAJC5 -1.43	EDN1 -1.15	EGLN1 2.07	EGLN2 -1.31	EGR1 -1.26
C	EIF4EBP1 -1.38	ENO1 1.01	EPO -4.17 B	ERO1A 1.24	F10 -2.08 B	F3 -1.25	FOS 1.34	GBE1 1.06	GPI 1.08	GYS1 1.21	HIF1A -1.54	HIF1AN -1.48
D	HIF3A -1.02 C	HK2 7.36	HMOX1 -1.03	HNF4A -54.57 A	IER3 -3.43 A	IGFBP3 -1.18	JMJD6 -1.28	LDHA 1.58	LGALS3 -1.39	LOX -1.24	MAP3K1 -1.58	MET -1.32
E	MIF -1.19	MMP9 -1.27 A	MXI1 1.23	NAMPT -1.17	NCOA1 -1.61	NDRG1 7.26	NFKB1 -1.49	NOS3 -1.61 B	ODC1 -1.52	P4HA1 2.01	P4HB -1.28	PDK1 4.06
F	PER1 1.25	PFKFB3 2.03	PFKFB4 5.35	PFKL 1.02	PFKP 1.04	PGAM1 1.13	PGF -1.20 B	PGK1 1.33	PIM1 -1.27	PKM -1.20	PLAU -1.48	RBPJ -1.23
G	RUVBL2 -1.53	SERPINE1 -1.29	SLC16A3 1.12 B	SLC2A1 2.19	SLC2A3 2.55	TFRC -4.53	TP53 1.27	TPI1 1.03	TXNIP -3.71	USF2 -1.31	VDAC1 -1.19	VEGFA 4.08

The Heat Map provides a visualization of the fold changes in expression between the selected groups for every gene in the array in the context of the array layout. The table provides the fold regulation data used for the map as well as the Comments associated with each one.

Treated Group	Control Group
Group 2	Control Group

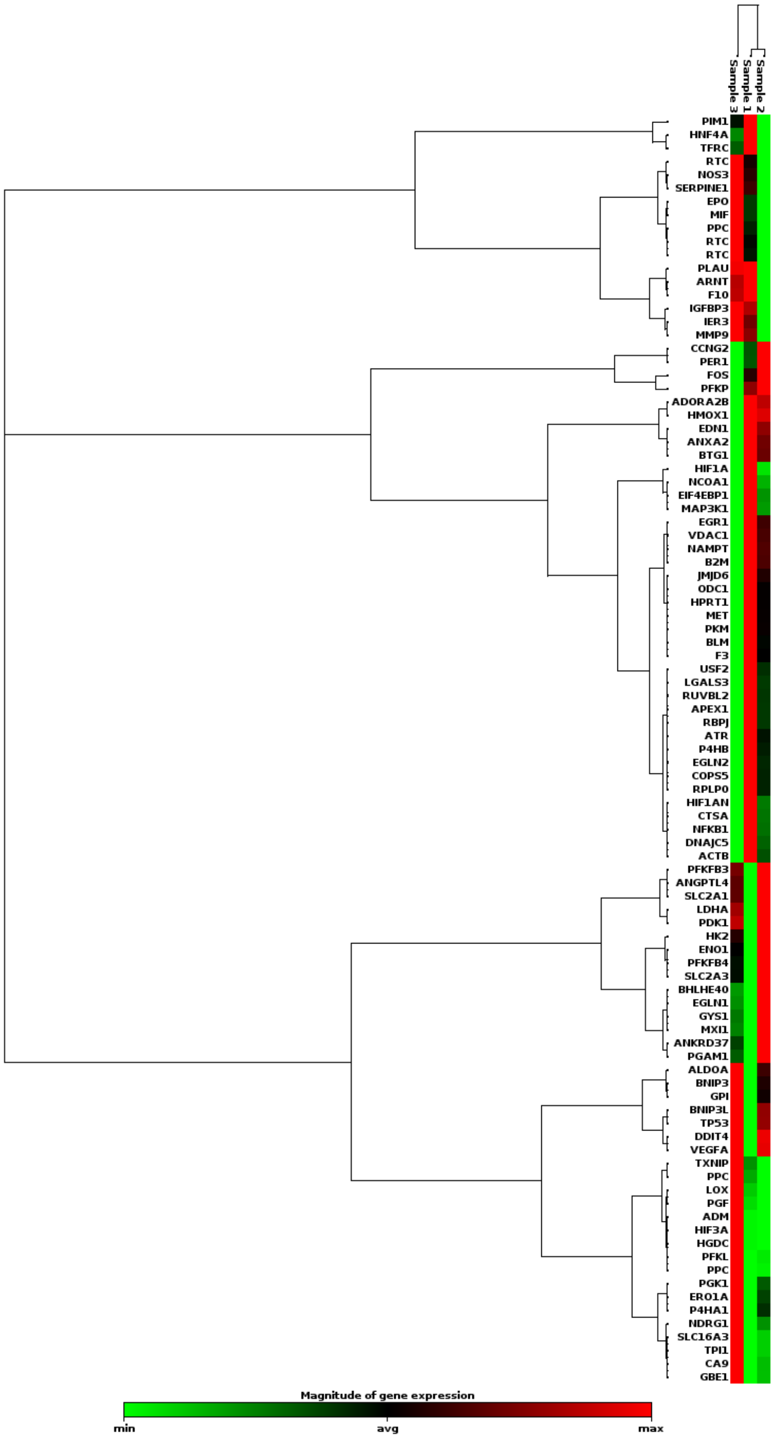


Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ADM 2.41 C	ADORA2B -1.91	ALDOA 1.14	ANGPTL4 3.78 A	ANKRD37 1.64	ANXA2 -1.49	APEX1 -2.60	ARNT -1.04	ATR -2.00	BHLHE40 1.17	BLM -2.35	BNIP3 4.79
B	BNIP3L 1.67	BTG1 -1.34	CA9 116.97 A	CCNG2 -1.20	COPS5 -2.27	CTSA -1.73	DDIT4 6.28 A	DNAJC5 -1.78	EDN1 -2.45	EGLN1 1.23	EGLN2 -1.71	EGR1 -2.19
C	EIF4EBP1 -1.53	ENO1 1.01	EPO 2.19 B	ERO1A 1.65	F10 -1.07 B	F3 -1.66	FOS -1.84 A	GBE1 1.53	GPI 1.15	GYS1 1.06	HIF1A -1.58	HIF1AN -1.79
D	HIF3A 2.41 C	HK2 4.63	HMOX1 -1.75	HNF4A -4.06 A	IER3 1.27	IGFBP3 1.03	JMJD6 -2.01	LDHA 1.47	LGALS3 -1.83	LOX 2.71	MAP3K1 -1.84 A	MET -1.99
E	MIF 1.25	MMP9 1.06 A	MXI1 1.06	NAMPT -1.75	NCOA1 -1.80	NDRG1 30.48	NFKB1 -1.85	NOS3 1.27 B	ODC1 -3.25	P4HA1 3.48	P4HB -1.66	PDK1 3.68
F	PER1 -1.14	PFKFB3 1.75	PFKFB4 3.05	PFKL 1.54	PFKP -1.14	PGAM1 1.04	PGF 3.34 B	PGK1 2.01	PIM1 -1.13	PKM -1.52	PLAU -1.01	RBPJ -1.44
G	RUVBL2 -2.31	SERPINE1 1.14	SLC16A3 2.30 B	SLC2A1 1.82	SLC2A3 1.74	TFRC -2.13	TP53 1.35	TPI1 1.30	TXNIP 3.71	USF2 -1.67	VDAC1 -1.80	VEGFA 4.23

The Heat Map provides a visualization of the fold changes in expression between the selected groups for every gene in the array in the context of the array layout. The table provides the fold regulation data used for the map as well as the Comments associated with each one.

ClusterGram

Sample	Dimension	Join Type	Color Coded
Array	2-D	Average	Genes



The clustergram performs non-supervised hierarchical clustering of the entire dataset to display a heat map with dendrograms indicating co-regulated genes across groups or individual samples.

What's next?

Thank you for using the RT² Profiler Data Analysis Software.

The Data Analysis software delivers a list of expression changes in the samples from the supplied data. However, this result often only starts an investigation into the underlying mechanisms at work. In order to assist in further analysis, the QIAGEN now utilizes the latest bioinformatics tools to analyze the data and suggest regulatory mechanisms and future experiments. Please review the results from the selected tools below.

Gene Expression: This tool will help define a panel of genes based of this experiment's results. This panel may represent a putative biomarker set, a target gene set or simply a collection of genes. The tool is designed to deliver a list of gene expression assays that would allow the user to follow-up the results of the analyzed experiment.

miRNA Regulation: This tool will identify candidate miRNA regulators in your experimental system. The tool is designed to deliver a list of miRNAs that could be targeting the genes that had observed changes in expression in your selected samples.

DNA Methylation: This tool will help define a panel of differentially expressed genes based on this experiment's results. Altered methylation patterns on the genes' promoters may be responsible for these gene expression changes. This tool is designed to deliver a list of available DNA methylation assays for those differentially expressed genes that would allow the user to follow-up their gene expression experiment with an epigenetic analysis.

Transcription Factor / Histone: This tool will help define a panel of differentially expressed genes based on this experiment's results. Altered transcription factor binding activity on the genes' promoters may be responsible for these gene expression changes. Altered histone modification patterns on the genes' promoters may also be responsible for these gene expression changes. This tool is designed to deliver a list of the transcription factors that might regulate the selected differentially expressed genes as well as the available respective gene-specific real-time PCR assays for DNA from anti-transcription factor or anti-histone chromatin immunoprecipitations. These assays would then allow the user to follow-up their gene expression experiment with an epigenetic analysis.

siRNA: This tool will help define a panel of differentially expressed genes based on this experiment's results. These differentially expressed genes may contribute to the observed differences between the tested sample groups. This tool is designed to deliver a list of differentially expressed genes and corresponding siRNA reagents to test the contribution of each differentially-expressed gene in the experiment. These siRNAs would then allow the user to follow-up their gene expression experiment with a functional analysis.

Somatic Mutation: This tool will help define a panel of genes based on this experiment's results. Mutations in these genes may affect whether their expression changes have any effect or different effects on the experimental model system. The tool is designed to deliver a list of available somatic mutation assays for these differentially expressed genes that would allow the user to follow-up the results of the analyzed experiment.

Gene Expression, DNA Methylation, RNAi, Somatic Mutation

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 1	Control Group	2	0.05

Position	Symbol	Fold Regulation	p-Value	RT2 qPCR Assay	EpiTect Methyl II qPCR Assay	FlexiTube siRNA	Somatic Mutation Assay
D04	HNF4A	-54.57	N/A	PPH05540A	Inquire	View siRNAs	Inquire
B03	CA9	15.67	N/A	PPH01751A	EPHS114317-1A	View siRNAs	Inquire
D02	HK2	7.36	N/A	PPH00983B	EPHS108346-1A	View siRNAs	Inquire
E06	NDRG1	7.26	N/A	PPH02202B	EPHS114068-1A	View siRNAs	Inquire
B07	DDIT4	6.11	N/A	PPH11172C	EPHS101697-1A	View siRNAs	Inquire
F03	PFKFB4	5.35	N/A	PPH14224A	EPHS110233-1A	View siRNAs	Inquire
A04	ANGPTL4	5.10	N/A	PPH02234F	EPHS106986-1A	View siRNAs	Inquire
G06	TFRC	-4.53	N/A	PPH00990H	EPHS110800-1A	View siRNAs	Inquire
C03	EPO	-4.17	N/A	PPH01338C	EPHS113348-1A	View siRNAs	Inquire
G12	VEGFA	4.08	N/A	PPH00251C	EPHS112506-1A	View siRNAs	Inquire
E12	PDK1	4.06	N/A	PPH20207A	EPHS108684-1A	View siRNAs	SMPH086715A
G09	TXNIP	-3.71	N/A	PPH02855A	Inquire	View siRNAs	Inquire
D05	IER3	-3.43	N/A	PPH10008F	EPHS112329-1A	View siRNAs	Inquire
A12	BNIP3	3.12	N/A	PPH00301C	EPHS102009-1A	View siRNAs	Inquire
A05	ANKRD37	2.69	N/A	PPH65925A	EPHS111391-1A	View siRNAs	Inquire
G05	SLC2A3	2.55	N/A	PPH02044B	Inquire	View siRNAs	Inquire
H10	PPC	-2.45	N/A		Inquire	View siRNAs	Inquire
G04	SLC2A1	2.19	N/A	PPH02043C	EPHS100481-1A	View siRNAs	Inquire
C05	F10	-2.08	N/A	PPH17879A	EPHS103896-1A	View siRNAs	Inquire
B10	EGLN1	2.07	N/A	PPH12056B	EPHS101371-1A	View siRNAs	Inquire

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 2	Control Group	2	0.05

Position	Symbol	Fold Regulation	p-Value	RT2 qPCR Assay	EpiTect Methyl II qPCR Assay	FlexiTube siRNA	Somatic Mutation Assay
B03	CA9	116.97	N/A	PPH01751A	EPHS114317-1A	View siRNAs	Inquire
E06	NDRG1	30.48	N/A	PPH02202B	EPHS114068-1A	View siRNAs	Inquire
B07	DDIT4	6.28	N/A	PPH11172C	EPHS101697-1A	View siRNAs	Inquire
A12	BNIP3	4.79	N/A	PPH00301C	EPHS102009-1A	View siRNAs	Inquire
D02	HK2	4.63	N/A	PPH00983B	EPHS108346-1A	View siRNAs	Inquire
G12	VEGFA	4.23	N/A	PPH00251C	EPHS112506-1A	View siRNAs	Inquire
D04	HNF4A	-4.06	N/A	PPH05540A	Inquire	View siRNAs	Inquire
A04	ANGPTL4	3.78	N/A	PPH02234F	EPHS106986-1A	View siRNAs	Inquire
G09	TXNIP	3.71	N/A	PPH02855A	Inquire	View siRNAs	Inquire
E12	PDK1	3.68	N/A	PPH20207A	EPHS108684-1A	View siRNAs	SMPH086715A
E10	P4HA1	3.48	N/A	PPH02052A	EPHS101701-1A	View siRNAs	Inquire
F07	PGF	3.34	N/A	PPH01155F	EPHS104212-1A	View siRNAs	Inquire
E09	ODC1	-3.25	N/A	PPH00987C	EPHS108037-1A	View siRNAs	Inquire
F03	PFKFB4	3.05	N/A	PPH14224A	EPHS110233-1A	View siRNAs	Inquire
H11	PPC	2.79	N/A		Inquire	View siRNAs	Inquire
D10	LOX	2.71	N/A	PPH01956A	EPHS111733-1A	View siRNAs	Inquire
A07	APEX1	-2.60	N/A	PPH02201A	EPHS103915-1A	View siRNAs	Inquire
B09	EDN1	-2.45	N/A	PPH00653A	Inquire	View siRNAs	Inquire
H06	HGDC	2.41	N/A		Inquire	View siRNAs	Inquire
A01	ADM	2.41	N/A	PPH02039C	EPHS102169-1A	View siRNAs	Inquire

miRNA Regulation

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 1	Control Group	2	0.05

Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
D04	HNF4A	-54.57	N/A
G06	TFRC	-4.53	N/A
C03	EPO	-4.17	N/A
G09	TXNIP	-3.71	N/A
D05	IER3	-3.43	N/A
H10	PPC	-2.45	N/A
C05	F10	-2.08	N/A

miRNA Regulating Genes Under-Expressed in Group 1 vs. Control Group

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-485-5p	-0.37	MS00006972	MSY0002175	MIN0002175	HNF4A
hsa-miR-545-3p	-0.37	MS00007777	MSY0003165	MIN0003165	HNF4A
hsa-miR-31-5p	-0.37	MS00003290	MSY0000089	MIN0000089	TFRC
hsa-miR-1291	-0.36	MS00014525	MSY0005881	MIN0005881	EPO
hsa-miR-544a	-0.32	MS00004585	MSY0003164	MIN0003164	TFRC
hsa-miR-142-3p	-0.30	MS00006664	MSY0000434	MIN0000434	IER3
hsa-miR-583	-0.29	MS00004851	MSY0003248	MIN0003248	TFRC
hsa-miR-450b-5p	-0.28	MS00009716	MSY0004909	MIN0004909	TFRC
hsa-miR-1827	-0.28	MS00014686	MSY0006767	MIN0006767	TFRC
hsa-miR-7-5p	-0.26	MS00006503	MSY0000252	MIN0000252	TFRC
hsa-miR-616-3p	-0.24	MS00008218	MSY0004805	MIN0004805	TFRC
hsa-miR-135a-5p	-0.24	MS00006650	MSY0000428	MIN0000428	TXNIP
hsa-miR-135b-5p	-0.24	MS00003472	MSY0000758	MIN0000758	TXNIP
hsa-miR-519d-3p	-0.24	MS00004508	MSY0002853	MIN0002853	TXNIP
hsa-miR-122-5p	-0.23	MS00003416	MSY0000421	MIN0000421	EPO
hsa-miR-302e	-0.23	MS00014693	MSY0005931	MIN0005931	TXNIP
hsa-miR-326	-0.22	MS00003948	MSY0000756	MIN0000756	HNF4A
hsa-miR-302d-3p	-0.21	MS00003920	MSY0000718	MIN0000718	TXNIP
hsa-miR-520b	-0.21	MS00007406	MSY0002843	MIN0002843	TXNIP
hsa-miR-520c-3p	-0.21	MS00007413	MSY0002846	MIN0002846	TXNIP

Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
B03	CA9	15.67	N/A
D02	HK2	7.36	N/A
E06	NDRG1	7.26	N/A
B07	DDIT4	6.11	N/A
F03	PFKFB4	5.35	N/A
A04	ANGPTL4	5.10	N/A
G12	VEGFA	4.08	N/A
E12	PDK1	4.06	N/A
A12	BNIP3	3.12	N/A
A05	ANKRD37	2.69	N/A
G05	SLC2A3	2.55	N/A
G04	SLC2A1	2.19	N/A
B10	EGLN1	2.07	N/A

miRNA Regulating Genes Over-Expressed in Group 1 vs. Control Group

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-578	-0.52	MS00004816	MSY0003243	MIN0003243	VEGFA
hsa-miR-361-5p	-0.49	MS00004032	MSY0000703	MIN0000703	VEGFA
hsa-miR-379-5p	-0.48	MS00004109	MSY0000733	MIN0000733	PDK1
hsa-miR-495-3p	-0.47	MS00004347	MSY0002817	MIN0002817	DDIT4
hsa-miR-153-3p	-0.47	MS00008771	MSY0000439	MIN0000439	DDIT4
hsa-miR-142-5p	-0.44	MS00006671	MSY0000433	MIN0000433	BNIP3
hsa-miR-889-3p	-0.44	MS00010710	MSY0004921	MIN0004921	VEGFA
hsa-miR-143-3p	-0.42	MS00003514	MSY0000435	MIN0000435	HK2
hsa-miR-205-5p	-0.40	MS00003780	MSY0000266	MIN0000266	VEGFA
hsa-miR-548p	-0.39	MS00014812	MSY0005934	MIN0005934	VEGFA
hsa-miR-299-3p	-0.39	MS00006783	MSY0000687	MIN0000687	VEGFA
hsa-miR-633	-0.38	MS00005194	MSY0003303	MIN0003303	HK2
hsa-miR-30a-5p	-0.38	MS00007350	MSY0000087	MIN0000087	DDIT4
hsa-miR-30e-5p	-0.38	MS00007357	MSY0000692	MIN0000692	DDIT4
hsa-miR-30d-5p	-0.38	MS00003283	MSY0000245	MIN0000245	DDIT4
hsa-miR-30c-5p	-0.37	MS00006580	MSY0000244	MIN0000244	DDIT4
hsa-miR-30b-5p	-0.37	MS00003276	MSY0000420	MIN0000420	DDIT4
hsa-miR-198	-0.36	MS00003724	MSY0000228	MIN0000228	SLC2A1
hsa-miR-593-3p	-0.36	MS00008211	MSY0004802	MIN0004802	HK2
hsa-miR-128-3p	-0.35	MS00008582	MSY0000424	MIN0000424	PDK1

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 2	Control Group	2	0.05

Genes Under-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
D04	HNF4A	-4.06	N/A
E09	ODC1	-3.25	N/A
A07	APEX1	-2.60	N/A
B09	EDN1	-2.45	N/A

miRNA Regulating Genes Under-Expressed in Group 2 vs. Control Group

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-548c-3p	-0.42	MS00010122	MSY0003285	MIN0003285	ODC1
hsa-miR-595	-0.38	MS00004928	MSY0003263	MIN0003263	ODC1
hsa-miR-545-3p	-0.37	MS00007777	MSY0003165	MIN0003165	HNF4A
hsa-miR-485-5p	-0.37	MS00006972	MSY0002175	MIN0002175	HNF4A
hsa-miR-587	-0.36	MS00004879	MSY0003253	MIN0003253	ODC1
hsa-miR-98-5p	-0.35	MS00003367	MSY0000096	MIN0000096	EDN1
hsa-let-7a-5p	-0.35	MS00006482	MSY0000062	MIN0000062	EDN1
hsa-let-7f-5p	-0.35	MS00006489	MSY0000067	MIN0000067	EDN1
hsa-let-7e-5p	-0.35	MS00003143	MSY0000066	MIN0000066	EDN1
hsa-let-7c-5p	-0.35	MS00003129	MSY0000064	MIN0000064	EDN1
hsa-let-7d-5p	-0.35	MS00003136	MSY0000065	MIN0000065	EDN1
hsa-let-7i-5p	-0.35	MS00003157	MSY0000415	MIN0000415	EDN1
hsa-let-7g-5p	-0.35	MS00003150	MSY0000414	MIN0000414	EDN1
hsa-let-7b-5p	-0.35	MS00003122	MSY0000063	MIN0000063	EDN1
hsa-miR-1294	-0.22	MS00014546	MSY0005884	MIN0005884	EDN1
hsa-miR-326	-0.22	MS00003948	MSY0000756	MIN0000756	HNF4A
hsa-miR-301b	-0.20		MSY0004958	MIN0004958	EDN1
hsa-miR-301a-3p	-0.20	MS00003892	MSY0000688	MIN0000688	EDN1
hsa-miR-130b-3p	-0.20	MS00003451	MSY0000691	MIN0000691	EDN1
hsa-miR-130a-3p	-0.20	MS00003444	MSY0000425	MIN0000425	EDN1

Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
B03	CA9	116.97	N/A
E06	NDRG1	30.48	N/A
B07	DDIT4	6.28	N/A
A12	BNIP3	4.79	N/A
D02	HK2	4.63	N/A
G12	VEGFA	4.23	N/A
A04	ANGPTL4	3.78	N/A
G09	TXNIP	3.71	N/A
E12	PDK1	3.68	N/A
E10	P4HA1	3.48	N/A
F07	PGF	3.34	N/A
F03	PFKFB4	3.05	N/A
H11	PPC	2.79	N/A
D10	LOX	2.71	N/A
H06	HGDC	2.41	N/A
A01	ADM	2.41	N/A

miRNA Regulating Genes Over-Expressed in Group 2 vs. Control Group

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-938	-0.53	MS00010857	MSY0004981	MIN0004981	ADM
hsa-miR-29c-3p	-0.53	MS00003269	MSY0000681	MIN0000681	VEGFA
hsa-miR-29b-3p	-0.53	MS00006566	MSY0000100	MIN0000100	VEGFA
hsa-miR-29a-3p	-0.53	MS00003262	MSY0000086	MIN0000086	VEGFA
hsa-miR-578	-0.52	MS00004816	MSY0003243	MIN0003243	VEGFA
hsa-miR-338-5p	-0.50	MS00009478	MSY0004701	MIN0004701	ADM
hsa-miR-361-5p	-0.49	MS00004032	MSY0000703	MIN0000703	VEGFA
hsa-miR-379-5p	-0.48	MS00004109	MSY0000733	MIN0000733	PDK1
hsa-miR-122-5p	-0.47	MS00003416	MSY0000421	MIN0000421	P4HA1
hsa-miR-495-3p	-0.47	MS00004347	MSY0002817	MIN0002817	DDIT4
hsa-miR-153-3p	-0.47	MS00008771	MSY0000439	MIN0000439	DDIT4
hsa-miR-576-5p	-0.46	MS00007798	MSY0003241	MIN0003241	VEGFA
hsa-miR-142-5p	-0.44	MS00006671	MSY0000433	MIN0000433	BNIP3
hsa-miR-889-3p	-0.44	MS00010710	MSY0004921	MIN0004921	VEGFA
hsa-miR-499a-5p	-0.43	MS00004375	MSY0002870	MIN0002870	P4HA1
hsa-miR-588	-0.43	MS00004886	MSY0003255	MIN0003255	TXNIP
hsa-miR-143-3p	-0.42	MS00003514	MSY0000435	MIN0000435	HK2
hsa-miR-205-5p	-0.40	MS00003780	MSY0000266	MIN0000266	VEGFA
hsa-miR-22-3p	-0.39	MS00003220	MSY0000077	MIN0000077	DDIT4
hsa-miR-548p	-0.39	MS00014812	MSY0005934	MIN0005934	VEGFA

Transcription Factor / Histone

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 1	Control Group	2	0.05

Genes Differentially Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
D04	HNF4A	-54.57	N/A	HOXA5, TBP, TFIID, MZF-1, Pax-2, Pax-2a, RFX1, LUN-1, Msx-1, CBF(2), CBF-A, CBF-B, CP1A, NF-Y, NF-YA, NF-YB, HNF-1, HNF-1A, CUTL1, Evi-1, Evi-1, GATA-1, AP-4, MyoD	GPH1008442(-)01A
B03	CA9	15.67	N/A	AP-2rep, CP2, ZIC2/Zic2, Zic3, C/EBPbeta, c-Myc, Max, E47, Tal-1, E47, Tal-1beta, Lmo2, c-Myc, Max1, MyoD, GATA-1, Lmo2, MRF-2, ARP-1, ZID, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Bach2, NF-E2, NF-E2 p45, p53, CP2, POU3F2	GPH1012980(-)01A
D02	HK2	7.36	N/A		GPH1007554(-)01A
E06	NDRG1	7.26	N/A	LyF-1, MyoD, C/EBPalpha, C/EBPbeta, Hlf	GPH1026403(-)01A
B07	DDIT4	6.11	N/A	NF-kappaB1, C/EBPalpha, CHOP-10, MAZR, FOXF2, FOXO1, FOXO1a, FOXO4, HSF1 (long), HSF1short, Sp1, MAZR, ER-alpha, Meis-1, Meis-1a, Meis-1b, RFX1, CBF(2), NF-Y, p300, SRF, SRF (504 AA)	GPH1001673(-)01A
F03	PFKFB4	5.35	N/A	Zic1, Zic3, Sp1, c-Ets-1, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C	GPH1022965(-)01A
A04	ANGPTL4	5.10	N/A	FOXO1, FOXO1a, FOXO1, FOXO1a, TBP	GPH1006507(-)01A
G06	TFRC	-4.53	N/A	ARP-1, E2F, E2F-1, E2F-2, E2F-3a, E2F-4, E2F-5, E47, Lmo2, MyoD, GCNF, GCNF-1, GCNF-2, HOXA3, Evi-1	GPH1023484(-)01A
C03	EPO	-4.17	N/A	AP-1, GATA-1, AP-4, YY1, E47, MZF-1, HOXA5, RREB-1, Pax-4a, Egr-1, Egr-2, Egr-3, Egr-4, AhR, Arnt, GATA-1, Spz1	GPH1012129(-)01A
G12	VEGFA	4.08	N/A	PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, E47, Hand1, HTF, XBP-1, AP-1, Pax-4a, LyF-1, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, C/EBPalpha	GPH1011376(-)01A
E12	PDK1	4.06	N/A	NF-kappaB1, NF-kappaB, C/EBPbeta, Elk-1	GPH1007880(-)01A
				GR, GR-alpha, GR-beta, AP-1, PPAR-gamma1, PPAR-gamma2, Evi-1, GATA-1, GATA-2, GATA-3,	

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
G09	TXNIP	-3.71	N/A	C/EBPalpha, CHOP-10, E2F, E2F-1, GR, GR-alpha, GR-beta, Pax-5, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, FOXO1, FOXO1a, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, N-Myc	GPH1000787(-)01A
D05	IER3	-3.43	N/A	c-Myb, c-Myb, c-Rel, RelA, p53	GPH1024778(-)01A
A12	BNIP3	3.12	N/A	Sp1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC	GPH1016023(-)01A
A05	ANKRD37	2.69	N/A	YY1	GPH1010345(-)01A
G05	SLC2A3	2.55	N/A		GPH1016929(-)01A
H10	PPC	-2.45	N/A		
G04	SLC2A1	2.19	N/A	PPAR-gamma1, PPAR-gamma2, Brachyury	GPH1014532(-)01A
C05	F10	-2.08	N/A	HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, Lmo2, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1	GPH1003755(-)01A
B10	EGLN1	2.07	N/A	NF-kappaB1, NF-kappaB, AP-1, AP-1, Evi-1, FOXF2	GPH1015440(-)01A

Test Group	Control Group	Fold Regulation Threshold	p-Value Threshold
Group 2	Control Group	2	0.05

Genes Differentially Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
B03	CA9	116.97	N/A	AP-2rep, CP2, ZIC2/Zic2, Zic3, C/EBPbeta, c-Myc, Max, E47, Tal-1, E47, Tal-1beta, Lmo2, c-Myc, Max1, MyoD, GATA-1, Lmo2, MRF-2, ARP-1, ZID, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Bach2, NF-E2, NF-E2 p45, p53, CP2, POU3F2	GPH1012980(-)01A
E06	NDRG1	30.48	N/A	LyF-1, MyoD, C/EBPalpha, C/EBPbeta, Hlf	GPH1026403(-)01A
B07	DDIT4	6.28	N/A	NF-kappaB1, C/EBPalpha, CHOP-10, MAZR, FOXF2, FOXO1, FOXO1a, FOXO4, HSF1 (long), HSF1short, Sp1, MAZR, ER-alpha, Meis-1, Meis-1a, Meis-1b, RFX1, CBF(2), NF-Y, p300, SRF, SRF (504 AA)	GPH1001673(-)01A
A12	BNIP3	4.79	N/A	Sp1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC	GPH1016023(-)01A
D02	HK2	4.63	N/A		GPH1007554(-)01A
G12	VEGFA	4.23	N/A	PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, PPAR-alpha, CREB, deltaCREB, E47, Hand1, HTF, XBP-1, AP-1, Pax-4a, LyF-1, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, C/EBPalpha	GPH1011376(-)01A
D04	HNF4A	-4.06	N/A	HOXA5, TBP, TFIID, MZF-1, Pax-2, Pax-2a, RFX1, LUN-1, Msx-1, CBF(2), CBF-A, CBF-B, CP1A, NF-Y, NF-YA, NF-YB, HNF-1, HNF-1A, CUTL1, Evi-1, Evi-1, GATA-1, AP-4, MyoD	GPH1008442(-)01A
A04	ANGPTL4	3.78	N/A	FOXO1, FOXO1a, FOXO1, FOXO1a, TBP	GPH1006507(-)01A
G09	TXNIP	3.71	N/A	GR, GR-alpha, GR-beta, AP-1, PPAR-gamma1, PPAR-gamma2, Evi-1, GATA-1, GATA-2, GATA-3, C/EBPalpha, CHOP-10, E2F, E2F-1, GR, GR-alpha, GR-beta, Pax-5, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, FOXO1, FOXO1a, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, N-Myc	GPH1000787(-)01A
E12	PDK1	3.68	N/A	NF-kappaB1, NF-kappaB, C/EBPbeta, Elk-1	GPH1007880(-)01A
E10	P4HA1	3.48	N/A	RFX1, Brachyury	GPH1015783(-)01A
F07	PGF	3.34	N/A	NF-kappaB1, NF-kappaB1, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4, MyoD	GPH1018061(-)01A
E09	ODC1	-3.25	N/A	CP2, CUTL1, Elk-1	GPH1021113(-)01A
F03	PFKFB4	3.05	N/A	Zic1, Zic3, Sp1, c-Ets-1, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1),	GPH1022965(-)01A

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
				POU2F2B, POU2F2C	
H11	PPC	2.79	N/A		
D10	LOX	2.71	N/A	POU2F1, POU2F1a, IRF-2, C/EBPalpha, FAC1, POU2F1, POU2F1a, NF-1, POU2F1, POU2F1a, HOXA3, Pax-4a, C/EBPalpha, C/EBPbeta, TBP, TFIID, STAT1, STAT1alpha, STAT1beta, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6, FOXC1, POU2F1, POU2F1a, FOXO4, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), IRF-1, IRF-2, IRF-2	GPH1024332(-)01A
A07	APEX1	-2.60	N/A	Max1, Max1, GR, GR-alpha, GR-beta, GR, GR-alpha, CREB, Max1, GR, GR-alpha, GR-beta, GR, GR-alpha, CREB, Max1, GR, GR-alpha, GR-beta, GR, GR-alpha, CREB, AREB6, Pax-3, CREB, deltaCREB, ATF, ATF-2, CRE-BP1, Pax-2, Pax-2a, Egr-1, Egr-2, Egr-3, Egr-4	GPH1003793(-)01A
B09	EDN1	-2.45	N/A	p53, Evi-1, CUTL1, POU2F1, POU2F1a, STAT3, E47, Hand1, Gfi-1, GATA-1, GATA-2, GATA-3, Arnt, CUTL1, AP-1, FOXJ2, FOXJ2 (long isoform), FOXO4, FOXO1, FOXO1a	GPH1011088(-)01A
H06	HGDC	2.41	N/A		
A01	ADM	2.41	N/A	GR, GR-alpha, GR-beta, Sp1, IRF-1, HNF-1, HNF-1A, POU2F1, POU2F1a, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), Pax-3, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), GATA-1, Arnt, Pax-2, Pax-2a, PPAR-gamma1, PPAR-gamma2, Sp1, RFX1	GPH1002144(-)01A

Next steps

After using QIAGEN's RT^2 Profiler PCR array, use the upregulated or downregulated qPCR assays to further validate your hypothesis.

You can use individual qPCR RT^2 assays or create custom RT^2 PCR arrays.

Further, you can use the assay and other products discussed above in the "What's next?" section to design additional studies on the expression and function of miRNAs regulating the differentially expressed genes, somatic mutations in those genes, epigenetic marks (such as modified histones, transcription factor binding, and DNA methylation) at the promoters of those genes, or study the genes' function using gene-specific siRNA.

Glossary

Comments

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30).These data mean that the gene's expression is relatively low in one sample and reasonably detected in the other sample suggesting that the actual fold-change value is at least as large as the calculated and reported fold-change result. This fold-change result may also have greater variations if p value > 0.05; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.

B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05).This fold-change result may also have greater variations; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.

C: This gene's average threshold cycle is either not determined or greater than the defined cut-off (default 35), in both samples meaning that its expression was undetected, making this fold-change result erroneous and un-interpretable.