



# RT<sup>2</sup> Profiler PCR Array Gene Expression Analysis Report

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# Introduction

## Cataloged arrays

RT<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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.

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# 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 RT<sup>2</sup> Profiler PCR Array (QIAGEN, Cat. no. PAHS-032Z) in combination with RT<sup>2</sup> SYBR® Green qPCR Mastermix (Cat. no. 330529).

C<sub>T</sub> values were exported to an Excel file to create a table of C<sub>T</sub> 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<sub>T</sub> 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<sub>T</sub> method, in which delta C<sub>T</sub> is calculated between gene of interest (GOI) and an average of reference genes (HKG), followed by delta-delta C<sub>T</sub> calculations (delta C<sub>T</sub> (Test Group)-delta C<sub>T</sub> (Control Group)). Fold Change is then calculated using  $2^{(-\text{delta delta } C_T)}$  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<sup>2</sup> 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 metalloproteinase 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	PK1	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

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# Data analysis setup

## Sample management

<b>Control Group</b>	<b>Group 1</b>	<b>Group 2</b>
Sample 1	Sample 2	Sample 3

## Pre-amplification

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

## Lower limit of detection

The C<sub>T</sub> cut-off was set to 35

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# 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	Check Samples: Sample 1

Test Performed	Sample 1
PCR Array Reproducibility	
Average $C_T$ (PPC)	19.63
Result	Pass
Reverse Transcription Control (RTC)	
Delta $C_T$ (Average RTC - Average PPC)	1.40
Result	Pass
Genomic DNA Contamination (GDC)	
$C_T$ (GDC)	34.13
Result	Inquiry

**Criteria for Genomic DNA Contamination (GDC):** If  $C_T$ (GDC)  $\geq$  35, then the GDC QC reports 'Pass'; if  $C_T$ (GDC)  $<$  35, then the GDC QC reports 'Inquiry'.

See the Troubleshooting Guide of the PCR Array User Manual/Handbook or Contact Technical Support at 888-503-3187 regarding samples with control(s) labeled 'Inquiry'.

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# Normalization analysis

## Automatic selection from HKG panel

Groups	Samples	GAPDH	Geometric Mean	Average Geometric Mean
Control Group	Sample 1	16.72	16.72	16.72
Group 1	Sample 2	16.99	16.99	16.99
Group 2	Sample 3	17.71	17.71	17.71

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	56.89	N/A	A
A05	ANKRD37	4.32	N/A	
A12	BNIP3	3.46	N/A	
B03	CA9	11.55	N/A	
B07	DDIT4	5.86	N/A	
B09	EDN1	-2.04	N/A	
B12	EGR1	-4.03	N/A	
C05	F10	-2.83	N/A	B
C06	F3	4.14	N/A	
C07	FOS	-2.39	N/A	
C11	HIF1A	-2.43	N/A	
D02	HK2	2.93	N/A	
D04	HNF4A	-6.68	N/A	A
D05	IER3	2.30	N/A	
E03	MXI1	10.41	N/A	
E06	NDRG1	12.82	N/A	
E10	P4HA1	-15608.02	N/A	A
E12	PDK1	2.27	N/A	
F02	PFKFB3	6.96	N/A	
F03	PFKFB4	8.06	N/A	
F07	PGF	7.46	N/A	
F11	PLAU	-2.22	N/A	
G03	SLC16A3	3.48	N/A	A
G04	SLC2A1	3.29	N/A	
G06	TFRC	-4.59	N/A	
G12	VEGFA	8.40	N/A	
H10	PPC	-2.11	N/A	

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

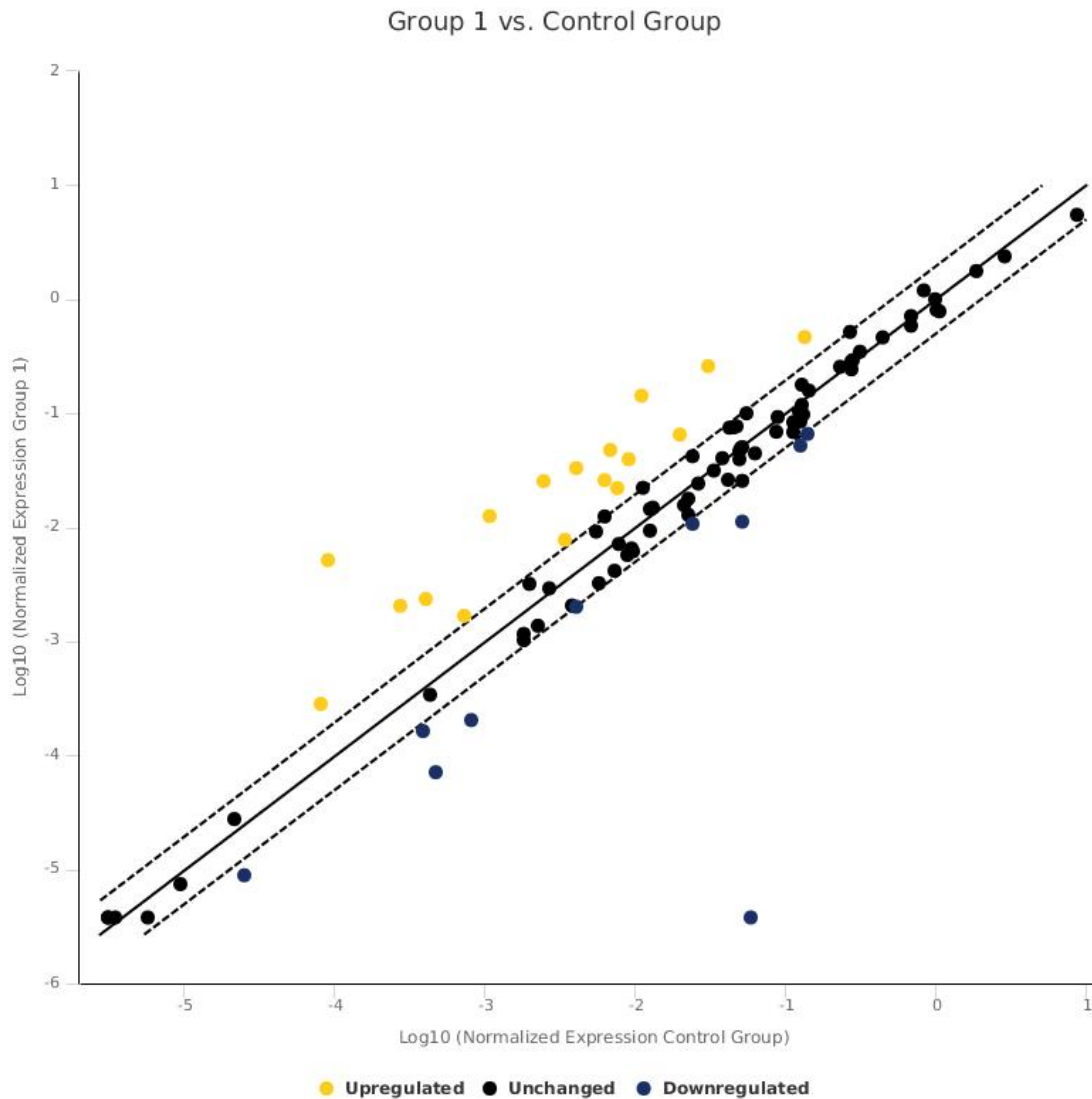
Position	Gene Symbol	Fold Regulation	p-Value	Comments
A04	ANGPTL4	9.19	N/A	A
A12	BNIP3	2.62	N/A	
B01	BNIP3L	2.46	N/A	
B03	CA9	30.91	N/A	
B11	EGLN2	-3.58	N/A	
B12	EGR1	3.16	N/A	
C03	EPO	2.10	N/A	B
C06	F3	-2.30	N/A	
C10	GYS1	2.69	N/A	
C11	HIF1A	-2.71	N/A	
D03	HMOX1	-2.07	N/A	
D04	HNFB4A	-14.52	N/A	A
D05	IER3	-2.27	N/A	
D10	LOX	-3.56	N/A	
D12	MET	-2.28	N/A	
E02	MMP9	77.17	N/A	A
E03	MXI1	5.74	N/A	
E06	NDRG1	5.66	N/A	
E07	NFKB1	-2.19	N/A	
E12	PDK1	2.89	N/A	
F02	PFKFB3	4.66	N/A	
F03	PFKFB4	5.43	N/A	
F05	PFKP	2.39	N/A	
F11	PLAU	-13.18	N/A	
G02	SERPINE1	-5.66	N/A	
G07	TP53	-6.87	N/A	
G10	USF2	-2.20	N/A	
G12	VEGFA	5.13	N/A	
H01	ACTB	-2.43	N/A	
H04	HPRT1	-2.39	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
A04	ANGPTL4	56.89	A	<a href="#">PPH02234F</a>
E06	NDRG1	12.82		<a href="#">PPH02202B</a>
B03	CA9	11.55		<a href="#">PPH01751A</a>
E03	MXI1	10.41		<a href="#">PPH11413A</a>
G12	VEGFA	8.40		<a href="#">PPH00251C</a>
F03	PFKFB4	8.06		<a href="#">PPH14224A</a>
F07	PGF	7.46		<a href="#">PPH01155F</a>
F02	PFKFB3	6.96		<a href="#">PPH00024F</a>
B07	DDIT4	5.86		<a href="#">PPH11172C</a>
A05	ANKRD37	4.32		<a href="#">PPH65925A</a>
C06	F3	4.14		<a href="#">PPH02227G</a>
G03	SLC16A3	3.48	A	<a href="#">PPH18809E</a>
A12	BNIP3	3.46		<a href="#">PPH00301C</a>
G04	SLC2A1	3.29		<a href="#">PPH02043C</a>
D02	HK2	2.93		<a href="#">PPH00983B</a>
D05	IER3	2.30		<a href="#">PPH10008F</a>
E12	PDK1	2.27		<a href="#">PPH20207A</a>

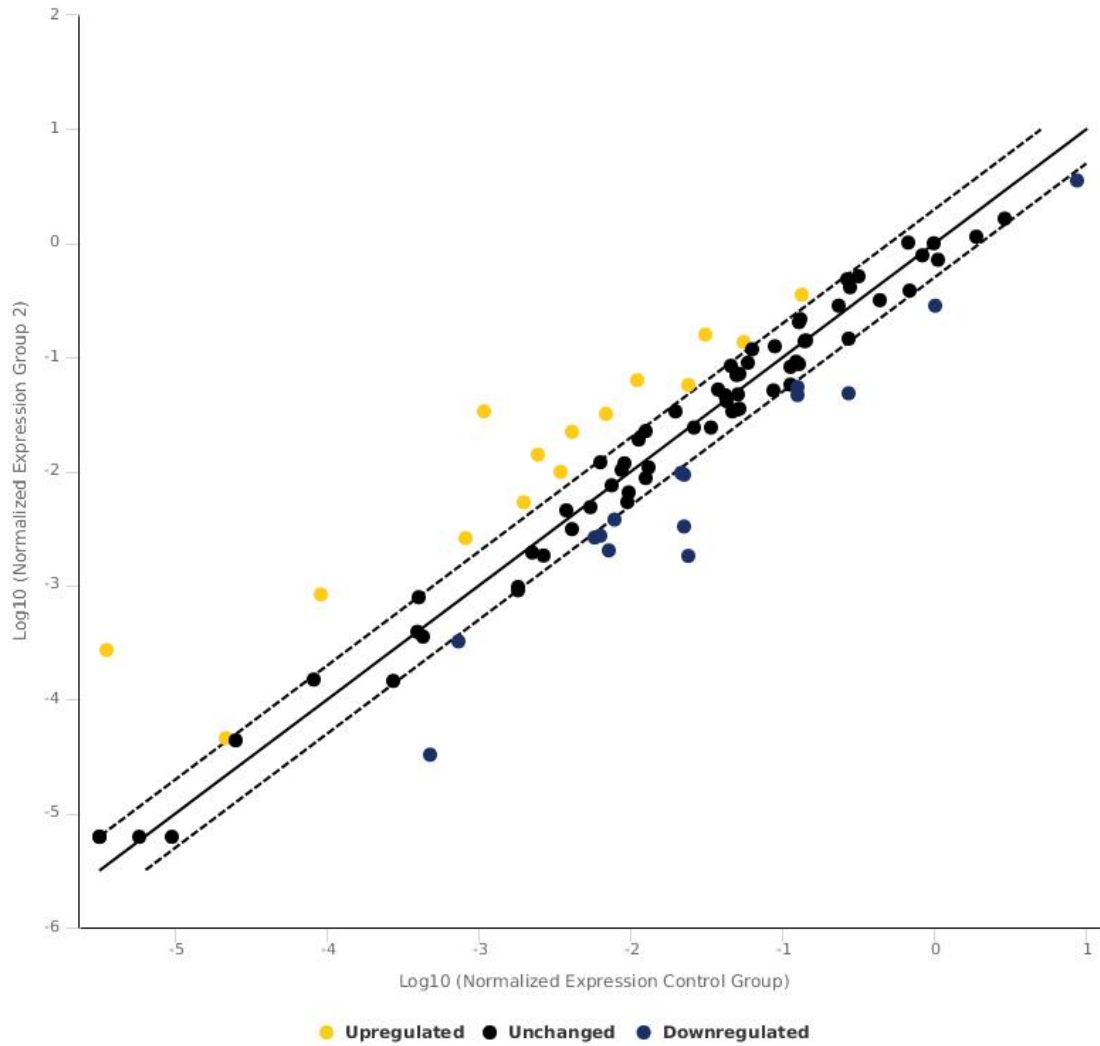
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## Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
E10	P4HA1	-15608.02	A	<a href="#">PPH02052A</a>
D04	HNF4A	-6.68	A	<a href="#">PPH05540A</a>
G06	TFRC	-4.59		<a href="#">PPH00990H</a>
B12	EGR1	-4.03		<a href="#">PPH00139A</a>
C05	F10	-2.83	B	<a href="#">PPH17879A</a>
C11	HIF1A	-2.43		<a href="#">PPH01361B</a>
C07	FOS	-2.39		<a href="#">PPH00094A</a>
F11	PLAU	-2.22		<a href="#">PPH00796C</a>
H10	PPC	-2.11		
B09	EDN1	-2.04		<a href="#">PPH00653A</a>

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

Group 2 vs. Control Group



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
E02	MMP9	77.17	A	<a href="#">PPH00152E</a>
B03	CA9	30.91		<a href="#">PPH01751A</a>
A04	ANGPTL4	9.19	A	<a href="#">PPH02234F</a>
E03	MXI1	5.74		<a href="#">PPH11413A</a>
E06	NDRG1	5.66		<a href="#">PPH02202B</a>
F03	PFKFB4	5.43		<a href="#">PPH14224A</a>
G12	VEGFA	5.13		<a href="#">PPH00251C</a>
F02	PFKFB3	4.66		<a href="#">PPH00024F</a>
B12	EGR1	3.16		<a href="#">PPH00139A</a>
E12	PDK1	2.89		<a href="#">PPH20207A</a>
C10	GYS1	2.69		<a href="#">PPH00988C</a>
A12	BNIP3	2.62		<a href="#">PPH00301C</a>
B01	BNIP3L	2.46		<a href="#">PPH12205C</a>
F05	PFKP	2.39		<a href="#">PPH08642E</a>
C03	EPO	2.10	B	<a href="#">PPH01338C</a>

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## Genes Under-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
D04	HNF4A	-14.52	A	<a href="#">PPH05540A</a>
F11	PLAU	-13.18		<a href="#">PPH00796C</a>
G07	TP53	-6.87		<a href="#">PPH00213F</a>
G02	SERPINE1	-5.66		<a href="#">PPH00215F</a>
B11	EGLN2	-3.58		<a href="#">PPH16948B</a>
D10	LOX	-3.56		<a href="#">PPH01956A</a>
C11	HIF1A	-2.71		<a href="#">PPH01361B</a>
H01	ACTB	-2.43		<a href="#">PPH00073G</a>
H04	HPRT1	-2.39		<a href="#">PPH01018C</a>
C06	F3	-2.30		<a href="#">PPH02227G</a>
D12	MET	-2.28		<a href="#">PPH00194A</a>
D05	IER3	-2.27		<a href="#">PPH10008F</a>
G10	USF2	-2.20		<a href="#">PPH01580A</a>
E07	NFKB1	-2.19		<a href="#">PPH00204F</a>
D03	HMOX1	-2.07		<a href="#">PPH00161F</a>

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## 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.

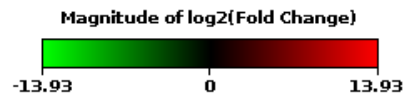
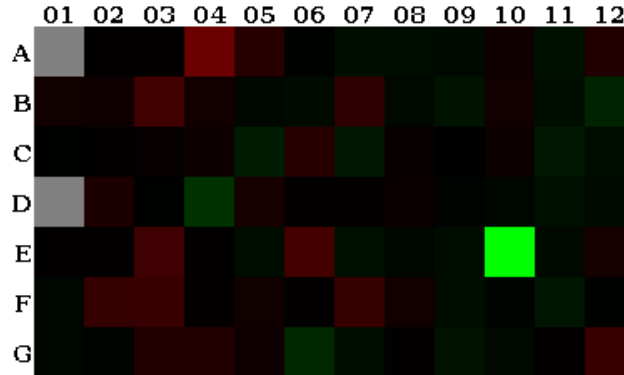
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<b>Test Group</b>	<b>Control Group</b>	<b>Fold Regulation Threshold</b>	<b>p-Value Threshold</b>
Group 2	Control Group	2	0.05

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

## Heat Map

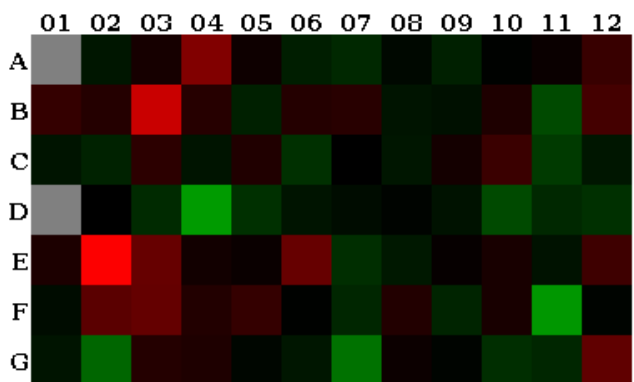
Treated Group	Control Group
Group 1	Control Group



Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ADM 1.21 C	ADORA2B 1.09	ALDOA 1.05	ANGPTL4 56.89 A	ANKRD37 4.32	ANXA2 -1.06	APEX1 -1.65	ARNT -1.64	ATR -1.44	BHLHE40 1.75	BLM -1.83	BNIP3 3.46
B	BNIP3L 1.82	BTG1 1.65	CA9 11.55	CCNG2 1.97	COP55 -1.27	CTSA -1.41	DDIT4 5.86	DNAJC5 -1.33	EDN1 -2.04	EGLN1 1.96	EGLN2 -1.74	EGR1 -4.03
C	EIF4EBP1 -1.07	ENO1 1.04	EPO 1.27 B	ERO1A 1.64	F10 -2.83 B	F3 4.14	FOS -2.39	GBE1 1.39	GPI 1.04	GYS1 1.61	HIF1A -2.43	HIF1AN -1.56
D	HIF3A 1.21 C	HK2 2.93	HMOX1 -1.09	HNF4A -6.68 A	IER3 2.30	IGFBP3 1.05	JMJD6 1.13	LDHA 1.42	LGALS3 -1.18	LOX -1.25	MAP3K1 -1.77	MET -1.47
E	MIF 1.10	MMP9 1.08 B	MXI1 10.41	NAMPT 1.06	NCOA1 -1.54	NDRG1 12.82	NFKB1 -1.78	NOS3 -1.27 B	ODC1 -1.60	P4HA1 -15608.02 A	P4HB -1.34	PDK1 2.27
F	PER1 -1.27	PFKFB3 6.96	PFKFB4 8.06	PFKL 1.16	PFKP 1.77	PGAM1 1.10	PGF 7.46	PGK1 1.93	PIM1 -1.56	PKM -1.16	PLAU -2.22	RBPJ -1.08
G	RUVBL2 -1.25	SERPINE1 -1.13	SLC16A3 3.48 A	SLC2A1 3.29	SLC2A3 1.68	TFRC -4.59	TP53 -1.75	TP11 1.09	TXNIP -2.00	USF2 -1.37	VDAC1 1.04	VEGFA 8.40

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.

<b>Treated Group</b>	<b>Control Group</b>
Group 2	Control Group

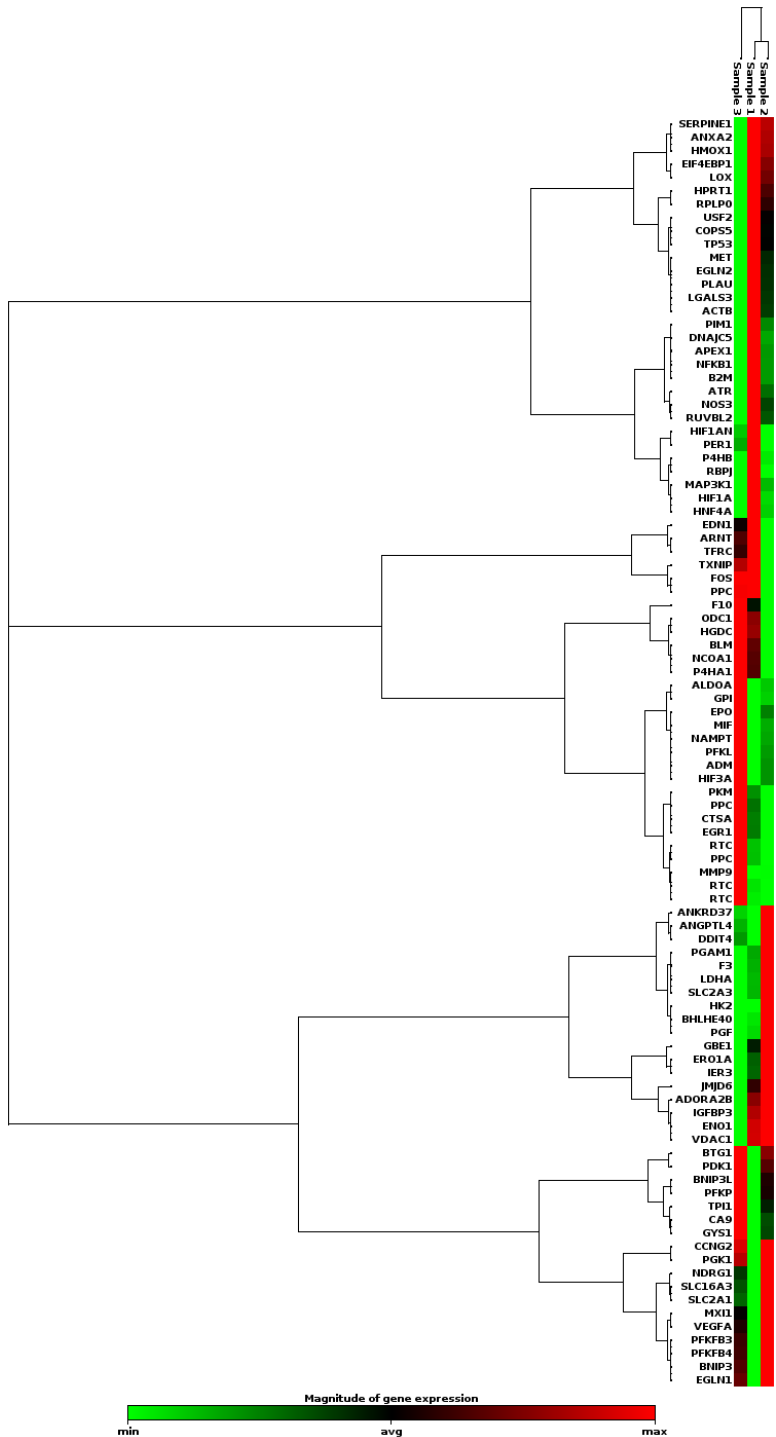


Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ADM	ADORA2B	ALDOA	ANGPTL4	ANKRD37	ANXA2	APEX1	ARNT	ATR	BHLHE40	BLM	BNIP3
	1.99	-1.46	1.47	9.19	1.28	-1.65	-1.96	-1.16	-1.75	-1.04	1.21	2.62
	C			A								
B	BNIP3L	BTG1	CA9	CCNG2	COPS5	CTSA	DDIT4	DNAJC5	EDN1	EGLN1	EGLN2	EGR1
	2.46	1.84	30.91	1.91	-1.71	1.87	1.96	-1.42	-1.32	1.67	-3.58	3.16
C	EIF4EBP1	ENO1	EPO	ERO1A	F10	F3	FOS	GBE1	GPI	GYS1	HIF1A	HIF1AN
	-1.39	-1.78	2.10	-1.40	1.74	-2.30	-1.00	-1.46	1.39	2.69	-2.71	-1.47
			B		B							
D	HIF3A	HK2	HMOX1	HNF4A	IER3	IGFBP3	JMJD6	LDHA	LGALS3	LOX	MAP3K1	MET
	1.99	-1.00	-2.07	-14.52	-2.27	-1.39	-1.22	-1.07	-1.34	-3.56	-2.00	-2.28
	C			A								
E	MIF	MMP9	MXI1	NAMPT	NCOA1	NDRG1	NFKB1	NOS3	ODC1	P4HA1	P4HB	PDK1
	1.64	77.17	5.74	1.38	1.17	5.66	-2.19	-1.52	1.11	1.52	-1.36	2.89
		A						B				
F	PER1	PFKFB3	PFKFB4	PFKL	PFKP	PGAM1	PGF	PGK1	PIM1	PKM	PLAU	RBPJ
	-1.21	4.66	5.43	1.80	2.39	-1.02	-1.89	1.80	-1.87	1.49	-13.18	-1.08
							A					
G	RUVBL2	SERPINE1	SLC16A3	SLC2A1	SLC2A3	TFRC	TP53	TPI1	TXNIP	USF2	VDAC1	VEGFA
	-1.42	-5.66	1.84	1.69	-1.13	-1.45	-6.87	1.21	-1.08	-2.20	-1.88	5.13
			B									

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.

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## What's next?

Thank you for using the RT<sup>2</sup> 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

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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
E10	P4HA1	-15608.02	N/A	<a href="#">PPH02052A</a>	<a href="#">EPHS101701-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
A04	ANGPTL4	56.89	N/A	<a href="#">PPH02234F</a>	<a href="#">EPHS106986-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
E06	NDRG1	12.82	N/A	<a href="#">PPH02202B</a>	<a href="#">EPHS114068-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B03	CA9	11.55	N/A	<a href="#">PPH01751A</a>	<a href="#">EPHS114317-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
E03	MXI1	10.41	N/A	<a href="#">PPH11413A</a>	<a href="#">EPHS101915-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G12	VEGFA	8.40	N/A	<a href="#">PPH00251C</a>	<a href="#">EPHS112506-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F03	PFKFB4	8.06	N/A	<a href="#">PPH14224A</a>	<a href="#">EPHS110233-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F07	PGF	7.46	N/A	<a href="#">PPH01155F</a>	<a href="#">EPHS104212-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F02	PFKFB3	6.96	N/A	<a href="#">PPH00024F</a>	<a href="#">EPHS101462-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
D04	HNF4A	-6.68	N/A	<a href="#">PPH05540A</a>	<a href="#">Inquire</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B07	DDIT4	5.86	N/A	<a href="#">PPH11172C</a>	<a href="#">EPHS101697-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G06	TFRC	-4.59	N/A	<a href="#">PPH00990H</a>	<a href="#">EPHS110800-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
A05	ANKRD37	4.32	N/A	<a href="#">PPH65925A</a>	<a href="#">EPHS111391-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
C06	F3	4.14	N/A	<a href="#">PPH02227G</a>	<a href="#">EPHS100727-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B12	EGR1	-4.03	N/A	<a href="#">PPH00139A</a>	<a href="#">EPHS111821-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G03	SLC16A3	3.48	N/A	<a href="#">PPH18809E</a>	<a href="#">EPHS106450-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
A12	BNIP3	3.46	N/A	<a href="#">PPH00301C</a>	<a href="#">EPHS102009-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G04	SLC2A1	3.29	N/A	<a href="#">PPH02043C</a>	<a href="#">EPHS100481-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
D02	HK2	2.93	N/A	<a href="#">PPH00983B</a>	<a href="#">EPHS108346-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
C05	F10	-2.83	N/A	<a href="#">PPH17879A</a>	<a href="#">EPHS103896-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>

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
E02	MMP9	77.17	N/A	<a href="#">PPH00152E</a>	<a href="#">EPHS109314-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B03	CA9	30.91	N/A	<a href="#">PPH01751A</a>	<a href="#">EPHS114317-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
D04	HNF4A	-14.52	N/A	<a href="#">PPH05540A</a>	<a href="#">Inquire</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F11	PLAU	-13.18	N/A	<a href="#">PPH00796C</a>	<a href="#">EPHS101714-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
A04	ANGPTL4	9.19	N/A	<a href="#">PPH02234F</a>	<a href="#">EPHS106986-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G07	TP53	-6.87	N/A	<a href="#">PPH00213F</a>	<a href="#">EPHS105711-1A</a>	<a href="#">View siRNAs</a>	<a href="#">SMPH014901A</a>
E03	MXI1	5.74	N/A	<a href="#">PPH11413A</a>	<a href="#">EPHS101915-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
E06	NDRG1	5.66	N/A	<a href="#">PPH02202B</a>	<a href="#">EPHS114068-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G02	SERPINE1	-5.66	N/A	<a href="#">PPH00215F</a>	<a href="#">Inquire</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F03	PFKFB4	5.43	N/A	<a href="#">PPH14224A</a>	<a href="#">EPHS110233-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
G12	VEGFA	5.13	N/A	<a href="#">PPH00251C</a>	<a href="#">EPHS112506-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
F02	PFKFB3	4.66	N/A	<a href="#">PPH00024F</a>	<a href="#">EPHS101462-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B11	EGLN2	-3.58	N/A	<a href="#">PPH16948B</a>	<a href="#">EPHS107507-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
D10	LOX	-3.56	N/A	<a href="#">PPH01956A</a>	<a href="#">EPHS111733-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B12	EGR1	3.16	N/A	<a href="#">PPH00139A</a>	<a href="#">EPHS111821-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
E12	PDK1	2.89	N/A	<a href="#">PPH20207A</a>	<a href="#">EPHS108684-1A</a>	<a href="#">View siRNAs</a>	<a href="#">SMPH086715A</a>
C11	HIF1A	-2.71	N/A	<a href="#">PPH01361B</a>	<a href="#">EPHS104131-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
C10	GYS1	2.69	N/A	<a href="#">PPH00988C</a>	<a href="#">EPHS107707-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
A12	BNIP3	2.62	N/A	<a href="#">PPH00301C</a>	<a href="#">EPHS102009-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>
B01	BNIP3L	2.46	N/A	<a href="#">PPH12205C</a>	<a href="#">EPHS113723-1A</a>	<a href="#">View siRNAs</a>	<a href="#">Inquire</a>

## 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
E10	P4HA1	-1.5608.02	N/A
D04	HNF4A	-6.68	N/A
G06	TFRC	-4.59	N/A
B12	EGR1	-4.03	N/A
C05	F10	-2.83	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-122-5p	-0.47	<a href="#">MS00003416</a>	<a href="#">MSY0000421</a>	<a href="#">MIN0000421</a>	P4HA1
hsa-miR-518f-3p	-0.46	<a href="#">MS00007735</a>	<a href="#">MSY0002842</a>	<a href="#">MIN0002842</a>	EGR1
hsa-miR-518b	-0.46	<a href="#">MS00004466</a>	<a href="#">MSY0002844</a>	<a href="#">MIN0002844</a>	EGR1
hsa-miR-518c-3p	-0.46	<a href="#">MS00004473</a>	<a href="#">MSY0002848</a>	<a href="#">MIN0002848</a>	EGR1
hsa-miR-518a-3p	-0.46	<a href="#">MS00007063</a>	<a href="#">MSY0002863</a>	<a href="#">MIN0002863</a>	EGR1
hsa-miR-518d-3p	-0.46	<a href="#">MS00009968</a>	<a href="#">MSY0002864</a>	<a href="#">MIN0002864</a>	EGR1
hsa-miR-499a-5p	-0.43	<a href="#">MS00004375</a>	<a href="#">MSY0002870</a>	<a href="#">MIN0002870</a>	P4HA1
hsa-miR-516b-5p	-0.41	<a href="#">MS00007749</a>	<a href="#">MSY0002859</a>	<a href="#">MIN0002859</a>	EGR1
hsa-miR-545-3p	-0.37	<a href="#">MS00007777</a>	<a href="#">MSY0003165</a>	<a href="#">MIN0003165</a>	HNF4A
hsa-miR-485-5p	-0.37	<a href="#">MS00006972</a>	<a href="#">MSY0002175</a>	<a href="#">MIN0002175</a>	HNF4A
hsa-miR-31-5p	-0.37	<a href="#">MS00003290</a>	<a href="#">MSY0000089</a>	<a href="#">MIN0000089</a>	TFRC
hsa-miR-544a	-0.32	<a href="#">MS00004585</a>	<a href="#">MSY0003164</a>	<a href="#">MIN0003164</a>	TFRC
hsa-miR-559	-0.30	<a href="#">MS00004683</a>	<a href="#">MSY0003223</a>	<a href="#">MIN0003223</a>	EGR1
hsa-miR-494-3p	-0.30	<a href="#">MS00004340</a>	<a href="#">MSY0002816</a>	<a href="#">MIN0002816</a>	P4HA1
hsa-miR-583	-0.29	<a href="#">MS00004851</a>	<a href="#">MSY0003248</a>	<a href="#">MIN0003248</a>	TFRC
hsa-miR-450b-5p	-0.28	<a href="#">MS00009716</a>	<a href="#">MSY0004909</a>	<a href="#">MIN0004909</a>	TFRC
hsa-miR-1827	-0.28	<a href="#">MS00014686</a>	<a href="#">MSY0006767</a>	<a href="#">MIN0006767</a>	TFRC
hsa-miR-524-5p	-0.27	<a href="#">MS00007119</a>	<a href="#">MSY0002849</a>	<a href="#">MIN0002849</a>	EGR1
hsa-miR-548a-5p	-0.27	<a href="#">MS00010108</a>	<a href="#">MSY0004803</a>	<a href="#">MIN0004803</a>	EGR1
hsa-miR-548i	-0.27	<a href="#">MS00014763</a>	<a href="#">MSY0005935</a>	<a href="#">MIN0005935</a>	EGR1

## Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
A04	ANGPTL4	56.89	N/A
E06	NDRG1	12.82	N/A
B03	CA9	11.55	N/A
E03	MXI1	10.41	N/A
G12	VEGFA	8.40	N/A
F03	PFKFB4	8.06	N/A
F07	PGF	7.46	N/A
F02	PFKFB3	6.96	N/A
B07	DDIT4	5.86	N/A
A05	ANKRD37	4.32	N/A
C06	F3	4.14	N/A
G03	SLC16A3	3.48	N/A
A12	BNIP3	3.46	N/A
G04	SLC2A1	3.29	N/A
D02	HK2	2.93	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-5481	-0.56	<a href="#">MS00014784</a>	<a href="#">MSY0005889</a>	<a href="#">MIN0005889</a>	F3
hsa-miR-29a-3p	-0.53	<a href="#">MS00003262</a>	<a href="#">MSY0000086</a>	<a href="#">MIN0000086</a>	VEGFA
hsa-miR-29c-3p	-0.53	<a href="#">MS00003269</a>	<a href="#">MSY0000681</a>	<a href="#">MIN0000681</a>	VEGFA
hsa-miR-29b-3p	-0.53	<a href="#">MS00006566</a>	<a href="#">MSY0000100</a>	<a href="#">MIN0000100</a>	VEGFA
hsa-miR-578	-0.52	<a href="#">MS00004816</a>	<a href="#">MSY0003243</a>	<a href="#">MIN0003243</a>	VEGFA
hsa-miR-361-5p	-0.49	<a href="#">MS00004032</a>	<a href="#">MSY0000703</a>	<a href="#">MIN0000703</a>	VEGFA
hsa-miR-495-3p	-0.47	<a href="#">MS00004347</a>	<a href="#">MSY0002817</a>	<a href="#">MIN0002817</a>	DDIT4
hsa-miR-1297	-0.47	<a href="#">MS00014567</a>	<a href="#">MSY0005886</a>	<a href="#">MIN0005886</a>	MXI1, PFKFB3
hsa-miR-153-3p	-0.47	<a href="#">MS00008771</a>	<a href="#">MSY0000439</a>	<a href="#">MIN0000439</a>	DDIT4
hsa-miR-26b-5p	-0.47	<a href="#">MS00003234</a>	<a href="#">MSY0000083</a>	<a href="#">MIN0000083</a>	MXI1, PFKFB3
hsa-miR-26a-5p	-0.47	<a href="#">MS00006559</a>	<a href="#">MSY0000082</a>	<a href="#">MIN0000082</a>	MXI1, PFKFB3
hsa-miR-142-5p	-0.44	<a href="#">MS00006671</a>	<a href="#">MSY0000433</a>	<a href="#">MIN0000433</a>	BNIP3
hsa-miR-889-3p	-0.44	<a href="#">MS00010710</a>	<a href="#">MSY0004921</a>	<a href="#">MIN0004921</a>	VEGFA
hsa-miR-143-3p	-0.42	<a href="#">MS00003514</a>	<a href="#">MSY0000435</a>	<a href="#">MIN0000435</a>	HK2
hsa-miR-224-5p	-0.41	<a href="#">MS00003878</a>	<a href="#">MSY0000281</a>	<a href="#">MIN0000281</a>	PFKFB3
hsa-miR-205-5p	-0.40	<a href="#">MS00003780</a>	<a href="#">MSY0000266</a>	<a href="#">MIN0000266</a>	VEGFA
hsa-miR-548p	-0.39	<a href="#">MS00014812</a>	<a href="#">MSY0005934</a>	<a href="#">MIN0005934</a>	VEGFA
hsa-miR-299-3p	-0.39	<a href="#">MS00006783</a>	<a href="#">MSY0000687</a>	<a href="#">MIN0000687</a>	VEGFA
hsa-miR-633	-0.38	<a href="#">MS00005194</a>	<a href="#">MSY0003303</a>	<a href="#">MIN0003303</a>	HK2
hsa-miR-30a-5p	-0.38	<a href="#">MS00007350</a>	<a href="#">MSY0000087</a>	<a href="#">MIN0000087</a>	DDIT4

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	-14.52	N/A
F11	PLAU	-13.18	N/A
G07	TP53	-6.87	N/A
G02	SERPINE1	-5.66	N/A
B11	EGLN2	-3.58	N/A
D10	LOX	-3.56	N/A
C11	HIF1A	-2.71	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-549a	-0.52	<a href="#">MS00004613</a>	<a href="#">MSY0003333</a>	<a href="#">MIN0003333</a>	HIF1A
hsa-miR-580-3p	-0.45	<a href="#">MS00004830</a>	<a href="#">MSY0003245</a>	<a href="#">MIN0003245</a>	HIF1A
hsa-miR-1825	-0.43	<a href="#">MS00014672</a>	<a href="#">MSY0006765</a>	<a href="#">MIN0006765</a>	SERPINE1
hsa-miR-150-5p	-0.41	<a href="#">MS00003577</a>	<a href="#">MSY0000451</a>	<a href="#">MIN0000451</a>	TP53
hsa-miR-889-3p	-0.40	<a href="#">MS00010710</a>	<a href="#">MSY0004921</a>	<a href="#">MIN0004921</a>	HIF1A
hsa-miR-485-5p	-0.37	<a href="#">MS00006972</a>	<a href="#">MSY0002175</a>	<a href="#">MIN0002175</a>	HNF4A
hsa-miR-545-3p	-0.37	<a href="#">MS00007777</a>	<a href="#">MSY0003165</a>	<a href="#">MIN0003165</a>	HNF4A
hsa-miR-181c-5p	-0.35	<a href="#">MS00003612</a>	<a href="#">MSY0000258</a>	<a href="#">MIN0000258</a>	PLAU, SERPINE1
hsa-miR-181a-5p	-0.35	<a href="#">MS00006685</a>	<a href="#">MSY0000256</a>	<a href="#">MIN0000256</a>	PLAU, SERPINE1
hsa-miR-181b-5p	-0.35	<a href="#">MS00006699</a>	<a href="#">MSY0000257</a>	<a href="#">MIN0000257</a>	PLAU, SERPINE1
hsa-miR-181d-5p	-0.35	<a href="#">MS00003619</a>	<a href="#">MSY0002821</a>	<a href="#">MIN0002821</a>	PLAU, SERPINE1
hsa-miR-18a-5p	-0.34	<a href="#">MS00007322</a>	<a href="#">MSY0000072</a>	<a href="#">MIN0000072</a>	HIF1A
hsa-miR-1202	-0.33	<a href="#">MS00014147</a>	<a href="#">MSY0005865</a>	<a href="#">MIN0005865</a>	EGLN2
hsa-miR-18b-5p	-0.32	<a href="#">MS00007329</a>	<a href="#">MSY0001412</a>	<a href="#">MIN0001412</a>	HIF1A
hsa-miR-1294	-0.32	<a href="#">MS00014546</a>	<a href="#">MSY0005884</a>	<a href="#">MIN0005884</a>	TP53
hsa-miR-1207-5p	-0.31	<a href="#">MS00014189</a>	<a href="#">MSY0005871</a>	<a href="#">MIN0005871</a>	EGLN2
hsa-miR-765	-0.31	<a href="#">MS00005432</a>	<a href="#">MSY0003945</a>	<a href="#">MIN0003945</a>	EGLN2
hsa-miR-134-5p	-0.30	<a href="#">MS00003465</a>	<a href="#">MSY0000447</a>	<a href="#">MIN0000447</a>	SERPINE1
hsa-miR-30b-5p	-0.29	<a href="#">MS00003276</a>	<a href="#">MSY0000420</a>	<a href="#">MIN0000420</a>	SERPINE1
hsa-miR-30c-5p	-0.29	<a href="#">MS00006580</a>	<a href="#">MSY0000244</a>	<a href="#">MIN0000244</a>	SERPINE1

## Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
E02	MMP9	77.17	N/A
B03	CA9	30.91	N/A
A04	ANGPTL4	9.19	N/A
E03	MXI1	5.74	N/A
E06	NDRG1	5.66	N/A
F03	PFKFB4	5.43	N/A
G12	VEGFA	5.13	N/A
F02	PFKFB3	4.66	N/A
B12	EGR1	3.16	N/A
E12	PDK1	2.89	N/A
C10	GYS1	2.69	N/A
A12	BNIP3	2.62	N/A
B01	BNIP3L	2.46	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-29c-3p	-0.53	<a href="#">MS00003269</a>	<a href="#">MSY0000681</a>	<a href="#">MIN0000681</a>	VEGFA
hsa-miR-29a-3p	-0.53	<a href="#">MS00003262</a>	<a href="#">MSY0000086</a>	<a href="#">MIN0000086</a>	VEGFA
hsa-miR-29b-3p	-0.53	<a href="#">MS00006566</a>	<a href="#">MSY0000100</a>	<a href="#">MIN0000100</a>	VEGFA
hsa-miR-548g-3p	-0.52	<a href="#">MS00014749</a>	<a href="#">MSY0005912</a>	<a href="#">MIN0005912</a>	BNIP3L
hsa-miR-578	-0.52	<a href="#">MS00004816</a>	<a href="#">MSY0003243</a>	<a href="#">MIN0003243</a>	VEGFA
hsa-miR-361-5p	-0.49	<a href="#">MS00004032</a>	<a href="#">MSY0000703</a>	<a href="#">MIN0000703</a>	VEGFA
hsa-miR-379-5p	-0.48	<a href="#">MS00004109</a>	<a href="#">MSY0000733</a>	<a href="#">MIN0000733</a>	PDK1
hsa-miR-1297	-0.47	<a href="#">MS00014567</a>	<a href="#">MSY0005886</a>	<a href="#">MIN0005886</a>	MXI1, PFKFB3
hsa-miR-26b-5p	-0.47	<a href="#">MS00003234</a>	<a href="#">MSY0000083</a>	<a href="#">MIN0000083</a>	MXI1, PFKFB3
hsa-miR-26a-5p	-0.47	<a href="#">MS00006559</a>	<a href="#">MSY0000082</a>	<a href="#">MIN0000082</a>	MXI1, PFKFB3
hsa-miR-518f-3p	-0.46	<a href="#">MS00007735</a>	<a href="#">MSY0002842</a>	<a href="#">MIN0002842</a>	EGR1
hsa-miR-518c-3p	-0.46	<a href="#">MS00004473</a>	<a href="#">MSY0002848</a>	<a href="#">MIN0002848</a>	EGR1
hsa-miR-518b	-0.46	<a href="#">MS00004466</a>	<a href="#">MSY0002844</a>	<a href="#">MIN0002844</a>	EGR1
hsa-miR-518d-3p	-0.46	<a href="#">MS00009968</a>	<a href="#">MSY0002864</a>	<a href="#">MIN0002864</a>	EGR1
hsa-miR-518a-3p	-0.46	<a href="#">MS00007063</a>	<a href="#">MSY0002863</a>	<a href="#">MIN0002863</a>	EGR1
hsa-miR-576-5p	-0.46	<a href="#">MS00007798</a>	<a href="#">MSY0003241</a>	<a href="#">MIN0003241</a>	VEGFA
hsa-miR-34b-3p	-0.44	<a href="#">MS00008190</a>	<a href="#">MSY0004676</a>	<a href="#">MIN0004676</a>	BNIP3L
hsa-miR-142-5p	-0.44	<a href="#">MS00006671</a>	<a href="#">MSY0000433</a>	<a href="#">MIN0000433</a>	BNIP3
hsa-miR-889-3p	-0.44	<a href="#">MS00010710</a>	<a href="#">MSY0004921</a>	<a href="#">MIN0004921</a>	VEGFA
hsa-miR-224-5p	-0.41	<a href="#">MS00003878</a>	<a href="#">MSY0000281</a>	<a href="#">MIN0000281</a>	PFKFB3

## 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
E10	P4HA1	-15608.02	N/A	RFX1, Brachyury	<a href="#">GPH1015783(-)01A</a>
A04	ANGPTL4	56.89	N/A	FOXO1, FOXO1a, FOXO1, FOXO1a, TBP	<a href="#">GPH1006507(-)01A</a>
E06	NDRG1	12.82	N/A	LyF-1, MyoD, C/EBPalpha, C/EBPbeta, Hlf	<a href="#">GPH1026403(-)01A</a>
B03	CA9	11.55	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	<a href="#">GPH1012980(-)01A</a>
E03	MX11	10.41	N/A	RFX1, Brachyury, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, Pax-5, Nkx5-1, E2F, E2F-1, E2F-2, E2F-3a, E2F-4, E2F-5	<a href="#">GPH1001880(-)01A</a>
G12	VEGFA	8.40	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, E47, Hand1, HTF, XBP-1, AP-1, Pax-4a, LyF-1, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, C/EBPalpha	<a href="#">GPH1011376(-)01A</a>
F03	PFKFB4	8.06	N/A	Zic1, Zic3, Sp1, c-Ets-1, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C	<a href="#">GPH1022965(-)01A</a>
F07	PGF	7.46	N/A	NF-kappaB1, NF-kappaB1, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4, MyoD	<a href="#">GPH1018061(-)01A</a>
F02	PFKFB3	6.96	N/A	CREB, deltaCREB, Elk-1, c-Ets-1, Arnt, USF-1, USF1, USF-1:USF-2, USF2, CREB, deltaCREB, SRF, SRF (504 AA), HNF-3beta, SEF-1 (1), PPAR-gamma1, PPAR-gamma2	<a href="#">GPH1001442(-)01A</a>
D04	HNF4A	-6.68	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	<a href="#">GPH1008442(-)01A</a>
B07	DDIT4	5.86	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)	<a href="#">GPH1001673(-)01A</a>

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
G06	TFRC	-4.59	N/A	ARP-1, E2F, E2F-1, E2F-2, E2F-3a, E2F-4, E2F-5, E47, Lmo2, MyoD, GCMF, GCMF-1, GCMF-2, HOXA3, Evi-1	<a href="#">GPH1023484(-)01A</a>
A05	ANKRD37	4.32	N/A	YY1	<a href="#">GPH1010345(-)01A</a>
C06	F3	4.14	N/A	NF-kappaB1, COMP1, Pax-5, c-Rel, RelA, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Bach2, AP-2rep, ISGF-3, IRF-1, IRF-1, IRF-2	<a href="#">GPH1014720(-)01A</a>
B12	EGR1	-4.03	N/A	Sp1, LCR-F1, AhR, Arnt, STAT3, NF-kappaB1, CREB, deltaCREB, AP-2alpha, AP-2alphaA, AP-2beta, AP-2gamma, STAT3, Elk-1, YY1, SRF, SRF (504 AA), TBP, HSF2, SRF, SRF (504 AA), SRF, SRF (504 AA), YY1, SRF, SRF (504 AA), Elk-1, c-Ets-1, NRF-2, LyF-1, Ikk-3, RelA, ATF6, ATF-2, c-Jun, CREB, deltaCREB, SRF, SRF (504 AA), Elk-1, c-Ets-1, SRF, SRF (504 AA), TBP, SRF, SRF (504 AA), CREB, deltaCREB, ATF, ATF-2, CRE-BP1, Egr-1, GATA-1, NF-kappaB1, AhR, Arnt	<a href="#">GPH1010751(-)01A</a>
G03	SLC16A3	3.48	N/A		<a href="#">GPH1006113(-)01A</a>
A12	BNIP3	3.46	N/A	Sp1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC	<a href="#">GPH1016023(-)01A</a>
G04	SLC2A1	3.29	N/A	PPAR-gamma1, PPAR-gamma2, Brachyury	<a href="#">GPH1014532(-)01A</a>
D02	HK2	2.93	N/A		<a href="#">GPH1007554(-)01A</a>
C05	F10	-2.83	N/A	HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, Lmo2, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1	<a href="#">GPH1003755(-)01A</a>

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
E02	MMP9	77.17	N/A	GR, GR-alpha, GR-beta, NF-E2, NF-E2 p45, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Pax-4a, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4, Pax-2, Pax-2a, AhR, Arnt, Bach2, c-Jun, AP-1, c-Fos, NF-E2, NF-E2 p45, MAZR, Sp1	<a href="#">GPH1008476(-)01A</a>
B03	CA9	30.91	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	<a href="#">GPH1012980(-)01A</a>
D04	HNF4A	-14.52	N/A	HOXA5, TBP, TFIID, MZF-1, Pax-2, Pax-2a, RFX1, LUN-1, Mx-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	<a href="#">GPH1008442(-)01A</a>
F11	PLAU	-13.18	N/A	Sp1	<a href="#">GPH1001684(-)01A</a>
A04	ANGPTL4	9.19	N/A	FOXO1, FOXO1a, FOXO1, FOXO1a, TBP	<a href="#">GPH1006507(-)01A</a>
G07	TP53	-6.87	N/A	Olf-1, NCX/Ncx	<a href="#">GPH1019297(-)01A</a>
E03	MXI1	5.74	N/A	RFX1, Brachyury, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, Pax-5, Nkx5-1, E2F, E2F-1, E2F-2, E2F-3a, E2F-4, E2F-5	<a href="#">GPH1001880(-)01A</a>
E06	NDRG1	5.66	N/A	LyF-1, MyoD, C/EBPalpha, C/EBPbeta, Hif	<a href="#">GPH1026403(-)01A</a>
G02	SERPINE1	-5.66	N/A	SREBP-1a, SREBP-1b, SREBP-1c, SREBP-1a, SREBP-1b, SREBP-1c, SREBP-1a, SREBP-1b, SREBP-1c, C/EBPbeta, TBP	<a href="#">GPH1012140(-)01A</a>
F03	PFKFB4	5.43	N/A	Zic1, Zic3, Sp1, c-Ets-1, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C	<a href="#">GPH1022965(-)01A</a>
G12	VEGFA	5.13	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, E47, Hand1, HTF, XBP-1, AP-1, Pax-4a, LyF-1, HNF-4alpha1, HNF-4alpha2, COUP, COUP-TF, COUP-TF1, C/EBPalpha	<a href="#">GPH1011376(-)01A</a>
F02	PFKFB3	4.66	N/A	CREB, deltaCREB, Elk-1, c-Ets-1, Arnt, USF-1, USF1, USF-1:USF-2, USF2, CREB, deltaCREB, SRF, SRF (504 AA), HNF-3beta, SEF-1 (1), PPAR-gamma1, PPAR-gamma2, C/EBPbeta, C/EBPalpha, GATA-1, IRF-1, IRF-2,	<a href="#">GPH1001442(-)01A</a>

Position	Gene Symbol	Fold Regulation	p-Value	Transcription Factors	EpiTect ChIP qPCR Assay
B11	EGLN2	-3.58	N/A	POU2F1, POU2F1a, POU2F1b, POU2F1c, ATF-2, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), POU2F1, POU2F1a, POU2F1b, POU2F1c, LCR-F1, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C, Arnt, c-Myc, Max1, SREBP-1a, SREBP-1b, SREBP-1c, N-Myc, USF-1, USF1, USF-1:USF-2, USF2	<a href="#">GPH1006881(-)01A</a>
D10	LOX	-3.56	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	<a href="#">GPH1024332(-)01A</a>
B12	EGR1	3.16	N/A	Sp1, LCR-F1, AhR, Arnt, STAT3, NF-kappaB1, CREB, deltaCREB, AP-2alpha, AP-2alphaA, AP-2beta, AP-2gamma, STAT3, Elk-1, YY1, SRF, SRF (504 AA), TBP, HSF2, SRF, SRF (504 AA), SRF, SRF (504 AA), YY1, SRF, SRF (504 AA), Elk-1, c-Ets-1, NRF-2, LyF-1, Ik-3, RelA, ATF6, ATF-2, c-Jun, CREB, deltaCREB, SRF, SRF (504 AA), Elk-1, c-Ets-1, SRF, SRF (504 AA), TBP, SRF, SRF (504 AA), CREB, deltaCREB, ATF, ATF-2, CRE-BP1, Egr-1, GATA-1, NF-kappaB1, AhR, Arnt	<a href="#">GPH1010751(-)01A</a>
E12	PDK1	2.89	N/A	NF-kappaB1, NF-kappaB, C/EBPbeta, Elk-1	<a href="#">GPH1007880(-)01A</a>
C11	HIF1A	-2.71	N/A	AhR, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, AhR, PPAR-gamma1, PPAR-gamma2, PPAR-gamma1, PPAR-gamma2, LyF-1, Pax-5, Arnt, c-Myc, Max, USF-1, USF1, c-Myc, Max1, SREBP-1a, SREBP-1b, SREBP-1c, CP2, AP-4, HEN1, NF-E2, NF-E2 p45, COMP1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, RREB-1, CP2, MAZR, Pax-4a	<a href="#">GPH1003965(-)01A</a>
C10	GYS1	2.69	N/A	C/EBPbeta, C/EBPalpha, IRF-7A, STAT1, AREB6, RFX1	<a href="#">GPH1020852(-)01A</a>
A12	BNIP3	2.62	N/A	Sp1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC	<a href="#">GPH1016023(-)01A</a>
B01	BNIP3L	2.46	N/A	ATF-2, c-Jun, NRF-2, SREBP-1a, SREBP-1b, SREBP-1c, c-Myc, Max1, ATF-2, c-Jun, CREB, deltaCREB	<a href="#">GPH1012553(-)01A</a>

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## Next steps

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

You can use individual qPCR RT<sup>2</sup> assays or create custom RT<sup>2</sup> 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.

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# 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.