RT² Profiler PCR Array Gene Expression Analysis Report

Customer Name

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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 12 samples with the PAHS-011Z.

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-011Z) 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

RT² Profiler™ PCR Array Human Inflammatory Cytokines & Receptors

Position	RefSeq Number	Symbol	Description
A01	NM_004757	AIMP1	Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1
A02	NM_001200	BMP2	Bone morphogenetic protein 2
A03	NM_001735	C5	Complement component 5
A04	NM_002981	CCL1	Chemokine (C-C motif) ligand 1
A05	NM_002986	CCL11	Chemokine (C-C motif) ligand 11
A06	NM_005408	CCL13	Chemokine (C-C motif) ligand 13
A07	NM_032965	CCL15	Chemokine (C-C motif) ligand 15
A08	NM_004590	CCL16	Chemokine (C-C motif) ligand 16
A09	NM_002987	CCL17	Chemokine (C-C motif) ligand 17
A10	NM_002982	CCL2	Chemokine (C-C motif) ligand 2
A11	NM_004591	CCL20	Chemokine (C-C motif) ligand 20
A12	NM_002990	CCL22	Chemokine (C-C motif) ligand 22
B01	NM_005064	CCL23	Chemokine (C-C motif) ligand 23
B02	NM_002991	CCL24	Chemokine (C-C motif) ligand 24
B03	NM_006072	CCL26	Chemokine (C-C motif) ligand 26
B04	NM_002983	CCL3	Chemokine (C-C motif) ligand 3
B05	NM_002984	CCL4	Chemokine (C-C motif) ligand 4
B06	NM_002985	CCL5	Chemokine (C-C motif) ligand 5
B07	NM_006273	CCL7	Chemokine (C-C motif) ligand 7
B08	NM_005623	CCL8	Chemokine (C-C motif) ligand 8
B09	NM_001295	CCR1	Chemokine (C-C motif) receptor 1
B10	NM_001123396	CCR2	Chemokine (C-C motif) receptor 2
B11	NM_001837	CCR3	Chemokine (C-C motif) receptor 3
B12	NM_005508	CCR4	Chemokine (C-C motif) receptor 4
C01	NM_000579	CCR5	Chemokine (C-C motif) receptor 5
C02	NM_004367	CCR6	Chemokine (C-C motif) receptor 6
C03	NM_005201	CCR8	Chemokine (C-C motif) receptor 8
C04	NM_000074	CD40LG	CD40 ligand
C05	NM_000757	CSF1	Colony stimulating factor 1 (macrophage)
C06	NM_000758	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
C07	NM_000759	CSF3	Colony stimulating factor 3 (granulocyte)
C08	NM_002996	CX3CL1	Chemokine (C-X3-C motif) ligand 1
C09	NM_001337	CX3CR1	Chemokine (C-X3-C motif) receptor 1
C10	NM_001511	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
C11	NM_001565	CXCL10	Chemokine (C-X-C motif) ligand 10
C12	NM_005409	CXCL11	Chemokine (C-X-C motif) ligand 11
D01	NM_000609	CXCL12	Chemokine (C-X-C motif) ligand 12
D02	NM_006419	CXCL13	Chemokine (C-X-C motif) ligand 13
D03	NM_002089	CXCL2	Chemokine (C-X-C motif) ligand 2
D04	NM_002090	CXCL3	Chemokine (C-X-C motif) ligand 3
D05	NM_002994	CXCL5	Chemokine (C-X-C motif) ligand 5
D06	NM_002993	CXCL6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
D07	NM_002416	CXCL9	Chemokine (C-X-C motif) ligand 9
D08	NM_000634	CXCR1	Chemokine (C-X-C motif) receptor 1
D09	NM_001557	CXCR2	Chemokine (C-X-C motif) receptor 2
D10	NM_000639	FASLG	Fas ligand (TNF superfamily, member 6)

Position	RefSeq Number	Symbol	Description
D11	NM_000605	IFNA2	Interferon, alpha 2
D12	NM_000619	IFNG	Interferon, gamma
E01	NM_001558	IL10RA	Interleukin 10 receptor, alpha
E02	NM_000628	IL10RB	Interleukin 10 receptor, beta
E03	NM_002188	IL13	Interleukin 13
E04	NM_000585	IL15	Interleukin 15
E05	NM_004513	IL16	Interleukin 16
E06	NM_002190	IL17A	Interleukin 17A
E07	NM_013278	IL17C	Interleukin 17C
E08	NM_052872	IL17F	Interleukin 17F
E09	NM_000575	IL1A	Interleukin 1, alpha
E10	NM_000576	IL1B	Interleukin 1, beta
E11	NM_000877	IL1R1	Interleukin 1 receptor, type I
E12	NM_000577	IL1RN	Interleukin 1 receptor antagonist
F01	NM_021803	IL21	Interleukin 21
F02	NM_145659	IL27	Interleukin 27
F03	NM_000588	IL3	Interleukin 3 (colony-stimulating factor, multiple)
F04	NM_033439	IL33	Interleukin 33
F05	NM_000879	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)
F06	NM_000564	IL5RA	Interleukin 5 receptor, alpha
F07	NM_000880	IL7	Interleukin 7
F08	NM_000584	CXCL8	Interleukin 8
F09	NM_000590	IL9	Interleukin 9
F10	NM_002186	IL9R	Interleukin 9 receptor
F11	NM_000595	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
F12	NM_002341	LTB	Lymphotoxin beta (TNF superfamily, member 3)
G01	NM_002415	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
G02	NM_005746	NAMPT	Nicotinamide phosphoribosyltransferase
G03	NM_020530	OSM	Oncostatin M
G04	NM_000582	SPP1	Secreted phosphoprotein 1
G05	NM_000594	TNF	Tumor necrosis factor
G06	NM_002546	TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b
G07	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10
G08	NM_003701	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11
G09	NM_003808	TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13
G10	NM_006573	TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b
G11	NM_003326	TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4
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
BRP1617P3A2_P1A3_C	BRP1617P3A2_P1A1_C	BRP1617P3A2_P1B1_C
BRP1617P3A2_P1A6_C	BRP1617P3A2_P1A2_C	BRP1617P3A2_P1B2_C
BRP1617P3A2_P1B3_C	BRP1617P3A2_P1A4_C	BRP1617P3A2_P1B4_C
BRP1617P3A2_P1B6_C	BRP1617P3A2_P1A5_C	BRP1617P3A2_P1B5_C

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	Check Samples: BRP1617P3A2_P1A2_C BRP1617P3A2_P1A4_C BRP1617P3A2_P1A5_C

Test Performed	**BRP1617P3A2_P1A2_C**	**BRP1617P3A2_P1A4_C**	**BRP1617P3A2_P1A5_C**
PCR Array Reproducibility			
Average C_T (PPC)	17.56	17.57	17.56
Result	Pass	Pass	Pass
Reverse Transcription Control (RTC)			
Delta C_T (Average RTC - Average PPC)	3.87	3.85	3.87
Result	Pass	Pass	Pass
Genomic DNA Contamination (GDC)			
C_T(GDC)	34.67	34.64	34.77
Result	Inquiry	Inquiry	Inquiry

Criteria for Genomic DNA Contamination (GDC): If C_T(GDC) >= 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'.

Normalization analysis

Automatic selection from HKG panel

Groups	**Samples**	**HPRT1**	**Geometric Mean**	**Average Geometric Mean**
Control Group	BRP1617P3A2_P1A3_C	24.59	24.59	24.61
Control Group	BRP1617P3A2_P1A6_C	24.56	24.56	
Control Group	BRP1617P3A2_P1B3_C	24.67	24.67	
Control Group	BRP1617P3A2_P1B6_C	24.61	24.61	
Group 1	BRP1617P3A2_P1A1_C	24.57	24.57	24.59
Group 1	BRP1617P3A2_P1A2_C	24.62	24.62	
Group 1	BRP1617P3A2_P1A4_C	24.61	24.61	
Group 1	BRP1617P3A2_P1A5_C	24.58	24.58	
Group 2	BRP1617P3A2_P1B1_C	24.69	24.69	24.70
Group 2	BRP1617P3A2_P1B2_C	24.74	24.74	
Group 2	BRP1617P3A2_P1B4_C	24.68	24.68	
Group 2	BRP1617P3A2_P1B5_C	24.71	24.71	

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**
A03	C5	-2.50	0.010061	
A11	CCL20	-4.10	0.001970	
B11	CCR3	-2.16	0.008105	
C06	CSF2	-7.70	0.000133	
C07	CSF3	-2.88	0.009959	
E10	IL1B	2.11	0.000003	

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
B07	CCL7	2.96	0.035185	
E06	IL17A	2.13	0.025846	

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

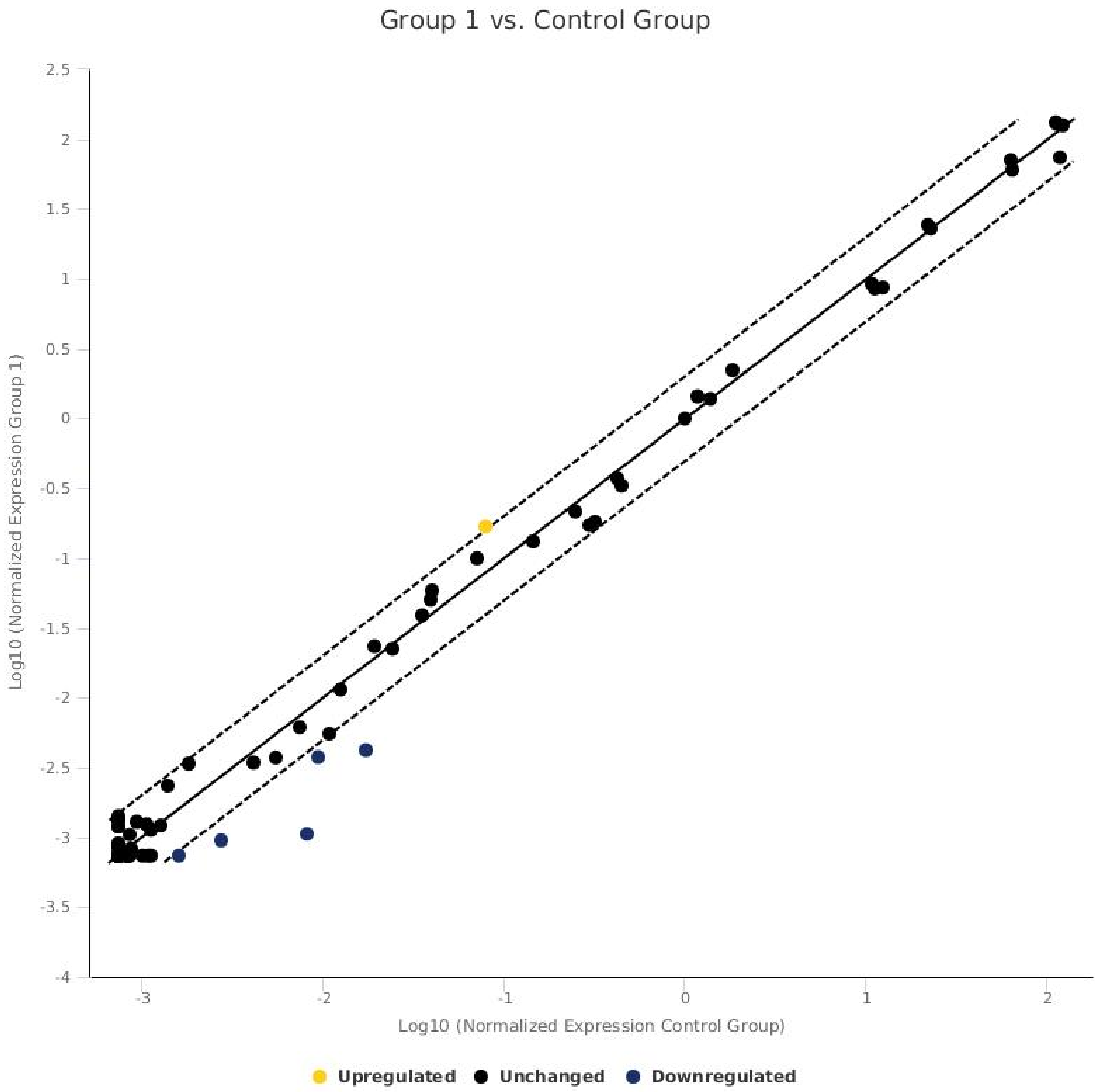
Position	**Gene Symbol**	**Fold Regulation**	**p-Value**	**Comments**
A03	C5	3.05	0.001214	
A11	CCL20	6.12	0.000013	A
C06	CSF2	5.04	0.001108	
C07	CSF3	2.45	0.011294	
D03	CXCL2	2.25	0.002617	
E06	IL17A	2.15	0.025103	
G11	TNFSF4	-2.15	0.017595	
H01	ACTB	2.06	0.000006	

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.

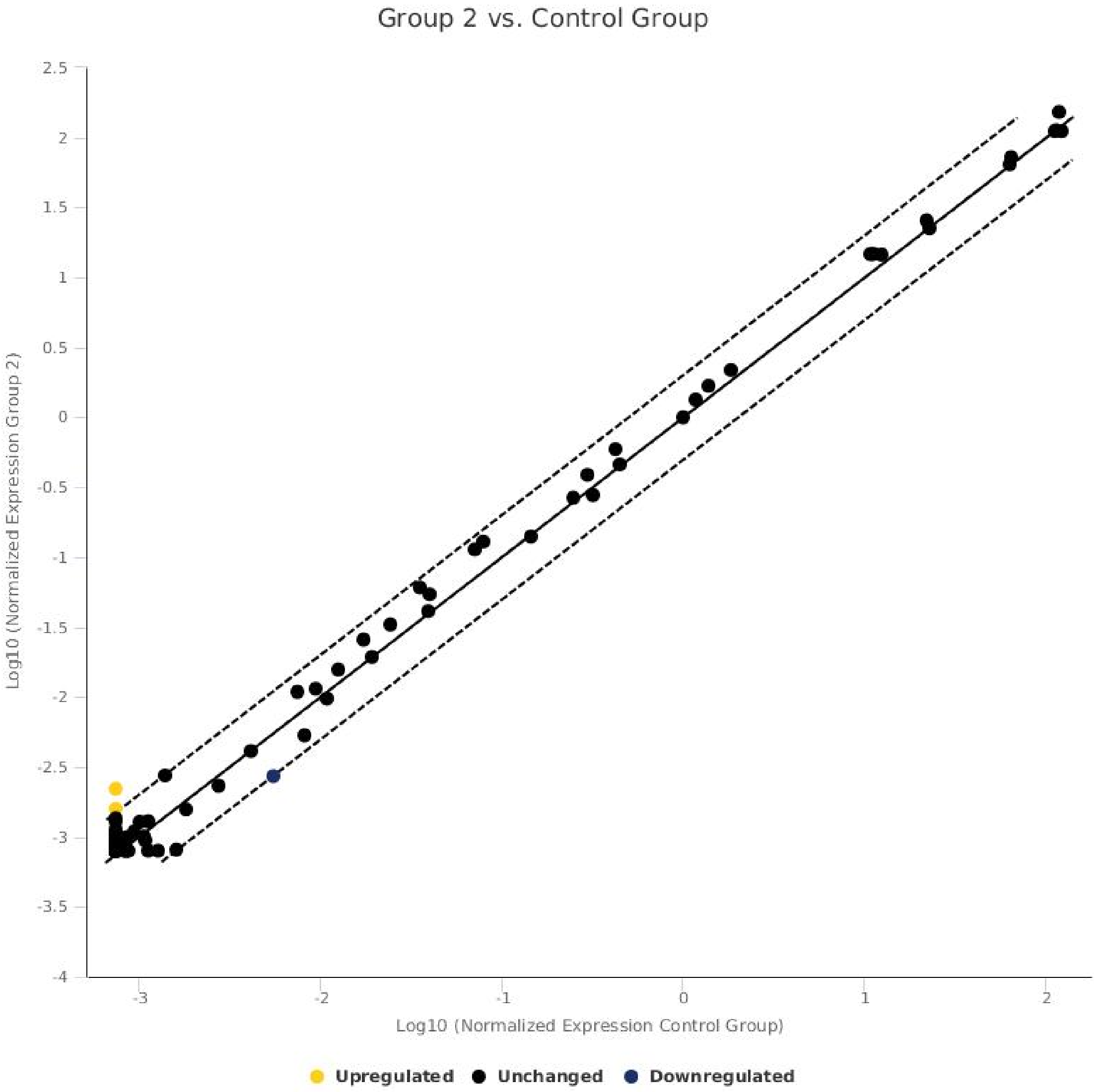
Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
E10	IL1B	2.11		PPH00171C

Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
C06	CSF2	-7.70		PPH00576C
A11	CCL20	-4.10		PPH00564C
C07	CSF3	-2.88		PPH00723B
A03	C5	-2.50		PPH07062C
B11	CCR3	-2.16		PPH00613B

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.

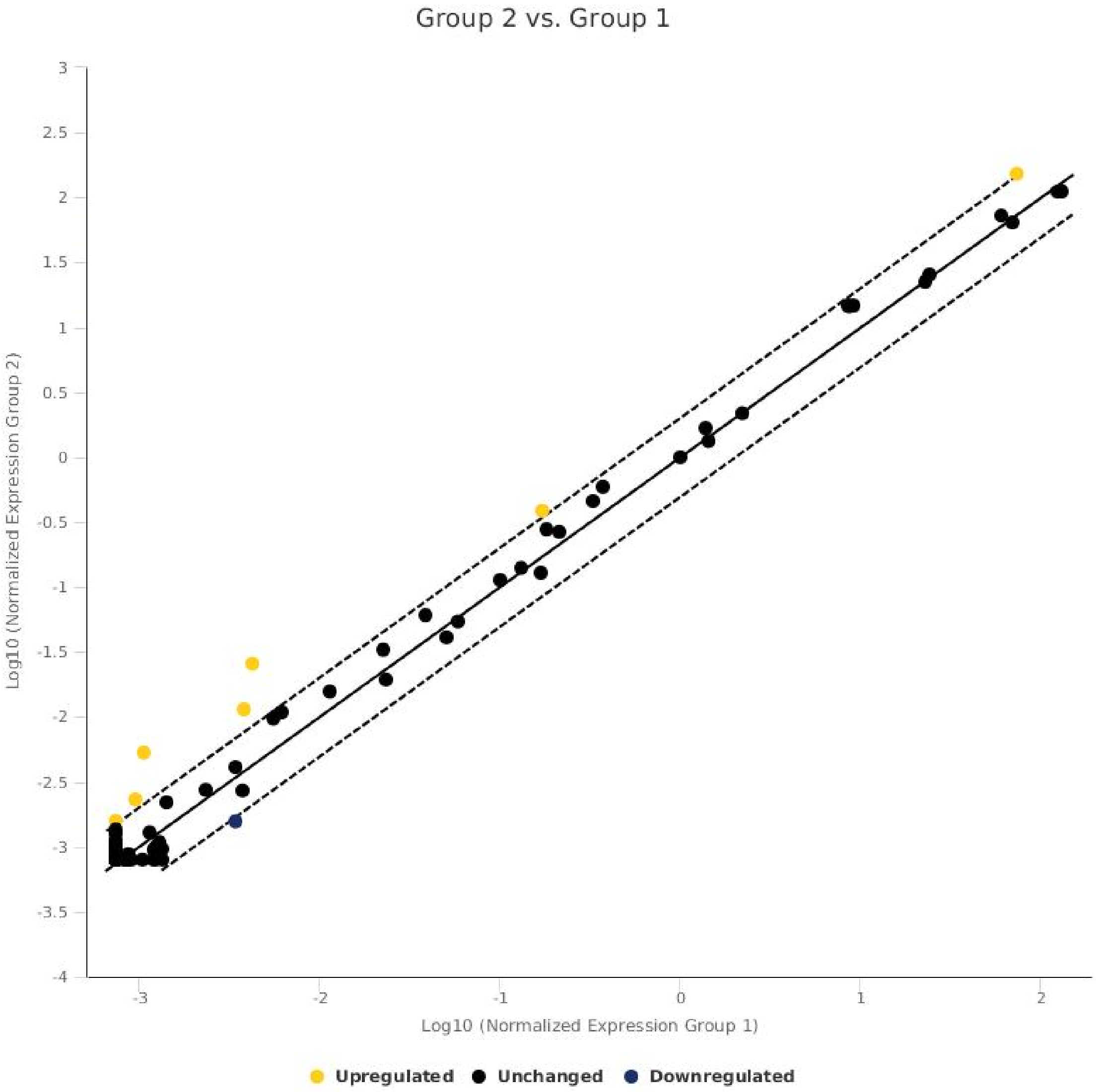
Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
B07	CCL7	2.96		PPH00575C
E06	IL17A	2.13		PPH00537C

Genes Under-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
E05	IL16	-2.01	B	PPH00586A

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

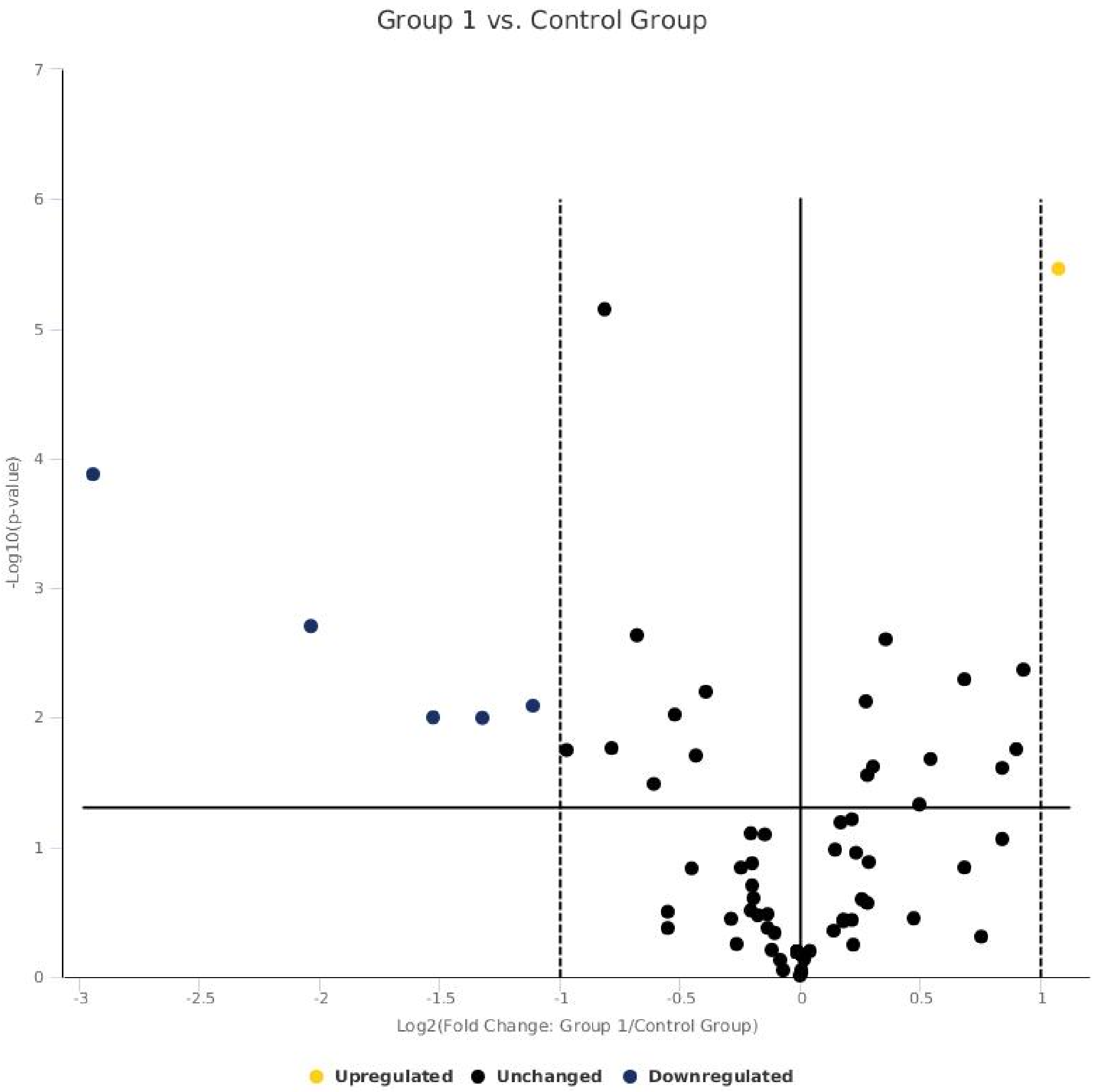
Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
A11	CCL20	6.12	A	PPH00564C
C06	CSF2	5.04		PPH00576C
A03	C5	3.05		PPH07062C
C07	CSF3	2.45		PPH00723B
D03	CXCL2	2.25		PPH00552F
E06	IL17A	2.15		PPH00537C
H01	ACTB	2.06		PPH00073G

Genes Under-Expressed in Group 2 vs. Group 1

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
G11	TNFSF4	-2.15		PPH00820D

Volcano Plot

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



The volcano plot helps quickly identify significant gene expression changes. The volcano plot displays statistical significance versus fold-change on the y- and x-axes, respectively. The volcano plot combines a p-value statistical test with the fold regulation change enabling identification of genes with both large and small expression changes that are statistically significant.

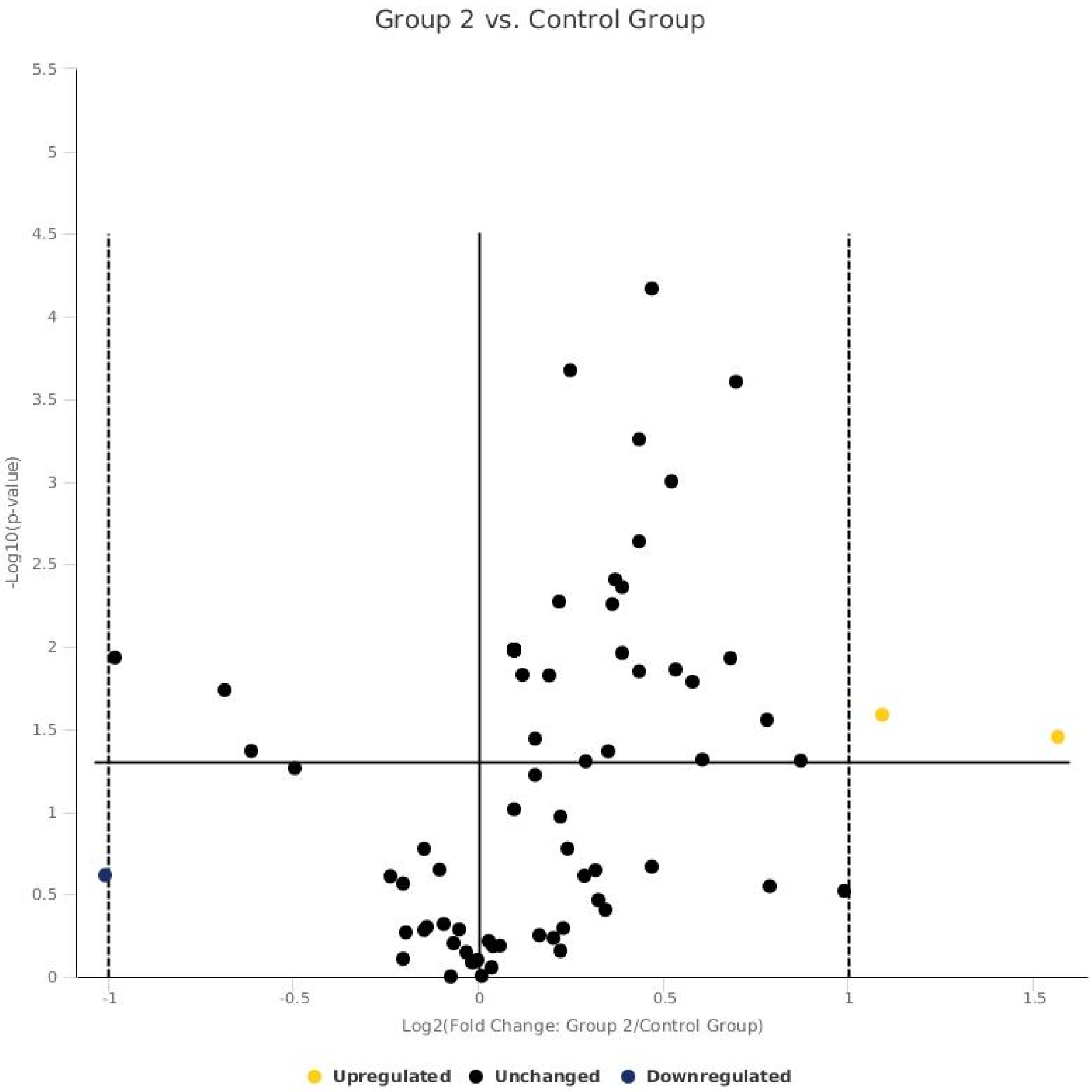
Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog
E10	IL1B	2.11	0.000003		PPH00171C

Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog
C06	CSF2	-7.70	0.000133		PPH00576C
A11	CCL20	-4.10	0.001970		PPH00564C
C07	CSF3	-2.88	0.009959		PPH00723B
A03	C5	-2.50	0.010061		PPH07062C
B11	CCR3	-2.16	0.008105		PPH00613B

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



The volcano plot helps quickly identify significant gene expression changes. The volcano plot displays statistical significance versus fold-change on the y- and x-axes, respectively. The volcano plot combines a p-value statistical test with the fold regulation change enabling identification of genes with both large and small expression changes that are statistically significant.

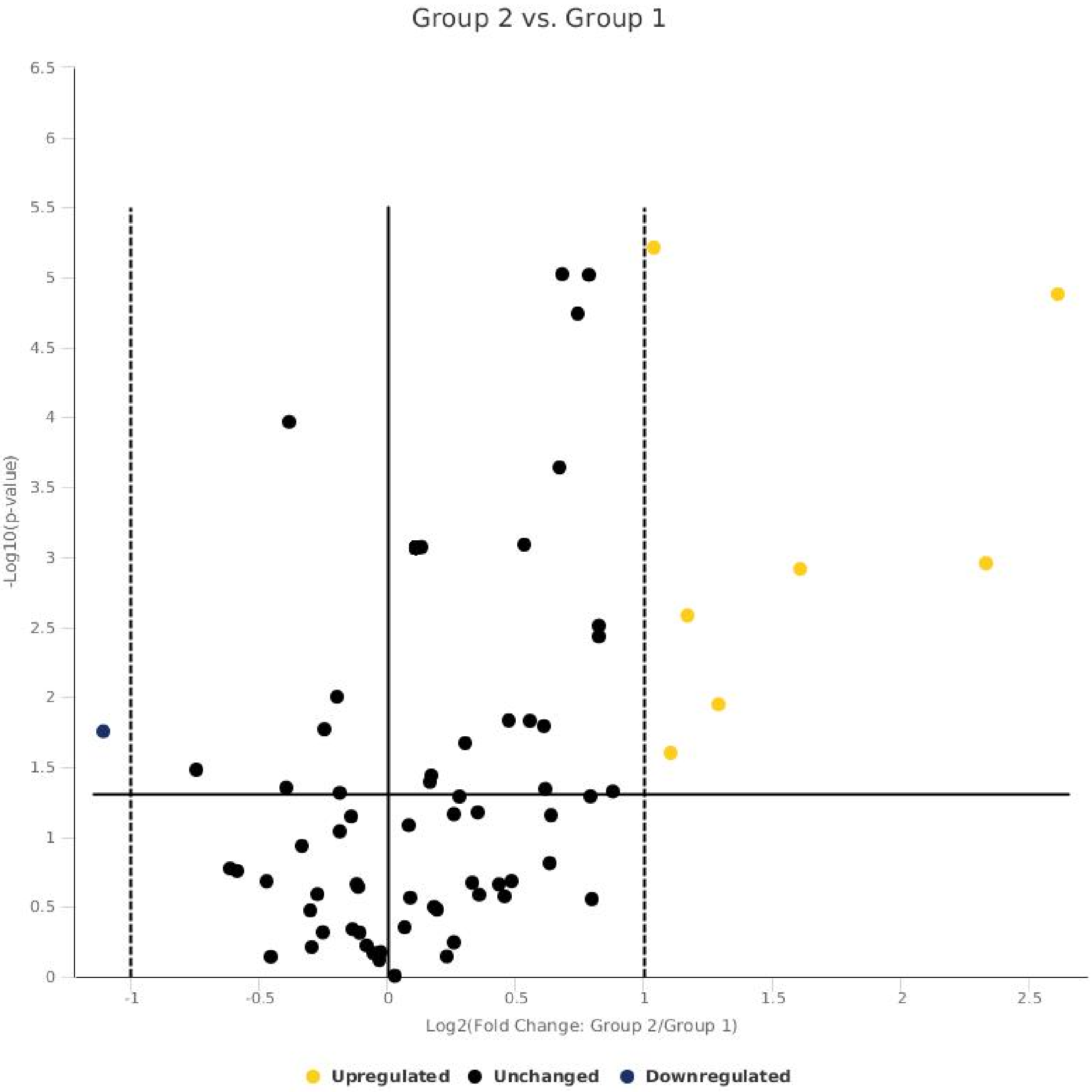
Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog
B07	CCL7	2.96	0.035185		PPH00575C
E06	IL17A	2.13	0.025846		PPH00537C

Genes Under-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog

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



The volcano plot helps quickly identify significant gene expression changes. The volcano plot displays statistical significance versus fold-change on the y- and x-axes, respectively. The volcano plot combines a p-value statistical test with the fold regulation change enabling identification of genes with both large and small expression changes that are statistically significant.

Genes Over-Expressed in Group 2 vs. Group 1

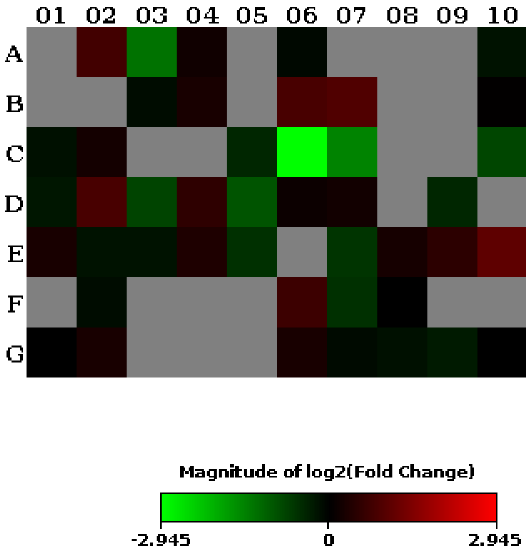
Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog
A11	CCL20	6.12	0.000013	A	PPH00564C
C06	CSF2	5.04	0.001108		PPH00576C
A03	C5	3.05	0.001214		PPH07062C
C07	CSF3	2.45	0.011294		PPH00723B
D03	CXCL2	2.25	0.002617		PPH00552F
E06	IL17A	2.15	0.025103		PPH00537C
H01	ACTB	2.06	0.000006		PPH00073G

Genes Under-Expressed in Group 2 vs. Group 1

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT2 Catalog
G11	TNFSF4	-2.15	0.017595		PPH00820D

Heat Map

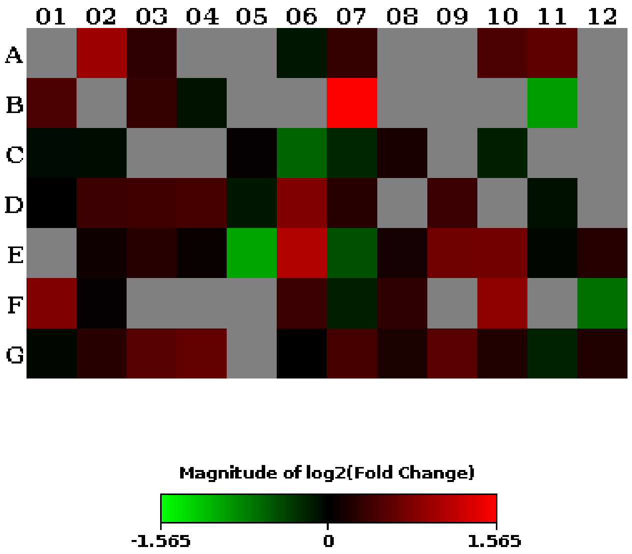
Treated Group	Control Group
Group 1	Control Group



Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	AIMP1 -1.01 C	BMP2 1.68 B	C5 -2.50	CCL1 1.13 B	CCL11 -1.01 C	CCL13 -1.06 B	CCL15 -1.01 C	CCL16 -1.01 C	CCL17 -1.01 C	CCL2 -1.15	CCL20 -4.10	CCL22 -1.01 C
B	CCL23 -1.01 C	CCL24 -1.01 C	CCL26 -1.10 B	CCL3 1.22 B	CCL4 -1.01 C	CCL5 1.79	CCL7 1.91	CCL8 -1.01 C	CCR1 -1.01 C	CCR2 1.01 B	CCR3 -2.16	CCR4 -1.01 C
C	CCR5 -1.13 B	CCR6 1.17 B	CCR8 -1.01 C	CD40LG -1.01 C	CSF1 -1.35	CSF2 -7.70	CSF3 -2.88	CX3CL1 -1.01 C	CX3CR1 -1.01 C	CXCL1 -1.76	CXCL10 1.60 B	CXCL11 -1.01 C
D	CXCL12 -1.20 B	CXCL13 1.79 B	CXCL2 -1.72	CXCL3 1.46	CXCL5 -1.96	CXCL6 1.10	CXCL9 1.16 B	CXCR1 -1.01 C	CXCR2 -1.37 B	FASLG -1.01 C	IFNA2 -1.15 B	IFNG -1.01 C
E	IL10RA 1.21	IL10RB -1.15	IL13 -1.15 B	IL15 1.28	IL16 -1.47 B	IL17A -1.01 C	IL17C -1.52	IL17F 1.19 B	IL1A 1.41	IL1B 2.11	IL1R1 -1.11	IL1RN 1.39 B
F	IL21 -1.01 C	IL27 -1.10 B	IL3 -1.01 C	IL33 -1.01 C	IL5 -1.01 C	IL5RA 1.60	IL7 -1.47 B	CXCL8 1.01	IL9 -1.01 C	IL9R -1.01 C	LTA -1.01 C	LTB -1.05 B
G	MIF -1.00	NAMPT 1.21	OSM -1.01 C	SPP1 -1.01 C	TNF -1.01 C	TNFRSF11B 1.21 B	TNFSF10 -1.09 A	TNFSF11 -1.14 B	TNFSF13 -1.22 B	TNFSF13B 1.01 B	TNFSF4 1.87	VEGFA 1.24

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.

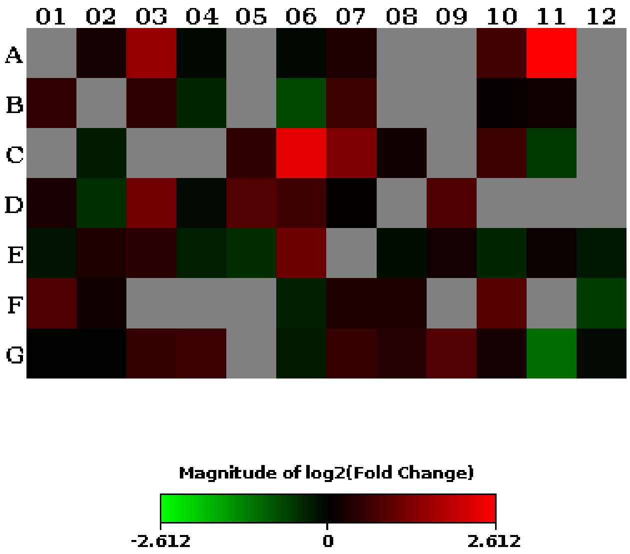
Treated Group	Control Group
Group 2	Control Group



Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	AIMP1 1.07 C	BMP2 1.98 B	C5 1.22 B	CCL1 1.07 C	CCL11 1.07 C	CCL13 -1.10 B	CCL15 1.25 B	CCL16 1.07 C	CCL17 1.07 C	CCL2 1.39	CCL20 1.49 A	CCL22 1.07 C
B	CCL23 1.39 B	CCL24 1.07 C	CCL26 1.25 B	CCL3 -1.08 B	CCL4 1.07 C	CCL5 1.07 C	CCL7 2.96	CCL8 1.07 C	CCR1 1.07 C	CCR2 1.07 C	CCR3 -1.97	CCR4 1.07 C
C	CCR5 -1.05 B	CCR6 -1.05 B	CCR8 1.07 C	CD40LG 1.07 C	CSF1 1.03	CSF2 -1.53	CSF3 -1.18 B	CX3CL1 1.11 B	CX3CR1 1.07 C	CXCL1 -1.15	CXCL10 1.07 C	CXCL11 1.07 C
D	CXCL12 -1.00 B	CXCL13 1.29	CXCL2 1.31	CXCL3 1.35	CXCL5 -1.11 B	CXCL6 1.72	CXCL9 1.18 B	CXCR1 1.07 C	CXCR2 1.27 B	FASLG 1.07 C	IFNA2 -1.07 B	IFNG 1.07 C
E	IL10RA 1.07 C	IL10RB 1.07	IL13 1.17 B	IL15 1.04	IL16 -2.01 B	IL17A 2.13	IL17C -1.41 B	IL17F 1.08	IL1A 1.60	IL1B 1.62	IL1R1 -1.04	IL1RN 1.17 B
F	IL21 1.73 B	IL27 1.03 B	IL3 1.07 C	IL33 1.07 C	IL5 1.07 C	IL5RA 1.28	IL7 -1.15 B	CXCL8 1.22	IL9 1.07 C	IL9R 1.83	LTA 1.07 C	LTB -1.61
G	MIF -1.02	NAMPT 1.19	OSM 1.44	SPP1 1.52	TNF 1.07 C	TNFRSF11B 1.01 B	TNFSF10 1.35	TNFSF11 1.12 B	TNFSF13 1.45	TNFSF13B 1.15 B	TNFSF4 -1.15 B	VEGFA 1.14

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	Group 1

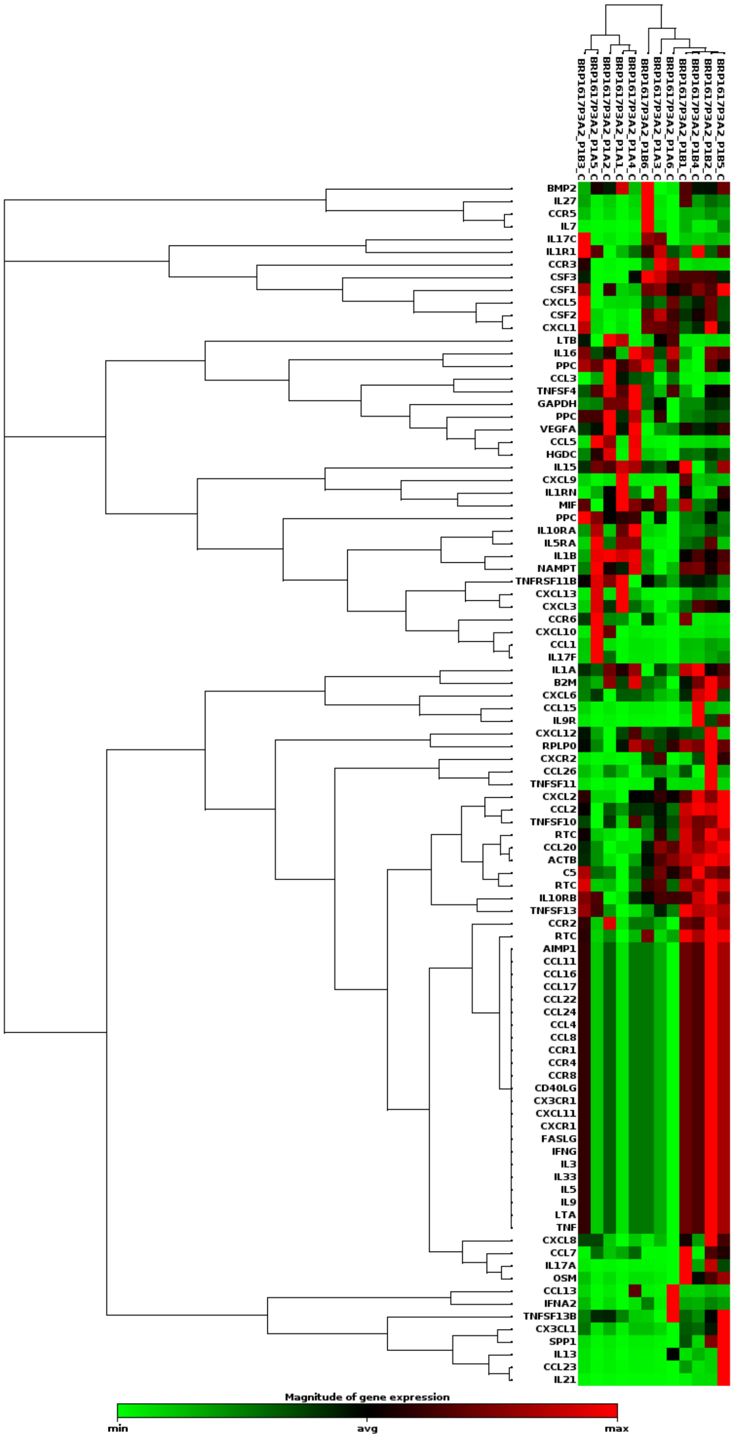


Layout	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
A	AIMP1 1.08 C	BMP2 1.18 B	C5 3.05	CCL1 -1.06 B	CCL11 1.08 C	CCL13 -1.04 B	CCL15 1.26 B	CCL16 1.08 C	CCL17 1.08 C	CCL2 1.59	CCL20 6.12 A	CCL22 1.08 C
B	CCL23 1.40 B	CCL24 1.08 C	CCL26 1.38 B	CCL3 -1.31	CCL4 1.08 C	CCL5 -1.68	CCL7 1.55 B	CCL8 1.08 C	CCR1 1.08 C	CCR2 1.06 B	CCR3 1.10	CCR4 1.08 C
C	CCR5 1.08 C	CCR6 -1.23 B	CCR8 1.08 C	CD40LG 1.08 C	CSF1 1.39	CSF2 5.04	CSF3 2.45	CX3CL1 1.12	CX3CR1 1.08 C	CXCL1 1.53	CXCL10 -1.50 B	CXCL11 1.08 C
D	CXCL12 1.20 B	CXCL13 -1.39 B	CXCL2 2.25	CXCL3 -1.08	CXCL5 1.77	CXCL6 1.56	CXCL9 1.02 B	CXCR1 1.08 C	CXCR2 1.73 B	FASLG 1.08 C	IFNA2 1.08 C	IFNG 1.08 C
E	IL10RA -1.13 B	IL10RB 1.23	IL13 1.35 B	IL15 -1.23	IL16 -1.37 B	IL17A 2.15	IL17C 1.08 C	IL17F -1.10 B	IL1A 1.13	IL1B -1.30	IL1R1 1.07	IL1RN -1.19 B
F	IL21 1.74 B	IL27 1.13	IL3 1.08 C	IL33 1.08 C	IL5 1.08 C	IL5RA -1.26 B	IL7 1.28 B	CXCL8 1.22	IL9 1.08 C	IL9R 1.84	LTA 1.08 C	LTB -1.53 B
G	MIF -1.02	NAMPT -1.02	OSM 1.45	SPP1 1.53	TNF 1.08 C	TNFRSF11B -1.21 B	TNFSF10 1.47 A	TNFSF11 1.28 B	TNFSF13 1.77	TNFSF13B 1.14 B	TNFSF4 -2.15	VEGFA -1.08

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.

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
C06	CSF2	-7.70	0.000133	PPH00576C	Inquire	View siRNAs	Inquire
A11	CCL20	-4.10	0.001970	PPH00564C	Inquire	View siRNAs	Inquire
C07	CSF3	-2.88	0.009959	PPH00723B	Inquire	View siRNAs	Inquire
A03	C5	-2.50	0.010061	PPH07062C	Inquire	View siRNAs	Inquire
B11	CCR3	-2.16	0.008105	PPH00613B	Inquire	View siRNAs	Inquire
E10	IL1B	2.11	0.000003	PPH00171C	Inquire	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
B07	CCL7	2.96	0.035185	PPH00575C	Inquire	View siRNAs	Inquire
E06	IL17A	2.13	0.025846	PPH00537C	Inquire	View siRNAs	Inquire

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

Position	Symbol	Fold Regulation	p-Value	RT2 qPCR Assay	EpiTect Methyl II qPCR Assay	FlexiTube siRNA	Somatic Mutation Assay
A11	CCL20	6.12	0.000013	PPH00564C	Inquire	View siRNAs	Inquire
C06	CSF2	5.04	0.001108	PPH00576C	Inquire	View siRNAs	Inquire
A03	C5	3.05	0.001214	PPH07062C	Inquire	View siRNAs	Inquire
C07	CSF3	2.45	0.011294	PPH00723B	Inquire	View siRNAs	Inquire
D03	CXCL2	2.25	0.002617	PPH00552F	EPHS111078-1A	View siRNAs	Inquire
G11	TNFSF4	-2.15	0.017595	PPH00820D	Inquire	View siRNAs	Inquire
E06	IL17A	2.15	0.025103	PPH00537C	Inquire	View siRNAs	Inquire
H01	ACTB	2.06	0.000006	PPH00073G	EPHS112951-1A	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
C06	CSF2	-7.70	0.000133
A11	CCL20	-4.10	0.001970
C07	CSF3	-2.88	0.009959
A03	C5	-2.50	0.010061
B11	CCR3	-2.16	0.008105

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-1322	-0.49	MS00014651	MSY0005953	MIN0005953	CCL20
hsa-miR-498	-0.46	MS00004368	MSY0002824	MIN0002824	C5
hsa-miR-590-5p	-0.42	MS00004900	MSY0003258	MIN0003258	CCL20
hsa-miR-21-5p	-0.41	MS00003213	MSY0000076	MIN0000076	CCL20
hsa-miR-577	-0.29	MS00004809	MSY0003242	MIN0003242	CSF3
hsa-miR-532-3p	-0.28	MS00010052	MSY0004780	MIN0004780	CSF3
hsa-miR-410-3p	-0.27	MS00004144	MSY0002171	MIN0002171	CSF2
hsa-miR-662	-0.24	MS00005397	MSY0003325	MIN0003325	CSF3
hsa-miR-890	-0.19	MS00010717	MSY0004912	MIN0004912	CSF3
hsa-miR-1272	-0.19	MS00014406	MSY0005925	MIN0005925	CCL20
hsa-miR-141-3p	-0.16	MS00003507	MSY0000432	MIN0000432	CSF3
hsa-miR-200a-3p	-0.14	MS00003738	MSY0000682	MIN0000682	CSF3

Genes Over-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
E10	IL1B	2.11	0.000003

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

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes

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

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

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes

Genes Over-Expressed in Group 2 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value
B07	CCL7	2.96	0.035185
E06	IL17A	2.13	0.025846

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-23a-3p	-0.51	MS00007336	MSY0000078	MIN0000078	CCL7
hsa-miR-23b-3p	-0.51	MS00007343	MSY0000418	MIN0000418	CCL7
hsa-miR-1266-5p	-0.43	MS00014371	MSY0005920	MIN0005920	IL17A
hsa-let-7c-5p	-0.36	MS00003129	MSY0000064	MIN0000064	CCL7
hsa-let-7a-5p	-0.36	MS00006482	MSY0000062	MIN0000062	CCL7
hsa-miR-98-5p	-0.36	MS00003367	MSY0000096	MIN0000096	CCL7
hsa-let-7b-5p	-0.36	MS00003122	MSY0000063	MIN0000063	CCL7
hsa-let-7f-5p	-0.36	MS00006489	MSY0000067	MIN0000067	CCL7
hsa-let-7e-5p	-0.36	MS00003143	MSY0000066	MIN0000066	CCL7
hsa-let-7d-5p	-0.36	MS00003136	MSY0000065	MIN0000065	CCL7
hsa-let-7i-5p	-0.36	MS00003157	MSY0000415	MIN0000415	CCL7
hsa-let-7g-5p	-0.36	MS00003150	MSY0000414	MIN0000414	CCL7
hsa-miR-202-3p	-0.10	MS00003759	MSY0002811	MIN0002811	CCL7

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

Genes Under-Expressed in Group 2 vs. Group 1

Position	Gene Symbol	Fold Regulation	p-Value
G11	TNFSF4	-2.15	0.017595

miRNA Regulating Genes Under-Expressed in Group 2 vs. Group 1

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-656-3p	-0.55	MS00005355	MSY0003332	MIN0003332	TNFSF4
hsa-miR-138-5p	-0.30	MS00006657	MSY0000430	MIN0000430	TNFSF4
hsa-miR-556-3p	-0.29	MS00010178	MSY0004793	MIN0004793	TNFSF4
hsa-miR-513b-5p	-0.29	MS00009919	MSY0005788	MIN0005788	TNFSF4
hsa-miR-125a-5p	-0.29	MS00003423	MSY0000443	MIN0000443	TNFSF4
hsa-miR-125b-5p	-0.29	MS00006629	MSY0000423	MIN0000423	TNFSF4
hsa-miR-516b-5p	-0.22	MS00007749	MSY0002859	MIN0002859	TNFSF4
hsa-miR-188-5p	-0.18	MS00003668	MSY0000457	MIN0000457	TNFSF4

Genes Over-Expressed in Group 2 vs. Group 1

Position	Gene Symbol	Fold Regulation	p-Value
A11	CCL20	6.12	0.000013
C06	CSF2	5.04	0.001108
A03	C5	3.05	0.001214
C07	CSF3	2.45	0.011294
D03	CXCL2	2.25	0.002617
E06	IL17A	2.15	0.025103
H01	ACTB	2.06	0.000006

miRNA Regulating Genes Over-Expressed in Group 2 vs. Group 1

miRNA Name	Strongest Strength Score	miScript Assay	miScript Mimic	miScript Inhibitor	Target Genes
hsa-miR-1322	-0.49	MS00014651	MSY0005953	MIN0005953	CCL20
hsa-miR-498	-0.46	MS00004368	MSY0002824	MIN0002824	C5
hsa-miR-1266-5p	-0.43	MS00014371	MSY0005920	MIN0005920	IL17A
hsa-miR-532-5p	-0.42	MS00004571	MSY0002888	MIN0002888	CXCL2
hsa-miR-590-5p	-0.42	MS00004900	MSY0003258	MIN0003258	CCL20
hsa-miR-145-5p	-0.41	MS00003528	MSY0000437	MIN0000437	ACTB
hsa-miR-21-5p	-0.41	MS00003213	MSY0000076	MIN0000076	CCL20
hsa-miR-577	-0.29	MS00004809	MSY0003242	MIN0003242	CSF3
hsa-miR-532-3p	-0.28	MS00010052	MSY0004780	MIN0004780	CSF3
hsa-miR-128-3p	-0.27	MS00008582	MSY0000424	MIN0000424	CXCL2
hsa-miR-410-3p	-0.27	MS00004144	MSY0002171	MIN0002171	CSF2
hsa-miR-641	-0.26	MS00005250	MSY0003311	MIN0003311	CXCL2
hsa-miR-1200	-0.26	MS00014133	MSY0005863	MIN0005863	ACTB
hsa-miR-662	-0.24	MS00005397	MSY0003325	MIN0003325	CSF3
hsa-miR-890	-0.19	MS00010717	MSY0004912	MIN0004912	CSF3
hsa-miR-1272	-0.19	MS00014406	MSY0005925	MIN0005925	CCL20
hsa-miR-141-3p	-0.16	MS00003507	MSY0000432	MIN0000432	CSF3
hsa-miR-200a-3p	-0.14	MS00003738	MSY0000682	MIN0000682	CSF3

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
C06	CSF2	-7.70	0.000133	AML1a, PPAR-gamma1, PPAR-gamma2, Nkx2-2, RREB-1, CP2, LUN-1, Olf-1, STAT5A, GATA-6, Lmo2, GATA-1, GATA-2, GATA-3, NF-kappaB1, NF-kappaB, NF-kappaB1, NF-kappaB, MZF-1, AML1a, CUTL1, Pbx1a	GPH1010705(-)01A
A11	CCL20	-4.10	0.001970	Cdc5, NF-kappaB1, NF-kappaB, NF-kappaB2, c-Rel, NF-kappaB1, RelA, NF-kappaB	GPH1008094(-)01A
C07	CSF3	-2.88	0.009959	Arnt, USF-1, USF1, AREB6, SREBP-1a, SREBP-1b, SREBP-1c, SEF-1 (1), Elk-1, IRF-7A, c-Ets-1, AREB6, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4, Bach1, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Bach2, c-Rel, NF-kappaB1, RelA, NF-kappaB, p53, Pax-5, CP2, AREB6, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C, POU2F1, POU2F1a, POU2F1b, POU2F1c, C/EBPalpha, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), STAT5A	GPH1005729(-)01A
A03	C5	-2.50	0.010061	S8, HNF-1, HNF-1A, Pax-4a, POU3F2, IRF-7A	GPH1026934(-)01A
B11	CCR3	-2.16	0.008105		GPH1009264(-)01A
E10	IL1B	2.11	0.000003	TBP, TBP, TFIID, C/EBPbeta, c-Rel, RelA, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4	GPH1021494(-)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
B07	CCL7	2.96	0.035185	TBP, TFIID	GPH1005668(-)01A
E06	IL17A	2.13	0.025846	STAT1, STAT1alpha, STAT1beta, C/EBPalpha, AML1a, TBP, TFIID	GPH1011406(-)01A

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

Genes Differentially Expressed in Group 2 vs. Group 1

Position	**Gene Symbol**	**Fold Regulation**	**p-Value**	**Transcription Factors**	**EpiTect ChIP qPCR Assay**
A11	CCL20	6.12	0.000013	Cdc5, NF-kappaB1, NF-kappaB, NF-kappaB2, c-Rel, NF-kappaB1, RelA, NF-kappaB	GPH1008094(-)01A
C06	CSF2	5.04	0.001108	AML1a, PPAR-gamma1, PPAR-gamma2, Nkx2-2, RREB-1, CP2, LUN-1, Olf-1, STAT5A, GATA-6, Lmo2, GATA-1, GATA-2, GATA-3, NF-kappaB1, NF-kappaB, NF-kappaB1, NF-kappaB, MZF-1, AML1a, CUTL1, Pbx1a	GPH1010705(-)01A
A03	C5	3.05	0.001214	S8, HNF-1, HNF-1A, Pax-4a, POU3F2, IRF-7A	GPH1026934(-)01A
C07	CSF3	2.45	0.011294	Arnt, USF-1, USF1, AREB6, SREBP-1a, SREBP-1b, SREBP-1c, SEF-1 (1), Elk-1, IRF-7A, c-Ets-1, AREB6, NF-AT, NF-AT1, NF-AT2, NF-AT3, NF-AT4, Bach1, c-Jun, AP-1, c-Fos, FosB, Fra-1, JunB, JunD, Bach2, c-Rel, NF-kappaB1, RelA, NF-kappaB, p53, Pax-5, CP2, AREB6, POU2F1, POU2F1a, Oct-B1, oct-B2, oct-B3, POU2F2, POU2F2 (Oct-2.1), POU2F2B, POU2F2C, POU2F1, POU2F1a, POU2F1b, POU2F1c, C/EBPalpha, POU3F2, POU3F2 (N-Oct-5a), POU3F2 (N-Oct-5b), STAT5A	GPH1005729(-)01A
D03	CXCL2	2.25	0.002617		GPH1023716(-)01A
G11	TNFSF4	-2.15	0.017595	Cart-1, aMEF-2, MEF-2, MEF-2A, FOXO4, FOXO1, FOXO1a, C/EBPalpha, POU6F1 (c2)	GPH1015165(-)01A
E06	IL17A	2.15	0.025103	STAT1, STAT1alpha, STAT1beta, C/EBPalpha, AML1a, TBP, TFIID	GPH1011406(-)01A
H01	ACTB	2.06	0.000006	p53, SRF, SRF (504 AA), TBP, TFIID, AP-2alpha, AP-2alphaA, AP-2beta, AP-2gamma, TBP, TBP, TFIID, SRF, SRF (504 AA), TBP, TFIID, Gfi-1, GATA-1, CBF(2), CBF-A, CBF-B, CBF-C, CP1A, CP1C, NF-Y, NF-YA, NF-YB, NF-YC, Bach2, RREB-1, LUN-1, p300, Egr-1	GPH1025353(-)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.