RT² Profiler PCR Array Gene Expression Analysis Report

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Introduction

Cataloged arrays

RT² Profiler PCR Arrays are highly reliable and sensitive gene expression profiling tools for analyzing focused panels of genes insignal transduction, biological processes or disease research pathways using real-time PCR. Each cataloged RT² Profiler PCR Array contains a list of the pathway-focused genes as well as five housekeeping (reference) genes on the array. In addition, eacharray 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 workunder standard conditions enabling a large number of genes to be assayed simultaneously. Their specificity is guaranteed when RT² SYBR Green qPCR Master Mixes are used as part of the complete PCR Array System protocol.

In this study, 96 genes were profiled on 6 samples with the PAHS-148Z.

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-148Z) 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 Manual Selection 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

Position	RefSeq Number	Symbol	Description
A01	NM_005163	AKT1	V-akt murine thymoma viral oncogene homolog 1
A02	NM_001639	APCS	Amyloid P component, serum
A03	NM_001165	BIRC3	Baculoviral IAP repeat containing 3
A04	NM_001725	BPI	Bactericidal/permeability-increasing protein
A05	NM_004345	CAMP	Cathelicidin antimicrobial peptide
A06	NM_032587	CARD6	Caspase recruitment domain family, member 6
A07	NM_052813	CARD9	Caspase recruitment domain family, member 9
A08	NM_033292	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
A09	NM_001228	CASP8	Caspase 8, apoptosis-related cysteine peptidase
A10	NM_002983	CCL3	Chemokine (C-C motif) ligand 3
A11	NM_002985	CCL5	Chemokine (C-C motif) ligand 5
A12	NM_000591	CD14	CD14 molecule
B01	NM_001278	CHUK	Conserved helix-loop-helix ubiquitous kinase
B02	NM_000567	CRP	C-reactive protein, pentraxin-related
B03	NM_001911	CTSG	Cathepsin G
B04	NM_001511	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
B05	NM_002089	CXCL2	Chemokine (C-X-C motif) ligand 2
B06	NM_004406	DMBT1	Deleted in malignant brain tumors 1
B07	NM_003824	FADD	Fas (TNFRSF6)-associated via death domain
B08	NM_001017963	HSP90AA1	Heat shock protein 90kDa alpha (cytosolic), class A member 1
B09	NM_024013	IFNA1	Interferon, alpha 1
B10	NM_002176	IFNB1	Interferon, beta 1, fibroblast
B11	NM_001556	IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
B12	NM_000882	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
C01	NM_002187	IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
C02	NM_001562	IL18	Interleukin 18 (interferon-gamma-inducing factor)
C03	NM_000576	IL1B	Interleukin 1, beta
C04	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
C05	NM_000584	CXCL8	Interleukin 8
C06	NM_001569	IRAK1	Interleukin-1 receptor-associated kinase 1
C07	NM_007199	IRAK3	Interleukin-1 receptor-associated kinase 3
C08	NM_001098629	IRF5	Interferon regulatory factor 5
C09	NM_001572	IRF7	Interferon regulatory factor 7
C10	NM_002228	JUN	Jun proto-oncogene

Position	RefSeq Number	Symbol	Description
C11	NM_004139	LBP	Lipopolysaccharide binding protein
C12	NM_005564	LCN2	Lipocalin 2
D01	NM_002343	LTF	Lactotransferrin
D02	NM_015364	LY96	Lymphocyte antigen 96
D03	NM_000239	LYZ	Lysozyme
D04	NM_002755	MAP2K1	Mitogen-activated protein kinase kinase 1
D05	NM_002756	MAP2K3	Mitogen-activated protein kinase kinase 3
D06	NM_003010	MAP2K4	Mitogen-activated protein kinase kinase 4
D07	NM_003188	MAP3K7	Mitogen-activated protein kinase kinase kinase 7
D08	NM_002745	MAPK1	Mitogen-activated protein kinase 1
D09	NM_001315	MAPK14	Mitogen-activated protein kinase 14
D10	NM_002746	MAPK3	Mitogen-activated protein kinase 3
D11	NM_002750	MAPK8	Mitogen-activated protein kinase 8
D12	NM_000243	MEFV	Mediterranean fever
E01	NM_000250	MPO	Myeloperoxidase
E02	NM_002468	MYD88	Myeloid differentiation primary response gene (88)
E03	NM_004536	NAIP	NLR family, apoptosis inhibitory protein
E04	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
E05	NM_020529	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
E06	NM_021209	NLRC4	NLR family, CARD domain containing 4
E07	NM_033004	NLRP1	NLR family, pyrin domain containing 1
E08	NM_183395	NLRP3	NLR family, pyrin domain containing 3
E09	NM_006092	NOD1	Nucleotide-binding oligomerization domain containing 1
E10	NM_022162	NOD2	Nucleotide-binding oligomerization domain containing 2
E11	NM_006218	PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
E12	NM_002777	PRTN3	Proteinase 3
F01	NM_003978	PSTPIP1	Proline-serine-threonine phosphatase interacting protein 1
F02	NM_013258	PYCARD	PYD and CARD domain containing
F03	NM_006908	RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
F04	NM_021975	RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)
F05	NM_003804	RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1
F06	NM_003821	RIPK2	Receptor-interacting serine-threonine kinase 2
F07	NM_000578	SLC11A1	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
F08	NM_003064	SLPI	Secretory leukocyte peptidase inhibitor
F09	NM_006704	SUGT1	SGT1, suppressor of G2 allele of SKP1 (S. cerevisiae)
F10	NM_182919	TICAM1	Toll-like receptor adaptor molecule 1
F11	NM_021649	TICAM2	Toll-like receptor adaptor molecule 2

Position	RefSeq Number	Symbol	Description
F12	NM_001039661	TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein
G01	NM_003263	TLR1	Toll-like receptor 1
G02	NM_003264	TLR2	Toll-like receptor 2
G03	NM_138554	TLR4	Toll-like receptor 4
G04	NM_003268	TLR5	Toll-like receptor 5
G05	NM_006068	TLR6	Toll-like receptor 6
G06	NM_017442	TLR9	Toll-like receptor 9
G07	NM_000594	TNF	Tumor necrosis factor
G08	NM_001065	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
G09	NM_019009	TOLLIP	Toll interacting protein
G10	NM_004620	TRAF6	TNF receptor-associated factor 6
G11	NM_001167	XIAP	X-linked inhibitor of apoptosis
G12	NM_030776	ZBP1	Z-DNA binding protein 1
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

Sample ID	Sample Name	Group
1	Saline 1	Control Group
2	Saline 2	Control Group
3	Saline 3	Control Group
4	UPEC 1	Group 1
5	UPEC 2	Group 1
6	UPEC 3	Group 1

Pre-amplification

A pre-amplification using the appropriate species- and pathway-specific RT^2 PreAMP Primer Mix was 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. Reverse Transcription Efficiency	All Samples Passed
3. Genomic DNA Contamination	All Samples Passed

Normalization analysis

Manual Selection

Groups	Samples	ACTB	B2M	GAPDH	HPRT1	RPLP0	Arithmetic Mean	Average Arithmetic Mean
Control Group	Saline 1	28.30	24.08	29.20	25.33	26.99	26.78	25.51
Control Group	Saline 2	26.80	22.49	27.60	22.65	24.43	24.79	
Control Group	Saline 3	25.88	21.58	27.70	24.00	25.60	24.95	
Group 1	UPEC 1	24.97	20.50	25.67	23.51	23.55	23.64	24.80
Group 1	UPEC 2	27.72	22.76	27.82	23.58	24.66	25.31	
Group 1	UPEC 3	27.09	21.60	27.96	24.82	25.81	25.46	

In the Manual Selection method, the arithmetic or geometric means of the data from the assays for the housekeeping / reference genes listed in the table were used to normalize the raw data.

Result

Fold regulation and p-value

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

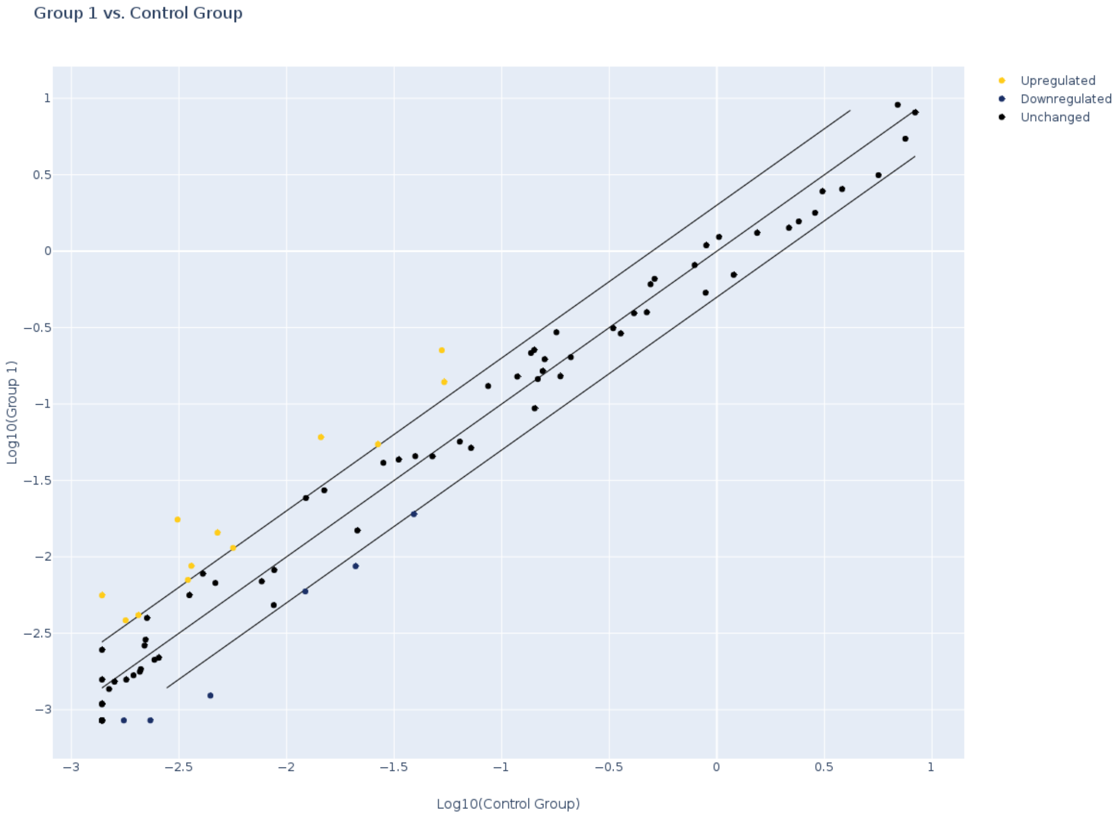
Position	Gene Symbol	Fold Regulation	p-Value	Comments
C08	IRF5	2.05	0.013674	A
D07	MAP3K7	2.42	0.009804	

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, and p values less than 0.05 are indicated in red. The p-value calculation used is based on parametric, unpaired, two-sample equal variance, two-tailed distribution – a method widely accepted in scientific literature.

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 PCR Array between the two selected groups by plotting them against one another to quickly visualize large gene expression changes. The center diagonal line indicates unchanged gene expression, while the outer diagonal lines indicate the selected fold regulation threshold. Genes with data points beyond the outer lines in the upper left and lower right corners are up-regulated or down-regulated, respectively, by more than the fold regulation threshold in the y-axis Group relative to the x-axis Group.

Genes Over-Expressed in Group 1 vs. Control Group

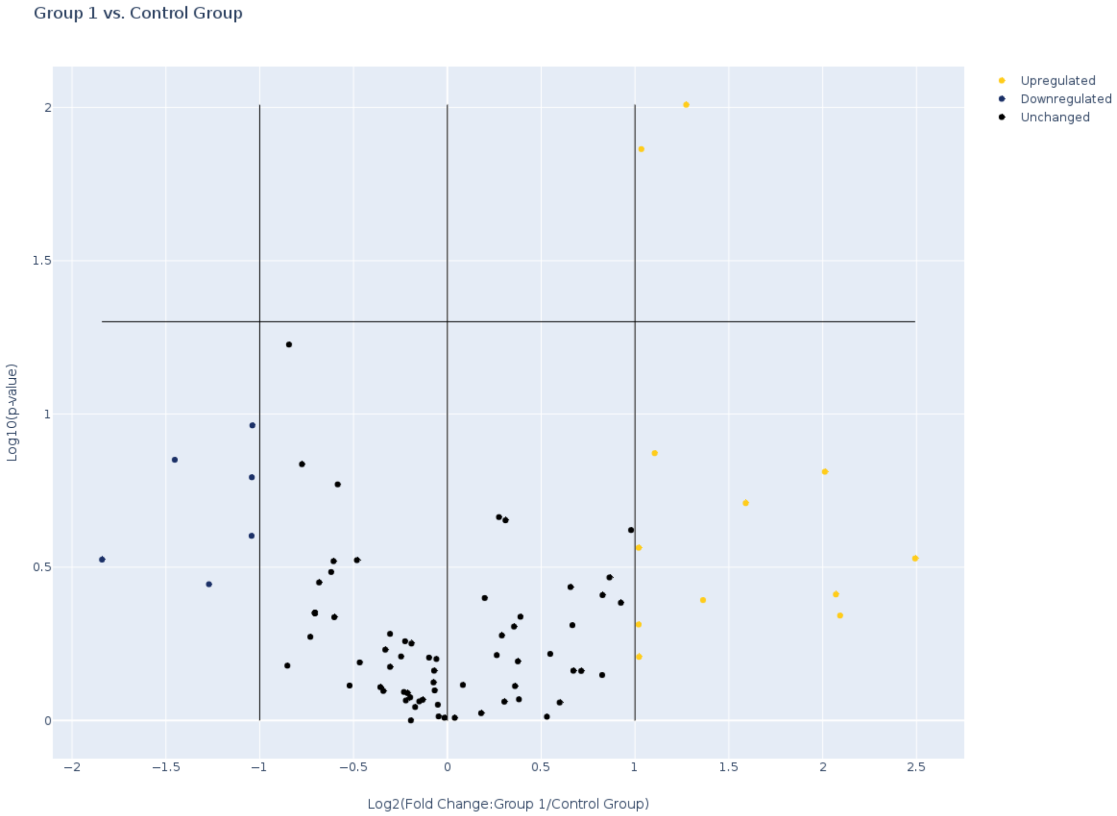
Position	Gene Symbol	Fold Regulation	Comments	RT² qPCR Assay Catalog #
A03	BIRC3	2.15	B	PPH00326B
A05	CAMP	4.20	A	PPH09430A
A08	CASP1	2.57		PPH00105C
B03	CTSG	5.63	B	PPH01358A
B09	IFNA1	2.03	B	PPH01321B
C08	IRF5	2.05	A	PPH02870A
C12	LCN2	4.27		PPH00446E
D07	MAP3K7	2.42		PPH00749C
E07	NLRP1	4.03	B	PPH06155E
E12	PRTN3	2.03	B	PPH07029A
G05	TLR6	2.03	B	PPH01798E
G07	TNF	3.01	B	PPH00341F

Genes Under-Expressed in Group 1 vs. Control Group

Position	**Gene Symbol**	**Fold Regulation**	**Comments**	**RT² qPCR Assay Catalog #**
A07	CARD9	-2.74	B	PPH06127F
B12	IL12A	-2.41	B	PPH00544B
E01	MPO	-3.58	B	PPH06082F
E11	PIK3CA	-2.06	B	PPH01355A
F09	SUGT1	-2.06	B	PPH05871A
G08	TNFRSF1A	-2.05	B	PPH00346C

Volcano Plot

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



The Volcano Plot identifies significant gene expression changes by plotting the log2 of the fold changes in gene expression on the x-axis versus their statistical significance on the y-axis. The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold. The horizontal line indicates the selected p-value threshold. Genes with data points in the far upper left (down-regulated) and far upper right (up-regulated) sections meet the selected fold regulation and p-value thresholds. By combining the fold change results with the p-value statistical test results, genes with both large and small expression changes that are statistically significant are easily visualized.

Genes Over-Expressed in Group 1 vs. Control Group

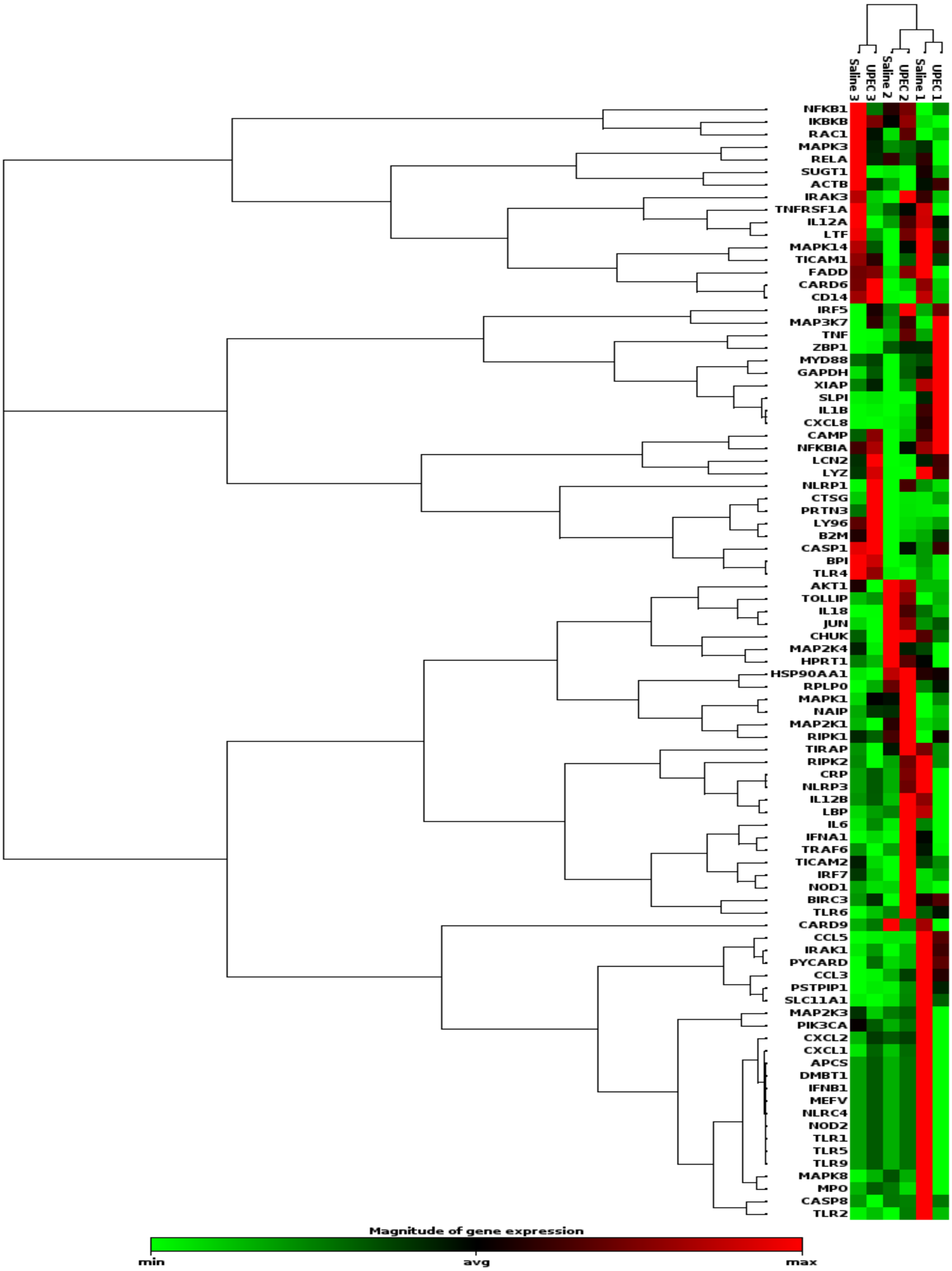
Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT² qPCR Assay Catalog #
A03	BIRC3	2.15	0.134280	B	PPH00326B
A05	CAMP	4.20	0.388068	A	PPH09430A
A08	CASP1	2.57	0.404806		PPH00105C
B03	CTSG	5.63	0.296140	B	PPH01358A
B09	IFNA1	2.03	0.620329	B	PPH01321B
C08	IRF5	2.05	0.013674	A	PPH02870A
C12	LCN2	4.27	0.454745		PPH00446E
D07	MAP3K7	2.42	0.009804		PPH00749C
E07	NLRP1	4.03	0.154395	B	PPH06155E
E12	PRTN3	2.03	0.486380	B	PPH07029A
G05	TLR6	2.03	0.273314	B	PPH01798E
G07	TNF	3.01	0.195453	B	PPH00341F

Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	p-Value	Comments	RT² qPCR Assay Catalog #
A07	CARD9	-2.74	0.141090	B	PPH06127F
B12	IL12A	-2.41	0.359328	B	PPH00544B
E01	MPO	-3.58	0.298691	B	PPH06082F
E11	PIK3CA	-2.06	0.249832	B	PPH01355A
F09	SUGT1	-2.06	0.160913	B	PPH05871A
G08	TNFRSF1A	-2.05	0.108971	B	PPH00346C

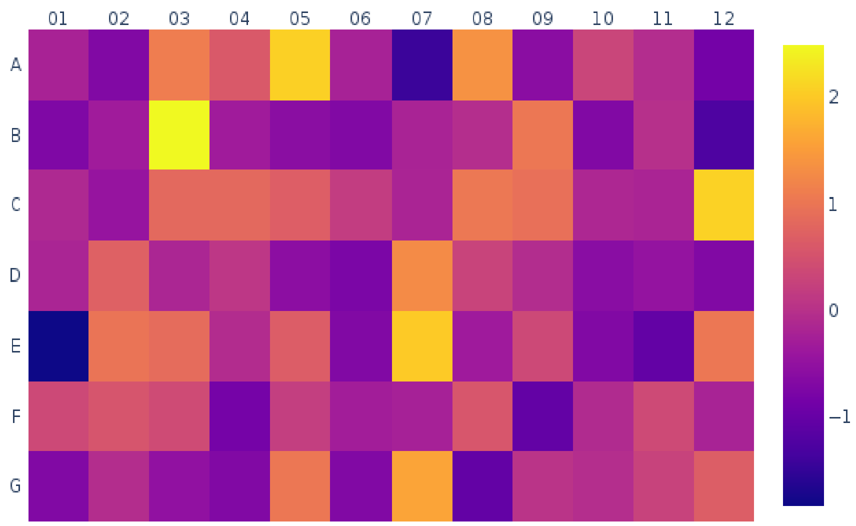
Clustergram

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



Heat Map

Test Group	Control Group
Group 1	Control Group



Visualization of log2(Fold Change)

Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	AKT1 / -1.17	APCS / -1.63 / C	BIRC3 / 2.15 / B	BPI / 1.51	CAMP / 4.2 / A	CARD6 / -1.17 / B	CARD9 / -2.74 / B	CASP1 / 2.57	CASP8 / -1.52	CCL3 / 1.23	CCL5 / -1.05	CD14 / -1.81 / B
B	CHUK / -1.66	CRP / -1.27 / B	CTSG / 5.63 / B	CXCL1 / -1.26	CXCL2 / -1.52	DMBT1 / -1.63 / C	FADD / -1.16 / B	HSP90AA1 / -1.03	IFNA1 / 2.03 / B	IFNB1 / -1.63 / C	IKBKB / -1.01	IL12A / -2.41 / B
C	IL12B / -1.1 / B	IL18 / -1.38	IL1B / 1.77 / B	IL6 / 1.77 / B	CXCL8 / 1.59	IRAK1 / 1.13 / B	IRAK3 / -1.15 / B	IRF5 / 2.05 / A	IRF7 / 1.9 / B	JUN / -1.11 / B	LBP / -1.14 / B	LCN2 / 4.27
D	LTF / -1.14 / B	LY96 / 1.64	LYZ / -1.13	MAP2K1 / 1.06	MAP2K3 / -1.5	MAP2K4 / -1.71	MAP3K7 / 2.42	MAPK1 / 1.21	MAPK14 / -1.05	MAPK3 / -1.54	MAPK8 / -1.4	MEFV / -1.63 / C
E	MPO / -3.58 / B	MYD88 / 1.97 / B	NAIP / 1.82 / A	NFKB1 / -1.05	NFKBIA / 1.58	NLRC4 / -1.63 / C	NLRP1 / 4.03 / B	NLRP3 / -1.28 / B	NOD1 / 1.28	NOD2 / -1.63 / C	PIK3CA / -2.06 / B	PRTN3 / 2.03 / B
F	PSTPIP1 / 1.28	PYCARD / 1.44 / B	RAC1 / 1.3 / A	RELA / -1.79	RIPK1 / 1.15 / A	RIPK2 / -1.24	SLC11A1 / -1.19	SLPI / 1.46 / A	SUGT1 / -2.06 / B	TICAM1 / -1.07 / B	TICAM2 / 1.3 / B	TIRAP / -1.17 / B
G	TLR1 / -1.63 / C	TLR2 / -1.04 / B	TLR4 / -1.44 / B	TLR5 / -1.63 / C	TLR6 / 2.03 / B	TLR9 / -1.63 / C	TNF / 3.01 / B	TNFRSF1A / -2.05 / B	TOLLIP / 1.03	TRAF6 / -1.04	XIAP / 1.2 / B	ZBP1 / 1.59 / B