



RT² Profiler PCR Array Gene Expression Analysis Report

Patti Champine

Tuesday, March 01, 2016

Table of Contents

Introduction..... 1

Summary and workflow 2

Data analysis setup..... 5

Data quality control (QC) 6

Normalization analysis 7

Results 8

 Fold regulation comparison and p-value 8

 Scatter Plot..... 10

Next steps 14

Glossary 15

Introduction

Cataloged arrays

RT² 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 RT² 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 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 2 samples with the PAHS-008Y.

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-008Y) 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 $2^{(-\text{delta delta } C_T)}$ 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

RT² Profiler™ PCR Array Human Mitochondrial Energy Metabolism Pathway Plus

Position	RefSeq Number	Symbol	Description
A01	NM_004046	ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
A02	NM_001686	ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
A03	NM_005174	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
A04	NM_001688	ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit B1
A05	NM_005175	ATP5G1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9)
A06	NM_001002031	ATP5G2	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C2 (subunit 9)
A07	NM_001689	ATP5G3	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C3 (subunit 9)
A08	NM_006356	ATP5H	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit d
A09	NM_007100	ATP5I	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit E
A10	NM_001685	ATP5J	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit F6
A11	NM_004889	ATP5J2	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit F2
A12	NM_006476	ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G
B01	NM_001697	ATP5O	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit
B02	NM_001861	COX4I1	Cytochrome c oxidase subunit IV isoform 1
B03	NM_004255	COX5A	Cytochrome c oxidase subunit Va
B04	NM_001862	COX5B	Cytochrome c oxidase subunit Vb
B05	NM_004373	COX6A1	Cytochrome c oxidase subunit VIa polypeptide 1
B06	NM_005205	COX6A2	Cytochrome c oxidase subunit VIa polypeptide 2
B07	NM_001863	COX6B1	Cytochrome c oxidase subunit Vib polypeptide 1 (ubiquitous)
B08	NM_004374	COX6C	Cytochrome c oxidase subunit VIc
B09	NM_001865	COX7A2	Cytochrome c oxidase subunit VIIa polypeptide 2 (liver)
B10	NM_004718	COX7A2L	Cytochrome c oxidase subunit VIIa polypeptide 2 like
B11	NM_001866	COX7B	Cytochrome c oxidase subunit VIIb
B12	NM_004074	COX8A	Cytochrome c oxidase subunit VIIIA (ubiquitous)
C01	NM_001916	CYC1	Cytochrome c-1
C02	NM_004541	NDUFA1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa
C03	NM_004544	NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
C04	NM_175614	NDUFA11	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 11, 14.7kDa
C05	NM_002488	NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa
C06	NM_004542	NDUFA3	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa
C07	NM_002489	NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa
C08	NM_005000	NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa
C09	NM_002490	NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa
C10	NM_014222	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa
C11	NM_005003	NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa
C12	NM_004548	NDUFB10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa
D01	NM_004546	NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa
D02	NM_002491	NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa
D03	NM_004547	NDUFB4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa
D04	NM_002492	NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa
D05	NM_182739	NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa
D06	NM_004146	NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa
D07	NM_005004	NDUFB8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa
D08	NM_005005	NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa
D09	NM_002494	NDUFC1	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa
D10	NM_004549	NDUFC2	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2, 14.5kDa

Position	RefSeq Number	Symbol	Description
D11	NM_005006	NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)
D12	NM_004550	NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)
E01	NM_004551	NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)
E02	NM_002495	NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)
E03	NM_004552	NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)
E04	NM_004553	NDUFS6	NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coenzyme Q reductase)
E05	NM_024407	NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)
E06	NM_002496	NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)
E07	NM_007103	NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa
E08	NM_021074	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa
E09	NM_021075	NDUFV3	NADH dehydrogenase (ubiquinone) flavoprotein 3, 10kDa
E10	NM_021129	PPA1	Pyrophosphatase (inorganic) 1
E11	NM_004168	SDHA	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
E12	NM_003000	SDHB	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)
F01	NM_003001	SDHC	Succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa
F02	NM_003002	SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein
F03	NM_006830	UQCRC1	Ubiquinol-cytochrome c reductase, complex III subunit XI
F04	NM_003365	UQCRC1	Ubiquinol-cytochrome c reductase core protein I
F05	NM_003366	UQCRC2	Ubiquinol-cytochrome c reductase core protein II
F06	NM_006003	UQCRC1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
F07	NM_006004	UQCRC1	Ubiquinol-cytochrome c reductase hinge protein
F08	NM_014402	UQCRC1	Ubiquinol-cytochrome c reductase, complex III subunit VII, 9.5kDa
F09	NM_020801	ARRDC3	Arrestin domain containing 3
F10	NM_001040445	ASB1	Ankyrin repeat and SOCS box containing 1
F11	NM_182580	CYB561D1	Cytochrome b-561 domain containing 1
F12	NM_006145	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1
G01	NM_001955	EDN1	Endothelin 1
G02	NM_015675	GADD45B	Growth arrest and DNA-damage-inducible, beta
G03	NM_005345	HSPA1A	Heat shock 70kDa protein 1A
G04	NM_005346	HSPA1B	Heat shock 70kDa protein 1B
G05	NM_182492	LRP5L	Low density lipoprotein receptor-related protein 5-like
G06	r4_NC_012920	MitoH1	Polycistronic_H1_3
G07	r3_NC_012920	MitoH2_12106	Polycistronic_H2_200_12106_1
G08	r2_NC_012920	MitoH2_14573	Polycistronic_H2_200_14573_3
G09	r1_NC_012920	MitoH2_4162	Polycistronic_H2_200_4162_1
G10	r5_NC_012920	MitoH2_5726	Polycistronic_H2_200_5726_3
G11	NR_004407	RNU11	RNU11
G12	NM_052901	SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25
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
MRC9 BX D21	H2172 BX D21

Pre-amplification

A pre-amplification using the appropriate species- and pathway-specific RT² 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: H2172 BX D21 MRC9 BX D21

Test Performed	H2172 BX D21	MRC9 BX D21
PCR Array Reproducibility		
Average C_T (PPC)	18.72	18.53
Result	Pass	Pass
Reverse Transcription Control (RTC)		
Delta C_T (Average RTC - Average PPC)	1.19	1.06
Result	Pass	Pass
Genomic DNA Contamination (GDC)		
C_T (GDC)	22.76	23.96
Result	Inquiry	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'.

Normalization analysis

Manual Selection

Groups	Samples	RPLP0	Arithmetic Mean	Average Arithmetic Mean
Control Group	MRC9 BX D21	12.26	12.26	12.26
Group 1	H2172 BX D21	12.4	12.40	12.40

This method allows researchers to select their own internal control / housekeeping / normalization genes for the analysis. Select either the use of the Geometric or Arithmetic Mean consistently across experiments. As a general guide, only select genes with small changes in their expression across different sample groups (differences in C_T values less than 1).

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
A02	ATP5B	4.26	0.000000	
A03	ATP5C1	2.69	0.000000	
A04	ATP5F1	3.07	0.000000	
A05	ATP5G1	3.32	0.000000	
A06	ATP5G2	3.32	0.000000	
A07	ATP5G3	4.29	0.000000	
A08	ATP5H	2.33	0.000000	
A09	ATP5I	3.25	0.000000	
A10	ATP5J	2.77	0.000000	
A11	ATP5J2	3.51	0.000000	
A12	ATP5L	22.63	0.000000	
B01	ATP5O	2.23	0.000000	
B02	COX4I1	2.08	0.000000	
B03	COX5A	3.84	0.000000	
B04	COX5B	8.34	0.000000	
B05	COX6A1	7.06	0.000000	
B06	COX6A2	12.38	0.000000	
B07	COX6B1	10.41	0.000000	
B08	COX6C	14.03	0.000000	
B09	COX7A2	2.95	0.000000	
B11	COX7B	2.75	0.000000	
B12	COX8A	4.00	0.000000	
C01	CYC1	6.36	0.000000	
C02	NDUFA1	4.14	0.000000	
C03	NDUFA10	7.06	0.000000	
C04	NDUFA11	2.57	0.000000	
C06	NDUFA3	2.28	0.000000	
C07	NDUFA4	2.41	0.000000	
C08	NDUFA5	3.16	0.000000	
C09	NDUFA6	2.41	0.000000	
C10	NDUFA8	4.47	0.000000	
C11	NDUFAB1	5.35	0.000000	
C12	NDUFB10	4.11	0.000000	
D01	NDUFB2	4.66	0.000000	
D02	NDUFB3	4.11	0.000000	
D03	NDUFB4	2.08	0.000000	
D05	NDUFB6	2.83	0.000000	
D06	NDUFB7	5.17	0.000000	
D08	NDUFB9	2.33	0.000000	
D11	NDUFS1	7.36	0.000000	
D12	NDUFS2	2.81	0.000000	
E01	NDUFS3	5.62	0.000000	

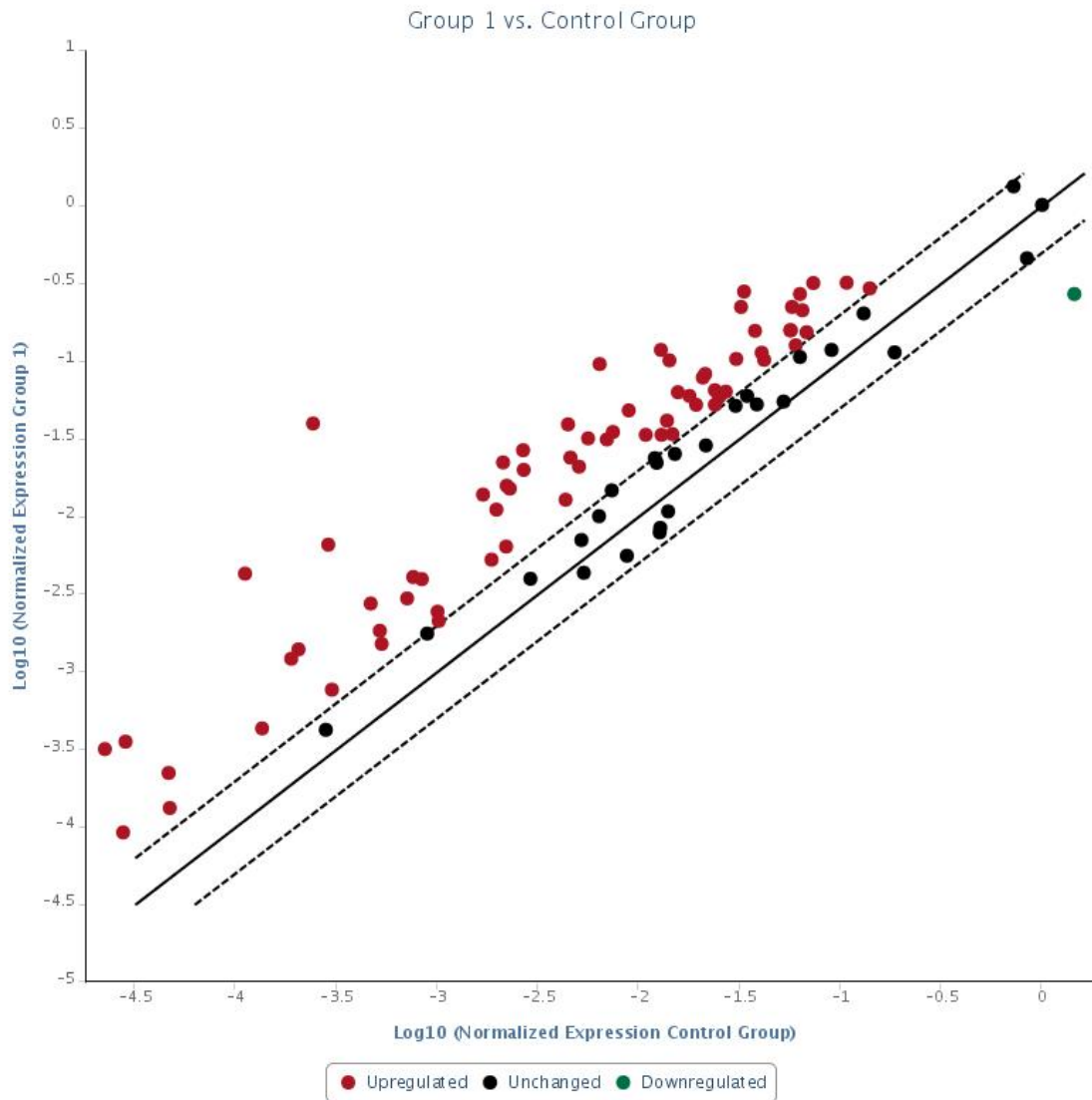
Position	Gene Symbol	Fold Regulation	p-Value	Comments
E04	NDUFS6	6.87	0.000000	
E05	NDUFS7	5.82	0.000000	
E06	NDUFS8	3.36	0.000000	
E07	NDUFV1	9.92	0.000000	
E08	NDUFV2	2.17	0.000000	
E09	NDUFV3	2.97	0.000000	
E11	SDHA	8.11	0.000000	
E12	SDHB	8.69	0.000000	
F01	SDHC	2.89	0.000000	
F02	SDHD	5.31	0.000000	
F04	UQCRC1	2.93	0.000000	
F05	UQCRC2	3.73	0.000000	
F06	UQCRFS1	9.06	0.000000	
F07	UQCRH	2.75	0.000000	
F10	ASB1	5.58	0.000000	
F11	CYB561D1	4.76	0.000000	
F12	DNAJB1	4.66	0.000000	
G01	EDN1	162.02	0.000000	
G02	GADD45B	6.73	0.000000	
G03	HSPA1A	14.83	0.000000	
G04	HSPA1B	38.05	0.000000	
G05	LRP5L	2.07	0.000000	
G08	MitoH2_14573	-5.39	0.000000	
G12	SLC25A25	2.79	0.000000	
H01	ACTB	3.84	0.000000	
H03	GAPDH	2.69	0.000000	
H04	HPRT1	6.54	0.000000	
H06	HGDC	2.53	0.000000	

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
G01	EDN1	162.02		PPH00653A
G04	HSPA1B	38.05		PPH01216B
A12	ATP5L	22.63		PPH11062B
G03	HSPA1A	14.83		PPH01193B
B08	COX6C	14.03		PPH17584D
B06	COX6A2	12.38		PPH15089A
B07	COX6B1	10.41		PPH20073D
E07	NDUFV1	9.92		PPH07537E
F06	UQCRCF1	9.06		PPH17737A
E12	SDHB	8.69		PPH06977A
B04	COX5B	8.34		PPH07071A
E11	SDHA	8.11		PPH20936F
D11	NDUFS1	7.36		PPH19871A
C03	NDUFA10	7.06		PPH14221A
B05	COX6A1	7.06		PPH13766A
E04	NDUFS6	6.87		PPH19394B
G02	GADD45B	6.73		PPH00149F
H04	HPRT1	6.54		PPH01018C
C01	CYC1	6.36		PPH00724A
E05	NDUFS7	5.82		PPH14500A
E01	NDUFS3	5.62		PPH05975A
F10	ASB1	5.58		PPH23303C
C11	NDUFAB1	5.35		PPH07347A
F02	SDHD	5.31		PPH17790A
D06	NDUFB7	5.17		PPH20190B
F11	CYB561D1	4.76		PPH07340A
F12	DNAJB1	4.66		PPH01207A
D01	NDUFB2	4.66		PPH08737A
C10	NDUFA8	4.47		PPH08929A
A07	ATP5G3	4.29		PPH06999B
A02	ATP5B	4.26		PPH19254A
C02	NDUFA1	4.14		PPH02585D
C12	NDUFB10	4.11		PPH14223A
D02	NDUFB3	4.11		PPH11130A
B12	COX8A	4.00		PPH20233A
H01	ACTB	3.84		PPH00073G
B03	COX5A	3.84		PPH19106C
F05	UQCRC2	3.73		PPH13592A
A11	ATP5J2	3.51		PPH14768A
E06	NDUFS8	3.36		PPH10450A
A05	ATP5G1	3.32		PPH10199A
A06	ATP5G2	3.32		PPH10413B
A09	ATP5I	3.25		PPH10323A
C08	NDUFA5	3.16		PPH02108B
A04	ATP5F1	3.07		PPH10213A
E09	NDUFV3	2.97		PPH14248B
B09	COX7A2	2.95		PPH09818B
F04	UQCRC1	2.93		PPH11441A
F01	SDHC	2.89		PPH20236B
D05	NDUFB6	2.83		PPH11124A
D12	NDUFS2	2.81		PPH13595A
G12	SLC25A25	2.79		PPH07341A
A10	ATP5J	2.77		PPH60000A

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
F07	UQCRH	2.75		PPH16023B
B11	COX7B	2.75		PPH20080A
H03	GAPDH	2.69		PPH00150F
A03	ATP5C1	2.69		PPH12974A
C04	NDUFA11	2.57		PPH19207A
H06	HGDC	2.53		
C07	NDUFA4	2.41		PPH09398A
C09	NDUFA6	2.41		PPH15585B
A08	ATP5H	2.33		PPH12984A
D08	NDUFB9	2.33		PPH08062A
C06	NDUFA3	2.28		PPH14220F
B01	ATP5O	2.23		PPH19444A
E08	NDUFV2	2.17		PPH09431A
D03	NDUFB4	2.08		PPH14669A
B02	COX4I1	2.08		PPH20181A
G05	LRP5L	2.07		PPH15486A

Genes Under-Expressed in Group 1 vs. Control Group

Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
G08	MitoH2_14573	-5.39		

Next steps

After using QIAGEN's RT² Profiler PCR array, use the upregulated or downregulated qPCR assays to further validate your hypothesis.

You can use individual qPCR RT² assays or create custom RT² 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.