qPCR-Array Analysis Service

Customer Name

Institute

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PO Number

Service Code

Report Date

Service Laboratory Department

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1. Overview

Service Content

Item	Requisition	Note
Total RNA Sample Number	9	
Reverse-transcription number	9	
Pathway	Human Inflammatory Cytokines & Receptors	
PCR Array Catalog	PAHS-011Z	
Technical repeat	1	
Total plate Amount	9	
Experimental design	9	

2. Sample: 9 human samples in 2C315041701

Item	Sample Name	Sample code
1	1	2C315041701-01A
2	2	2C315041701-02A
3	3	2C315041701-03A
4	4	2C315041701-04A
5	5	2C315041701-05A
6	6	2C315041701-06A
7	7	2C315041701-13A
8	8	2C315041701-14A
9	9	2C315041701-15A

3. Reverse-transcription

Start by converting the experimental RNA samples into PCR template with the RT^2 First Strand Kit (QIAGEN, 330401), according to the standard protocol of the supplier. Each sample was used 1µg RNA per 20µl reaction.

5X Buffer BC3 (µl)	Control P2 (µl)	RE3 Reverse Transcription Mix (µl)	ddH2O (µl)	RNA (1µg) volume with RNase-free water (µl)	Buffer GE (µl)	Total volume (µl)
4	1	2	3	8	2	20

4. Plate-information

Brand	PCR Array Catalog	pathway	Lot No.
SABiosciences, Qiagen	PAHS-011Z	Human Inflammatory Cytokines & Receptors	GG17.3-R1
For more detailed information (primer info, gene accession number…etc.), please see the Gene table sheet in PCRArrayDataset excel file.			

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AIMP1 A01	BMP2 A02	C5 A03	CCL1 A04	CCL11 A05	CCL13 A06	CCL15 A07	CCL16 A08	CCL17 A09	CCL2 A10	CCL20 A11	CCL22 A12
CCL23 B01	CCL24 B02	CCL26 B03	CCL3 B04	CCL4 B05	CCL5 B06	CCL7 B07	CCL8 B08	CCR1 B09	CCR2 B10	CCR3 B11	CCR4 B12
CCR5 C01	CCR6 C02	CCR8 C03	CD40LG C04	CSF1 C05	CSF2 C06	CSF3 C07	CX3CL1 C08	CX3CR1 C09	CXCL1 C10	CXCL10 C11	CXCL11 C12
CXCL12 D01	CXCL13 D02	CXCL2 D03	CXCL3 D04	CXCL5 D05	CXCL6 D06	CXCL9 D07	CXCR1 D08	CXCR2 D09	FASLG D10	IFNA2 D11	IFNG D12
IL10RA E01	IL10RB E02	IL13 E03	IL15 E04	IL16 E05	IL17A E06	IL17C E07	IL17F E08	IL1A E09	IL1B E10	IL1R1 E11	IL1RN E12
IL21 F01	IL27 F02	IL3 F03	IL33 F04	IL5 F05	IL5RA F06	IL7 F07	IL8 F08	IL9 F09	IL9R F10	LTA F11	LTB F12
MIF G01	NAMPT G02	OSM G03	SPP1 G04	TNF G05	TNFRSF11B G06	TNFSF10 G07	TNFSF11 G08	TNFSF13 G09	TNFSF13B G10	TNFSF4 G11	VEGFA G12
ACTB H01	B2M H02	GAPDH H03	HPRT1 H04	RPLP0 H05	HGDC H06	RTC H07	RTC H08	RTC H09	PPC H10	PPC H11	PPC H12

5. qPCR-Array

5.1 Every reaction well was performed by using 10 ng cDNA per 25 µl reaction volume.

5.2 Quantitative PCR was performed by the condition: 95°C, 10min; 40 cycles of (95°C, 15sec; 60°C, 1min).

5.3 The 10min for 95°C is required to activate the Hotstart Taq DNA polymerase.

5.4 The assays were performed on a Bio-Rad CFX Connect™ Real-Time PCR Detection System.

5.5 RT2 qPCR master mix (QIAGEN, 330523) is used for qPCR-array assay.

Mix the following components in a PCR tube:

Pre-Mix (µl)	RT2 SYBR Green qPCR Master Mix (µl)	Nuclease-free H20 (µl)	Template cDNA (10 ng/µl)
2700	1350	1248	102

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6. Experimental design

	Control		Treat	
	Control		**Treat**	
1	01A	1	13A	10
2	04A	2	13A	11
3	02A	3	14A	12
4	05A	4	14A	13
5	03A	5	15A	14
6	06A	6	15A	15
7	13A	7	14A	16
8	14A	8	15A	17
9	13A	9	15A	18

7. qPCR-array quality check result

Item (Sample Name)	PCR Array Reproducibility	AVG PPC (Ct) (Wells H10~H12)	AVG RTC (Ct) (Wells H7~H9)	RT Efficiency (Delta Ct, AVG RTC-AVG PPC)	Genomic DNA contamination	Result
1	Inquiry	22.56	25.24	2.68 (Pass)	Pass	Inquiry
2	Inquiry	22.37	25.23	2.86 (Pass)	Pass	Inquiry
3	Inquiry	22.30	25.21	2.91 (Pass)	Pass	Inquiry
4	Inquiry	22.16	25.16	3.00 (Pass)	Pass	Inquiry
5	Inquiry	22.49	25.30	2.81 (Pass)	Pass	Inquiry
6	Inquiry	22.23	25.05	2.82 (Pass)	Pass	Inquiry
7	Inquiry	22.29	25.96	3.67 (Pass)	Pass	Inquiry
8	Inquiry	22.19	25.35	3.16 (Pass)	Pass	Inquiry
9	Inquiry	22.36	25.39	3.03 (Pass)	Pass	Inquiry

PCR Array Reproducibility

Criteria：If the Average PPC C_t is 20$\pm$2 and no two arrays have Average PPC C_t are > 2 away from one another then the sample and group Pass.

RTC (Reverse Transcription Controls)

Criteria：A value of ($Ct^{RTC} - Ct^{PPC}$) less than five (5) indicates no apparent inhibition.
A value greater than five (5) provides evidence of impurities that inhibited the reverse transcription phase of the procedure. Double check the quality, purity, and integrity of the RNA samples.

PPC (Positive PCR Control)

Criteria：Values of C_t^{PPC} = 20 $\pm$2 on each PCR Array indicate a lack of PCR inhibitors. Higher values and more widely variable values across arrays indicate the presence of inhibitors with different concentrations in or effects on the samples.

GDC (genomic DNA contamination)

Criteria：If C_t(GDC) >= 35, then the GDC QC reports 'Pass'; if C_t(GDC) < 35, then the GDC QC reports 'Inquiry'.

8. **The fold change & fold regulation analysis**

There are two reference genes in this plate, the reference genes with stable Ct values in all control and test samples were selected to normalize the target genes. We selected the **ACTB** and **RPLP0** as reference genes.

Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in the Test Sample divided the normalized gene expression (2^(- Delta Ct)) in the Control Sample.

Fold-Regulation represents fold-change results in a biologically meaningful way. Fold-change values greater than one indicate 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.

Genes with expression fold regulations between samples larger than 2 are selected as differential genes.

RT^2 profiler PCR Array Data analysis software (v3.5) was used for PCR array data analysis. http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php

The Ct determination: The Bio-Rad CFX Manager Software was used to determine the Ct value. In this report, we used automatic threshold and automatic baseline setting for Ct determination.

8.1 The raw data's combine analysis for all genes.

Fold-regulation greater than 2 indicate a positive or an up-regulation by color Red.

Fold-regulation less than -2 indicate a negative or down-regulation by color Blue.

Comments:

A: This gene average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30).
B: This gene average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples.
C: This gene average threshold cycle is either not determined or greater than the defined cut-off value (default 35), in both samples meaning that its expression was undetected.

(1)

Analysis Design		
	Control	Treat
1	01A 1	13A 10

Position	Gene Symbol	Fold Change (comparing to control group)		Fold Regulation (comparing to control group)
		Fold Change	Comments	Fold Regulation
A01	AIMP1	18.42	A	18.42
A02	BMP2	4.09	A	4.09
A03	C5	0.13	B	-7.53
A04	CCL1	2.76	B	2.76
A05	CCL11	0.24	C	-4.17
A06	CCL13	0.4	B	-2.5
A07	CCL15	0.33	B	-3.04
A08	CCL16	0.24	C	-4.17
A09	CCL17	0.24	C	-4.17
A10	CCL2	5.36	A	5.36
A11	CCL20	3176.91	A	3176.91
A12	CCL22	0.02	B	-41.77
B01	CCL23	0.05	A	-20.44
B02	CCL24	0.2	B	-4.9
B03	CCL26	0.24	C	-4.17
B04	CCL3	41.34	OKAY	41.34
B05	CCL4	88.12	A	88.12
B06	CCL5	9.61	OKAY	9.61
B07	CCL7	7.59	B	7.59
B08	CCL8	0.24	C	-4.17
B09	CCR1	1.93	A	1.93
B10	CCR2	0	A	-680.5
B11	CCR3	0.24	C	-4.17
B12	CCR4	0.24	C	-4.17
C01	CCR5	0	A	-404.05
C02	CCR6	0.24	C	-4.17
C03	CCR8	0.24	C	-4.17
C04	CD40LG	0.24	C	-4.17
C05	CSF1	15.48	A	15.48
C06	CSF2	2.29	B	2.29
C07	CSF3	36.47	A	36.47
C08	CX3CL1	0.24	C	-4.17
C09	CX3CR1	0.01	B	-83.1
C10	CXCL1	268.25	A	268.25
C11	CXCL10	6.01	B	6.01
C12	CXCL11	5.08	B	5.08
D01	CXCL12	0.17	B	-5.77
D02	CXCL13	3.7	B	3.7
D03	CXCL2	143.09	A	143.09
D04	CXCL3	573.35	A	573.35
D05	CXCL5	25.54	A	25.54
D06	CXCL6	115.82	A	115.82
D07	CXCL9	0.24	C	-4.17

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D08	CXCR1	0.24	C	-4.17
D09	CXCR2	0.02	A	-53.96
D10	FASLG	0.24	C	-4.17
D11	IFNA2	0.24	C	-4.17
D12	IFNG	0.24	C	-4.17
E01	IL10RA	0.52	OKAY	-1.92
E02	IL10RB	0.78	OKAY	-1.27
E03	IL13	0.24	C	-4.17
E04	IL15	1.71	B	1.71
E05	IL16	0.07	B	-15.07
E06	IL17A	0.24	C	-4.17
E07	IL17C	0.24	C	-4.17
E08	IL17F	0.24	C	-4.17
E09	IL1A	10.2	A	10.2
E10	IL1B	4970.08	A	4970.08
E11	IL1R1	15.41	A	15.41
E12	IL1RN	25.96	OKAY	25.96
F01	IL21	0.24	C	-4.17
F02	IL27	0.24	C	-4.17
F03	IL3	0.24	C	-4.17
F04	IL33	0.93	B	-1.08
F05	IL5	0.24	C	-4.17
F06	IL5RA	0.24	C	-4.17
F07	IL7	0.62	B	-1.62
F08	IL8	230.1	OKAY	230.1
F09	IL9	0.24	C	-4.17
F10	IL9R	0.24	C	-4.17
F11	LTA	0.32	B	-3.14
F12	LTB	0.43	B	-2.34
G01	MIF	0.22	OKAY	-4.56
G02	NAMPT	21.36	A	21.36
G03	OSM	0.01	A	-149.94
G04	SPP1	4237.87	A	4237.87
G05	TNF	0.57	OKAY	-1.75
G06	TNFRSF11B	0.24	C	-4.17
G07	TNFSF10	3.76	A	3.76
G08	TNFSF11	0.34	B	-2.92
G09	TNFSF13	0.62	B	-1.61
G10	TNFSF13B	2.87	A	2.87
G11	TNFSF4	1.59	B	1.59
G12	VEGFA	3.38	OKAY	3.38
H01	ACTB	0.48	OKAY	-2.06
H02	B2M	34.08	OKAY	34.08
H03	GAPDH	0.17	OKAY	-5.81
H04	HPRT1	0.58	OKAY	-1.74
H05	RPLP0	0.17	OKAY	-5.89

The differential gene analysis

Comments:

|	
A: This gene average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30).
B: This gene average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples.
C: This gene average threshold cycle is either not determined or greater than the defined cut-off value (default 35), in both samples meaning that its expression was undetected.

1.

Arrays included in Test Group:	11
Arrays included in Control Group:	1

Genes Over-Expressed in Group 1 vs. Control Group				
Position	**Gene Symbol**	**Fold Regulation**	**Comments**	**RT2 Catalog**
A01	AIMP1	18.4167	A	PPH01171C
A02	BMP2	4.0874	A	PPH00549C
A04	CCL1	2.7585	B	PPH00701B
A10	CCL2	5.3631	A	PPH00192F
A11	CCL20	3176.9145	A	PPH00564C
B04	CCL3	41.3438	OKAY	PPH00566F
B05	CCL4	88.118	A	PPH00563B
B06	CCL5	9.6109	OKAY	PPH00703B
B07	CCL7	7.5897	B	PPH00575C
C05	CSF1	15.4835	A	PPH00124B
C06	CSF2	2.2949	B	PPH00576C
C07	CSF3	36.4656	A	PPH00723B
C10	CXCL1	268.2532	A	PPH00696C
C11	CXCL10	6.0068	B	PPH00765E
C12	CXCL11	5.0761	B	PPH00506A
D02	CXCL13	3.7042	B	PPH01168E
D03	CXCL2	143.0933	A	PPH00552F
D04	CXCL3	573.3526	A	PPH00557C
D05	CXCL5	25.5362	A	PPH00698B
D06	CXCL6	115.8244	A	PPH00704B
E09	IL1A	10.2032	A	PPH00690A
E10	IL1B	4970.0817	A	PPH00171C
E11	IL1R1	15.4074	A	PPH00274A
E12	IL1RN	25.963	OKAY	PPH00555G
F08	IL8	230.097	OKAY	PPH00568A
G02	NAMPT	21.3594	A	PPH16287B
G04	SPP1	4237.8705	A	PPH00582E
G07	TNFSF10	3.7598	A	PPH00242F
G10	TNFSF13B	2.8669	A	PPH01180B
G12	VEGFA	3.3798	OKAY	PPH00251C
H02	B2M	34.0769	OKAY	PPH01094E

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Genes Under-Expressed in Group 1 vs. Control Group				
Position	Gene Symbol	Fold Regulation	Comments	RT2 Catalog
A03	C5	-7.5269	B	PPH07062C
A05	CCL11	-4.1702	C	PPH00570B
A06	CCL13	-2.5024	B	PPH00578B
A07	CCL15	-3.0441	B	PPH01161B
A08	CCL16	-4.1702	C	PPH00579B
A09	CCL17	-4.1702	C	PPH00543C
A12	CCL22	-41.766	B	PPH00697F
B01	CCL23	-20.4369	A	PPH00699A
B02	CCL24	-4.9036	B	PPH01162B
B03	CCL26	-4.1702	C	PPH01163F
B08	CCL8	-4.1702	C	PPH01167B
B10	CCR2	-680.5024	A	PPH00612F
B11	CCR3	-4.1702	C	PPH00613B
B12	CCR4	-4.1702	C	PPH00614A
C01	CCR5	-404.052	A	PPH00615F
C02	CCR6	-4.1702	C	PPH00616F
C03	CCR8	-4.1702	C	PPH00618F
C04	CD40LG	-4.1702	C	PPH00259C
C08	CX3CL1	-4.1702	C	PPH00689C
C09	CX3CR1	-83.0973	B	PPH00620A
D01	CXCL12	-5.7705	B	PPH00528B
D07	CXCL9	-4.1702	C	PPH00700B
D08	CXCR1	-4.1702	C	PPH01040F
D09	CXCR2	-53.9577	A	PPH00608F
D10	FASLG	-4.1702	C	PPH00142C
D11	IFNA2	-4.1702	C	PPH00379A
D12	IFNG	-4.1702	C	PPH00380C
E03	IL13	-4.1702	C	PPH00688F
E05	IL16	-15.0668	B	PPH00586A
E06	IL17A	-4.1702	C	PPH00537C
E07	IL17C	-4.1702	C	PPH01074F
E08	IL17F	-4.1702	C	PPH01680F
F01	IL21	-4.1702	C	PPH01684A
F02	IL27	-4.1702	C	PPH18307B
F03	IL3	-4.1702	C	PPH00691B
F05	IL5	-4.1702	C	PPH00692B
F06	IL5RA	-4.1702	C	PPH00605E
F09	IL9	-4.1702	C	PPH00693A
F10	IL9R	-4.1702	C	PPH00609A
F11	LTA	-3.1418	B	PPH00337F
F12	LTB	-2.3428	B	PPH00342F
G01	MIF	-4.5643	OKAY	PPH00548E
G03	OSM	-149.9436	A	PPH02646B
G06	TNFRSF11B	-4.1702	C	PPH01049B
G08	TNFSF11	-2.916	B	PPH01048F
H01	ACTB	-2.0639	OKAY	PPH00073G
H03	GAPDH	-5.806	OKAY	PPH00150F
H05	RPLP0	-5.8944	OKAY	PPH21138F
H06	HGDC	-4.1702	C	

9. Clustering Analysis

The correlation of expression profiles between samples and treatment conditions was demonstrated by unsupervised hierarchical clustering analysis.
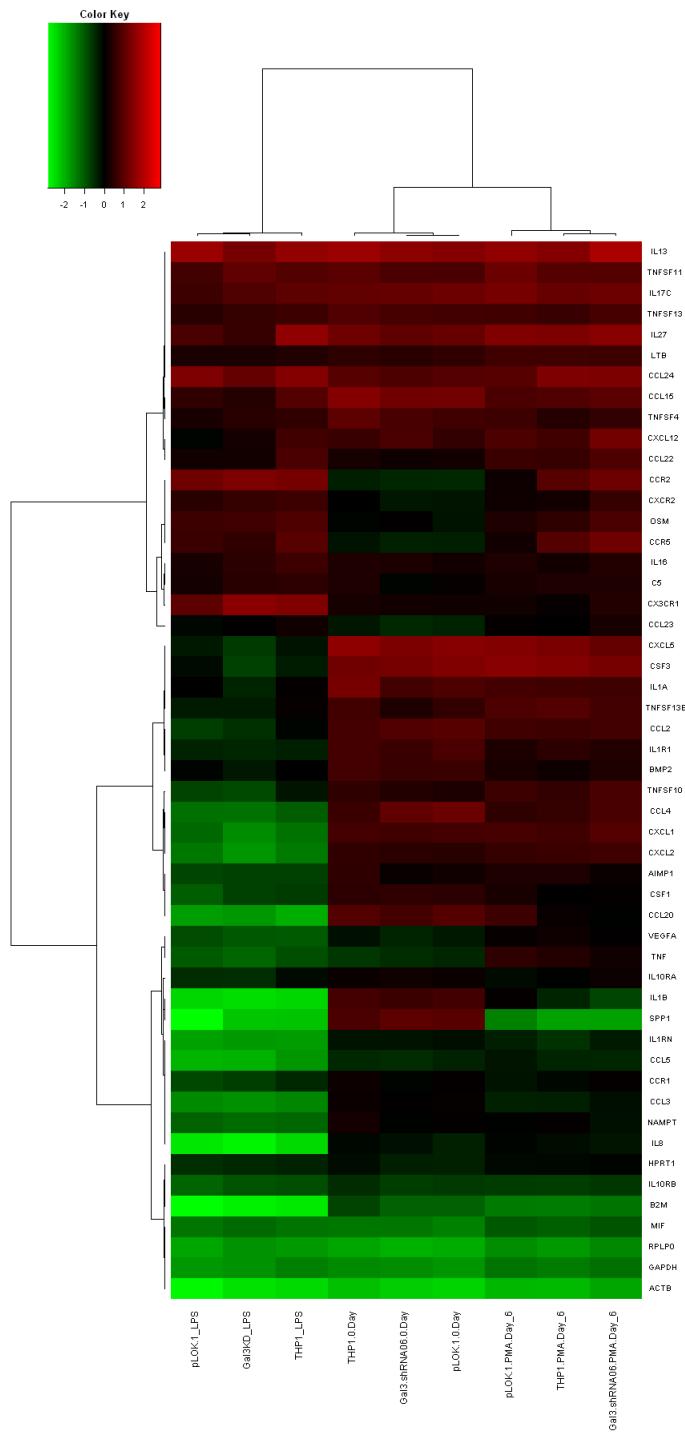
Clustering analysis

Clustering was performed to visualize the correlations among the varying sample conditions. Up- and down-regulated genes are represented in red and green colors, respectively. All of the genes were selected for the clustering analysis.

If you have any questions, please contact us.

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