

SYBR Green qPCR Service

Customer Information

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Service Code: 1P314010101
Date: 2014/01/10

1. Samples: 2 Human samples

Item	Sample Name	qPCR Sample code
1	X	
2	Y	

Comparisons:

	Treatment	Control
set1	Y	X

2. Primers

This qPCR service request included 2 target genes. We used the GAPDH as reference gene.

2.1 primer name

Gene Name	Primer name	Primer code	Primer sequence
a	a-F	1	ACATGGGAACAGAGGTGTCG
	a-R	2	CCACGTAGGCAATCTGTGAT
b	b-F	3	TGTGATACCGACACCATCCA
	b-R	4	ATGTGGGAAAGCAGCCTCTG
GAPDH	GAPDH-F	5	CATGAGAAGTATGACAACAGCCT
	GAPDH-R	6	AGTCCTCCACGATACCAAAGT

3. Quantitative PCR

Each sample was tested in triplicate. Each reaction included 20 ng cDNA, 500nM of forward and reverse primers, and 2X Fast SYBR Green PCR Master Mix (Applied Biosystems, 4385612).

10µl reaction volumes were used according to the table below:

Fast SYBR master mix(µl)	nuclease-free water(µl)	RT product (20 ng/µl)	F+R primer (10µM)	total volume (µl)
5	3	1	1	10

A BIO-RAD CFX Connect real-time PCR machine was used with the following program: (95°C → 20sec), and 39 cycles (95°C → 5sec, 60°C → 30sec).

4. Data Analysis

BIO-RAD CFX Manager Version 3.0 software was used for experimental setup and data analysis. Target gene qPCR data were normalized to reference genes, GAPDH.

4.1 mean Cq values

set1	control	treatment
	01A	02A
Mean Cq	X	Y
A	25.17	23.68
b	21.03	21.25
GAPDH	16.40	16.27

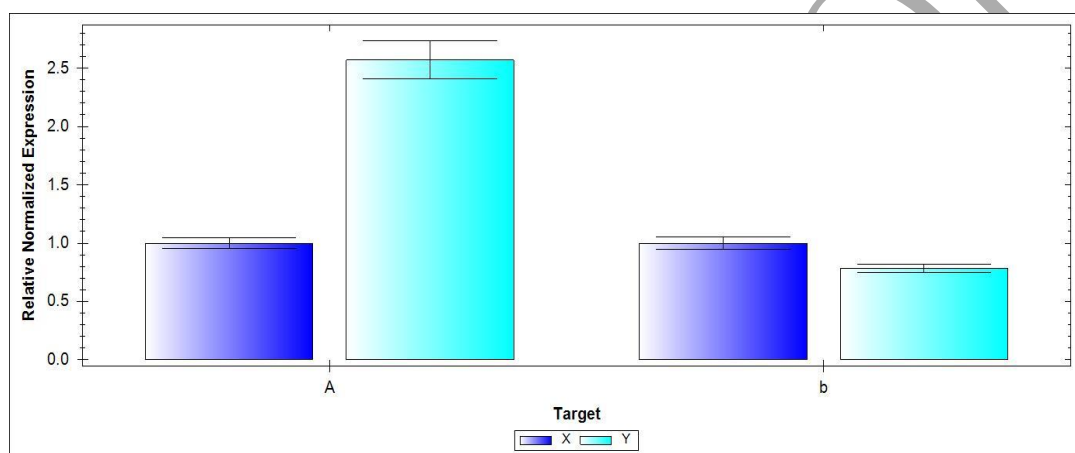
4.2 SD

set1	control	treatment
	01A	02A
Cq SEM	X	Y
A	0.05	0.07
b	0.07	0.03
GAPDH	0.03	0.04

4.3 Fold change analysis – fold changes are relative to the control group to easily determine the degree of up- or down-regulation in the treatment group.

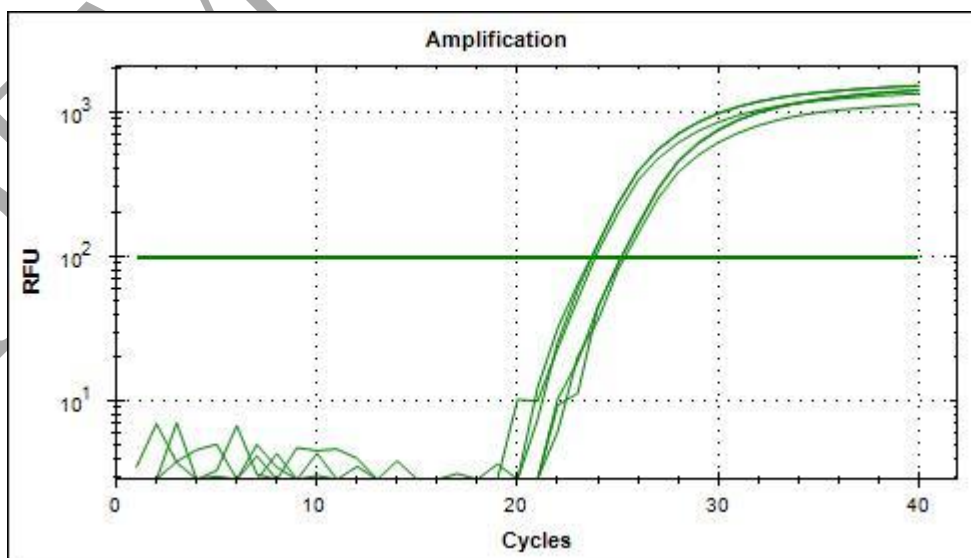
Set1

set1	control	treatment
	01A	02A
Fold change	X	Y
A	1.00	2.57
b	1.00	0.79

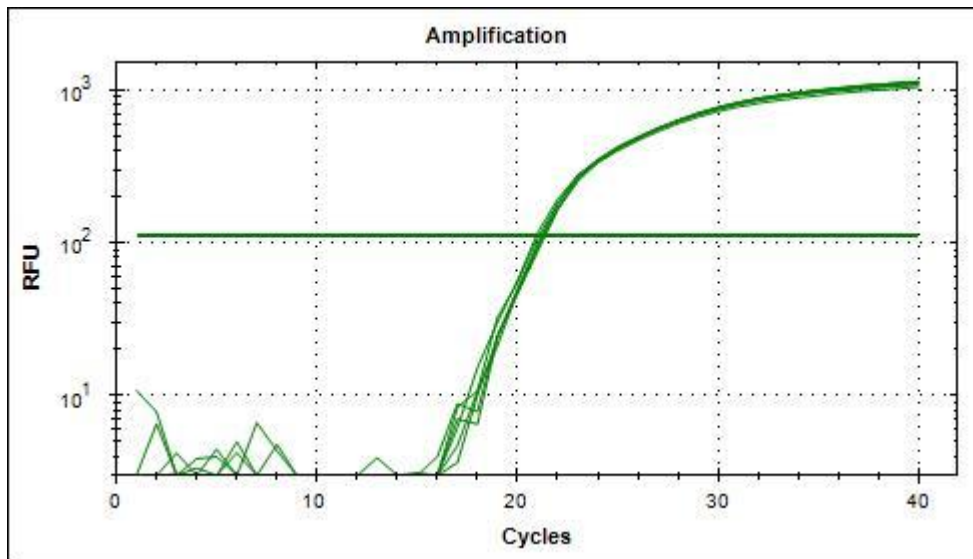


4.4 Amplification curve result

4.4.1 A

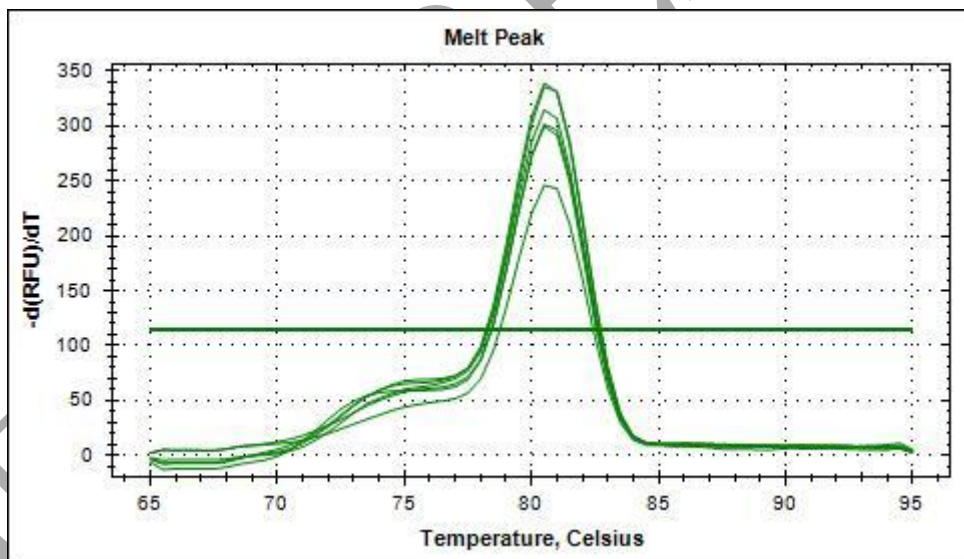


4.4.2 B



4.5 Melting curve result

4.5.1 A

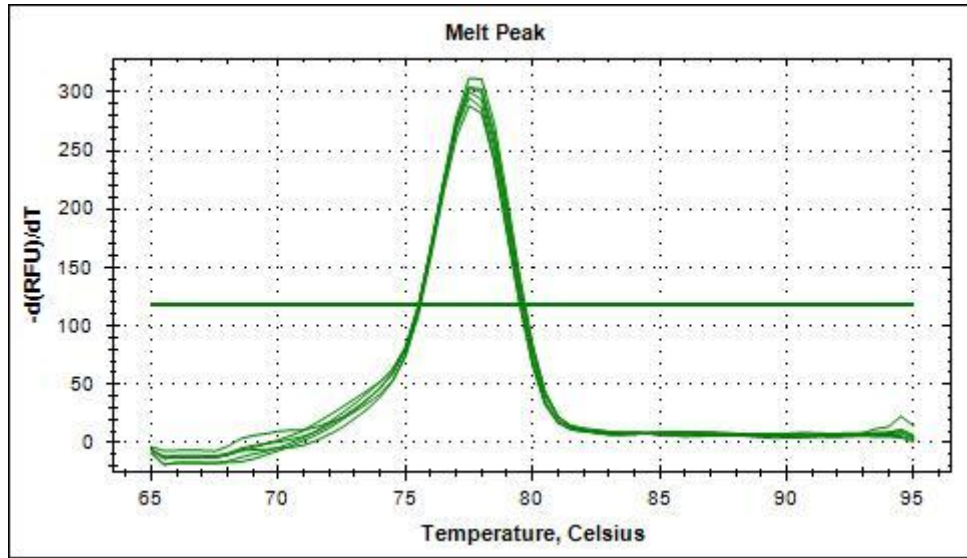


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4.5.2 B



DEMO REPORT

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