Quantitative real-time polymerase chain reaction (qRT – PCR) for determination of human OGG1 mRNA level in human cells

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Abstract

The main goal of this experiment was to measure the expression of the human OGG1 gene by measuring the mRNA concentration in cells through the use of quantitative real-time-PCR amplification of cDNA made during the previous experiment. Based on the obtained results, the fold change of a normal cell is higher than the fold change of a cancer cell, while the mRNA level in a normal cell is lower than in a cancer cell.

Introduction

RT – qPCR, or real-time quantitative polymerase chain reaction, is a common method applied to measure gene expression using a small amount of RNA. This technique is typically derived from the sample of conventional PCR.

qPCR is usually processed faster and gives more detailed real-time results to quantify nucleic acids, while regular real-time RCR is used for cDNA amplification and detection. PRC products can be detected using CYBR Green or specific DNA probes. CYBR Green is a specific fluorescent dye, which intercalates with any double-stranded DNA, while specific DNA probes, due to their oligonucleotide nature, allow the detection of fluorescence signals through the hybridization of the probe with its complementary sequence in a cDNA. cDNAs are important in this experiment because they can be isolated as a single fragment, which does not contain introns, and most eukaryotic genes contain mainly introns, non-coding sequences. Thus, cDNAs become a great template for exponential amplification, which later can be used for gene cloning.

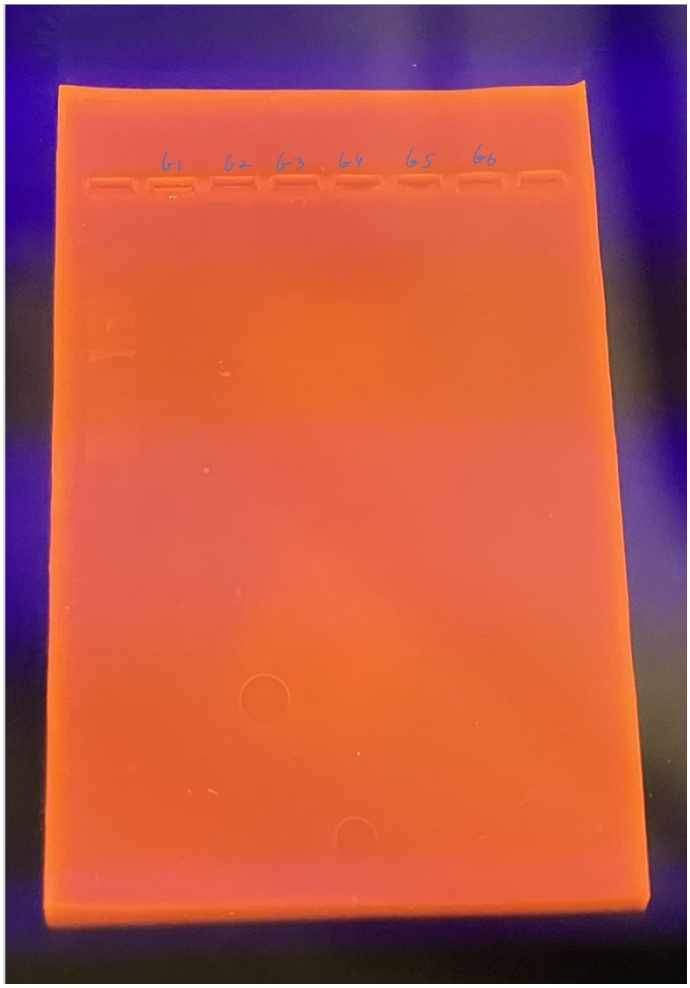
Once the experiment was performed, qPCR results can be shown as the cycle number of the thresholds illustrating the number of fluorescence signals, C_T. The larger the C_T value, the smaller the level of mRNA of a gene. However, it is necessary to normalize the qPCR, because of the number of possible errors that

could happen during the experiment. To do so, a specific gene that is being studied needs to be quantified in relation to internal control gene. This ratio will allow to compare the former without knowing its normal level of expression.

Procedures

First, the PCR reagents were prepared by thawing the master mix on ice. Once the master mix is thawed, the tube was gently tabbed to mix the reagents. Next, the DNA samples and primers were thawed on ice, vortexed to mix, and briefly centrifuged. Next, the PCR reactions were prepared. Reaction number 1, RT-qPCR for OGG1, was made by mixing 2 ul cDNA samples from Lab 7, 1 ul 10 pmol/u forward primer for human OGG1, 1 ul 10 pmol/ul reverse primer for human OGG1, 10 ul PowerTrack SYBR Green Master Mix, and 6ul DEPC-treated water. The total reagent contained 20 ul solution. The second reaction, RT – qPCR for beta-actin (internal control) was made by mixing 2 ul cDNA samples from Lab 7, 1 ul 10 pmol/u forward primer for human beta-actin, 1 ul 10 pmol/u reverse primer for human beta-actin, 10 ul PowerTrack SYBR master Mix, and 6 ul DEPC-treated water. The amount of total solution was 20 ul. Later, the TA put the samples into a real-time PCR thermal cylinder, and the results were analyzed.

Results



No bands appeared for any groups. PCR product is expected to not be pure because such a result is usually caused by the mismatched amount of the cDNA, which brings impurities.

Well	Fluor	Target	Content	Sample	Cq
K01	SYBR	Actin	Unkn	Group 3.1-HEK	30.26
K02	SYBR	Actin	Unkn	Group 3.2-HEK	25.49
K03	SYBR	Actin	Unkn	Group 3.3-Hela	23.93
K04	SYBR	Actin	Unkn	Group 3.4-Hela	N/A
K05	SYBR	Actin	Unkn	Group 3.5-HEK	21.25
K06	SYBR	Actin	Unkn	Group 3.6-HEK	23.67
K07	SYBR	Actin	Unkn	MO-HEK	22.24
L01	SYBR	pol B	Unkn	Group 3.1-HEK	32.01
L02	SYBR	pol B	Unkn	Group 3.2-HEK	32.88
L03	SYBR	pol B	Unkn	Group 3.3-Hela	33.59
L04	SYBR	pol B	Unkn	Group 3.4-Hela	N/A
L05	SYBR	pol B	Unkn	Group 3.5-HEK	29.77
L06	SYBR	pol B	Unkn	Group 3.6-HEK	29.54
L07	SYBR	pol B	Unkn	MO-HEK	26.24
M01	SYBR	Actin	Unkn	Group 4.1-Hela	22.39
M02	SYBR	Actin	Unkn	Group 4.2-HEK	31.57
M03	SYBR	Actin	Unkn	Group 4.3-Hela	28.02
M04	SYBR	Actin	Unkn	Group 4.4-Hela	23.09
M05	SYBR	Actin	Unkn	Group 4.5-Hela	20.14
M06	SYBR	Actin	Unkn	Group 4.6-HEK	25.47
M07	SYBR	Actin	Unkn	empty	N/A
N01	SYBR	pol B	Unkn	Group 4.1-Hela	30.70
N02	SYBR	pol B	Unkn	Group 4.2-HEK	33.77
N03	SYBR	pol B	Unkn	Group 4.3-Hela	31.94
N04	SYBR	pol B	Unkn	Group 4.4-Hela	29.69
N05	SYBR	pol B	Unkn	Group 4.5-Hela	28.46
N06	SYBR	pol B	Unkn	Group 4.6-HEK	32.83
N07	SYBR	pol B	Unkn	empty	N/A

C_q *and* C_T are the same things, and both stand for quantification cycle.

HEK cell:

Group 3
Cq1 = 30.26 (Actin)
Cq 2 = 32.01 (pol B)
dCq = 32.01 – 30.26 = 1.74

Group 4
Cq1= 31.57 (Actin)
Cq2 = 33.77 (pol B)
dCq = 33.77 – 31.57 = 2.20

ddCq = 2.20 – 1.74 = 0.46

$2^{-ddCq}= 2^{-0.46}$=0.727

Hela cell:
Group 3
Cq 1 = 23.93 (Actin)

Cq 2= 33.59 (pol B)
dCq = 33.59 – 22.93 = 10.66

Group 4

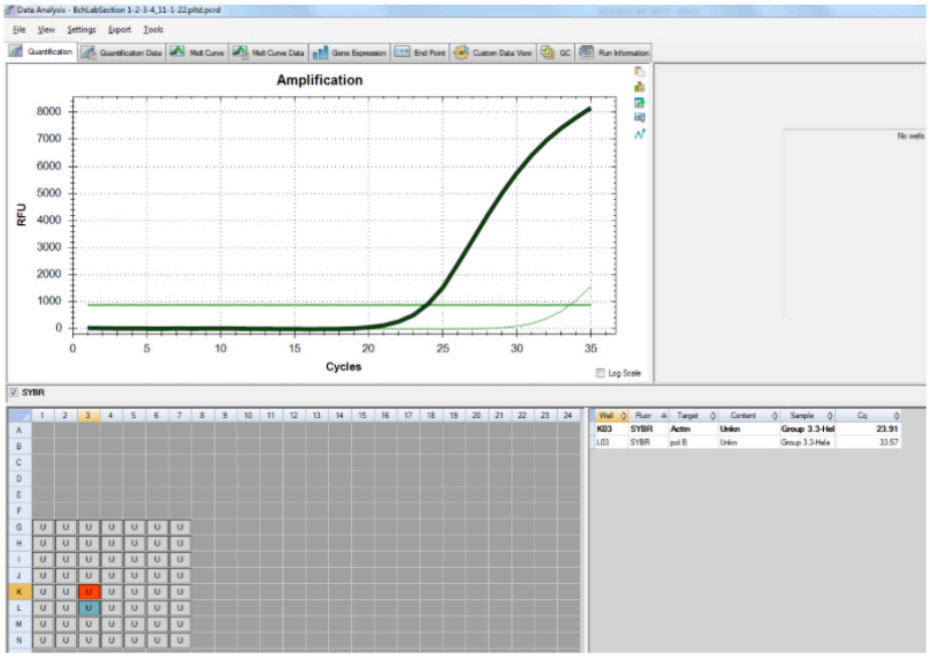
Cq 1 = 28.03 (Actin)
Cq 2= 31.94 (pol B)

dCq = 31.94-28.03 = 3.91

ddCq = 10.66 – 3.91 = 6.75

$2^{-ddCq} = 2^{-6.75} = 0.0093$

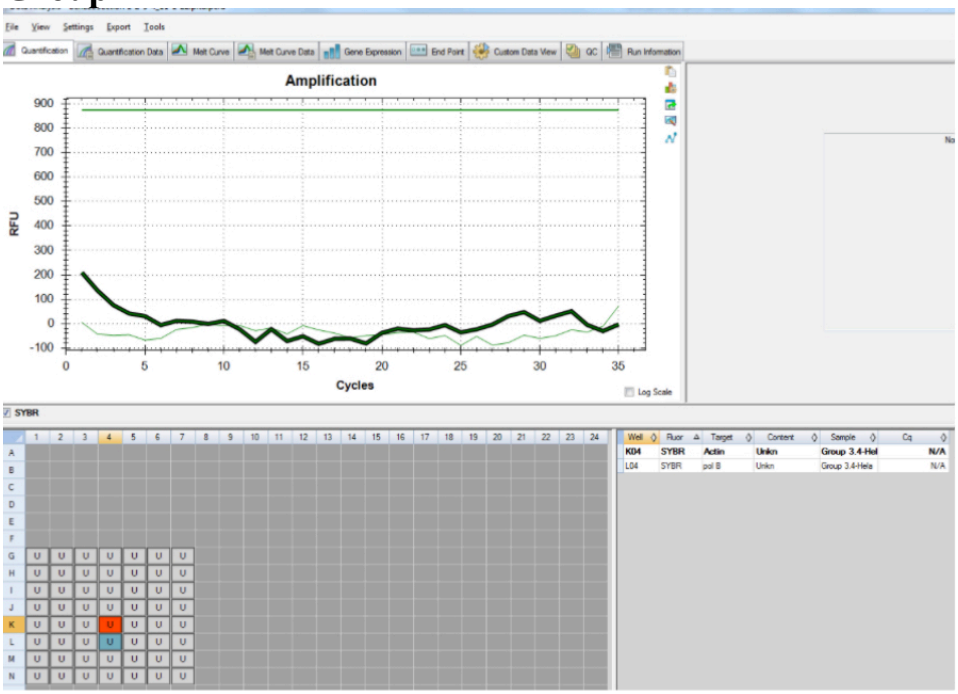
Group 3



Cq for Actin = 23.91
Cq for pol B = 33.57
Threshold = 1000 RFU

Pol B has a higher Cq value because the gene is being expressed at a higher quantit

Group 4

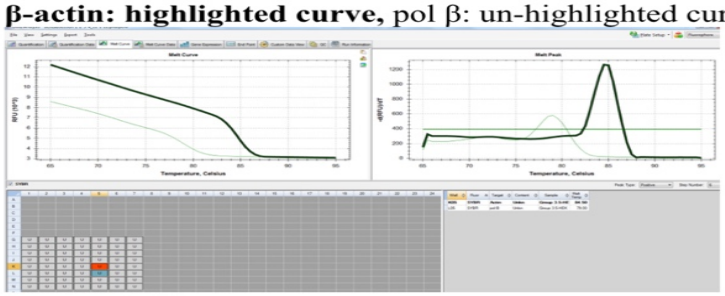


Actin Cq = N/A
Pol B Cq = N/A
Threshold line = 900 RFU

The Cq values are not available because both curves lie under the threshold line.

Melt curve.

β-actin: highlighted curve, pol β: un-highlighted curve



HEK cell Cq melt temp = 84.50 (Actin)
HEK cell Cq melt temp = 79.00 (pol B)

Actin has a higher melting temperature.

Discussion

Based on the gel results, no bands could be seen. This is usually caused by the problem with the DNAs. There is either too much or too little of the cDNAs. Thus, it will affect the PCR results, because cDNAs serve as a template for the polymerase chain reaction. Excess amounts of the cDNA can, first, result in a wasting of the material and, second, increase the polymerase chain reaction inhibition by impurities. If there is not enough cDNA, the reaction will not occur as there is no template for it. The possible source of error for the mismatched amount of cDNA is inaccurate measurement of the volume using a micropipette. Thus, the pipette should be better calibrated in the future.

The fold change of the target gene expression in a sample relative to a reference sample, normalized to the reference gene for the HEK cell is 0.727, while the obtained value for the Hela cell was 0.0093. Thus, the fold change of a normal cell is higher than the fold change of a cancer cell. However, since the C_T value for the normal cell is larger than the value of a cancer cell, the level of mRNA in a normal cell is lower. The fold change illustrates whether a gene is up- or down- regulated. If the value of a fold change is greater than 0, then the gene is up-regulated, or, in other words, expressed in a higher quantity. As known, if a gene is not expressed

in a proper amount, it may result in a mutation, which can cause cancer development.

Looking at the graph, the Cq values for group 3 from the table are close to the Cq values obtained for the graph. However, for the group 4 graph, results are not available, although the numbers can be found in the table. This is because both actin and pol B curves don't intercept the threshold line, which is a systematic error.

The melt curve graphs usually indicate the change when double-stranded DNA is "melting" into single-stranded DNA. The obtained results are- 84.50 degrees Celsius for Actin and 79.00 degrees Celsius for pol B. The expected values should be 85 degrees Celsius for the Actin, and 78 degrees Celsius for pol B. Obtained values are slightly different from the expected value, which could be explained by possible contaminations or inaccurate measurements of the components, but still close to each other. The main sources of error could happen during sample preparation as it could have been contaminated or lost.

Reference

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Notebook pages