All-in-One™ qPCR Primer Array Data Analysis Operation Guidance

1. qPCR Data Analysis Principle

Web-based All-in-One™ qPCR Primer Array Data Analysis Soft use ΔΔCT Method to perform relative quantification of genes between different samples. ΔΔCT data analysis, a relative quantitative analysis technique, is the most common and direct method for gene-expression analysis. The method requires stable expression from reference genes to normalize the variation introduced by each step, including sample collections, RNA isolations, reverse transcriptions and amplifications. Typically housekeeping genes such as GAPDH, ACTB and B2M are used as reference genes. In miRNA detections, some constantly expressed small RNAs such as U6, SNORD44 and SNORD48, are used.

In Real-Time PCR, as in many amplification-based techniques, the amount of amplification products (N) is calculated as follows:

$N = N_0 \times (1+E)^{CT}$ (N_0: number of template molecules, CT: threshold cycle, E: amplification efficiency)

When the amplification efficiency E is 100%, the number of template molecules in pre-amplification mix is calculated as follows:

$N_0 = N \times 2^{-CT}$.

To analyze the fold differences in expression level for the gene of interest in multiple samples using the ΔΔCT method, the amount of the amplification template from different samples is normalized by dividing the expression level of the gene of interest (x) with the reference factor (r) as follows:

$N_{rel} = N_{0x}/N_{0r} = N \times 2^{-CTx} / N \times 2^{-CTr} = 2^{-(CTx - CTr)} = 2^{-\Delta CT}$

The changes in normalized expression levels of the gene of interest (x) between experimental sample (sample 1) and the control sample (sample 2) is as follows:

$Nrel_1/Nrel_2 = 2^{-\Delta CT1}/2^{-\Delta CT2} = 2^{-(\Delta CT1 - \Delta CT2)} = 2^{-\Delta\Delta CT}$

The value of $2^{-\Delta\Delta CT}$ is the fold difference in expression level of the gene of interest between different samples.

2. Obtain the C_T or Cp values

The baseline is the noise level in early cycles. Generally, the baseline and threshold are automatically calculated by the qPCR program. . However, if the lowest Ct is less than the upper limit of the baseline setting, then the baseline should be manually adjusted. Use the “Linear View” of the amplification plot to determine the earliest visible amplification, and then set the baseline from cycle 2 to 2 cycles before the earliest visible amplification. Normally it is between 2 to 10 cycles. Do not use cycles greater than 15.

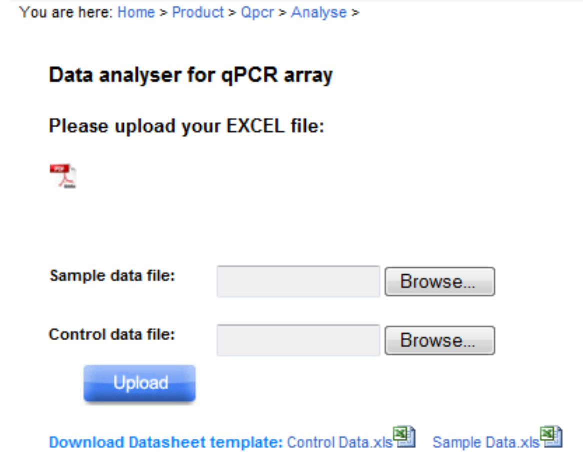
Ensure that baseline settings are the same across all PCR runs in the same analysis to allow comparison of results.

Correct placement of the threshold is the next crucial step in data analysis. To adjust the threshold properly, set the threshold value within the exponential phase of all amplification plots when viewed using the logarithmic scale for the y axis.

The Ct is defined as the cycle when sample fluorescence exceeds a chosen threshold above background fluorescence. This is also known as the Cp or crossing point. Export the data. Most qPCR instruments provide a function for exporting Ct or Cp values to Excel.

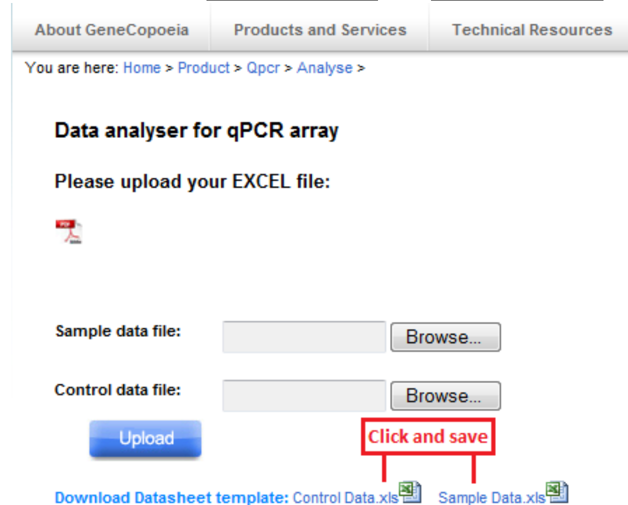
3． Analyze the qPCR results with GeneCopoeia's online qPCR Primer Array Data Analysis Soft

a. Access the web-based data analysis tools at http://www.genecopoeia.com/product/qpcr/analyse/ with the following interface:



b. Download the data analysis template forms

Click and save the Sample Data.xls and Control Data.xls.



* This Data Analysis System uses the ΔΔCT method to perform fold-change analysis for each gene between test samples and control samples. So two data forms are needed, one is for control samples and the other for test samples.

* Current template forms are for Human Wnt Signaling 96 Q-PCR Array (Cat＃: HAQPA-102-01).

c． Import the Ct (Cp) values into the corresponding data forms

Import the Ct (Cp) values into the corresponding data forms (Sample Data.xls and Control Data.xls). Open the download data analysis template forms, only import the data in white cells according your array layout and results. Don't change the content in the grey cells.

Fill out the white cells as indicated in the template forms:

1). Input the array Catalog#;

2). Input the test sample name;

3). Import the gene's Accession# and Symbol information to B and C columns from your array's layout information form.

4). Copy and Paste the Ct values from your real-time PCR results to the "Ct Value-Columns"(D,E,F…) .

5). When finished check again to make sure that the Catalog#, Accession# and Symbol# information from information in Sample Data.xls and Control Data.xls are the same.

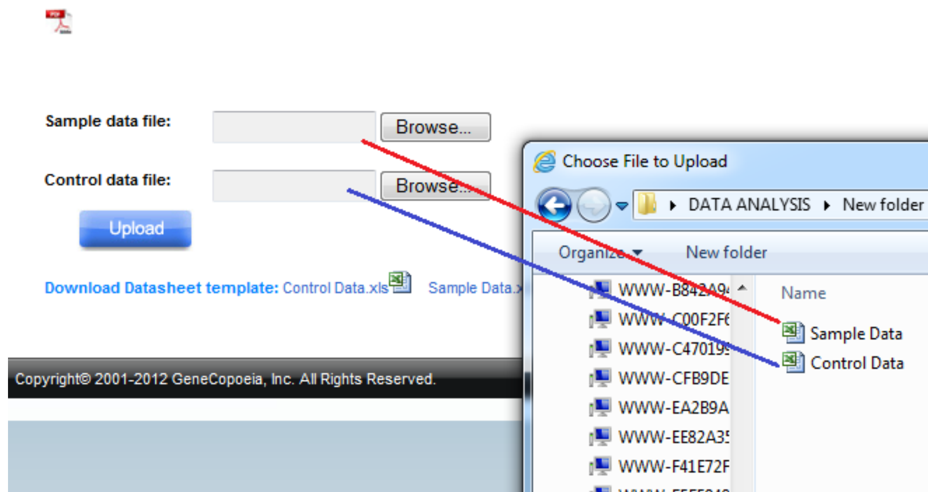
PCR Array Catalog#		HAQPA-102-01			
No.	Accession#	Symbol	Homo Liver Assay Data(Control)		
			Ct Value-1	Ct Value-2	Ct Value-3
1	NM_000038	APC	33.99	33.95	33.74
2	NM_001037954	DIXDC1	29.54	29.73	29.59
3	NM_001429	EP300	25.80	25.91	25.82
4	NM_001463	FRZB	29.45	29.50	29.51
5	NM_001664	RHOA	21.92	22.11	22.01
6	NM_001896	CSNK2A2	25.10	25.38	25.17
7	NM_001904	CTNNB1	23.53	23.61	23.37
8	NM_002093	GSK3B	26.92	27.35	26.96
9	NM_002336	LRP6	25.53	25.81	25.81
10	NM_002467	MYC	28.04	28.09	28.20

Control data.xls

PCR Array Catalog#		HAQPA-102-01			
No.	Accession#	Symbol	Homo Placenta Assay Data(Sample)		
			Ct Value-1	Ct Value-2	Ct Value-3
1	NM_000038	APC	32.30	32.74	32.31
2	NM_001037954	DIXDC1	32.92	32.80	32.84
3	NM_001429	EP300	25.99	25.61	25.72
4	NM_001463	FRZB	24.36	24.17	24.18
5	NM_001664	RHOA	22.85	22.63	22.77
6	NM_001896	CSNK2A2	25.10	25.03	25.32
7	NM_001904	CTNNB1	24.28	23.91	24.05
8	NM_002093	GSK3B	27.80	27.90	27.89
9	NM_002336	LRP6	27.64	27.04	27.21
10	NM_002467	MYC	26.89	26.94	27.09

Sample Data.xls

d. Upload the template forms containing your PCR data



Browse and select the template forms containing your PCR data. Click "Upload".

! Caution: import the “sample date.xls” to “Sample Excel” and “control date.xls” to “Control Excel”. Don’t switch them.

e. Choose the correct reference factors and perform the specified analysis.

When the data files are uploaded, the analysis interface allows you to select your preferred housekeeping genes (whose expression level is constant between samples) for data normalization by clicking the corresponding checkboxes.

Data Analyse

Perfect match! Please Choose the reference factors.

No.	Accession#	Symbol	Control factor	Analytical factor
1	NM_000038	APC	○	◉
2	NM_001037954	DIXDC1	○	◉
3	NM_001429	EP300	○	◉
4	NM_001463	FRZB	○	◉
5	NM_001664	RHOA	○	◉
6	NM_001896	CSNK2A2	○	◉
7	NM_001904	CTNNB1	○	◉
8	NM_002093	GSK3B	○	◉
9	NM_002336	LRP6	○	◉
10	NM_002467	MYC	○	◉
11	NM_003012	SFRP1	○	◉
12	NM_003181	T	○	◉

!Caustion： One or more housekeeping genes can be chosen as reference factors. The arithmetic mean of the selected housekeeping genes is used for normalization.

f. Data Analysis Results Page

When the reference factor is chosen, click the "set icon" to get the analysis result page which shows the fold-change and P-value for each gene of interest *("Fold change" and "P-Value" Column)*. The full analysis results can be downloaded in Excel file format.

PCR Array Catal	HAQPA-102-01																		
No.	Accession#	Symbol	Homo Placenta Assay Data(Sample)			Homo Liver Assay Data(Control)			Homo Placenta Assay Data(Sample) ΔCt				Homo Liver Assay Data(Control) ΔCt					Fold Change	T test
			Ct Value-1	Ct Value-2	Ct Value-3	Ct Value-1	Ct Value-2	Ct Value-3	ΔCt− 1	ΔCt− 2	ΔCt− 3	Ave.ΔCt	ΔCt− 1	ΔCt− 2	ΔCt− 3	Ave.ΔCt	ΔΔCt	2^-ΔΔCt	P_Value
1	NM_00003	APC	32.3026	32.7437	32.3129	33.9894	33.9464	33.7433	11.503	11.995	11.531	11.676	14.599	14.591	14.29	14.493	-2.817	7.046	0.0001187
2	NM_00103	DIXDC1	32.925	32.8024	32.8389	29.5358	29.7282	29.5889	12.125	12.054	12.057	12.079	10.145	10.372	10.136	10.218	1.861	0.275	2.10E-05
3	NM_00142	EP300	25.9943	25.6088	25.7176	25.7962	25.9133	25.8233	5.194	4.861	4.936	4.997	6.405	6.557	6.37	6.444	-1.447	2.727	0.000239
4	NM_00146	FRZB	24.3618	24.1736	24.1778	29.4482	29.4952	29.5123	3.562	3.425	3.396	3.461	10.057	10.139	10.059	10.085	-6.624	98.649	3.00E-08
5	NM_00166	RHOA	22.8497	22.6266	22.7707	21.9192	22.1095	22.0109	2.05	1.878	1.989	1.972	2.528	2.754	2.558	2.613	-0.641	1.559	0.0017828
6	NM_00189	CSNK2A	25.0981	25.0349	25.3215	25.0988	25.3772	25.172	4.298	4.287	4.54	4.375	5.708	6.021	5.719	5.816	-1.441	2.715	0.0003969
7	NM_00190	CTNNB1	24.2796	23.9112	24.0493	23.5267	23.614	23.3681	3.48	3.163	3.268	3.303	4.136	4.258	3.915	4.103	-0.8	1.741	0.0042868
8	NM_00209	GSK3B	27.8019	27.9039	27.888	26.9213	27.352	26.9574	7.002	7.156	7.106	7.088	7.531	7.996	7.504	7.677	-0.589	1.504	0.0238587
9	NM_00233	LRP6	27.6384	27.0367	27.2111	25.5266	25.8089	25.8117	6.838	6.288	6.43	6.519	6.136	6.453	6.359	6.316	0.203	0.869	0.345248
10	NM_00246	MYC	26.8867	26.9418	27.0945	28.0378	28.0948	28.198	6.087	6.194	6.313	6.198	8.647	8.739	8.745	8.71	-2.513	5.706	4.17E-06
11	NM_00301	SFRP1	27.4413	27.3211	27.6404	28.7343	28.4652	28.6388	6.641	6.573	6.859	6.691	9.344	9.109	9.186	9.213	-2.522	5.743	2.18E-05

* Data in Yellow cells in the information about Catalog#, Accession#, Symbol and Ct values provided by the user.
* All Ct values reported as greater than 36 or as N/A (not detected) are converted to 36 for total analysis.
* "ΔCT" value is the Ct value difference between the target gene and the reference gene for both the test sample and the control sample.
* "Ave ΔCT" value is the arithmetic mean for each replicate's ΔCT values.
* "ΔΔCT" value is calculated by the formula ΔΔCT = Ave ΔCT (test) – Ave ΔCT (control)
* "Fold Change" value is the fold difference of the target gene in the test sample relative to the control sample. Red font means increased expression and green font indicates decreased expression.
* "p_valve" is generated from T Test to show the Statistical significance difference between the test sample and the control sample. Red font is used when $P<0.05$, otherwise black font is used. If the replicates are less than 3, T-Test analysis is omitted.