For Research Use

TaKaRa

PrimerArray® Analysis Tool for Embryonic Stem Cells

Manual

v201801

Table of Contents

The PrimerArray Analysis Tool for Embryonic Stem Cells is a software tool for analysis of data obtained using Takara Bio's PrimerArray for Embryonic Stem Cells (Cat. #PH016, PN016), a primer set for real-time RT-PCR analysis of gene expression related to ES cell differentiation. The tool allows comparison between data obained for an unknown and control sample and performs relative quantitative analysis using Ct values exported from real-time PCR instrument software by the △△ Ct method. Results are displayed in a graphical format.

＊ The PrimerArray Analysis Tool for Embryonic Stem Cells uses a Microsoft Office Excel format file containing macros. Its performance has been validated in the following operating systems and versions of Microsoft Office Excel:
Windows XP operating system
Microsoft Office Excel 2003
Microsoft Office Excel 2007

＊ The PrimerArray Analysis Tool for Embryonic Stem Cells is available for download from the Takara Bio website.

I. Calculating and exporting Ct values

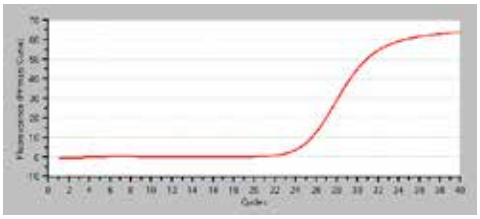
Set the analysis parameters using the real-time PCR instrument software, and calculate Ct values. Refer to the instruction manual of the real-time PCR analysis software for specific details of the analysis procedure.

(1) Setting analysis parameters

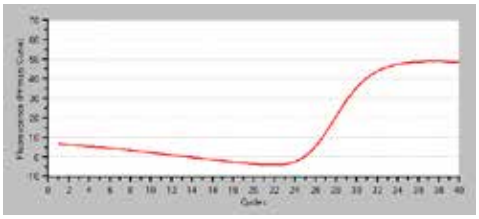
The analysis parameters are automatically set in most real-time PCR analysis software. However, settings should be reviewed to ensure that those parameters are correct. If they are incorrect, the parameters will need to be re-set manually.

Baseline region

Set the flat region before amplification curve begins to rise as the baseline region. If this region is not long enough, the baseline will not be properly normalized. In contrast, if this region is too long, it may cause amplification curve which can lower progressively (refer to the graphs below).



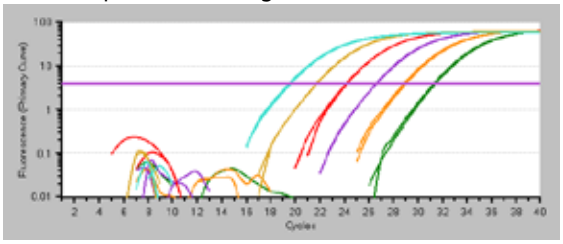
Correct Baseline



Baseline Region is Too Wide

Threshold

Set the threshold within the region of exponential PCR amplification. This is the region where the amplification curve becomes linear when vertical axis of the curve is plotted on a log scale.



Correct Threshold

(2) Calculation of Ct value
Most real-time PCR analysis software automatically calculates the Ct value.

(3) Output of the data
Output of the Ct values is generally in Microsoft Office Excel or CSV format. The output form varies depending on the analysis software used.

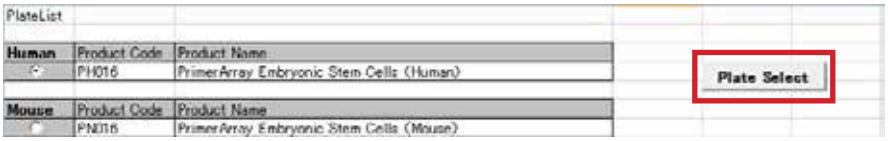
＊ Some real-time PCR analysis software packages do not output data from wells where sample information is not set, or from wells omitted from the analysis. In this case, errors are likely to happen during the data input into the PrimerArray Analysis Tool for Embryonic Stem Cells. Please ensure data from all wells is exported before using the analysis tool.

II. Relative quantification

Below is a protocol to perform relative quantitative analysis using the △△ Ct method with the PrimerArray Analysis Tool for Embryonic Stem Cells.

(1) Starting the PrimerArray Analysis Tool for Embryonic Stem Cells
Open the PrimerArray Analysis Tool for Embryonic Stem Cells (PrimerArray Analysis Tool for Embryonic Stem Cells.xls) file.

(2) Select a plate
Choose PrimerArray plate used for your experiment, then click the "Plate Select" button.



(3) Input Control Sample Data
After clicking "Plate Select" button, a sheet for control sample data will appear. Input Ct values in exp1 (C column), exp 2 (D column), exp 3 (E column), etc. This can generally be done by copying and pasting the Ct value output from the real-time PCR analysis software. Data for up to 10 repeated experiments can be entered.

	A	B	C	D	E	F	G	H	I
1									C
2	Symbol	Well	exp1	exp2	exp3	exp4	exp5	exp6	exp7
3	CTNNB1	A01	26.16	26.45	26.57				
4	FLT1	A02	26.5	26.56	26.55				
5	FN1	A03	28.39	28.43	28.49				
6	LAMA1	A04	20.51	20.58	20.56				
7	PDX1	A05	31.11	30.95	31.04				
8	PTEN	A06	22.56	22.41	22.52				
9	CCL2	A07	34.61	34.28	34.81				
10	ACTC1	A08	33.89	33.92	34.36				
11	AFP	A09	22.36	22.35	22.59				
12	CCR7	A10	33.48	33.95	33.83				
13	BRIX1	A11	23.63	23.62	23.72				
14	GUSB	A12	23.87	23.76	24.04				
15	CD34	B01	31.59	31.54	31.3				
16	CD9	B02	24.69	25.09	25.39				
17	CDH5	B03	30.78	31.45	31.1				
18	CDX2	B04	26.11	26.18	26.18				
19	COL1A1	B05	28.44	28.48	28.66				

PlateSelect PlateInfo GeneID TestSampleData CntrlSampleData PCR_amp.eff normalization.factors scatter.p

(4) Input Test Sample Data

Select the sheet "TestSampleData" for Test Sample data input. Input the data in the same way as the Control Sample. After inputting the data, click the "set sample data" button.

Clearing data

If you need to re-input data, click the "clear" button. This will delete all of the data.

Setting the Ct value cutoff

Once a Ct value cutoff is set, Ct values beyond a certain level will be excluded from analysis. The default cutoff is set at 35 cycles, and will exclude Ct values greater than 35. To change this cutoff level, change the "Ct cutoff value" .

(5) Calculation of the Normalization Factor

Click on "Set Sample Data" . The sheet "normalization_factors" should open for calculation of the Normalization Factor. Select housekeeping gene (HKG)*1 for normalization by checking the box in the column A, and then clicking the "NF value" button. The Normalization Factor is calculated and relative quantitative analysis will be performed automatically.

	HKG	Test Sample		Control Sample	
		Quantity	SD_Q	Quantity	SD_Q
☑	GUSB	4.55E-08	2.68E-09	6.43E-08	6.29E-09
☑	HPRT1	9.16E-08	8.76E-09	1.28E-07	8.23E-09
☑	PGK1	4.87E-07	2.11E-08	8.71E-07	5.61E-08
☑	ACTB	2.50E-05	3.66E-06	3.29E-05	4.82E-06
☑	GAPDH	3.51E-06	1.75E-07	5.90E-06	2.49E-07
☑	TBP	1.56E-08	1.14E-09	2.82E-08	1.68E-09
☑	B2M	4.17E-06	2.46E-07	2.70E-06	2.47E-07
☑	PPIA	3.06E-06	0.00E+00	3.84E-06	0.00E+00

NF value

normalization factors	Quantity	SD_Q
NF Test		
NF Control		

＊1 Selection of housekeeping gene:

The normalization factor is the coefficient used to normalize the template quantities used in the reaction. A housekeeping gene (HKG) whose expression level is stable among the samples is used as the index for this calculation. Care should be taken in selecting the housekeeping gene, because incorrect results can be obtained if a gene having differing expression levels among samples is used as an index. To select an appropriate housekeeping gene, confirm stable expression experimentally or use known information (biological insight, published literature, microarray analysis results, etc.).

If there is no known information suggesting an appropriate gene, use all of the control housekeeping genes as a reference. Alternatively, perform the analysis without normalization of the RNA amount (without Housekeeping Gene Normalization).

References

- Housekeeping Gene Primer Set (Cat. # 3790/3791/3792)*2
- geNorm manual
  http://medgen.ugent.be/~jvdesomp/genorm/geNorm_manual.pdf
- Vandesompele J, *et al*. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* (2002) Jun18; **3**(7): RESEARCH0034. Epub 2002 Jun 18.

＊2 Not available in all geographic locations. Check for availability in your area.

(6) Confirmation of the analysis results

After the analysis, a 3D profile of the Fold Differences will appear. Select each sheet to view the additional results.

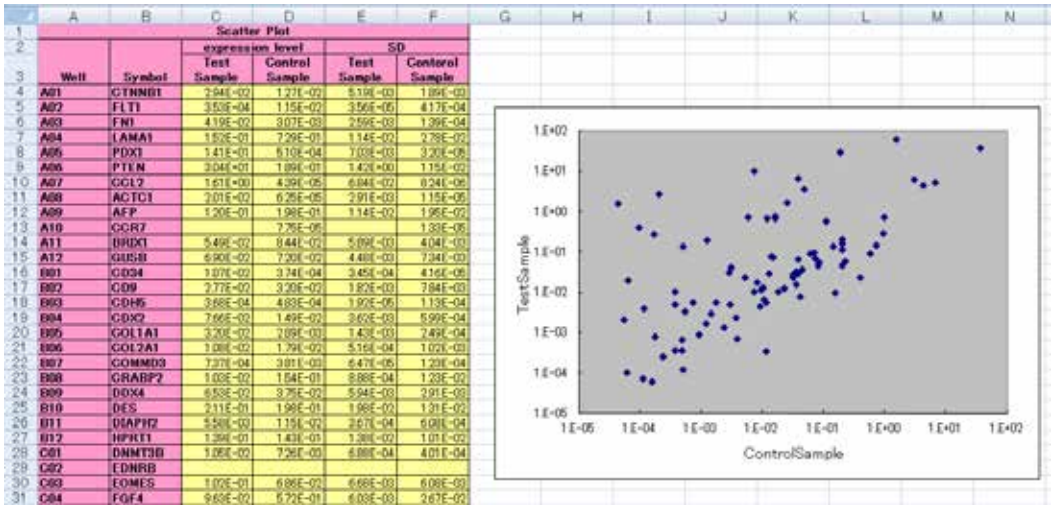
Fold Difference

The list will show the relative quantification values (fold difference) and standard deviation of the Test Sample, with the Control Sample set to 1.

Fold Difference					
		expression level		SD	
Well	Symbol	Test Sample	Contorol Sample	Test Sample	Control Sample
A01	CTNNB1	2.31	1.00E+00	4.08E-01	1.49E-01
A02	FLT1	0.03	1.00E+00	3.11E-03	3.64E-02
A03	FN1	13.64	1.00E+00	8.42E-01	4.52E-02
A04	LAMA1	0.21	1.00E+00	1.56E-02	3.81E-02
A05	PDX1	276.28	1.00E+00	1.38E+01	6.26E-02
A06	PTEN	160.90	1.00E+00	7.54E+00	6.11E-02
A07	CCL2	3.66E+04	1.00E+00	1.56E+03	1.88E-01
A08	ACTC1	321.80	1.00E+00	4.66E+01	1.85E-01
A09	AFP	0.61	1.00E+00	5.73E-02	9.84E-02
A10	CCR7		1.00E+00		1.72E-01

Scatter plot

The left table shows a list of values and standard deviations before relative quantification with the Control Sample. The values are shown in Scatter plot in the graph on the right.



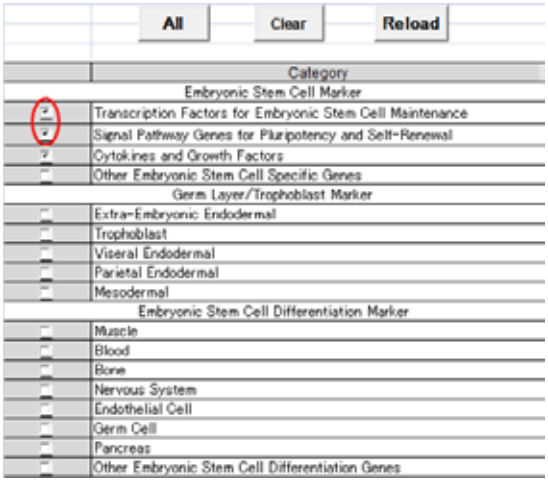
3D Profile

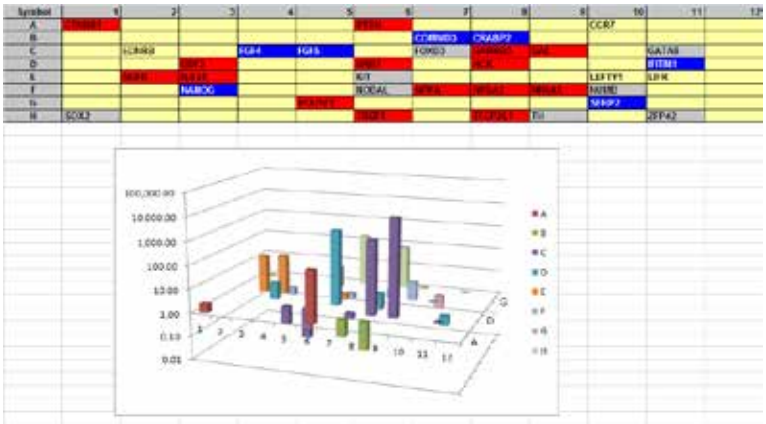
A table listing the Fold Difference of the Test Sample and gene symbols is shown, with the placement of the data corresponding to the position on the plate. The color is indicative of the degree of expression difference: red, increased expression (fold difference>2); gray, minimal change (fold difference 0.5 - 2); blue, no change or reduced expression (fold difference<0.5).

3D Profile	1	2	3	4	5	6	7	8	9	10	11	12
A	2.31E+00	3.08E-02	1.36E+01	2.09E-01	2.76E+02	1.61E+02	3.66E+04	3.22E+02	6.07E-01		6.51E-01	9.59E-01
B	2.86E+01	8.65E-01	7.63E-01	5.13E+00	1.11E+01	6.03E-01	1.93E-01	6.65E-02	1.74E+00	1.06E+00	4.86E-01	9.73E-01
C	1.44E+00		1.48E+00	1.68E-01	5.79E-02	8.59E-01	5.86E-01	1.37E+03	1.33E+04		9.73E-01	7.58E-01
D		1.23E+02	5.24E+00	7.56E+01		1.83E+03		4.76E+00	4.51E-01	4.36E+00	4.03E-01	1.03E+00
E	1.34E+01	3.89E+01	4.82E+01	4.25E+01		1.69E+00	8.59E-01	5.81E+01	1.44E+00			8.07E-01
F	6.60E-01	3.23E+00	4.86E-01	2.48E-01	4.31E+01	5.66E-01	5.62E+00	2.19E+00	6.23E+00	9.27E-01	1.28E+00	7.53E-01
G	6.60E-01	1.44E+00	1.71E+02	2.58E-01	7.73E+00	3.14E-01	3.45E+01	5.99E-01	1.69E+00	2.68E-01	6.60E-01	2.10E+00
H	8.01E-01	1.25E+00		2.16E+00	1.55E+00	1.58E+02	1.92E-01	6.76E+01	1.11E+00	4.63E+00	9.79E-01	1.08E+00

Symbol	1	2	3	4	5	6	7	8	9	10	11	12
A	CTNNB1	FLT1	FN1	LAMA1	POX1	PTEN	CCL2	ACTC1	AFP	CCR7	BRIX1	GUSB
B	CD34	CD9	CDH5	CDX2	COL1A1	COL2A1	COMMD3	CRABP2	DDX4	DES	DNAPH2	HPRT1
C	DNMT3B	EDNRB	EOMES	FGF4	FGF5	FOXA2	FOXD3	GABRB3	GAL	GATA4	GATA6	PGK1
D	GCG	GCM1	GDF3	GFAP	DCN	GRB7	HBZ	HCK	MNX1	IAPP	IFITM1	ACTB
E	IFITM2	NGFR	IL6ST	IGF2BP2	ISL1	KIT	KRT1	LAMB1	LAMC1	LEFTY1	LIFR	GAPDH
F	LIN28A	LCK	NANOG	NES	NEUROD1	NODAL	NPPA	NR5A2	NR6A1	NUMB	OLIG2	TBP
G	PAX4	PAX6	PECAM1	PODXL	POU5F1	REST	RUNX2	SEMA3A	SERPINA1	SFRP2	SOX17	B2M
H	SOX2	SYCP3	SYP	T	TAT	TDGF1	TERT	TFCP2L1	TH	WT1	ZFP42	PPIA

Specify a Category, then click the "Reload" button. Changes in expression of genes in that category are displayed in the table, with the color corresponding to the degree of expression difference, and as a bar graph. To display all of the data, click "All" to select all of the categories. Click "Clear" to remove all selected categories.





Analysis is complete. When continuing the analysis with a different data set, erase the data by clicking the "clear" button on the "TestSampleData" sheet. Begin again at step (2) Select a Plate.

III. Troubleshooting

- Security alert appears.
  PrimerArray Analysis Tool for Embryonic Stem Cells includes a macro, and a security alert may appear. In this case, enable macros.

  Microsoft Office Excel 2007
  (1) Click "Options" on the security warning.

(2) Select the "Enable this content" (2), and then click the OK button.

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