For Research Use

TaKaRa

PrimerArray® Analysis Tool Ver. 2.2

Manual

v202012

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The PrimerArray Analysis Tool Ver. 2.2 is a software tool for analysis of data obtained using Takara Bio's PrimerArray series (Cat. # PH001-PH007, PH009-PH015, PN001-PN015), primer sets for real-time RT-PCR for analysis of gene expression related to specific biological pathways. The tool allows comparison of data obtained for an unknown and control sample and performs relative quantification 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 Ver. 2.2 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 Ver. 2.2 is available for download from the Takara Bio website.

I. Calculating and Exporting Ct Values

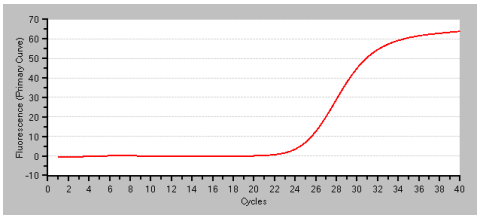
Set the analysis parameters using the real-time PCR instrument software, and calculate Ct values. Refer to the instruction manual of the real-time PCR analysis software for specific details of the analysis procedure.

(1) Setting analysis parameters

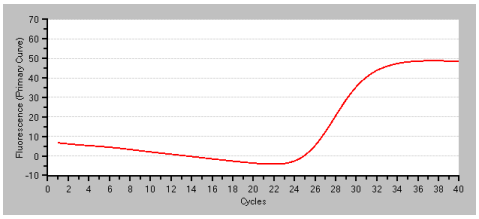
The analysis parameters are automatically set in most real-time PCR analysis software. However, settings should be reviewed to ensure that those parameters are correct. If they are incorrect, the parameters will need to be re-set manually.

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).



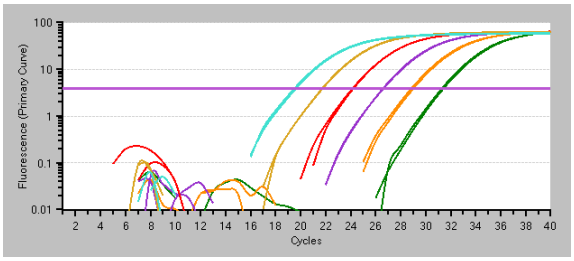
Correct Baseline



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 during the data input into the PrimerArray Analysis Tool Ver. 2.2. 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 Ver. 2.2.

(1) Starting the PrimerArray Analysis Tool Ver. 2.2

Open the PrimerArray Analysis Tool Ver. 2.2 (PrimerArray Analysis Tool Ver.2.2.xls) file.

(2) Select a plate

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

PlateList

Human	Product Code	Product Name
○	PH001	PrimerArray® Cytokine-cytokine receptor interaction(Human)
○	PH002	PrimerArray® Cell cycle(Human)
○	PH003	PrimerArray® Cell adhesion molecules(Human)
○	PH004	PrimerArray® Jak-STAT signaling pathway(Human)
○	PH005	PrimerArray® Natural killer cell mediated cytotoxicity(Human)
○	PH006	PrimerArray® Axon guidance(Human)
○	PH007	PrimerArray® Focal adhesion(Human)
○	PH008	PrimerArray® T cell receptor signaling pathway(Human)
○	PH009	PrimerArray® TGF-beta signaling pathway(Human)
○	PH010	PrimerArray® Wnt signaling pathway(Human)
●	PH011	PrimerArray® Colorectal Cancer & Pancreatic Cancer (Human)
○	PH012	PrimerArray® Prostate Cancer & Melanoma (Human)
○	PH013	PrimerArray® Small Cell Lung Cancer & Non-small Cell Lung Cancerr (Human)
○	PH014	PrimerArray® Asthma & Rheumatoid arthritis (Human)
○	PH015	PrimerArray® Diabetes mellitus, TypeI & TypeII (Human)

Plate Select

Mouse	Product Code	Product Name
○	PN001	PrimerArray® Cytokine-cytokine receptor interaction(Mouse)
○	PN002	PrimerArray® Cell cycle(Mouse)
○	PN003	PrimerArray® Cell adhesion molecules(Mouse)
○	PN004	PrimerArray® Jak-STAT signaling pathway(Mouse)
○	PN005	PrimerArray® Natural killer cell mediated cytotoxicity(Mouse)
○	PN006	PrimerArray® Axon guidance(Mouse)
○	PN007	PrimerArray® Focal adhesion(Mouse)
○	PN008	PrimerArray® T cell receptor signaling pathway(Mouse)
○	PN009	PrimerArray® TGF-beta signaling pathway(Mouse)
○	PN010	PrimerArray® Wnt signaling pathway(Mouse)
○	PN011	PrimerArray® Colorectal Cancer & Pancreatic Cancer (Mouse)
○	PN012	PrimerArray® Prostate Cancer & Melanoma (Mouse)
○	PN013	PrimerArray® Small Cell Lung Cancer & Non-small Cell Lung Cancer (Mouse)
○	PN014	PrimerArray® Asthma & Rheumatoid arthritis (Mouse)
○	PN015	PrimerArray® Diabetes mellitus, TypeI & TypeII (Mouse)

(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	J	K
1	Symbol	Well	Control Samp								
2			exp1	exp2	exp3	exp4	exp5	exp6	exp7	exp8	exp9
3	AKT3	A01	26.16	26.45	26.57						
4	CDK4	A02	26.5	26.56	26.55						
5	CDK6	A03	28.39	28.43	28.49						
6	TNFRSF10B	A04	20.51	20.58	20.56						
7	APC2	A05	31.11	30.95	31.04						
8	RALBP1	A06	22.56	22.41	22.52						
9	CHUK	A07	34.61	34.28	34.81						
10	CTNNB1	A08	33.89	33.92	34.36						
11	DCC	A09	22.36	22.35	22.59						
12	E2F1	A10	33.48	33.95	33.83						
13	E2F2	A11	23.63	23.62	23.72						
14	GUSB	A12	23.87	23.76	24.04						
15	E2F3	B01	31.59	31.54	31.3						
16	EGF	B02	24.69	25.09	25.39						
17	EGFR	B03	30.78	31.45	31.1						
18	ERBB2	B04	26.11	26.18	26.18						
19	AKT1	B05	28.44	28.48	28.66						
20	AKT2	B06	25.84	25.89	25.98						
21	FIGF	B07	28.11	28.14	28.15						

PlateSelect / PlateInfo / GeneII / ControlSampleData / estSampleData / 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

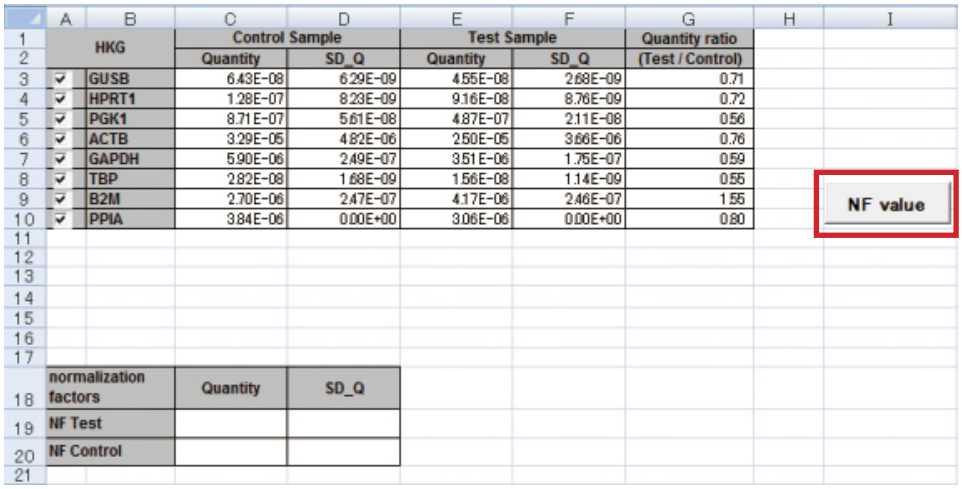
If you need to re-input data, click the "clear" button. This will delete all of the data.

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	Control Sample		Test Sample		Quantity ratio
		Quantity	SD_Q	Quantity	SD_Q	(Test / Control)
☑	GUSB	6.43E-08	6.29E-09	4.55E-08	2.68E-09	0.71
☑	HPRT1	1.28E-07	8.23E-09	9.16E-08	8.76E-09	0.72
☑	PGK1	8.71E-07	5.61E-08	4.87E-07	2.11E-08	0.56
☑	ACTB	3.29E-05	4.82E-06	2.50E-05	3.66E-06	0.76
☑	GAPDH	5.90E-06	2.49E-07	3.51E-06	1.75E-07	0.59
☑	TBP	2.82E-08	1.68E-09	1.56E-08	1.14E-09	0.55
☑	B2M	2.70E-06	2.47E-07	4.17E-06	2.46E-07	1.55
☑	PPIA	3.84E-06	0.00E+00	3.06E-06	0.00E+00	0.80

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 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
- Vandesompele J, *et al.* Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. (2002) Jun 18; **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

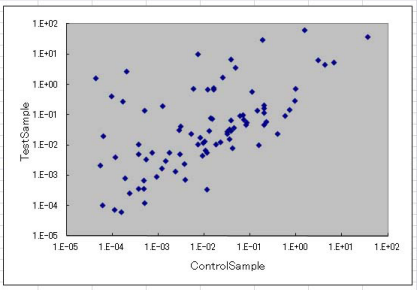
The list will show the relative quantification values (fold difference) and standard deviation of the Test Sample, with the Control Sample set to 1.

	A	B	C	D	E	F
1	Fold Difference					
2			expression level		SD	
3	Well	Symbol	Test Sample	Contorol Sample	Test Sample	Control Sample
4	A01	AKT3	2.31E+00	1.00E+00	4.08E−01	1.49E−01
5	A02	CDK4	3.08E−02	1.00E+00	3.11E−03	3.64E−02
6	A03	CDK6	1.36E+01	1.00E+00	8.42E−01	4.52E−02
7	A04	TNFRSF10B	2.09E−01	1.00E+00	1.56E−02	3.81E−02
8	A05	APC2	2.76E+02	1.00E+00	1.38E+01	6.26E−02
9	A06	RALBP1	1.61E+02	1.00E+00	7.54E+00	6.11E−02
10	A07	CHUK	3.66E+04	1.00E+00	1.56E+03	1.88E−01
11	A08	CTNNB1	3.22E+02	1.00E+00	4.66E+01	1.85E−01
12	A09	DCC	6.07E−01	1.00E+00	5.73E−02	9.84E−02
13	A10	E2F1		1.00E+00		1.72E−01
14	A11	E2F2	6.51E−01	1.00E+00	6.98E−02	4.78E−02
15	A12	GUSB	9.59E−01	1.00E+00	6.23E−02	1.02E−01
16	B01	E2F3	2.86E+01	1.00E+00	9.25E−01	1.11E−01
17	B02	EGF	8.65E−01	1.00E+00	5.70E−02	2.45E−01
18	B03	EGFR	7.63E−01	1.00E+00	3.98E−02	2.34E−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.

	A	B	C	D	E	F
1	Scatter Plot					
2			expression level		SD	
3	Well	Symbol	Test Sample	Control Sample	Test Sample	Control Sample
4	A01	AKT3	2.94E−02	1.27E−02	5.19E−03	1.89E−03
5	A02	CDK4	3.53E−04	1.15E−02	3.56E−05	4.17E−04
6	A03	CDK6	4.19E−02	3.07E−03	2.59E−03	1.39E−04
7	A04	TNFRSF10E	1.52E−01	7.29E−01	1.14E−02	2.28E−02
8	A05	APC2	1.41E−01	5.10E−04	7.03E−03	3.20E−05
9	A06	RALBP1	3.04E+01	1.89E−01	1.42E+00	1.15E−02
10	A07	CHUK	1.61E+00	4.39E−05	6.84E−02	8.24E−06
11	A08	CTNNB1	2.01E−02	6.25E−05	2.91E−03	1.15E−05
12	A09	DCC	1.20E−01	1.98E−01	1.14E−02	1.95E−02
13	A10	E2F1		7.75E−05		1.33E−05
14	A11	E2F2	5.49E−02	9.44E−02	5.89E−03	4.04E−03
15	A12	GUSB	6.90E−02	7.20E−02	4.48E−03	7.34E−03
16	B01	E2F3	1.07E−02	9.74E−04	3.45E−04	4.16E−05
17	B02	EGF	2.77E−02	3.20E−02	1.82E−03	7.84E−03
18	B03	EGFR	9.66E−04	4.83E−04	1.92E−05	1.13E−04
19	B04	ERBB2	7.66E−02	1.49E−02	3.62E−03	5.99E−04
20	B05	AKT1	3.20E−02	2.89E−03	1.43E−03	2.49E−04
21	B06	AKT2	1.08E−02	1.79E−02	5.16E−04	1.02E−03
22	B07	FKGF	7.37E−04	3.81E−03	6.47E−05	1.23E−04
23	B08	FOS	1.10E−02	1.54E−01	8.88E−04	1.23E−02
24	B09	PIK3R5	6.53E−02	3.75E−02	5.94E−03	2.91E−03
25	B10	APPL1	2.11E−01	1.98E−01	1.98E−02	1.31E−02
26	B11	GSK3B	5.58E−03	1.15E−02	3.67E−04	6.08E−04
27	B12	HPRT1	1.39E−01	1.43E−01	1.38E−02	1.01E−02
28	C01	MSH6	1.05E−02	7.26E−03	6.88E−04	4.01E−04
29	C02	APC				
30	C03	BIRC5	1.02E−01	6.86E−02	6.68E−03	6.08E−03
31	C04	IKBKB	9.63E−02	5.72E−01	6.03E−03	2.67E−02
32	C05	ARAF	2.31E−02	3.99E−01	2.26E−03	1.82E−02
33	C06	JAK1	1.69E−01	1.97E−01	1.06E−02	6.28E−03

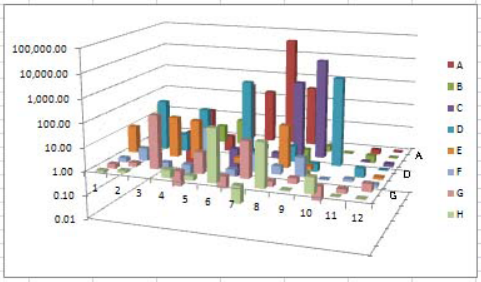


3D Profile

The Fold Difference is shown as a bar graph. Above the graph, a table listing the Fold Difference of the Test Sample and gene symbols is shown, with the placement of the data corresponding to their positions 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	3.86E+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		1.53E+03		4.38E+00	4.51E-01	4.36E+03	4.03E-01	1.03E+00
E	1.34E+01	3.89E+01	4.82E+01	4.23E+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	2.13E+00	6.23E+00	9.27E-01	1.28E+00	7.53E-01
G	6.60E-01	1.44E+00	1.71E+02	2.38E-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		2.18E+00	1.55E+00	1.58E+02	1.92E-01	6.76E+01	1.11E+00	4.53E+00	9.79E-01	1.08E+00

Symbol	1	2	3	4	5	6	7	8	9	10	11	12
A	AKT3	CDK4	CDK6	TNFR SF10B	APC2	RALBP1	CHUK	CTNNB1	DCC	E2F1	E2F2	GUSB
B	E2F3	EGF	EGFR	ERBB2	AKT1	AKT2	FIGF	FOS	PIK3R5	APPL1	GSK3B	HPRT1
C	MSH6	APC	BIRC5	IKBKB	ARAF	JAK1	JUN	KRAS	RHOA	SMAD2	SMAD3	PGK1
D	SMAD4	MLH1	MSH2	MSH3	MYC	NFKB1	LEF1	PGF	PIK3CA	PIK3CG	PIK3R1	ACTB
E	PLD1	CYCS	MAPK1	MAPK8	MAP2K1	BAD	RAC1	RAC2	RAD51	RAF1	RALA	GAPDH
F	RALB	RALGDS	RB1	CCND1	BCL2	RELA	BCL2L1	BRAF	STAT1	STAT3	TCF7	TBP
G	TCF7L2	TGFA	TGFB1	TGFB2	TGFB3	TGFBR1	TGFBR2	TP53	VEGFA	VEGFC	AXIN1	B2M
H	AXIN2	TCF7L1	CASP3	CASP9	PIK3R3	IKBKG	ARHGEF6	CDC42	BRCA2	VEGFB	THBS1	PPIA



KEGG_pathway

Takara Bio Inc. is a authorized service provider of KEGG

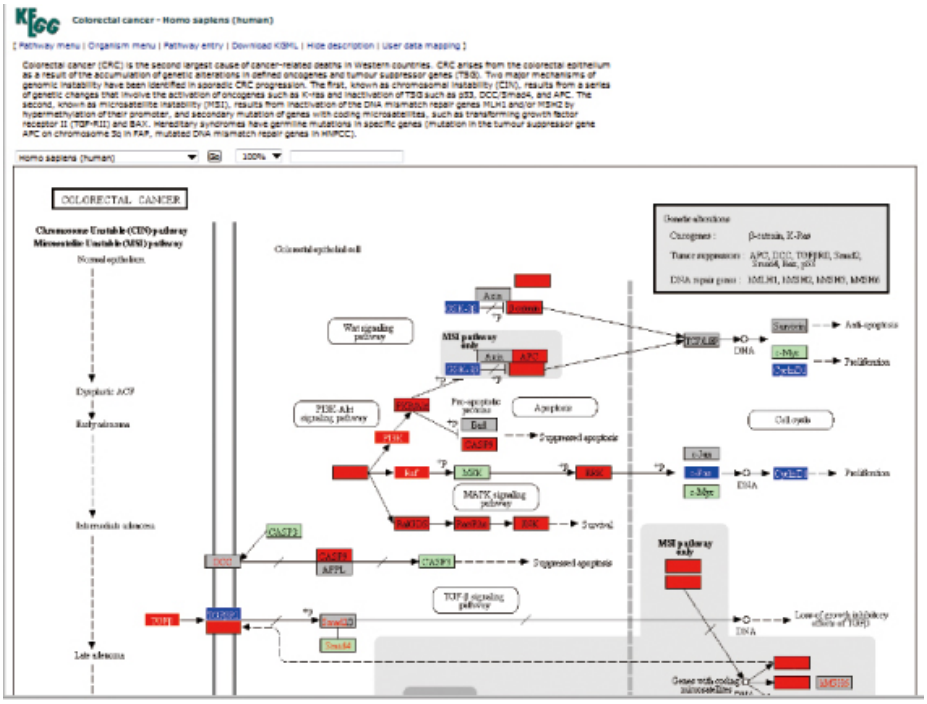
Click the "KEGG_pathway" button. At first, a color-coded legend based on the difference of expression on the KEGG pathway map is shown.
Then, click "KEGG pathway" on the screen; a pathway map displaying the relative expression levels of the genes will appear.

KEGG pathway
hsa05210 : Colorectal cancer
hsa05212 : Pancreatic cancer

Definition of node color when behavior is analyzed

	Relation between behavior and point color of gene	character color	background color	Example
A	Up Gene Point	Black	Red	1,2,3,4
B	Down Gene Point	White	Blue	1,2,3,4
C	No_change Gene Point	Black	Gray	1,2,3,4
A+B*	Up Gene and Down Gene Point	White	Red	1,2,3,4

* Plural GeneID might be included in one node that displayed in map of KEGG pathway

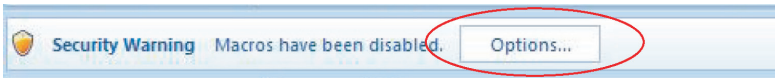


Analysis is complete. When continuing the analysis with a different data set, erase the data by clicking the "clear" button on the "TestSampleData" sheet. Begin again at step (2) Select a Plate.

III. Troubleshooting

- Security alert appears.
  PrimerArray Analysis Tool Ver. 2.2 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.