For Research Use

TaKaRa

# PrimerArray® Analysis Tool Ver. 2.2

Manual

v201801

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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

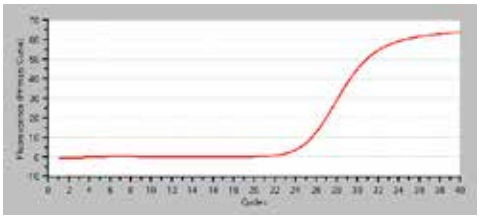
Set the analysis parameters using the real-time PCR instrument software, and calculate Ct values. Refer to the instruction manual of the real-time PCR analysis software for specific details of the analysis procedure.

(1) Setting analysis parameters

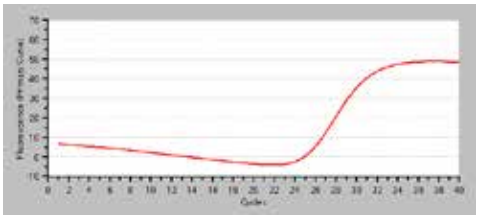
The analysis parameters are automatically set in most real-time PCR analysis software. However, settings should be reviewed to ensure that those parameters are correct. If they are incorrect, the parameters will need to be re-set manually.

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).



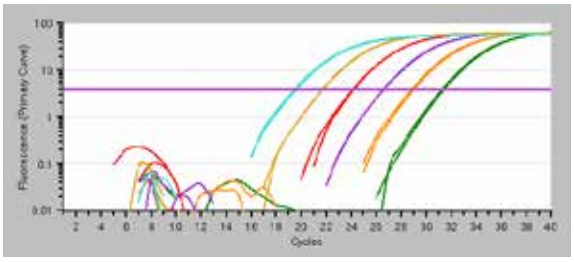
Correct Baseline



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

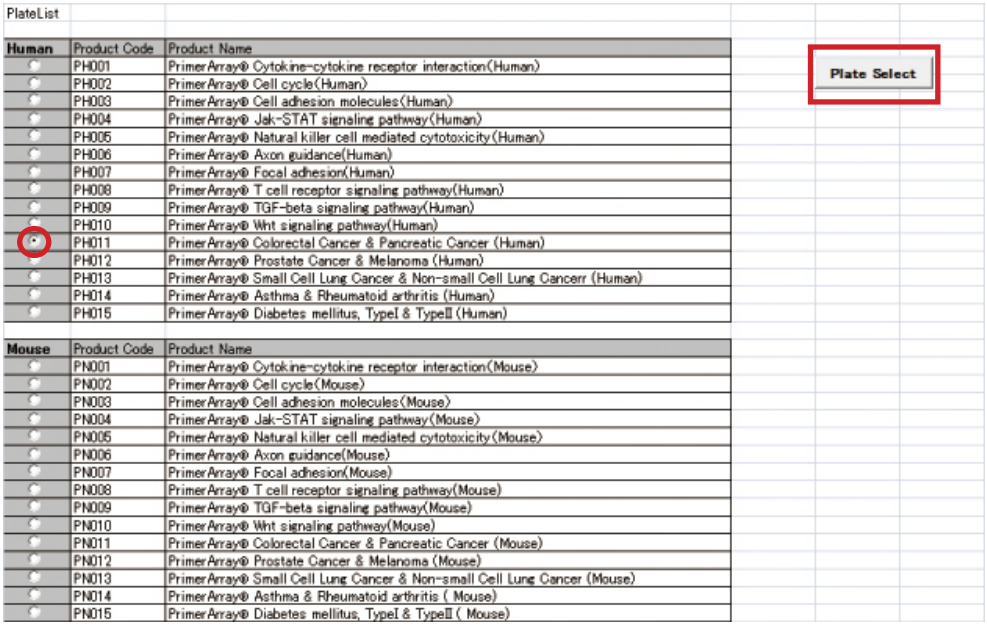
Below is a protocol to perform relative quantitative analysis using the △△ CT method with the PrimerArray Analysis Tool Ver. 2.2.

(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) |

| 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) |

Plate Select

(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 | 28.46 | 28.66 | | | | | | |
| 20 | AKT2 | B06 | 25.84 | 25.89 | 25.98 | | | | | | |
| 21 | FIGF | B07 | 28.11 | 28.14 | 28.15 | | | | | | |

PlateSelect PlateInfo Gene ControlSampleData TestSampleData PCR amp.eff normalization factors scatter

(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

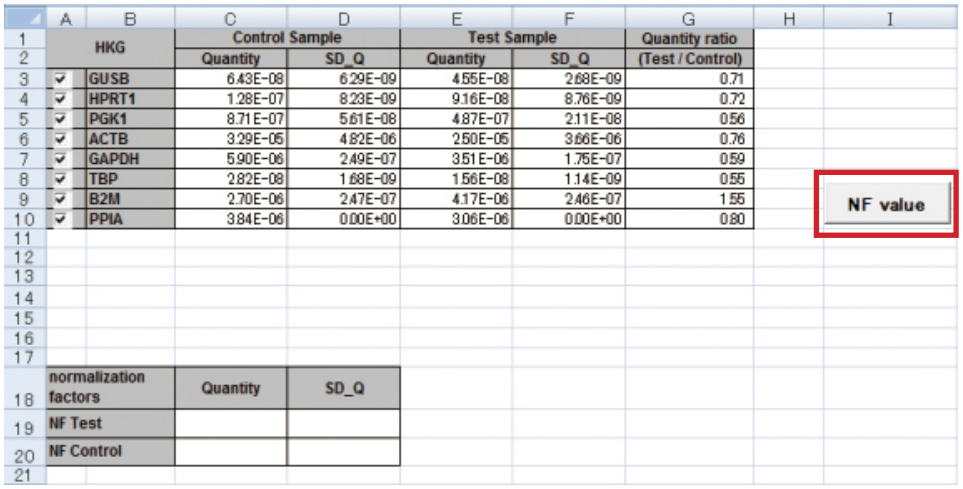
If you need to re-input data, click the "clear" button. This will delete all of the data.

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
- 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) 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.

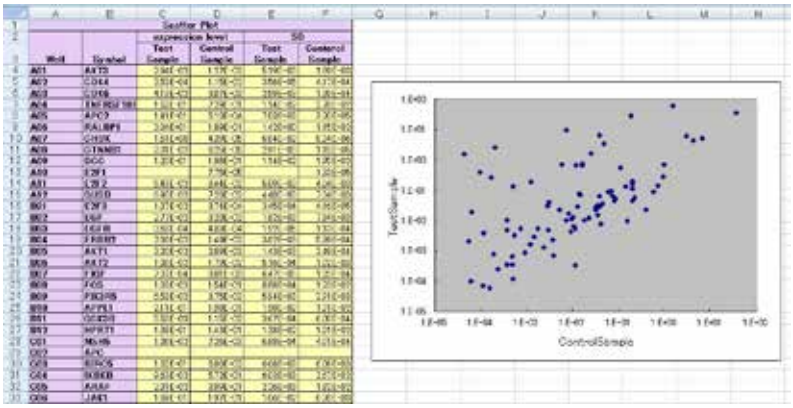
Fold Difference

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 | 3.03E-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 | 2.03E-01 | 1.00E+00 | 1.56E-02 | 3.81E-02 |
| 8 | A05 | APC2 | 2.75E+02 | 1.00E+00 | 1.38E+01 | 5.26E-02 |
| 9 | A06 | RALBP1 | 1.51E+02 | 1.00E+00 | 7.54E+00 | 5.11E-02 |
| 10 | A07 | CHUK | 3.65E+01 | 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 |

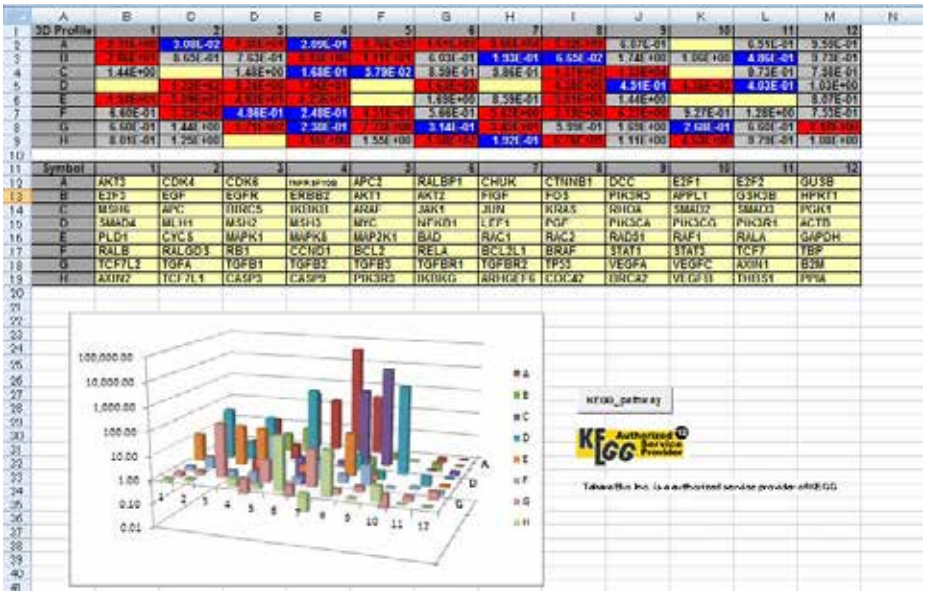
Scatter plot

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.



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).



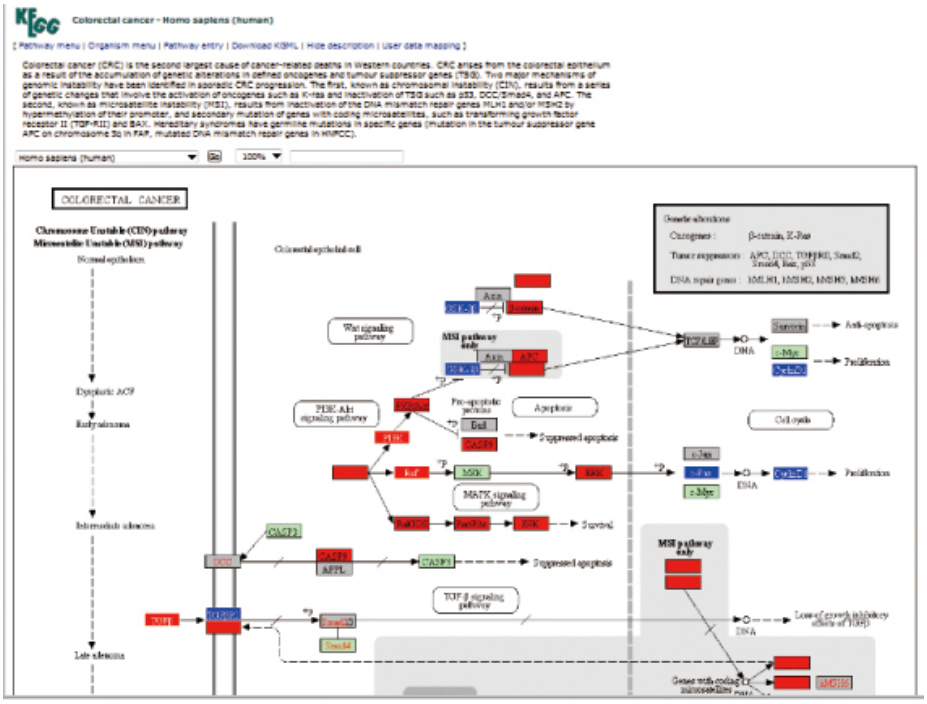
Click the "KEGG_pathway" button. At first, a color-coded legend based on the difference of expression on the KEGG pathway map is shown.
Then, click "KEGG pathway" on the screen; a pathway map displaying the relative expression levels of the genes will appear.

**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.

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