**RefGenuine Manual**

**What's RefGenuine?**

RefGenuine is a software tool to screen reliable reference genes for gene expression analyses. This tool incorporates qRT-PCR data of some candidate reference genes across multiple samples and evaluates how stable the genes and gene combinations are at gene expression level by comparison of Ct values among them. Users can set how many genes are combined as a reference at max between one to ten as following users' preference. The fundamental of the gene stability evaluation was documented by Silver et al. (2006). We will show an example of reference gene identification in a research paper (in preparation).

Silver, Nicholas, et al. "Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR." BMC molecular biology 7.1 (2006): 33.

**Software platform**

Perl

**Contact information**

If you have any questions or suggestions about this tool, please make a contact below.

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**Preparation for use**

Input file has to be given in a tab-delimitated text file (please see the example file "singlect.txt")

# file name: singlect.txt

# text encoding: SJIS code text

# The 1st row: header (no processed)

# The 2nd row later : data content

# The 1st column : Gene identifier (user-defined)

# The 2nd column and later : Ct value in each sample

# The input file must be put at the same directory with this tool

\* I recommend a text editor "Sakura-Editor". This software is available at http://sakura-editor.sourceforge.net/

\* If you want to avoid using command line interface, you can use ActivePerl (http://www.activestate.com/activeperl). ActivePerl can start any Perl script by only clicking the Perl script file.

**Implementation**

1) Windows OS for command line

a) Start your terminal application for handling command line.

>Start menu >Programs >Accessories >Windows PowerShell >

Windows PowerShell

b) Be sure that Perl language has installed in your PC.

Command; perl -v

c) Move the current directory to "RefGenuine". Please use the command "cd".

d) Put your Ct value data onto the same directory with RefGenuine.

e) Set the parameter "$combi" in Line 14 of RefGenuine and save. This should be

made by a text editor and be sure that the text file should be saved in SJIS

encoding.

f) Command; perl RefGenuine\_v1.0.pl

g) Check result files after finishing the run.

2) Linux & Mac OS for command line

a) Start your terminal application for handling command line.

b) Be sure that Perl language has installed in your PC.

c) Move the current directory to "RefGenuine"

d) Put your Ct value data onto the same directory with RefGenuine.

e) Set the parameter "$combi" in Line 14 of RefGenuine and save.

f) Command; perl RefGenuine\_v1.0.pl

g) Check result files after finishing the run.