

REST 2005

A new standalone software for efficiency-corrected relative quantification.

Matthew Herman (1), Valin Reja (1), Michael W. Pfaffl (2)

(1) Corbett Research, Mortlake, NSW, Australia

(2) Lehrstuhl für Physiologie, Wissenschaftszentrum Weihenstephan, TUM, Germany

<http://rest.gene-quantification.info> michael.pfaffl@wzw.tum.de

REST 2005: Relative Expression Software Tool 2005 is a new standalone software tool to estimate efficiency-corrected relative gene quantification results, while incorporating multiple sources of experimental variation. The algorithm addresses issues surrounding the measurement of non-linear variation from traditionally difficult distributions such as expression ratios and standard dilutions, by using randomisation and bootstrapping techniques.

Efficiency Correction: An efficiency corrected relative quantification is calculated. Amplification efficiency can be imported or calculated via the dilution method.

Normalization: Housekeeper normalization over multiple genes using the geometric mean, called Housekeeping Gene Index (HKG Index), provides further control over the experiment.

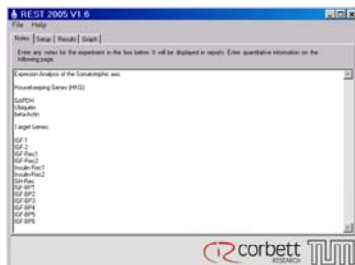
$$R = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}} (MEAN \text{ control} - MEAN \text{ sample})}}{(E_{\text{HKG index}})^{\Delta CP_{\text{HKG index}} (MEAN \text{ control} - MEAN \text{ sample})}}$$

Statistics: The hypothesis test has been enhanced to incorporate variation in the efficiency measurement, and by increasing the number of iterations from 2,000 to a fixed 50,000, now achieving a level of consistency on par with traditional statistical tests. New confidence intervals for expression levels also allow scientists to measure not only the statistical significance of deviations, but also their likely magnitude, even in the presence of outliers.

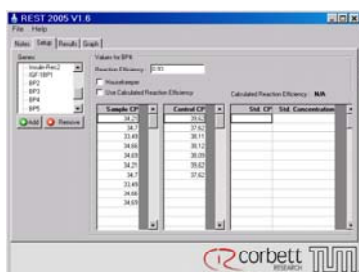
Graphical Output: Expression ratio results are presented in a whisker box-plot, providing a visual representation of variation for each gene that highlights potential issues such as distribution skew.

Application: REST 2005 beta version 1.6 can be tested in the qPCR Matrix Workshop.

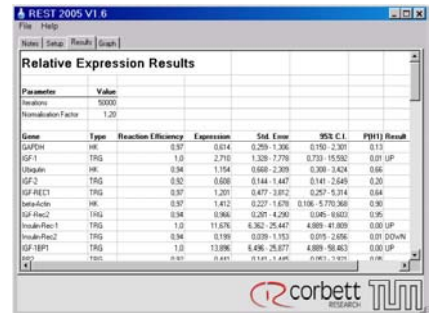
Start Window: Notes for REST 2005 beta version 1.6



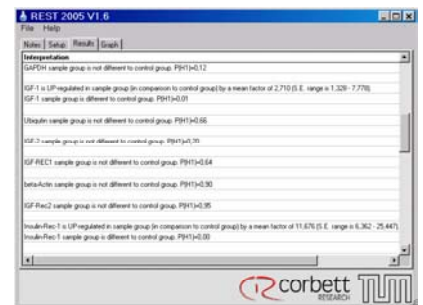
Setup Window: Naming of the genes
Efficiency import or Efficiency calculation
Input of Sample Group CPs and Control Group CPs



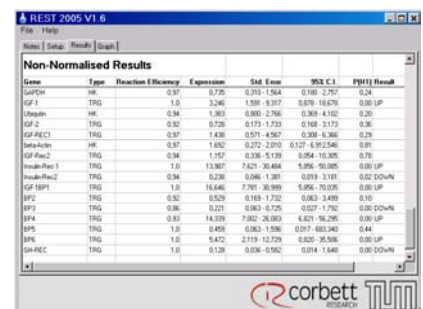
Result Window: relative expression results



Result Window: data interpretation



Result Window: non-normalized results



Graphical Output:

