Mean new microRNA data analysis method gives sharper results

June 16 2009

Our understanding of the importance of microRNAs in regulating gene expression is expanding, and with it our requirement for robust methods to measure their expression levels. Now a new method published in BioMed Central's open access journal *Genome Biology* helps researchers to better understand the delicate interplay between differences in microRNA expression levels and their target genes.

Following their highly cited method for the analysis of reverse transcription quantitative PCR (RT-qPCR) expression data for mRNA transcripts, published in Genome Biology in 2007, Jo Vandesompele of the Center for Medical Genetics, Ghent University Hospital, Belgium and colleagues have created an innovative, straightforward and universally applicable method for quantitative RT-PCR data normalization for microRNAs. The researchers also provide a workflow for proper data normalization of both large scale (whole miRNome) and small-scale microRNA profiling experiments in their paper.

Vandesompele's team profiled 430 microRNAs along with 18 small RNA controls for a number of independent tissue sample sets. They assessed the use of the mean expression value of all expressed microRNAs in a given sample as a normalization factor for microRNA real-time quantitative PCR data, and then compared this method with the currently adopted approach of relying on one or two small RNA controls.

The mean expression value outperforms the current normalization

strategy by better reducing technical variation and more accurately appreciating biological changes. Furthermore, normalization using microRNAs that resemble the mean expression value is platform independent and closely mimics normalization using the mean expression value.

"The mean expression value of all expressed microRNAs performs better than one based on only those microRNAs that are expressed in all samples," says Vandesompele. "This suggests a more accurate representation of input RNA fluctuations when all microRNAs are considered."

"Changes in microRNA expression are often very subtle but biologically relevant," says Pieter Mestdagh, working on the method in Vandesompele's lab. "Because microRNAs regulate genes acting in different pathways, deregulated microRNA expression can trigger a cascade of events that alter the biology of the cell. As a consequence, a proper normalization strategy that enables the detection of small changes is of utmost importance, especially when dealing with heterogeneous patient samples."

Reverse transcription quantitative PCR (RT-qPCR) has become the method of choice for measuring gene expression levels in terms of accuracy and specificity, both for coding and non-coding RNAs. However, result accuracy is largely dependent on effective data normalization.

Source: BioMed Central (news : web)