Raw fluorescence readings taken from the demonstration databases

To facilitate testing and comparison with other qPCR methods that use curve analysis, the raw fluorescence readings from the two demonstration databases have been provided as Excel workbooks. Note that these files can also be used for manual data import into the LRE Analyzer. These databases were taken from a study investigating the precision of gene expression profiling in which the reproducibility of reverse transcription and run-to-run variance were assessed.

Assessing quantitative accuracy

As described previously (Rutledge and Stewart 2008, 2010), assessing quantitative accuracy was a central aspect during development of LRE qPCR. As outlined in the help documentation, this included using limiting dilution (LDA), which is based on Poisson distribution, to independently quantify two of the targets within the COL1 RT sample. This provides a foundation from which to assessing the quantitative accuracy of any method that enables absolute quantification, as is summarized in this table:

LDA-COL1

	12240	46630
Dilution:	4,000	6,300
# of Replicates	24	16
# of Nil Reactions:	15	9
Nav:	0.47	0.58
LDA No:	1,880	3,664
LRE No	2,087	3,088
% Difference:	11.0%	15.7%

Refer to the “Assessing Quantitative Accuracy” section in the LRE Analyzer help documentation for additional details.

Methods

Four Arabidopsis reference genes were quantified within three replicate runs conducted over a three day period, allowing run to run variance to be examined. The three samples (COL1-3) are replicate reverse transcriptase preparations made with an identical RNA sample, allowing the variance of reverse transcription to be examined. Optical calibration was conducted with CAL1 using six replicate PCR reactions for each determination. Although only three replicate reactions are normally conducted, increasing to six provides a more in depth insight into the reproducibility of optical calibration.

The three runs were conducted on an Applied Biosystems 7500 instrument (normal ramping) using QuantiTect in a 10 µl reaction volume containing 500 nM of primers, in 96 well white plates (ABgene) sealed with MicroAmp film (Applied Biosystems), amplified using a cycling regime of 15' activation at 95 oC, followed by 50 cycles of 95 oC-10 sec, 65 oC-120 sec.

Literature

Rutledge RG, Stewart D (2008) A kinetic-based sigmoidal model for the polymerase chain reaction and its application to high-capacity absolute quantitative real-time PCR. BMC Biotechnol 8: 47

Rutledge RG, Stewart D (2010) Assessing the Performance Capabilities of LRE-Based Assays for Absolute Quantitative Real-Time PCR. PLoS ONE 5(3): e9731. doi:10.1371/journal.pone.0009731