

Advanced quantitative real-time PCR in clinical diagnostics and cDNA micro-array validation

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Real-time PCR is the method of choice for quantitative studies of gene expression. The method has been an important tool in basic research for some years, and has now also started to replace more conventional methods in clinical diagnostics. Real-time PCR is characterized by a wide dynamic range of quantification, high sensitivity and high precision. One of the major problems in DNA quantification is to account for PCR inhibitions appropriately. We have developed an in situ calibration method based on either addition of known amount of target DNA or dilution of the test sample to determine sample specific PCR efficiencies. Relative gene expression in clinical samples and cDNA microarray validations are applications particular suitable for in situ calibration, resulting in high accuracy. In situ calibration is particular suitable for a few samples per gene investigations, for example: cDNA microarray validation and relative gene expression in clinical samples. Further, the efficiency and reproducibility of various reverse transcription assays has been carefully evaluated. Our results suggest that sample to sample variation in reverse transcription is significantly higher than in real-time PCR, except when quantifying very low copy numbers. The efficiency of reverse transcription differs significantly between genes and priming strategy. The reproducibility of reverse transcription and real-time PCR suggest that the least difference in mRNA that can be significantly measured is ~50 % when comparing two genes in one sample and ~100 % when comparing expression of genes in two samples.