Comparative Methods Evaluation for qPCR Analysis

Relative quantification with Real-Time qPCR between two or more samples is a method currently used in gene expression analysis. The aim of this Bachelor Thesis is a theoretical and statistical comparison of different mathematical methods used in qPCR.
The standard-curve method, the linear and the nonlinear regression are methods used to calculate real PCR-efficiencies. The baseline signal of the fluorescence, used to visualize amplicons, and various mathematical models to describe the amplification curves are applied to calculate the Cq-values. The efficiencies and the Cq-values are then used to calculate relative quantities. Here one can choose between methods assuming perfect efficiencies or efficiency-corrected quantifications, and between one or more reference targets.
By evaluating the CV across triplicates of different samples, the statistical analysis showed very good values using nonlinear regression (Miner) for calculation of efficiencies and using the SDM method after describing the amplification curves with logistic models (Miner) for calculation of Cq-values. When choosing efficiencies and Cq-values calculated with Miner for relative quantification, best results were obtained by using the comparative Cq-method by Livak/Schmittgen. When choosing efficiencies calculated with serial dilutions and Cq-values calculated with SDS, best results were obtained by using the efficiency-corrected methods.

KEYWORDS: qPCR, efficiency, Cq-Value, relative quantification, statistical comparison