Using the variance of efficiency for quality assessment in real-time PCR

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1 Introduction

Proper quantification with real-time PCR requires similar efficiencies in the compared samples, a non-trivial task given the sensitivity of the method to a vast range of inhibitors (Figure 1).

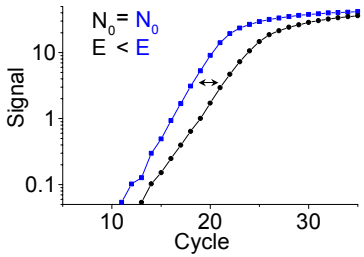


Figure 1: Two samples with similar initial number of DNA molecules (N_0) but different efficiencies (E) reach the threshold at different Cycle of Threshold (↔).

2 Materials and Methods

Equation [1] was fitted to each amplification curve.

$$y = y_b + \frac{a}{1+e^{-(x-x0)/b}} \qquad [1]$$

The 18 data points around the inflection point were re-fitted (Equalized fit, Figure 2).

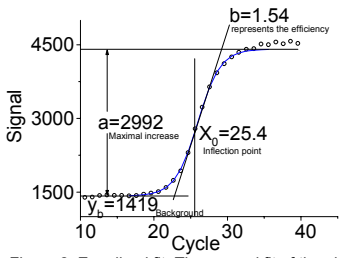


Figure 2: Equalized fit. The second fit of the sigmoidal equation is done in all curves with equal number of data points around the inflection point (blue line). Efficiency was calculated by equation [2], where y_x is the calculated signal at cycle x. The efficiency was calculated at cycle x=1.

$$E = \frac{y_x - y_{x-1}}{y_{x-1} - y_b} \qquad [2]$$

3 Experimental design

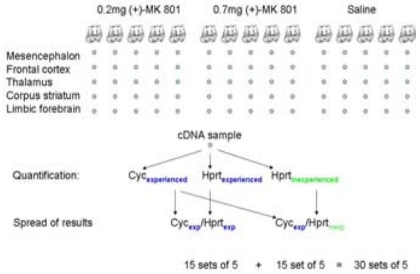


Figure 3: Using standard curves, the expression of *Hprt* and *Cyclophilin* was quantified in 75 cDNA samples from 5 different parts of 15 brains of rats treated with different doses of (+)-MK 801 hydrogen maleate per kg body weight. *Hprt* expression was quantified by inexperienced and again by experienced worker. *Cyclophilin* expression was quantified by an experienced worker only.

4 Results

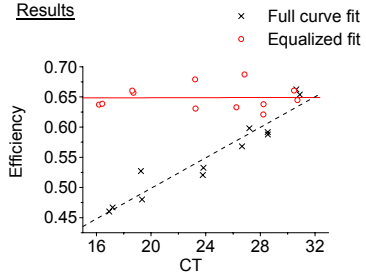
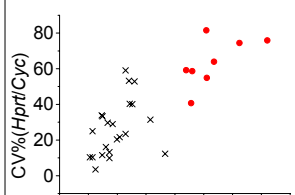


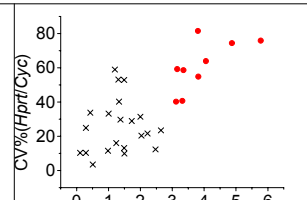
Figure 4: An example of the improvement in efficiency estimation with equalized fit in one set of standard curve samples. Fitting large number of amplification curves with the two methods, equalized fit reduced:

1. The correlation between efficiency and Cycle of Threshold (CT)
2. The variance of the efficiency to $7.3*10^{-4}$
3. The bias between the average estimated efficiency and the efficiency obtained from the standard curve's slope

5 Two approaches to the variance of efficiency

	Nominal variance similarity test	Comparative variance similarity test
Compare	Var(Eff) of test samples to a given nominal value based on characterization study (see 4).	Var(Eff) of test samples to the Var(Eff) of high quality samples from the same run (typically the samples consisting the standard curve)
Statistics 	n = number of experimental samples-1 S^2 = Estimated variance of efficiency of experimental samples σ^2 = Nominal value of variance of efficiency to compare with $\chi^2 = \frac{(n-1)s^2}{\sigma_0^2}$	Var(Eff) = Variance of efficiency $F = Var(Eff_{experimental})/Var(Eff_{standards})$
Test	Chi-square test	F-test, degrees of freedom= sizes of compared sets-1
Empirical suggestions:	Use σ^2=0.035 for SYBR green on iCycler	Variances' ratio > 3 may signify low quality set
Graphical presentation	CV% = 100*Standard deviation/Average, • Low quality sets	





6 Conclusion

The variance of efficiencies could be used to draw attention of the user to suspected sets of samples.