

# Quantification Strategies in real time qRT-PCR

M. W. Pfaffl, *BioSpektrum* 2004 (Sonderausgabe PCR)

## absolute quantification

external calibration curve  
one color detection system  
SYBR Green I

external calibration curve  
two color detection system  
e.g. Probes, ROX

ROX

### external calibration curve

- RT-PCR product
- plasmid DNA
- *in vitro* transcribed RNA
- synthetic DNA Oligos
- synthetic RNA Oligos

## relative quantification

### normalisation

via one reference gene

via reference gene index  
>3 HKG

external calibration curve **without** any reference gene

ROX

without real-time PCR efficiency correction

$2^{-\Delta\Delta CP}$

with real-time PCR efficiency correction

REST, qGene  
LC software

# Gene Quantification

**G Orfield**



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