Chapter 10

Glossary

This chapter describes the terms used throughout this manual.

Definitions

Absolute quantification: Absolute quantification describes a real-time PCR experiment in which samples of known quantity are serially diluted and then amplified to generate a standard curve. Unknown samples are then quantified by comparison with this curve.

Amplification factor: The amplification factor (AF) is a measure of the average efficiency at which the DNA target or SPC sequences are copied and detected by their respective primer and probe assays during each cycle of the qPCR reaction. It is used in comparative cycle threshold calculation methods like ddCT. AF values can range from 1 (0 % of sequences copied and detected) to 2 (100 % of sequences copied and detected) and are calculated from the standard curve.

Amplification plot: The plot of cycle numbers versus fluorescence signal which correlates with the initial amount of target nucleic acid during the exponential phase of PCR.

Baseline: Baseline is the initial cycles of PCR during which there is a little change in fluorescence signal. It is set to distinguish relevant amplification signal from the background and by default, usually, q PCR instrument software automatically sets the threshold at 10 times the standard deviation of the fluorescence value of the baseline. However, the positioning of the threshold can be set at any point in the exponential phase of PCR. When modified manually, it is recommended that the baseline should eliminate the "noise" of the reaction due to the chemistry occurring at the beginning of the reaction. The baseline may cover the initial cycles before the actual amplification but it should not be strong enough to cover the signal of the actual amplification. Comparing the Ct values of same assays of different runs would be useful to decide for setting up the baseline.

Calibrator: A single reference sample used as the basis for relative-fold increase during the PCR.

Comparative quantification algorithms

ΔCt:

This is comparative quantification in its most basic form. A Ct is obtained for expression of the gene of interest from both a test and calibrator sample, and the difference between them is the ΔCt. The fold difference is then simply 2 to the power of ΔCt. Fold difference = 2ΔCt This basic method is inadequate because it does not control for differences in sample quantity, sample quality, or reaction efficiency.

ΔΔCt:

The ΔΔCt method is another technique that compares results from experimental samples with both a calibrator (e.g., untreated or wild-type sample) and a normalizer (e.g., reference DNA). With this method, Cts for the gene of interest in both the test sample(s) and calibrator sample are now adjusted in

relation to a normalizer gene Ct from the same two samples. The resulting ΔΔCt value is incorporated to determine the fold difference in expression.

Correlation coefficient (R2): The square of a correlation coefficient of regression reflects the linearity of the standard curve, therefore indicates how precisely the line fits the data. The R2 in qPCR analysis should be greater than 0.99, indicating that the standard curve was constructed precisely.

FAM: (6-carboxy fluorescein) Most commonly used quencher at the 5' end of TaqMan® probe.

Log-dilution: Serial dilutions in powers of 10.

No template control: includes all the qPCR reagents except the template. No product should be synthesize at the end of the reaction.

Quencher: the molecule that absorbs the emission of fluorescent reporter when in close vicinity.

Real-time PCR: The continuous collection of fluorescent signal from polymerase chain reaction throughout cycles.

Relative quantification: Relative quantification describes a real-time PCR experiment in which the expression of a gene of interest in one sample (i.e., treated) is compared to expression of the same gene in another sample (i.e., untreated). The results are expressed as fold change (increase or decrease) in expression of the treated in relation to the untreated. A normalizer gene (such as β-actin) is used as a control for experimental variability in this type of quantification.

Reporter dye: The fluorescent dye used to monitor amplicon accumulation. This can be attached to specific probe.

Slope (Efficiency): The slope is a measure of reaction efficiency. The efficiency should be as close to 100% as possible, which is equivalent to a slope of -3.32.

The efficiency is calculated from the formula:

$$E = 10^{(-1/slope)}-1$$

Standard curve: Obtained by plotting Ct values against log-transformed concentrations of serial ten-fold dilutions of the target nucleic acid. Standard curve is obtained for qPCR and the range of concentrations included should cover the expected unknown concentrations range.

TAMRA: (6-carboxy tetra methyl rhodamine): Most commonly used quencher at the 3' end of TaqMan ®probe.

TaqMan® probe: A dual-labeled specific hydrolysis probe designed to bind to a target sequence with a fluorescent reporter dye at one end and a quencher at the other.

Threshold cycle (Ct) : The threshold cycle (Ct) is the cycle number at which the fluorescence generated within a reaction crosses the threshold. A threshold is usually 10X the standard deviation of reporter signal for early PCR cycles (baseline). Therefore it is useful for calculations. The Ct is negatively cor-

ralled to the logarithm of the initial copy number. As the template concentration decreases, the cycle number increases.

Unknown: A sample containing an unknown quantity of template. This is the sample of interest whose quantity is being determined.

Y-intercept (Sensitivity): The y-intercept is less reproducible than the slope but gives some indication of the sensitivity of the assay. The y-intercept corresponds to the theoretical limit of detection of the reaction, or the Ct value expected if the lowest copy number of target molecules denoted on the x-axis gave rise to statistically significant amplification. The y-intercept value may be useful for comparing different amplification systems and targets.

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