Ct value interpretation

Method 2

Appendix of Real-time quantitative PCR training course.

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This document explains how you can interpret the Ct values from ΔΔCt method and how you can determine the ΔΔCt method is applicable for your experiment instead of the conventional calibration curve method.

If you are not sure which method to use, use calibration curve method because it is more reliable for most of the real-time PCR applications.

Ct value interpretation

Experimental condition

- Sample subject: Control (Cont) and Experimental (Exp)
- Target gene: *Tar*
- Reference gene: *Ref*

Results

- Control sample

  Ct for *Tar* ($ContCt^{Tar}$) was 22, Ct for *Ref* ($ContCt^{Ref}$) was 19.
- Experimental sample

  Ct for *Tar* ($ExpCt^{Tar}$) was 25, Ct for *Ref* ($ExpCt^{Ref}$) was 18.

Ct value interpretation

ΔExpCt = (2^ExpCt^{Tar} /2^ExpCt^{Ref})

= (2^{25} / 2^{18}) = (2^{25-18}) = 2^{7}

This calculation assumes that the DNA product becomes 2-fold after each PCR cycle (means 100% PCR efficiency).

This step normalizes the amount of *Tar* by the amount of *Ref* in Exp. (In other wards, *Tar* was 2^{7}-times more than *Ref* in Exp).

ΔContCt = (2^ContCt^{Tar} /2^ContCt^{Ref})

= (2^{22}/ 2^{19}) = (2^{22-19}) = 2^{3}

In the same way, the expression difference is 2^{3} = 8-fold.

This is also assuming 100% PCR efficiency.

This step normalizes the amount of *Tar* by the amount of *Ref* in Cont. (*Tar* was 2^{3}-times more than *Ref* in Cont)

Ct value interpretation

$\Delta\Delta Ct = (\Delta ExpCt / \Delta ContCt) = (2^7 / 2^3) = 2^{7-3} = 2^4$

This means *Tar* in Exp requires 4 more rounds of PCR cycle to reach the same amount of *Tar* in Cont.

Therefore Exp would have had 2^4 = 16-fold smaller amount of *Tar* compared to that in Cont.

When the amount of *Tar* in Cont is set as 1.0, the amount of *Tar* in Exp is 0.0625 (= 1/16).

Attention!!:

This calculation is applicable only when the PCR efficiency of both *Tar* and *Ref* is 100% (DNA product becomes 2-fold after each PCR cycle).

Ct value interpretation

If the PCR efficiency is less than 100%, you have to change the calculations.

- Hypothetical results:

  The efficiency was 95% for *Tar* and 93% for *Ref*, meaning that DNA becomes 1.95-fold for *Tar* and 1.93-fold for *Ref* after each PCR cycle.

Ct value interpretation

The calculations change as follows:

ΔExpCt = (1.95^ExpCt^{Tar} / 1.93^ExpCt^{Ref}) = (1.95^{25} / 1.93^{18})

ΔContCt = (1.95^ContCt^{Tar} / 1.93^ContCt^{Ref}) = (1.95^{22} / 1.93^{19})

$\Delta\Delta$Ct = (ΔExpCt /ΔContCt) = (1.95^{25} / 1.93^{18}) / (1.95^{22} / 1.93^{19})

= (1.95^{25-22} / 1.93^{18-19}) = (1.95^{3} / 1.93^{-1})

≒ (7.41 / 0.52) ≒ 14.31

This means that the amount of *Tar* in Exp was 14.31-fold less than that in Cont so that if the amount of *Tar* in Cont is set as 1.0, the amount of *Tar* in Exp is approximately 0.070 (= 1/14.31).

Ct value interpretation

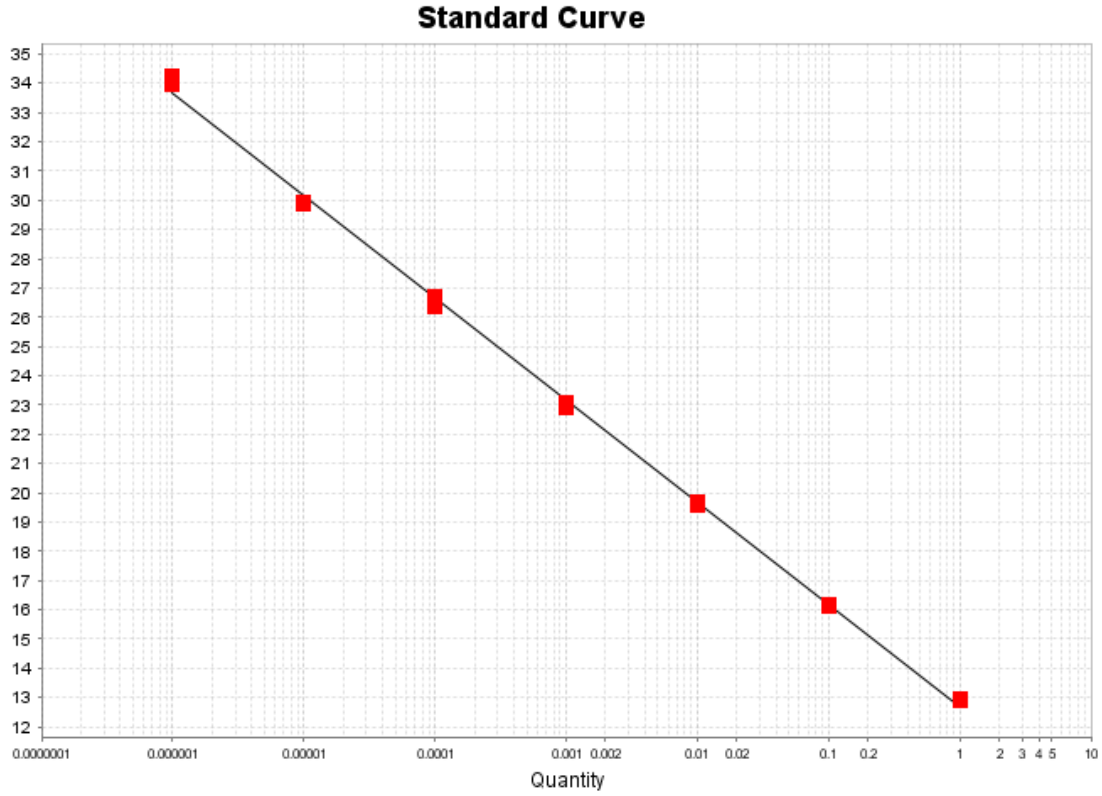
Important rules:

You can use the ΔΔCt method only when the PCR efficiency of *Tar* and *Ref* is steady, high and almost equal.

How to evaluate the PCR efficiency?

- When you make calibration curves for *Tar* and *Ref*, you will have values of the steadiness (R^2), the amplification efficiency and the slope for each calibration curve (refer to the next page).
- If the efficiency is 90% or higher and R^2 is 0.95 or higher (0.99 or higher is preferred) for both *Tar* and *Ref* and at the same time the difference of slope values between *Tar* and *Ref* is less than 0.1 (means 0.999 is OK but 0.100 is not OK), the experimental condition is good enough to use the ΔΔCt method, otherwise you have to use the calibration curve method.

Ct value interpretation



Target: GeneA Slope: -3.494 Y-Inter: 12.69 R^2: 0.999 Eff%: 93.288

Slope R^2 Efficiency