Ct value interpretation

Method 1

Appendix of Real-time quantitative PCR training course.

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This document explains how you can interpret the Ct values from $\Delta\Delta$Ct method and how you can determine the $\Delta\Delta$Ct method is applicable for your Experimental 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

Experimentalal 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

ΔCt^{Tar} = (2^ExpCt^{Tar} /2^ContCt^{Tar})

= (2^{25} / 2^{22}) = (2^{25-22}) = 2^3

This calculation assumes that the *Tar* DNA becomes 2-fold more after each PCR cycle (when 100% PCR efficiency) so that the expression difference is 2^3 = 8-fold.

Experimental had 8-fold less *Tar* DNA compared to that of Cont, because high Ct value means less DNA at the starting point.

ΔCt^{Ref} = (2^ExpCt^{Ref} /2^ContCt^{Ref})

= (2^{18} / 2^{19}) = (2^{18-19}) = 2^{-1}

In the same manner the expression difference is 2^{-1} = 1/2-fold, meaning Exp had 1/2 times less (meaning 2-fold more) *Ref* DNA compared to that of Cont at the starting point.

This is also assuming that *Ref* has 100% PCR efficiency.

Ct value interpretation

$\Delta\Delta Ct = (\Delta Ct^{Tar} / \Delta Ct^{Ref}) = (2^{3} / 2^{-1}) = 2^{3-(-1)} = 2^{4} = 16$

This is compensating the amount of *Tar* using the amount of *Ref* gene.

Real expression difference is 16-fold.

This means the amount of *Tar* in Exp was 16-fold less than that in Cont so that if you set the amount of *Tar* in Cont 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 more for *Tar* and 1.93-fold more for *Ref* after each PCR cycle.

Ct value interpretation

The calculations change as follows:

ΔCt^{Tar} = (1.95^$ExpCt^{Tar}$ / 1.95^$ContCt^{Tar}$) = (1.95^{25} / 1.95^{22})

= (1.95^{25-22}) = 1.95^{3}

ΔCt^{Ref} = (1.93^$ExpCt^{Ref}$ / 1.93^$ContCt^{Ref}$) = (1.93^{18} / 1.93^{19})

= (1.93^{18-19}) = 1.93^{-1}

$\Delta\Delta Ct$ = (ΔCt^{Tar} /ΔCt^{Ref}) = (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 you set the amount of *Tar* in Cont as 1.0, the amount of *Tar* in Exp is approximately 0.070 (= 1/14.31)

Ct value interpretation

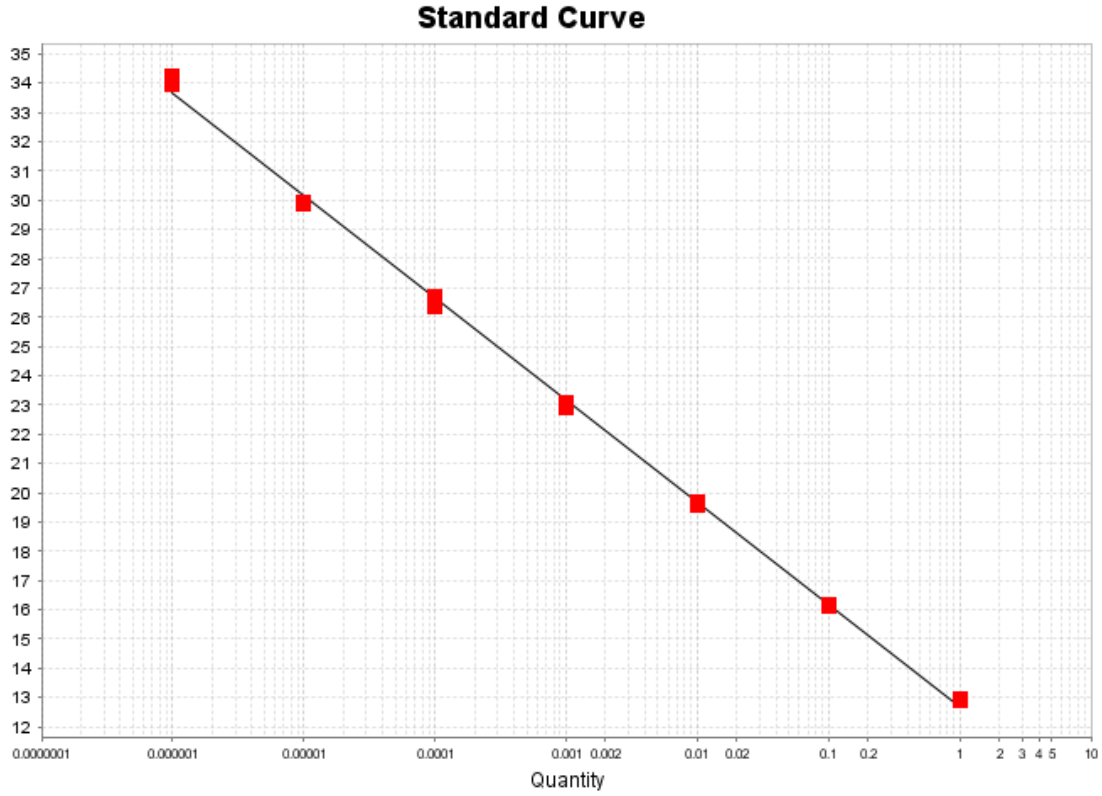
Important rules:

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 Experimentalal 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