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Comparative Ct method quantification (2$^{-\Delta Ct}$ method). 🔗

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May 07, 2019

Working

ABSTRACT

For the evaluation of the expression level of each miRNA was used the Ct (threshold cycle) method quantification. The threshold cycle (Ct) is defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold. This method allows to quantify the absolute expression of each miRNAs in each sample analyzed and then to calculate the different expression of each miRNA in sample versus the controls.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0216363

Evaluation of the absolute expression of miRNAs in each sample analyzed:

1. For each sample is obtained an amplification curve in which the Ct is inversely proportional to the initial sample quantity;

2. Normalize the data to an internal control and calculate DCt = (Ct gene of interest - Ct internal control);

3. Calculate 2$^{-\Delta Ct}$ value that is the measure of the microRNA expression level in each sample analyzed.

Evaluation of the expression of each miRNA in two different samples (2$^{-\Delta\Delta Ct}$ value):

4. Calculate DDCt= [(Ct gene of interest-Ct internal control) sample A] − [(Ct gene of interest-Ct internal control) sample B];

5. Calculate 2$^{-\Delta\Delta Ct}$ value that is the measure of the microRNA expression in each sample analyzed compared with control sample.