Delta Ct (ΔCt) Calculation Protocol

For Bio-Rad RT-qPCR Data Analysis

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Overview

This protocol outlines the steps for calculating ΔCt values from RT-qPCR data generated on Bio-Rad CFX systems (CFX96, CFX Connect, CFX Opus, etc.). The ΔCt method normalizes target gene expression to reference genes, allowing for accurate comparison of gene expression across samples.

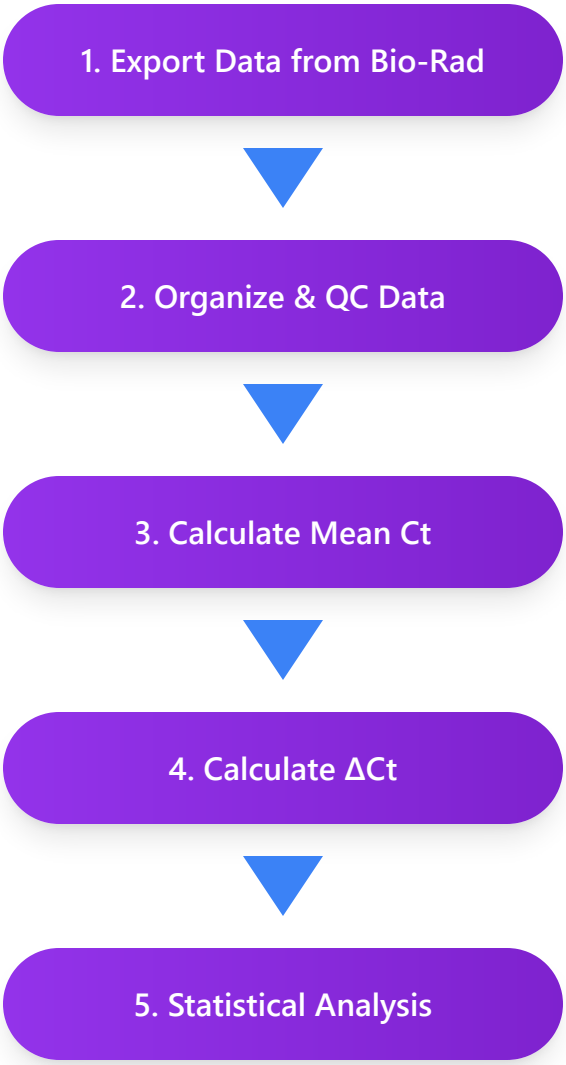
What is ΔCt?

ΔCt (Delta Ct) represents the difference between the Ct value of your target gene and a reference gene. It normalizes gene expression to account for differences in RNA input amount and quality.

Workflow Overview

1. Export data from Bio-Rad CFX Maestro software
2. Organize data in spreadsheet
3. Calculate mean Ct values with quality control
4. Calculate ΔCt (target Ct - reference Ct)
5. Calculate ΔCt standard deviation
6. Interpret ΔCt values
7. Optional: Calculate ΔΔCt for fold change analysis

Complete Workflow


1. Export Data from Bio-Rad
2. Organize & QC Data
3. Calculate Mean Ct
4. Calculate ΔCt
5. Statistical Analysis