

Livak Method, Step One

BIO-RAD

Example

Sample	Gene	
	C _T p53 (Target)	C _T GAPDH (Reference)
Control (calibrator)	15.0	16.5
Tumor (test)	12.0	15.9

1. Normalize C_T of the target gene to the C_T of the reference gene:

- $\Delta C_T(\text{calibrator}) = C_T(\text{target, cal}) - C_T(\text{reference, cal})$
 $= \Delta C_T(\text{control}) = 15.0 - 16.5 = -1.5$

- $\Delta C_T(\text{test}) = C_T(\text{target, test}) - C_T(\text{reference, test})$
 $= \Delta C_T(\text{tumor}) = 12.0 - 15.9 = -3.9$

$$\Delta C_T(\text{calibrator}) = 15.0 - 16.5 = -1.5$$

$$\Delta C_T(\text{test}) = 12.0 - 15.9 = -3.9$$

2. Normalize ΔC_T of the test sample to the ΔC_T of the calibrator:

$$\Delta\Delta C_T = \Delta C_T(\text{test}) - \Delta C_T(\text{calibrator})$$

$$= -3.9 - (-1.5) = -2.4$$

$$\Delta\Delta C_T = -2.4$$

3. Calculate the fold difference in expression:

$$2^{-\Delta\Delta C_T} = \text{Normalized expression ratio}$$

$$2^{-(-2.4)} = 5.3$$

Tumor cells express p53 at a **5.3-fold** higher level than control cells.