The following data was obtained in a qPCR experiments designed to determine the relative expression of two genes in leaf or flower tissue of lavender plants. Using the Livak method, calculate the expression fold differences for Gene1 and Gene2 in flower relative to leaf tissue. Notes: 1) Use the average CT value for each gene in each tissue. 2) Actin was used as a reference gene.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | Replication | Tissue | CT | Average CT |
| Actin | 1 | Leaf | 24.00 | 23.91 |
| Actin | 2 | Leaf | 23.90 |
| Actin | 3 | Leaf | 23.84 |
| Gene1 | 1 | Leaf | 35.39 | 35.01 |
| Gene1 | 2 | Leaf | 35.01 |
| Gene1 | 3 | Leaf | 34.62 |
| Gene2 | 1 | Leaf | 29.69 | 29.65 |
| Gene2 | 2 | Leaf | 29.44 |
| Gene2 | 3 | Leaf | 29.83 |
| Actin | 1 | Flower | 21.28 | 21.28 |
| Actin | 2 | Flower | 21.39 |
| Actin | 3 | Flower | 21.16 |
| Gene1 | 1 | Flower | 25.26 | 25.15 |
| Gene1 | 2 | Flower | 25.11 |
| Gene1 | 3 | Flower | 25.07 |
| Gene2 | 1 | Flower | 31.99 | 31.90 |
| Gene2 | 2 | Flower | 31.84 |
| Gene2 | 3 | Flower | 31.87 |

Fold Change = 2-∆∆CT = 2 -((CT target – CT reference) in sample – (CT target – CT reference) in calibrator), where Sample is flower tissue; Calibrator is leaf tissue; Target is Gene1 or Gene2; Reference gene is Actin.

Hints:

1) Calculate the average CT value for each gene in both flower and leaf tissue.

2) **Normalize** the CT of target to that of reference (ΔCT) in both sample (flower) and calibrator (leaf) tissue:

ΔCT (sample) = CT (target) – CT (reference)

ΔCT (calibrator) = CT (target in calibrator) – CT (reference calibrator)

3) Calculate the difference between the ΔCT of the test sample and the ΔCT of the calibrator, also called **ΔΔCT**:

ΔΔCT = ΔCT (sample) – ΔCT (calibrator)

4) Calculate the **expression ratio (fold difference)**:

Expression ratio (folds) = 2–ΔΔCT

5) Interpret the value you obtained.

**Answer – Trevor Fox**

Sample: Flower

Calibrator: Leaf

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Sample (Flower) | | Calibrator (Leaf) | |
| G1 | G2 | G1 | G2 |
| Reference Avg | 21.28 | | 23.91 | |
| Average | 25.15 | 31.90 | 35.01 | 29.65 |
| ΔCT | 3.87 | 10.62 | 11.1 | 5.74 |

ΔΔCT (Gene 1): -7.23

ΔΔCT (Gene 2): 4.88

ER (Gene 1): 2-(-7.23) = 150.122873502 folds

ER (Gene 2): 2-(4.88) = 0.0339604644539 folds

By these results, one can see that the presence of Gene 1 is increased in the flower 150.123-fold compared to the reference gene. This means that Gene 1 is upregulated in flowers.

Inversely, Gene 2 is decreased 0.034-fold in the flower, meaning that the gene is downregulated in the flower in comparison to the leaf.