

BIOINFORMATICS ASSIGNMENT 1 (Day 1 - 5)

Note: You will be added in a slack community of Biversity for further doubts and communications

1. Gene Name:

F2 – coagulation factor II, thrombin

2. Function of the Gene:

***-The F2 gene helps in formation of blood clots in response to injury.
-The F2 gene provides instructions for making a protein called prothrombin (also called coagulation factor II)***

3. NCBI accession number:

>NC_000011.10

4. Forward Primer:

TGAATGGGAGAACTGCTGGT

5. Reverse primer:

TTCACATGCCCTCGGTAGTT

6. Features of primers:

***- Length of primer – 18 to 28 bases
- GC Content of primer – 40% to 60%
- Melting Temperature of primer (T_m) – 65 degree celsius – 72 degree celsius
- Annealing Temperature of primer- 4 degree celsius***

7. Amplicon length and sequence:

Amplicon Size – 207

Amplicon Sequence – TGAATGGGAGAACTGCTGGTTGCAGAGGAAGAGG

**GGCTGGGTGAATGCAGGTTTCAGGATTGTGGACCTGCATGAGCTGGGAGGTGGGGGA
TAGA**

**CAACTTTGCAGGGAGAGAGGAAATAAGTCCCCAGGCTCCAAGGCTGACCGGGGTGG
GGTC**

TCCGCAGGTAAGTGTGCTGAGGGTCTGGGTACGAACTACCGAGGGCATGTGAA

qPCR Data analysis (DAY 5)

	Ct values			Ct values	
Housekeeping genes(GAPDH)	Ct 1	Ct 2	Gene of interest (HER2)	Ct 1	Ct 1
Untreated (control)	18.5	18.5	Untreated(control)	23.3	22.5
Untreated (control)	17.8	17.8	Untreated(control)	22.5	22.2
Untreated (control)	17.5	17.5	Untreated(control)	21.2	21.9
Treated	18.3	18.3	Treated	25.3	25.3
Treated	18.5	18.5	Treated	26.5	26.5
Treated	18.2	18.2	Treated	27.5	27.5

The following data are results of qPCR from cancer cell lines. HER2 stands for human epidermal growth factor. It's healthy in normal amounts, but too much may be a sign of a certain type of breast cancer. Calculate the 2 Delta Ct values for the following data and plot the values on a graph using graphpad prism.

excel 1.e - Excel (Product Activation Failed)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1			Housekeeping Gene			Gene of Interest (HER2)			Average Ct Value for Housekeeping gene		Average Ct Value for HER2			delta Ct		double delta Ct		Fold Change			
2			Ct Values			Ct values												2 ^{ΔΔ} (double delta Ct)			
3			Ct 1	Ct 2		Ct 1	Ct 2														
4	Untreated(control)		18.5	18.5		23.3	22.5		18.5		22.9			4.4		0.066667		0.954842			
5	Untreated(control)		17.8	17.8		22.5	22.2		17.8		22.35			4.55		4.833333		0.035077			
6	Untreated(control)		17.5	17.5		21.2	21.9		17.5		21.55			4.05		-0.28333		1.217004			
7	Treated		18.3	18.3		25.3	25.3		18.3		25.3			7		2.666667		0.15749			
8	Treated		18.5	18.5		26.5	26.5		18.5		26.5			8		3.666667		0.078745			
9	Treated		18.2	18.2		27.5	27.5		18.2		27.5			9.3		4.966667		0.03198			
10																					
11																					
12									Avg delta Ct of control							4.333333					
13																					
14																					
15																					
16																					
17																					
18																					
19																					
20																					
21																					
22																					
23																					

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-Average Ct Value for Housekeeping gene -

18.5
17.8
17.5
18.3
18.5
18.2

-Average Ct Value for HER2 -

22.9
22.35
21.55
25.3
26.5

27.5

-delta Ct -

Avg delta Ct of control- 4.333333333

4.4

4.55

4.05

7

8

9.3

-double delta Ct -

0.066666667

4.833333333

-0.283333333

2.666666667

3.666666667

4.966666667

- Fold Change

$2^{-(\text{double delta Ct})}$ -

0.954841604

0.035076939

1.217003514

0.157490131

0.078745066

0.031980434