BIOINFORMATICS ASSIGNMENT 1 (Day 1 - 5)

1.   Gene Name: BRCA2 gene.

2.   Function of the Gene: BRCA2 are involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair

3. NCBI accession number: NC_000013.11

4.   Forward Primer: GGGATGACACAGCTGCAAAA

5.   Reverse primer: TGGGCCTTAACAGCATACCA

6.   Features of primers:

        a) Product size: 160
        b) GC content: forward primer-50%; reverse primer-50%
        c) Tm temperature: forward-59.04°C; reverse-59.00°C

Primer3 Output

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PRIMER PICKING RESULTS FOR NC_000013.11:32315508-32400268 Homo sapiens chromosome 13, GRCh38.p14 Primary Assembly

Template masking not selected
No mispriming library specified
Using 1-based sequence positions
OLIGO          start  len      tm     gc%  any_th  3'_th hairpin seq
LEFT PRIMER    47728   20   59.04   50.00   15.27   0.00    0.00 GGGATGACACAGCTGCAAAA
RIGHT PRIMER   47887   20   59.00   50.00    4.53   0.00    0.00 TGGGCCTTAACAGCATACCA
SEQUENCE SIZE: 84761
INCLUDED REGION SIZE: 84761
```

7.   Amplicon sequence & length: 160bp

UCSC In-Silico PCR

>chr13:32363235+32363394 160bp GGGATGACACAGCTGCAAAA TGGGCCTTAACAGCATACCA
GGGATGACACAGCTGCAAAAacacttgttctctgtgtttctgacataatt
tcattgagcgcaaatatatctgaaacttctagcaataaaactagtagtgc
agatacccaaaaagtggccattattgaacttacagatgggTGGTATGCTG
TTAAGGCCCA

Amplicon Sequence.

qPCR &Data analysis (DAY 5)

Housekeeping genes(GAPDH)	Ct values	
	Ct 1	Ct 2
Untreated (control)	18.5	18.5
Untreated (control)	17.8	17.8
Untreated (control)	17.5	17.5
Treated	18.3	18.3
Treated	18.5	18.5
Treated	18.2	18.2

Gene of interest (HER2)	Ct values	
	Ct 1	Ct 1
Untreated(control)	23.3	22.5
Untreated(control)	22.5	22.2
Untreated(control)	21.2	21.9
Treated	25.3	25.3
Treated	26.5	26.5
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.

	P20														
	A	B	D	E	F	G	H	I	J	K	L	M	N	O	P	Q

	A	B	D	E	F	G	H	K	N	O	P
1	**qPCR & DATA ANALYSIS….**										
2											
3			HOUSEKEEPING GENES		GENE OF INTEREST		Average ct value for HG		Average ct value for GOI	Δct value	ΔΔct value	Fold change
4			C1	C2	C1	C2						
5	Control		18.5	18.5	23.3	22.5	18.5		22.9	4.4	0	1
6	Control		17.8	17.8	22.5	22.2	17.8		22.3	4.5	0	1
7	Control		17.5	17.5	21.2	21.9	17.5		21.5	4	0	1
8	Treated		18.3	18.3	25.3	25.3	18.3		25.3	7	2.7	0.153893
9	Treated		18.5	18.5	26.5	26.5	18.5		26.5	8	3.7	0.076947
10	Treated		18.2	18.2	27.5	27.5	18.2		27.5	9.3	5	0.03125
11											
12											