

BIOINFORMATICS ASSIGNMENT 1 (Day 1 - 5)

Rasitha Arafa R

rasithaarafarafiudeen@gmail.com

1. Gene Name: Cytochrome b5 type B (CYB5B) [*Homo sapiens*]
2. Function of the Gene
 - Enables heme binding activity.
 - Contributes to nitrite reductase activity.
 - Involved in nitric oxide biosynthetic process.
 - Located in membrane of mitochondria.
 - Part of nitric-oxide synthase complex.
3. NCBI accession number: NC_000016.10
4. Forward Primer: GTCAGCGGTTTGTCTTGTGT
5. Reverse primer: ACAGGGCATAGCTTTGGAGT
6. Features of primers
 - Left primer (Forward): Length – 20 bases, GC% - 50%, Tm – 58.99 degree Celsius
 - Right primer (Reverse): Length – 20 bases, GC% - 50%, Tm – 59 degrees Celsius
7. Amplicon length: 250 bp
8. Amplicon sequence:

TTTATATTTGTGTTTACTCAAGTAGGACTGCTTTTTGAAACATTTTTCTTAACAAGAGAAGTTAC
AAAGTATTTACTTTTTCCCAAGCAAAAATCCTATTTTTCTGGAATTTGGACTCAGTATCATCTCA
GGAATAAAAGAATAGCTGAGTCTTGAACAGTAGGAAACATTTTGCTAATGCCTTTATACGCTTTT
TTTTTTAACTGAAACTCCAAAGCTATGCCCTGT

qPCR Data analysis (DAY 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.

	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

SOLUTION:

	House Keeping Genes(GAPDH)		Gene of Interest (HER2)		Average ct value of HG	Average ct value of GOI	Δct value	ΔΔct value	Fold Change
	ct1	ct2	ct1	ct2					
Untreated (control) 1	18.5	18.5	23.3	22.5	18.5	22.9	4.4	0	1
Untreated (control) 2	17.8	17.8	22.5	22.2	17.8	22.35	4.55	0	1
Untreated (control) 3	17.5	17.5	21.2	21.9	17.5	21.55	4.05	0	1
Treated 1	18.3	18.3	25.3	25.3	18.3	25.3	7	2.6	0.16493849
Treated 2	18.5	18.5	26.5	26.5	18.5	26.5	8	3.45	0.09150536
Treated 3	18.2	18.2	27.5	27.5	18.2	27.5	9.3	5.25	0.02627801

NCBI – Primer BLAST Results

Primer-BLAST» JOB ID:QEqf0sJaz_LozNXJ2Knx-6Ky4MmPofvUjg

Primer-BLAST Results

Input PCR template

NC_000016.10 Homo sapiens chromosome 16, GRCh38.p14 Primary Assembly

Range

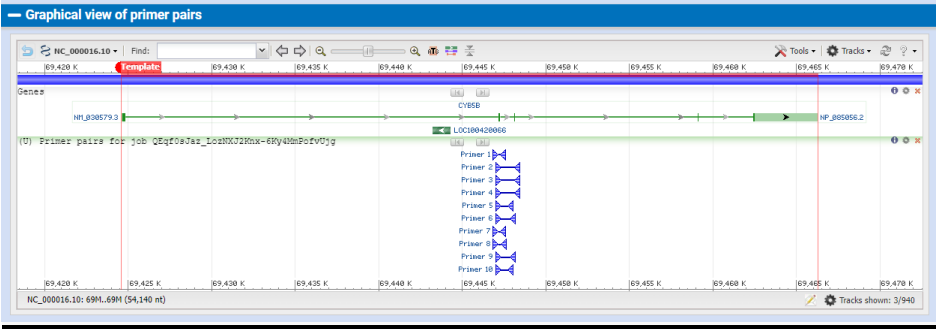
69424619 - 69466264

Specificity of primers

Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)

Other reports

Search Summary



Detailed primer reports

Primer pair 1									
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTGTAGCACCCCTGGAGGAGA	Plus	20	69447144	69447163	59.88	55.00	4.00	0.00
Reverse primer	TCTGCATAAGGGCTCCCATC	Minus	20	69447313	69447294	59.23	55.00	4.00	2.00
Product length	170								
Primer pair 2									
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGATGGGAGCCCTTATGCAG	Plus	20	69447293	69447312	59.23	55.00	4.00	2.00
Reverse primer	CCGCTGACTTCTTACCTTGC	Minus	20	69448159	69448140	58.92	55.00	2.00	2.00
Product length	867								
Primer pair 3									
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGAGATGGGAGCCCTTATGC	Plus	20	69447291	69447310	58.94	55.00	4.00	2.00
Reverse primer	CCGCTGACTTCTTACCTTGC	Minus	21	69448159	69448139	60.34	52.38	2.00	0.00
Product length	869								
Primer pair 4									
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGGGAGCCCTTATGCAGAG	Plus	21	69447294	69447314	60.27	57.14	4.00	2.00
Reverse primer	ACACAAGACAAACCGCTGACT	Minus	21	69448171	69448151	60.41	47.62	3.00	1.00
Product length	878								
Primer pair 5									
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAGAGATGGGAGCCCTTATGC	Plus	21	69447290	69447310	60.27	57.14	4.00	2.00
Reverse primer	GCTACCTGGCAAAGAAGAAGC	Minus	21	69447761	69447741	59.53	52.38	4.00	2.00
Product length	472								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACTATCAGAGATGGGAGCCCT	Plus	21	69447285	69447305	59.49	52.38	4.00	2.00
Reverse primer	AGGAGCTTGCAAAACAAGCA	Minus	21	69447888	69447868	59.52	42.86	6.00	2.00
Product length	604								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTGTAGCACCCCTGGAGGA	Plus	20	69447142	69447161	60.91	60.00	4.00	2.00
Reverse primer	AAGGGCTCCCATCTCTGATAGT	Minus	22	69447306	69447285	60.09	50.00	4.00	1.00
Product length	165								

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTCCTGTAGCACCCCTGGAG	Plus	20	69447140	69447159	59.02	55.00	3.00	2.00
Reverse primer	TCCTGTTCTGCAGCTGTTAAGT	Minus	22	69447342	69447321	59.90	45.45	6.00	1.00
Product length	203								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGGGAGCCCTTATGCAGAGA	Plus	21	69447295	69447315	60.70	52.38	4.00	2.00
Reverse primer	AGCAAGGAGCTTGCAAAACA	Minus	21	69447892	69447872	59.52	42.86	7.00	0.00
Product length	598								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGGAGCCCTTATGCAGAGAA	Plus	21	69447296	69447316	60.91	52.38	4.00	1.00
Reverse primer	ACATGTGCTACCTGGCAAAG	Minus	20	69447767	69447748	58.46	50.00	6.00	2.00
Product length	472								

Primer 3 Results

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PRIMER PICKING RESULTS FOR NC_000016.10:69424619-69466264 Homo sapiens chromosome 16, 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 23534 20  58.99 50.00 0.00 0.00 0.00  GTCAGCGTTTGTCTTGTGT
RIGHT PRIMER 23783 20  59.00 50.00 6.29 0.00 0.00  ACAGGGCATAGCTTTGGAGT
SEQUENCE SIZE: 41646
INCLUDED REGION SIZE: 41646

PRODUCT SIZE: 250, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
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ADDITIONAL OLIGOS
      start  len  tm  gc%  any_th  3' th  hairpin  seq
1 LEFT PRIMER 37842 20  59.02 50.00 7.64 0.00 0.00  AGGCGCTGTTCTCTTAGGTT
  RIGHT PRIMER 38049 20  59.01 50.00 7.01 0.00 0.00  TGAGGTGATGTCTGGCCAAT
  PRODUCT SIZE: 208, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

2 LEFT PRIMER 7324 20  59.02 55.00 0.00 0.00 0.00  CTGGGAAAGCCTCTGTGAGA
  RIGHT PRIMER 7504 20  59.01 50.00 5.51 0.00 0.00  AGCTCTCTGGCCATTCTTGT
  PRODUCT SIZE: 181, PAIR ANY_TH COMPL: 11.70, PAIR 3'_TH COMPL: 6.38

3 LEFT PRIMER 38120 20  59.04 50.00 5.05 0.00 0.00  ACAATTGCCGGTGTTTCCTC
  RIGHT PRIMER 38358 20  59.01 55.00 0.00 0.00 0.00  CCACCACACCTAGCCCTAA
  PRODUCT SIZE: 239, PAIR ANY_TH COMPL: 2.92, PAIR 3'_TH COMPL: 0.00

4 LEFT PRIMER 22374 20  59.04 50.00 0.00 0.00 0.00  GCTGTGTTCTTCCATTGCA
  RIGHT PRIMER 22541 20  59.02 55.00 0.00 0.00 0.00  CTCCAGGGTGCTACAAGGAA
  PRODUCT SIZE: 168, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
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