

Sequence Analysis Task

You have been provided with the following nucleotide sequence:

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ACATTTGCTTCTGACACAACCTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCTGA
GGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGC
AGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGAGACAGAGAAG
ACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGG
TGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGG
CAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGAC
AACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACT
TCAGGGTGAGTCTATGGGACGCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCATAG
GAAGGGGATAAGTAACAGGGTACAGTTTAGAATGGGAAACAGACGAATGATTGCATCAGTGTGGAAGTCT
CAGGATCGTTTTAGTTTTCTTTTATTTGCTGTTTCATAACAATTGTTTTCTTTTGTAAATTCTTGCTTTCT
TTTTTTTTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAACATTGTGTATAACAAAAGGAAATA
TCTCTGAGATACATTAAGTAACCTAAAAAAAACCTTACACAGTCTGCCTAGTACATTACTATTTGGAAT
ATATGTGTGCTTATTTGCATATTCATAATCTCCCTACTTTATTTTTCTTTTATTTTAAATTGATACATAAT
CATTATACATATTTATGGGTTAAAGTGTAATGTTTTAATATGTGTACACATATTGACCAAATCAGGGTAA
TTTTGCATTTGTAATTTTAAAAAATGCTTTCTTCTTTTAAATATACTTTTTTGTATCTTATTTCTAATA
CTTTCCCTAATCTCTTTCTTTTCAGGGCAATAATGATACAATGTATCATGCCTCTTTGCACCATTCTAAAG
AATAACAGTGATAATTTCTGGGTAAAGGCAATAGCAATATCTCTGCATATAAATATTTCTGCATATAAAT
TGTAAGTATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTTATTTT
ATGTTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTTCATACCTCTT
ATCTTCCTCCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCA
CCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCAAGTATCA
CTAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGTTTCTTTGTTCCCTAAGTCCAATACTAACT
GGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAA
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Using bioinformatics tools, analyze the given sequence and answer the following questions:

1. Gene Identification

- To which gene does this sequence belong?
- Which organism does it originate from?
- What is the length of the sequence?

2. Genomic Location

- How many exons does this gene have?
- On which chromosome is it located? Provide the precise location.

3. Sequence Alignment

- Align this sequence with the sequence of the following accession number:
(NM_001164428.1)
- What is the taxonomy of the organism corresponding to the subject sequence?
- Based on similarity percentages and query coverage, describe the relationship between the two sequences.
- Explain the significance of the dot plot in comparing these sequences.
- Why are there multiple alignment ranges in the result?
- What are the gap numbers in each range?

4. Global Alignment Evaluation

- What is the identity percentage based on global alignment?
- Describe the graphical summary of the global alignment.
- What is the total number of gaps in the global alignment?
- How would you evaluate the relationship between the two genes based on global alignment?

Use appropriate bioinformatics tools such as **NCBI & BLAST** to perform these analyses.

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Primer design part:

You have been provided with the following accession number: (NM_001382762.1)

Using Primer-BLAST, design forward and reverse primers with the following criteria:

- The forward primer must be located within the first 600 nucleotides of the provided sequence.
- The reverse primer must be located within the second 600 nucleotides of the provided sequence.
- The PCR product length should not be less 200 bp.
- Maximum melting temperature should be 60.
- Allow the program to choose primers that amplify mRNA splice variants.
- No additional preferences or constraints are required.

Instructions:

1. **Modify the Primer-BLAST settings to ensure the designed primers meet the above criteria. And provide a screenshot of the modified settings you used in Primer-BLAST.**
2. **Generate 5 sets of primer pairs using the tool. And save the 5 primer sets in a CVS format and attach it with your report.**
3. **Select the best primer pair from your results and justify your selection.**
This set has the best value for the self-3' complementarity value.
4. **If you want your primer within two exons which option, you are going to choose?**

