Introduction to applied

bioinformatics

Exam

Tuesday 6.5.2025 13:10

Moodle: files with the version

open "task" for submission (like for homeworks).

Usual Exam test:

2 parts, each five points

Maximum 10 points

8.5-10p: 1

7-8p: 2

5-6.5p: 3

Version 1

Find human protein sequence FGF3

- · What is the accession number and function of this protein?
- · How many valines does the sequence contain?
- How long is the longest fragment after trypsin digestion?
- How many human similar reference proteins are in the databases?
- · Does the sequence contain any transmembrane regions?

Download segence NM_001757.3

- · What does this sequence encode?
- Is there a complementary sequence to primer R1?

>R1 GCTCTGACGCTCATGATGC

- · In which exon is the complemetary sequence for this primer?
- Is the primer suitable for PCR with Ta=60°C?
- · Design suitable F primer for this experiment.

Last year Exam

MNWELLLWLLVLCALLLLLVQLLRFLRADGDLTLLWAEWQGRRPEWELTDMVVWVTGASS
GIGEELAYQLSKLGVSLVLSARRVHELERVKRRCLENGNLKEKDILVLPLDLTDTGSHEA
ATKAVLQEFGRIDILVNNGGMSQRSLCMDTSLDVYRKLIELNYLGTVSLTKCVLPHMIER
KQGKIVTVNSILGIISVPLSIGYCASKHALRGFFNGLRTELATYPGIIVSNICPGPVQSN
IVENSLAGEVTKTIGNNGDQSHKMTTSRCVRLMLISMANDLKEVWISEQPFLLVTYLWQY
MPTWAWWITNKMGKKRIENFKSGVDADSSYFKIFKTKHD

- Identify the sequence.
- Does this protein have any transmembrane regions?
- How many isoforms this protein has? Align them.
- How many cysteins does the sequence have?
- Design primers to amplify the CDS of the respective gene.

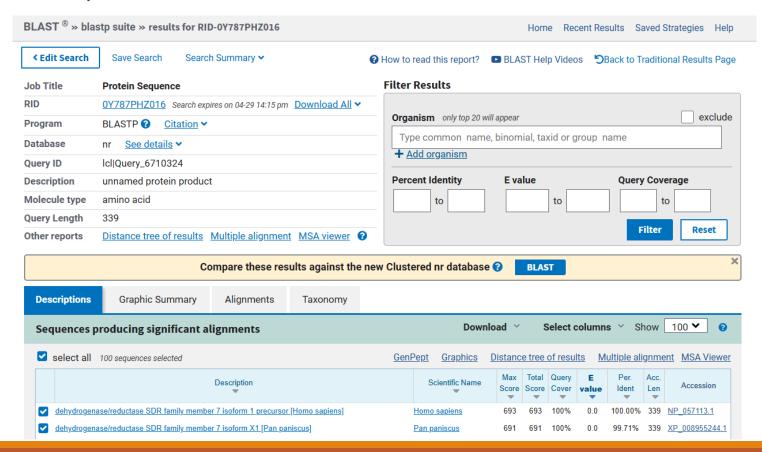
MNWELLLWLLVLCALLLLLVQLLRFLRADGDLTLLWAEWQGRRPEWELTDMVVWVTGASS
GIGEELAYQLSKLGVSLVLSARRVHELERVKRRCLENGNLKEKDILVLPLDLTDTGSHEA
ATKAVLQEFGRIDILVNNGGMSQRSLCMDTSLDVYRKLIELNYLGTVSLTKCVLPHMIER
KQGKIVTVNSILGIISVPLSIGYCASKHALRGFFNGLRTELATYPGIIVSNICPGPVQSN
IVENSLAGEVTKTIGNNGDQSHKMTTSRCVRLMLISMANDLKEVWISEQPFLLVTYLWQY
MPTWAWWITNKMGKKRIENFKSGVDADSSYFKIFKTKHD

- Identify the sequence.
 BLASTp
 Does this protein have any transmembrane regions?
 TMHMM, TopCons, Phobius...
 not Protter (just figure)
- How many isoforms this protein has? Align them. Uniprot for isoforms, multalin for alingment
- How many cysteins does the sequence have?

 SMS: Protein Stats
- Design primers to amplify the CDS of the respective gene. Manual design (OligoCalc for check-similar Tm)
 SMS:Reverse complement for R primer

Identify the sequence.

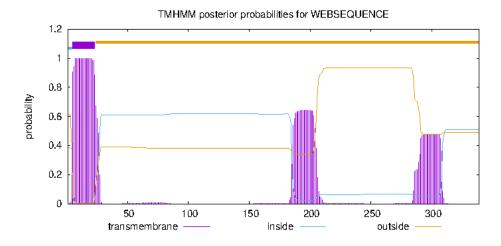
DHRS7



Does this protein have any transmembrane regions?

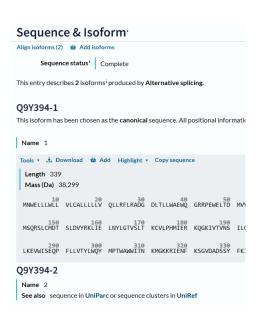
Yes 1: TMHMM result

```
# WEBSEQUENCE Length: 339
# WEBSEQUENCE Number of predicted TMHs: 1
# WEBSEQUENCE Exp number of AAs in TMHs: 44.121
# WEBSEQUENCE Exp number, first 60 AAs: 20.90194
# WEBSEQUENCE Total prob of N-in:
# WEBSEQUENCE POSSIBLE N-term signal sequence
               TMHMM2.0
                              inside
                                         1
WEBSEQUENCE
               TMHMM2.0
                              TMhelix
                                        5 23
WEBSEQUENCE
               TMHMM2.0
                              outside
                                        24 339
```



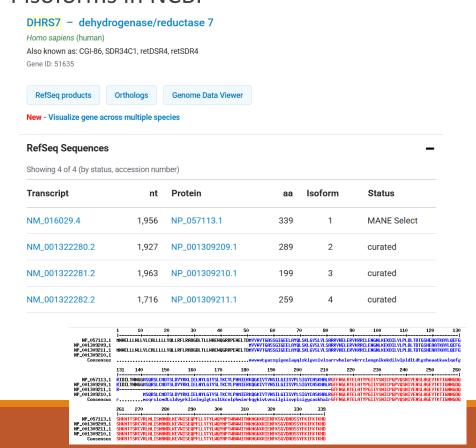
How many isoforms this protein has? Align them.

2 isoforms in uniprot





4 isoforms in NCBI



How many cysteins does the sequence have?

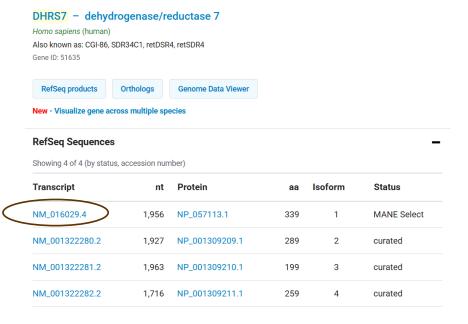
7 cysteins: Sequence Manipulation Suite:

Protein Stats

Protein Stats returns the number of occurrences of each residue in the sequence you enter. Percentage totals are also given for each res

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 500,000,000 characters. MNWELLLWLLVLCALLLLLVQLLRFLRADGDLTLLWAEWQGRRPEWELTDMVVWVTGASS ... GIGEELAYOLSKLGVSLVLSARRVHELERVKRRCLENGNLKEKDILVLPLDLTDTGSHEA ATKAVLQEFGRIDILVNNGGMSQRSLCMDTSLDVYRKLIELNYLGTVSLTKCVLPHMIER KOGKIVTVNSILGIISVPLSIGYCASKHALRGFFNGLRTELATYPGIIVSNICPGPVOSN IVENSLAGEVTKTIGNNGDOSHKMTTSRCVRLMLISMANDLKEVWISEOPFLLVTYLWOY MPTWAWWITNKMGKKRIENFKSGVDADSSYFKIFKTKHD Clear Reset Submit *This page requires JavaScript. See browser compatibility. *You 🕻 🌐 Sequence Manipulation Suite – Škola – Microsoft Edge I cita about:blank Protein Stats results Results for 339 residue sequence "Untitled" starting "MNWELLLWLL" Pattern: Percentage: Times found: 4.72 10.00 2.06 11/1 113

Design primers to amplify the CDS of the respective gene.



F: ATG AAC TGG GAG CTG CTG C

R: TCA GTC ATG TTT TGT CTT AAA GAT TTT

FASTA -

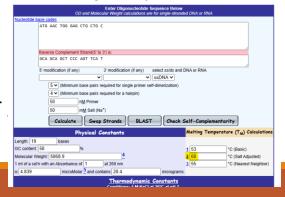
Homo sapiens dehydrogenase/reductase 7 (DHRS7),

NCBI Reference Sequence: NM_016029.4

GenBank Graphics

 $>NM_016029.4:90-1109$ Homo sapiens dehydrogenase/reductase 7 (DHRS7), transcrimRNA

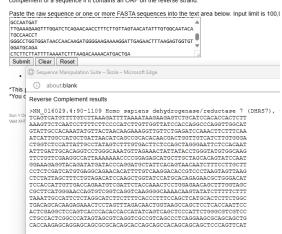
Oligonucleotide Properties Calculator



Sequence Manipulation Suite:

Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement complement of a sequence if it contains an ORF on the reverse strand.



Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below OD and Molecular Weight calculations are for single-stranded DNA or RNA	
Nucleotide base codes	
TCA GTC ATG TIT TGT CTT AAA GAT TTT AA	
Reverse Complement Strand(5' to 3') is:	
TTA AAA TCT TTA AGA CAA AAC ATG ACT GA	
5' modification (if any) 3' modification (if any) select ss/ds an	id DNA or RNA
▼ ssDNA ▼	
5 (Minimum base pairs required for single primer self-dimerization) 4 (Minimum base pairs required for a hairpin)	
50 nM Primer	
50 m <u>M</u> Sait (Na ⁺)	
Calculate Swap Strands BLAST Check Self-Complementarity	
Physical Constants	Melting Temperature (T _M) Calculations
Length: 29 bases	
GC content: 24 %	1 52 °C (Basic)
Molecular Weight: 8886.9	2 60 °C (Salt Adjusted)
1 ml of a sol'n with an Absorbance of 1 at 260 nm	3 54 °C (Nearest Neighbor)

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- Is the primer suitable for PCR with Ta=60°C?
- · Design suitable F primer for this experiment.

- What does this mRNA encode?
- How long is the second exon?
- Will the CDS of this sequence be cleaved by the following enzymes: BamHI, EcoRI or Scal?
- What fragments are formed after cleavage by all these enzymes at once?
- Compare how similar is the corresponding protein to the respective mouse homologue?

- What does this mRNA encode? —— Finding the sequnce in NCBI
- How long is the second exon?
 NCBI: highlit sequence features or Graphic
- Will the CDS of this sequence be cleaved by the following enzymes: BamHI, EcoRI or Scal? Get CDS only and SMS: Restriction summary
- What fragments are formed after cleavage by all these enzymes at once?
- Compare how similar is the corresponding protein to the respective mouse homologue?

Get protein and mouse protein and Laling or Needle pairwise comparison

What does this mRNA encode?

FM01

GenBank -

Homo sapiens flavin containing dimethylaniline monoxygenase 1 (FMO1), transcript variant 1, mRNA

NCBI Reference Sequence: NM 001282692.1

FASTA Graphics

Go to: ✓

LOCUS NM_001282692 2198 bp mRNA linear PRI 09-FEB-2025

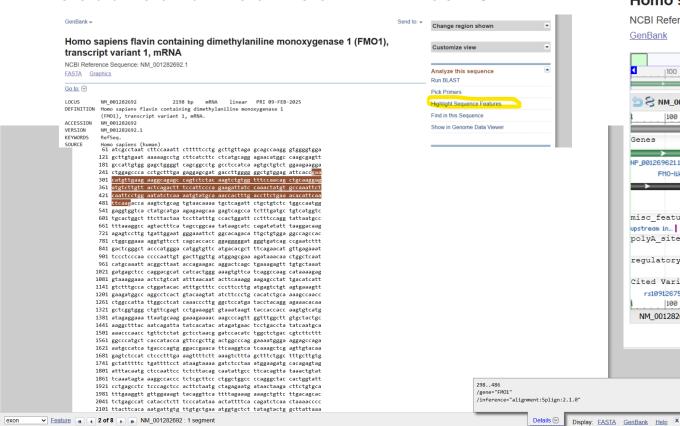
DEFINITION Homo sapiens flavin containing dimethylaniline monoxygenase 1

(FMO1), transcript variant 1, mRNA.

ACCESSION NM 001282692

How long is the second exon?

298..486 : 486-298+1 = 189nt

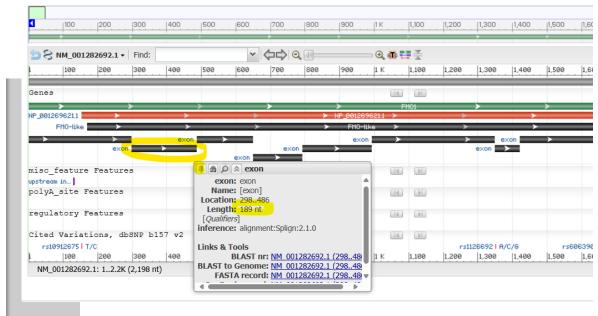


Graphics -

GenBank FASTA

Homo sapiens flavin containing dimethylaniline monoxygenase 1 (FMO1), trar

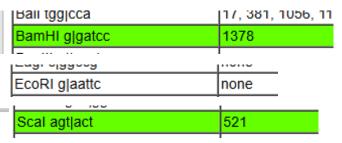
NCBI Reference Sequence: NM 001282692.1



 Will the CDS of this sequence be cleaved by the following enzymes: BamHI, EcoRI or Scal?

BamHI, Scal- cuts 1x

EcoRI –does not cut

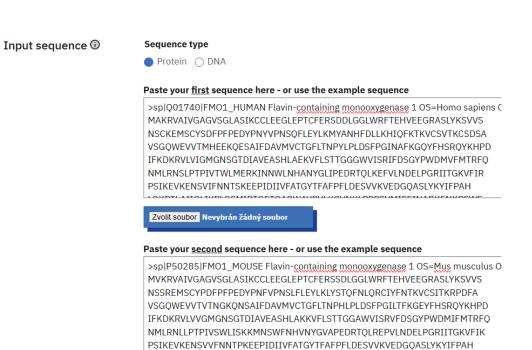


 What fragments are formed after cleavage by all these enzymes at once?

Three fragments: 857 + 520 +234 bp

Sequence Manipulation Suite: Restriction Digest cleaves a DNA sequence in a virtual restriction digest, with one, two, or three restriction ei that produced them. You can digest linear or circular molecules, and even a mixture of molecules (by enterin Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100,000,00 GCCAGAAAT GCCATCATGACCCAGTGGGACCGAACATTCAAGGTCATCAAAGCTCGAGTTGTACAAGAGT CTCCATCTC CCTTTGAAAGTTTTCTTAAAGTCTTTAGCTTTCTGGCTTTGCTTGTGGCTATTTTTCTGAT TTTCCTATA Submit Clear Reset Treat sequences as linear ➤ molecules. *This Sequence Manipulation Suite – Škola – Microsoft Edge (i) about:blank Restriction Digest results >857 bp linear fragment from linear parent NM 001282692.1:154-1764 Homo sa ACTITICATAGCCGGCAATATAAGCATCCAGATATATTTAAGGACAAGAGAGTCCTTGTGA TGTTCCTCAGCACCACCGGAGGGGGATGGGTGATCAGCCGAATCTTTGACTCGGGCTACC CAATTGTGACTTGGTTGATGGAGCGAAAGATAAACAACTGGCTCAATCATGCAAATTACG GCTTAATACCAGAAGACAGGACTCAGCTGAAAGAGTTTGTGCTAAATGATGAGCTCCCAG GACGCATCATCACTGGGAAAGTGTTCATCAGGCCAAGCATAAAAGAGGTAAAGGAAAACT CTGTCATATTTAACAATACTTCAAAGGAAGAGCCTATTGACATCATTGTCTTTGCCACTG GATACACATTTGCTTTCCCCTTCCTTGATGAGTCTGTAGTGAAAGTTGAAGATGGCCAGG CCTCACTGTACAAGTATATCTTCCCTGCACATCTGCAAAAGCCAACCCTGGCCATTATTG GCCTCATCAAACCCTTGGGCTCCATGATACCTACAGGAGAAACACAAGCTCGGTGGGCTG TTCGAGTCCTGAAAGGTGTAAATAAGTTACCACCACCAAGTGTCATGATAGAGGAAATTA ATGCAAGGAAAGAAACAAGCCCAGTTGGTTTGGCTTGTGCTACTGCAAGGCTTTACAAT CAGATTATATCACATACATAGATGAACTCCTGACCTATATCAATGCAAAACCCAACCTGT TCTCTATGCTCCTAACG >520 bp linear fragment from linear parent NM 001282692.1:154-1764 Homo sa ATGCAGGAGAACATGGCCAAGCGAGTTGCCATTGTGGGAGCTGGGGTCAGCGGCCTGGCC TCCATCAAGTGCTGTCTGGAAGAAGGACTGGAGCCCACCTGCTTTGAGAGGAGCGATGAC CTTGGGGGGCTGTGGAGATTCACCGAACATGTTGAAGAAGGCAGAGCCAGTCTCTACAAG GATTATCCAAACTATGTGCCAAATTCTCAATTCCTGGAATATCTCAAAATGTATGCAAAC CACTTTGACCTTCTGAAACACATTCAATTCAAGACCAAAGTCTGCAGTGTAACAAAATGC TCAGATTCTGCTGTCTCTGGCCAATGGGAGGTGGTCACTATGCATGAAGAAGCAAGAG TCAGCCATCTTGATGCTGTCATGGTCTGCACTGGCTTTCTTACTAATCCTTATTTGCCA CTGGATTCCTTTCCAGGTATTAATGCCTTTAAAGGCCAGT >234 bp linear fragment from linear parent NM 001282692.1:154-1764 Homo sa GATCCACATCTGGCTCTGACCGTCTTCTTTGGCCCATGCTCACCATACCAGTTCCGCTTG ACTGGCCCAGGAAAATGGGAAGGAGCCAGAAATGCCATCATGACCCAGTGGGACCGAACA

 Compare how similar is the corresponding protein to the respective mouse homologue?



```
# Aligned_sequences: 2
# 1: FM01 HUMAN
# 2: FM01 MOUSE
# Matrix: EBLOSUM62
# Gap penalty: 10.0
# Extend penalty: 0.5
# Length: 532
# Identity:
               443/532 (83,3%)
                 0/532 ( 0.0%)
# Gaps:
# Score: 2396.0
FM01 HUMAN
                  1 MAKRVAIVGAGVSGLASIKCCLEEGLEPTCFERSDDLGGLWRFTEHVEEG
FM01_M0USE
                  1 MVKRVAIVGAGVSGLASIKCCLEEGLEPTCFERSSDLGGLWRFTEHVEEG
FM01_HUMAN
                 51 RASLYKSVVSNSCKEMSCYSDFPFPEDYPNYVPNSQFLEYLKMYANHFDL
FM01_M0USE
                 51 RASLYKSVVSNSSREMSCYPDFPFPEDYPNFVPNSLFLEYLKLYSTQFNL
FM01_HUMAN
                101 LKHIQFKTKVCSVTKCSDSAVSGQWEVVTMHEEKQESAIFDAVMVCTGFL
                    .:.|.|.||||:||..|.||||||||||
FM01 MOUSE
                101 ORCIYFNTKVCSITKRPDFAVSGOWEVVTVTNGKONSAIFDAVMVCTGFL
```

Previous tasks-hints (solutions in other pdf)

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How long is the longest fragment after trypsin digestion? Peptide cutter-include the third table

How many human similar reference proteins are in the databases? BLASTp-looking for RefSeq in homo sapiens

Does the sequence contain any transmembrane regions?

TMHMM, TopCons, Phobius...
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