

Problem set #9- **due April 12th, 2007 at 11:55 pm**

**PLEASE NOTE** This problem set is also posted as a Word file so you can easily copy and paste the nucleotide and amino acid sequences into the websites listed in the questions

1. Albinism-deafness syndrome is a rare X-linked syndrome that causes profound deafness and albinism. Linkage studies with DNA probes indicate that the gene that causes albinism-deafness syndrome when mutant is on the q (long) arm of the X chromosome. Which of the following experiments would provide the most definitive information that you have found the gene that when mutant causes albinism-deafness syndrome?

- Calculating the allele frequency of albinism-deafness syndrome in a population afflicted by this disease
- Using a microarray to show that the gene you identified is expressed in ear and skin cells of normal individuals
- Showing that a mutation occurs in the gene in affected individuals from the same family
- Showing that a mutation occurs in the gene in affected individuals from several different families
- Using a FISH probe to show that the gene you identified is on the X chromosome

2. The opossum genome is being sequenced in order to understand more about marsupials. Unlike most mammals, marsupials have babies that are born early and complete their development in a protective pouch. You are wondering if opossums have a beta hemoglobin gene, so you BLAST the nucleotide sequence of the human beta hemoglobin gene, which when mutant causes sickle cell anemia, against the nucleotide sequences from the opossum genome. This opossum nucleotide sequence that has the closest match to the human beta hemoglobin sequence is:

```
ATGGATGGTGCACCTGACTCCTGAGGAGAAGAAGTGTATCACCAGCCTCTGGTCTAAGGTGGCAGTTGACCAGACTG
GTGGTGAGGCCCTTGGCAGGATGCTCGTCTACCCCTGGACCACAAGGTTTTTTGGGAGCTTTGGTGATCTGTCC
TCTGCTGGCGCTGTCATGTCAAATGCTAAGGTTCAAGCCATGGTGCTAAGGTGCTGACTTCCTTCGGTGAAGCTGT
CAAGCATTGGACAACCTGAAGGGTACTTATGCCAAGCTGAGTGAGCTCCACTGTGACAACTGCATGTGGACCCTG
AGAAGTTCAAGATGCTGGGAATATCATTGTTATCTGCCTGGCTGAGCACTTTGGCAAAGAGTTTACTCCTGAATGTC
AGGTGGCTTGGCAGAAGCTGGTGGCTGGAGTTGCCCATGCCCTGGCCCACAAGTACCACTAA
```

To find the open reading frame in this sequence, copy and paste the opossum sequence above into the text box of the following website: <http://www.vivo.colostate.edu/molkit/translate/index.html> (website addresses linked to the MCDB2150 course website) and click on the Translate DNA button. According to the Graphic and Text Output views, which frame provides the longest open reading frame?

- Forward Frame 1
- Forward Frame 2
- Forward Frame 3
- Reverse Frame 1
- Reverse Frame 2
- Reverse Frame 3

3. Based on the open reading frame prediction, you determine that part of the amino acid sequence for the opossum beta hemoglobin protein is:

```
VQAHGAKVLTSFGEAVKHLNLDNLKGTYAKLSELHCDKLHVDPENFKMLGNIIVICLAEHFGKEFTPECQVAWQKLVAGVAHA
LAHKYH
```

You BLAST the opossum amino acid sequence and find out that the E value comparing the opossum and wallaby beta hemoglobin amino acid sequences is  $2e-66$  and the E value comparing the opossum and human beta hemoglobin amino acid sequences is  $4e-58$ . Here are the amino acid alignments (note the middle sequence shows you which amino acids match exactly between the two organisms, and the + indicates that the two amino acids have similar properties):

```
Opossum    VQAHGAKVLTSFGEAVKHLNLDNLKGTYAKLSELHCDKLHVDPENFKMLGNIIVICLAEHFG
           V AHGAKVL +FG+A+K+LDNLKGT+AKLSELHCDKLHVDPENFK+LGNIIVICLAEHFG
Wallaby    VLAHGAKVLVAFGDAIKNLDNLKGTFAKLSELHCDKLHVDPENFKLLGNIIVICLAEHFG

Opossum    KEFTPECQVAWQKLVAGVAHALAHKYH
           KEFT + QVAWQKLVAGVA+ALAHKYH
Wallaby    KEFTIDAQVAWQKLVAGVANALAHKYH
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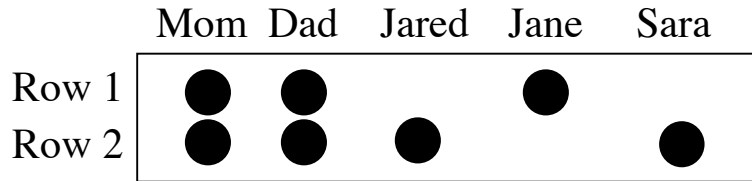
```
Opossum    VQAHGAKVLTSFGEAVKHLNLDNLKGTYAKLSELHCDKLHVDPENFKMLGNIIVICLAEHFG
           V+AHG KVL +F + + HLDNLKGT+A LSELHCDKLHVDPENF++LGN++V LA HFG
Human      VKAHGKVKLVGAFSDGLAHLNLDNLKGTFAFATLSELHCDKLHVDPENFRLGNIIVLCVLAHFFG

Opossum    KEFTPECQVAWQKLVAGVAHALAHKYH
           K+FTP Q A+QK+VAGVA+ALAHKYH
Human      KKFTPPVQAAYQKVVAGVANALAHKYH
```

From this result you conclude that:

- The amino acid sequence of the opossum beta hemoglobin protein is more similar to the amino acid sequence of the wallaby beta hemoglobin sequence than the human beta hemoglobin sequence
- The amino acid sequence of the opossum beta hemoglobin protein is more similar to the amino acid sequence of the human beta hemoglobin sequence than the wallaby beta hemoglobin sequence
- The amino acid sequence of the wallaby beta hemoglobin protein is more similar to the amino acid sequence of the human beta hemoglobin sequence than the opossum beta hemoglobin sequence

4. On problem set #7 you learned about Jared, who is homozygous for the most common missense mutation that causes sickle cell anemia. Genomic DNA from Jared, his parents, and his new twin sisters (Jane and Sara) was analyzed by Allele Specific Oligonucleotide (ASO) hybridization. Which row was hybridized with an ASO for the wild type allele of the beta hemoglobin gene?

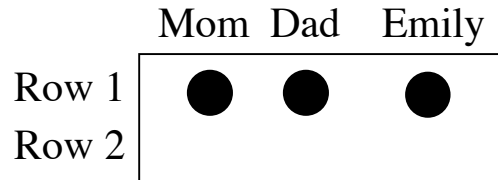


- Row 1
- Row 2
- Both rows
- Neither row

5. Based on the ASO analysis, Jane and Sara are:

- fraternal, dizygotic twins
- identical, monozygotic twins
- you cannot distinguish between answers a and b based on the ASO analysis

6. Jared and his parents meet Emily through a support group. Emily has similar symptoms as Jared; she is anemic and is having episodes of intense pain. Emily and her parents also had their genomic DNA analyzed with the same ASO probes described in question #4 (please note the ASO probes used in row 1 and row 2 are the same in questions 4 and 6, the order has not been switched). What can you conclude based on this analysis?



- Emily has sickle cell anemia
- Emily has the same beta hemoglobin mutation as Jared
- Emily is adopted
- Emily does not have any mutations in her beta hemoglobin gene
- None of the above

7. A deletion mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in humans results in the production of a defective CFTR protein and consequently the disease cystic fibrosis.

Below is the partial amino acid sequence of a protein that is similar to the human CFTR protein:

MAGNLVSWACKLCRSPGFGPISFYGDFTQCFIDGVILNLSAIFMITFGIRDLVNLCKKKHSGIKYRRNWIIVSRMALVLEIA  
FVSLASLNISKEEAENFTIVSQYASTMLSLFVALAL

To find out what organism this amino acid sequence comes from, you need to perform a BLAST search on this website (note a link to this website is posted on the course website):

[http://www.ncbi.nlm.nih.gov/BLAST/beta/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST\\_PROGRAMS=blastp&AGE\\_TYPE=BlastSearch&SHOW\\_DEFAULTS=on](http://www.ncbi.nlm.nih.gov/BLAST/beta/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&AGE_TYPE=BlastSearch&SHOW_DEFAULTS=on)

Copy and paste the amino acid sequence into the upper left textbox (textbox says enter accession number, gi, or FASTA sequence). You do not need to change any of the other menus, just scroll down to the bottom of the page and press the blue BLAST button on the left side of the screen. It may take a few minutes to get your results back, but once you do, scroll down past the box with the colorful lines and click on the number under the column heading "Sequences producing significant alignments"

Sequences producing significant alignments:		Score (Bits)	E Value
<a href="#">Click here</a> → <a href="#">gb AAA50353.1 </a>	metal resistance protein	<a href="#">246</a>	4e-64
<a href="#">ref NP_010419.1 </a>	Vacuolar glutathione S-conjugate transporter...	<a href="#">245</a>	4e-64
<a href="#">ref XP_449053.1 </a>	unnamed protein product [redacted] >...	<a href="#">174</a>	1e-42
<a href="#">ref XP_455982.1 </a>	unnamed protein product [redacted] ...	<a href="#">127</a>	2e-28
<a href="#">ref NP_986712.1 </a>	AGR047Wp [redacted] ATCC 10895] >gb AA...	<a href="#">64.3</a>	2e-09
<a href="#">ref XP_721319.1 </a>	vacuolar metal resistance ABC transporter [redacted] ..	<a href="#">50.8</a>	2e-05
<a href="#">ref XP_460066.1 </a>	hypothetical protein DEHA0E18392g [redacted]	<a href="#">50.8</a>	2e-05
<a href="#">ref XP_504380.1 </a>	hypothetical protein [redacted] >...	<a href="#">48.9</a>	7e-05
<a href="#">ref XP_001382516.1 </a>	predicted protein [redacted] CBS 60...	<a href="#">48.1</a>	1e-04

What organism is this amino acid sequence from:

- Drosophila melanogaster* (fruit fly)
- Oryza sativa* (rice)
- Saccharomyces cerevisiae* (baker's yeast)
- Pan troglodytes* (Chimpanzee)

8. According to your BLAST search, which organism has the next most similar amino acid sequence to the amino acid sequence written in question 7?

- Kluyveromyces lactis*
- Ashbya gossypii*
- Candida glabrata*
- Candida albicans*

9. You have made a mouse knockout of the CFTR gene. The knockout mice are unable to clear bacteria from their lungs, which leads to mucus retention and subsequent lung disease. You put a human CFTR gene into this type of knockout mouse and discover that the CFTR knockout mice with the human gene are able to clear bacteria from their lungs and no longer show phenotypic similarities to cystic fibrosis in humans. From this experiment, you can conclude that:

- The mouse CFTR gene and human CFTR gene are evolutionarily conserved
- The nucleotide sequence of the mouse CFTR gene and human CFTR gene are identical
- The mRNA sequence of the mouse CFTR gene and human CFTR gene are identical
- The amino acid sequence of the mouse CFTR protein and the human CFTR protein are identical
- Answers A and D
- All of the above

10. Patau syndrome, which typically results from the duplication of chromosome 13, normally causes a fetus to self-abort prior to coming to term. If the pregnancy does reach full term, the child has many birth defects. A pregnant woman has been referred to the local OB/GYN for an amniocentesis test. The lab analyzes the chromosomes from cells derived from the amniocentesis test using a FISH probe that is specific for nucleotide sequences on chromosome 13. Some of the cells have chromosomes that have three fluorescent spots, while others have two fluorescent spots. The doctor is confused, what would you tell him is the most likely reason for the different FISH results?

- The probe must have failed to anneal to some of the DNA
- The fetus has some cells that have yet to replicate the third copy of Chromosome 13
- The fetus is a somatic mosaic
- The amniotic cells that were used in the FISH analysis must have been contaminated