POSTGRADUATE INSTITUTE OF MEDICINE
UNIVERSITY OF COLOMBO

POSTGRADUATE DIPLOMA IN MOLECULAR MEDICINE
(SEMESTER II) EXAMINATION – JUNE, 2010

Date: 8 June 2010 **Time :** 9.00 a.m. -- 12.00 noon.

SEQ PAPER II
(BIOINFORMATICS AND MOLECULAR THERAPEUTICS MODULE)

Answer all **six (06)** questions.
Answer each question in a separate answer book.

Note: For all the questions given below, web addresses are NOT required

1. Your molecular laboratory has been involved in genome sequencing of a Sri Lankan strain of the *Leishmania* parasite. At the end of this exercise, you find that you have a pBluescriptII clone with a 1290 bp DNA fragment which you think has not been reported previously.

1.1 Briefly mention the most appropriate approach to check whether this DNA sequence has been previously found in *Leishmania* spp? (25 marks)

1.2 After re-sequencing your new parasite clone to eliminate any sequencing errors, your sequence is now ready to be deposited in a databank. Name 3 databases where you can potentially deposit your nucleotide sequence data. (15 marks)

1.3 You have used the NCBI Blast server to look for a match for the above sequence and the best alignment contained the following section of the alignment: What do the Xs signify in the alignment? (20 marks)

```
Hit from database: XXXXXXXXXXXXXXXXXXXXNAGCGTACGT
query sequence:    AAAGCTAAAGTTAAACTAATGAGCGTACGT
```

1.4 One database search algorithm of an online data mining resource returned a large number of hits with high *E* values for the above *Leishmania* sequence. What can you say about the sensitivity and selectivity of the algorithm? (20 marks)

1.5 For each of the following instances, state which BLAST strategy is best suited?

1.5.1 To find out the function of this hypothetical protein coded by your new sequence. (10 marks)

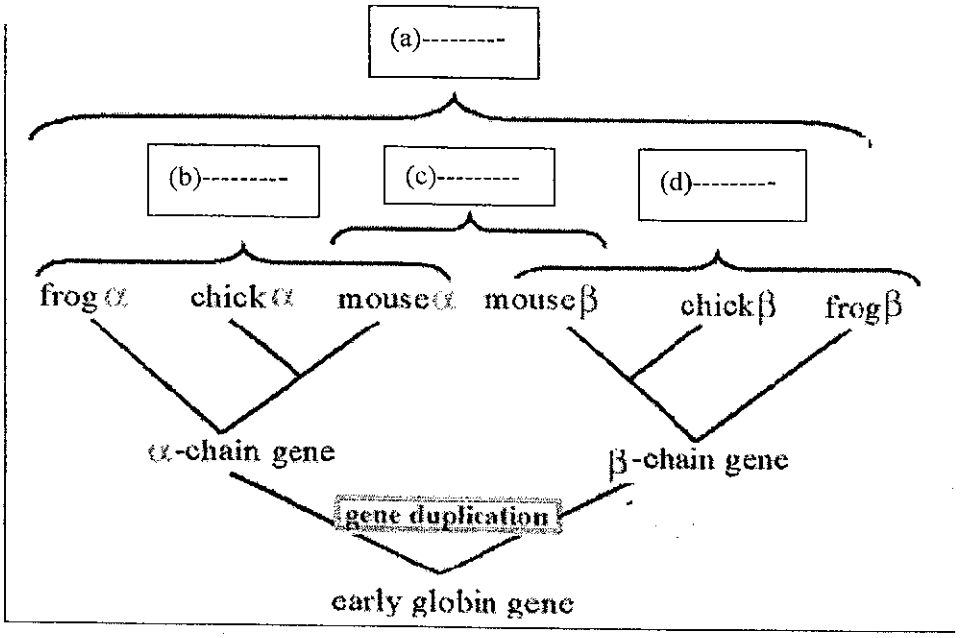
1.5.2 To discover new genes encoding similar proteins (to your hypothetical protein). (10 marks)

2.

2.1 State two reasons why one should make "local alignments" in pair- wise sequence comparison. (10 marks)

2.2 The schematic diagram given below depicts some evolutionary events pertaining to early globin genes (α and β chains). State the relationship of different genetic components given below (marked (a), (b), (c) and (d)), in terms of their evolutionary origin. (20 marks)

(a)
(b)
(c)
(d)



2.3 Consider the two DNA sequences CAGCAT and CGACAT. These sequences are aligned using a global alignment algorithm where the score is 1 for a match and 0 for a mismatch and there is a penalty of 0.2 for each gap.

2.3.1 Indicate the optional alignments possible. (15 marks)

2.3.2 What are the scores you get for the alignments above? (15 marks)

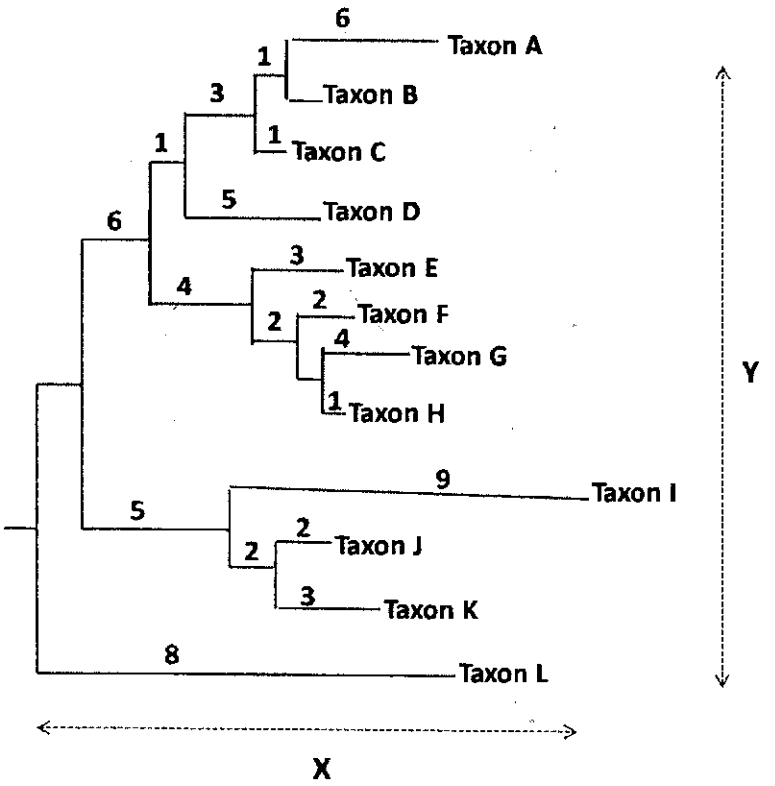
2.3.3 What would be the optimal alignment from the above options? (10 marks)

2.4 To build up a multiple sequence alignment (MSA), state 2 important parameters you must consider in choosing the right sequences to incorporate in the multiple sequence alignment. (10 marks)

2.5 In case if you do not have an adequate number of raw sequences from your wet lab experiments to build up a meaningful multiple sequence alignment, what can you do to remedy the situation? (20 marks)

3.

3.1 Given below is a phylogentic tree constructed using internal transcribed spacer (ITS2) rDNA region of a medically important fungus.



Answer the questions given below using the information given in the phylogenetic tree above.

3.1.1 What does the vertical line (X) mean in a phylogenetic tree? (07 marks)

3.1.2 What does the horizontal line (Y) mean in a phylogenetic tree? (07 marks)

3.1.3 To which taxon is taxon B more related ? (07 marks)

3.1.4 To which taxon is taxon B most similar ? (07 marks)

3.1.5 How many different cluster/s is/ are in this polygenetic tree? (07 marks)

3.1.6 Indicate the common ancestor of taxons A, B and C in the phylogenetic tree. (07 marks)

3.1.7 Which taxon would be the ideal candidate to be the outgroup of this phylogenetic tree? Give reasons for your answer. (10 marks)

3.1.8 Which is the fast evolving taxon? (08 marks)

3.2

3.2.1 Explain why comparative modeling is possible for predicting the protein structure. (20 marks)

3.2.2 State what are the assumptions made in comparative protein modeling? (20 marks)

4.

4.1 Explain briefly, what is meant by "*Ex vivo*" gene therapy. (20 marks)

4.2 State two common technical difficulties encountered with regard to delivery and stability of therapeutic genes involving "*In vivo*" gene therapy protocols? (16 marks)

4.3 State three desirable characteristics of a viral vector that enable gene transfer into a target tissue. (18 marks)

4.4 Outline briefly using a schematic diagram, how would you engineer a retro viral genome as a gene transfer vector. (36 marks)

4.5 Once the retro viral vector construct carrying the therapeutic gene is engineered, it is transfected into a packaging cell line carrying a helper pro-virus. To prevent packaging of helper pro viral constructs into daughter virion particles, which segment of the helper pro-viral element, should be removed from its genome? (10 marks)

5.

5.1 List three methods that could be potentially used to prevent the expression of a mutated gene at the post transcriptional level. (15 marks)

5.2 Briefly mention two potential applications of Ribozymes as therapeutic agents. (20 marks)

5.3

5.3.1 Name the mechanism of gene repair for mutated genes that function in the "dominant negative" manner? (10 marks)

5.3.2 What do you understand by the term "dominant negative mutation"? (10 marks)

5.4 Stem cells which are genetically identical to cells of donor patients can potentially be recovered from cloned embryos in a similar fashion to Dolly, the cloned sheep and can be used as therapeutic interventions. State the main steps involved in this procedure that generates embryonic stem cells. (25 marks)

5.5 Explain briefly, the term "cell targeted suicide gene therapy" as a therapeutic means for cancer. (20 marks)

6.

6.1

6.1.1 List three expression systems which can be employed for the production of recombinant therapeutic proteins. (12 marks)

6.1.2 Recombinant follicles stimulating hormone (rFSH) is used for the treatment of fertility disorders. Select the type of expression system (from those listed in 6.1.1) you would use to produce the above recombinant hormone and explain the reasons for your choice. (12 marks)

6.1.3 List four important analytical methods which can be used for determining the safety and efficacy of a recombinant therapeutic protein. (16 marks)

6.2

6.2.1 Briefly, describe the major structural differences between the chimeric and the humanized monoclonal antibodies. (20 marks)

6.2.2 State three potential modes of action of recombinant monoclonal antibodies for the treatment of cancers in humans. (20 marks)

6.3

6.3.1 Describe a DNA vaccine. (10 marks)

6.3.2 List four major advantages of DNA vaccines over the conventional vaccines. (10 marks)

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(SEMESTER II) EXAMINATION
JUNE 2010

Date : 10th June 2010 **Time :** 9.00 a.m. – 12.00 noon.

ESSAY PAPER

Write essays on four (04) topics selecting at least ONE TOPIC from each part.
100 marks each)
Answer each essay in a separate answer book.

PART A

(1) Apoptosis
(2) Cytoskeletal disorders
(3) Molecular basis of cancer
(4) Histone chaperones in chromatin assembly

PART B

(1) Benefits and disadvantages of prenatal diagnosis for genetic disorders
(2) Genomic Medicine
(3) Micro RNA
(4) Transgenic plants as bioreactors