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POSTGRADUATE INSTITUTE OF MEDICINE
UNIVERSITY OF COLOMBO

POSTGRADUATE DIPLOMA IN MOLECULAR MEDICINE
EXAMINATION 1 – MODULE I – APRIL 2013

Date :- 2nd April 2013 **Time :-** 9.00 a.m. 12.00 noon

SEQ PAPER
(Molecular Cell Biology and Cytogenetics)

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

1.

1.1. Describe briefly the first week of development after formation of the zygote. (30 marks)

1.2. Describe prophase I in meiosis I. (50 marks)

1.3. Compare the differences seen in prophase I between males and females. (20 marks)

2. Answer any **two** (02) of the following.

2.1 Explain the biochemical basis of the following :

2.1.1 whooping cough caused by pertussis toxin. (25 marks)

2.1.2. Diarrhoea caused by cholera toxin. (25 marks)

2.2.

2.2.1. List the sequence of events occurring at the replication origin in DNA replication of prokaryotes. (20 marks)

2.2.2. Distinguish between DNA damage caused by UV radiation and ionizing radiation. (30 marks)

Contd.../2-

2.3. Explain the following :

2.3.1. C-value paradox. (15 marks)

2.3.2. Pseudogenes. (15 marks)

2.3.3. Satellite DNA. (20 marks)

3. Answer any **two (02)** of the following.

3.1. Explain how the following influence gene expression in eukaryotes.

3.1.1. Histone modification. (25 marks)

3.1.2. Nucleosome remodeling. (25 marks)

3.2. Explain the following post-transcriptional events (state an example for each event).

3.2.1. Alternative poly (A) site selection. (25 marks)

3.2.2. Alternative splicing. (25 marks)

3.3. Explain the following :

3.3.1. Iron uptake by the cell and iron storage are tightly regulated by iron response element binding protein (IRE-BP). (30 marks)

3.3.2. Expression of apo B (*APOB*) gene in the liver and small intestine gives rise to apo B100 and apoB 48 respectively. (20 marks)

Contd..../3-

4. Briefly explain the following:

4.1. Role of "TATA box" in prokaryotic gene expression. (15 marks)

4.2. How lactose and glucose regulate the *lac* operon in prokaryotes. (35 marks)

4.3. The mechanism employed by the bacteria for positioning of the ribosome during initiation of protein synthesis. (20 marks)

4.4. The "wobble hypothesis" and its significance in protein synthesis. (30 marks)

5. Answer any **two (02)** of the following.

5.1. Describe briefly, how microarray technique is used to study differential gene expression in two strains of a bacterium. (50 marks)

5.2. How Real Time PCR makes viral load quantification possible over conventional PCR. (50 marks)

5.3. Write a brief account on development of technology to increase the throughput of DNA sequencing. (50 marks)

6. Answer any **four (04)** of the following.

6.1. Draw a labeled schematic diagram to illustrate an expression vector cassette and explain the function of each component. (25 marks)

6.2. Distinguish between transient and stable gene expression. (25 marks)

6.3. Outline the steps involved in expressing recombinant somatostatin in *E. coli.* (25 marks)

6.4. State the disadvantages of expressing recombinant human insulin in prokaryotic hosts when compared with eukaryotic hosts. (25 marks)

6.5. What is a fusion protein? Comment on the use of fusion proteins in genetic engineering. (25 marks)

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POSTGRADUATE DIPLOMA IN MOLECULAR MEDICINE
EXAMINATION 1 – MODULE I – APRIL 2013

Date :- 2nd April 2013 **Time :-** 1.00 p.m. – 3.00 p.m.

PRACTICAL PAPER
(Molecular Cell Biology and Cytogenetics)

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

1. Answer either 1.1. **OR** 1.2.

1.1.

1.1.1. State the applications of restriction enzyme mapping. (15 marks)

1.1.2. Name four (04) parameters that determine the rate of migration of DNA fragments during agarose gel electrophoresis, and briefly explain how two (02) of them affect migration. (25 marks)

1.1.3. You have isolated two restriction enzymes for the first time from *Streptomyces avidini* which have potential commercial use; one is a 4-base-pair cutter which forms blunt ends and the other is a 6-base-pair cutter which forms 3' overhangs. You want to patent them.

(a) Name the enzymes you isolated (according to standard nomenclature procedures). (10 marks)

(b) For each enzyme, write a hypothetical recognition sequence and indicate cleavage sites that will produce the ends described above. (30 marks)

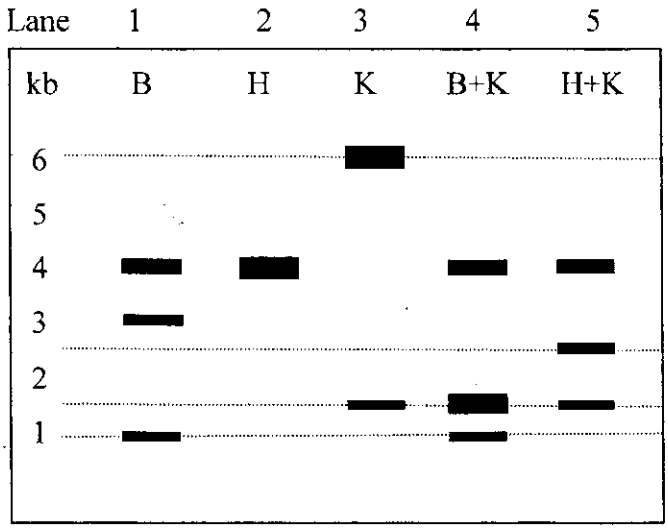
1.1.4. You have carried out restriction enzyme digestions of genomic and plasmid DNA. You are ready to clone! Your supervisor says you must eliminate all restriction enzyme activity from your DNA samples before you ligate. State the importance of eliminating restriction enzyme activity and briefly explain how you would achieve this. (20 marks)

OR

Contd.../2-

1.2. A genomic DNA fragment (8 kb) had been cloned into the *Sal* I unique restriction site of a circular cloning vector. The recombinant clone was cleaved with *Sal* I enzyme to release the cloned insert. This 8 kb linear fragment was purified, and a series of restriction digestions were carried out using *Bam*HI (B), *Hin*dIII (H) and *Kpn*I (K). DNA fragments were separated by agarose gel electrophoresis.

The figure below depicts the schematic diagram of the gel photograph. The thickness of the bands indicates approximately, the intensity of the DNA bands visualized.



1.2.1. State the applications of restriction enzyme mapping. (15 marks)

1.2.2. For each lane, draw schematic diagrams of *all possible* restriction maps that explain the restriction fragments obtained. (50 marks)

1.2.3. Draw a complete restriction map of the linear DNA fragment that explains all the digestions shown in the 'gel photograph'. (35 marks)

Contd...../3-

2. You are ready to construct a genomic DNA library.

2.1. Briefly explain why you need to prepare 'competent bacterial cells' in your cloning experiments? (20 marks)

2.2. In your cloning experiment, you conduct the following control experiments. Explain what each of them intends to test. (50 marks)

2.2.1. Competent cells 'transformed' with sterile distilled water and plated on plain LB agar plates.

2.2.2. Competent cells 'transformed' with sterile distilled water and plated on ampillicin+ LB agar plates.

2.2.3. Restriction digested and alkaline phosphatase treated vector alone is "ligated" (without insert), transformed and plated on ampicillin+LB agar plates.

2.3. Explain the principle of blue/white colour selection of recombinants. (30 marks)

3. Sickle cell anemia caused by a point mutation of the beta globin gene can be identified by Southern blotting followed by hybridization using a gene specific probe. The point mutation creates a loss of *Mst*II restriction site in the beta globin gene.

3.1. The following are some reagents you used for extraction of DNA. Explain the function of each. (05 marks each)

3.1.1. EDTA.
3.1.2. SDS.
3.1.3. Proteinase K.
3.1.4. Absolute ethanol.

3.2. List the steps involved in carrying out Southern blotting starting with the DNA extracted from the patient. (30marks)

Contd...../4-

3.3. State two (02) types of labeling techniques used in labeling the gene specific probe. (10 marks)

3.4. State two (02) types of labels that can be used and the principle of detection of each label (experimental details are not necessary) (10 marks)

3.5. Prior to hybridization with the labeled probe, prehybridization was carried out in 6xSSC in the presence of salmon sperm DNA. Give reasons. (10 marks)

3.6. After hybridization, the blot was washed in 2XSSC. Give reasons. (10 marks)

3.7. State two (02) disadvantages in carrying out Southern blot analysis in diagnosis of genetic diseases. (10 marks)

4.

4.1. State briefly how you would prepare a 100 µl solution of 10 mM dNTP mix for PCR. You are provided with the following solutions: 100 mM dATP, 100 mM dTTP, 100 mM dCTP, 100 mM dGTP and distilled de-ionized water. (40 marks)

4.2. The forward and the reverse primers (FP and RP) designed for a novel PCR based detection assay for *Mycobacterium tuberculosis* (MTB) are given below. Both primers are 100 % specific for MTB and have identical melting temperatures (Tm = 60 ℃). However, PCR amplification of reference control MTB DNA, carried out at 5 different annealing temperatures using these primers, resulted in a low yield of the desired 400 bp product and a strong DNA band visible in the agarose gel below the 100 bp marker.

Comment on the incompatibility of these primers for the intended PCR assay. (30 marks)

FP: 5' GCTTACGTGTACAATCAGTAG 3'
RP: 5' TAATCCGCGTACTACTGATTG 3'

4.3. List the constituents of a reverse –transcription polymerase chain reaction (RT-PCR). (30 marks)

Contd...../5-

5.

5.1. Describe the process of chromosome culture and karyotyping.
(85 marks)

5.2. Name three (03) categories of chromosomal disorders that can be detected from karyotype . (15 marks)

6. Design an experiment to detect induction of apoptosis by a toxic chemical.
(100 marks)

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POSTGRADUATE DIPLOMA IN MOLECULAR MEDICINE
EXAMINATION 1 – MODULE II – APRIL 2013

Date :- 3rd April 2013 **Time :-** 9.00 a.m. 12.00 noon

SEQ PAPER
(Human Biology, Medical Microbiology and Parasitology)

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

1.

1.1. Define the term 'cardiac output' and outline the mechanisms involved in increasing the cardiac output. (30 marks)

1.2. Write an account on the embryological basis of the patent foramen ovale. (35 marks)

1.3. Outline the transport of oxygen in blood and explain the factors affecting delivery of oxygen to tissues. (35 marks)

2.

2.1. Describe briefly the structure of the liver lobule. (35 marks)

2.2. Outline the role of insulin in regulating the blood glucose concentration. (35 marks)

2.3. Outline the function of bile salts in digestion and absorption of lipids. (30 marks)

Contd...../2-

3.

3.1. Outline the structural adaptations in the trachea to convey clean, humidified air to the lungs. (35 marks)

3.2. Using a labeled diagram, outline the electrical and chemical changes taking place in a neurone during an action potential. (35 marks)

3.3. Outline the forces involved in plasma filtration at the renal glomerulus. (30 marks)

4.

4.1. Explain how genetic defects in LDL metabolism lead to hypercholesterolaemia. (35 marks)

4.2. Explain the role of glutamate in the liver, kidney and the brain. (35 marks)

4.3. Explain the biochemical basis of hyperuricaemia resulting from complete deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) enzyme. (30 marks)

5.

5.1. Name three (03) parasitic diseases which are transmitted by ingesting faecally contaminated food or water. (15 marks)

5.2. Name the infective stages of the parasitic disease/s mentioned above. (15 marks)

5.3. Name the investigation that would be performed to diagnose a parasitic infection transmitted by ingestion of contaminated food or water. (05 marks)

5.4. Name three methods by which parasitic infections, transmitted by faecally contaminated food and water can be prevented. (15 marks)

5.5. Briefly describe the life cycle of a virus with the aid of a diagram. (30 marks)

5.6. Define and outline the cytopathic effects seen in cells infected with virus. (20 marks)

Contd..../3-

6.

6.1. Outline briefly the structure of cytoplasmic membrane of bacteria and list two (02) functions. (20 marks)

6.2. What are dimorphic fungi and give two (02) examples of these. (20 marks)

6.3. List four (04) different types of fungal asexual spores giving one example for each type (20 marks)

6.4. List two (02) specimens you collect to arrive at microbiological diagnosis from a patient with community acquired pneumonia. State the factors you consider when collecting and transporting **one (01)** of the specimens to the laboratory of microbiology. (20 marks)

6.5. Describe the steps you would carry out when a sample of pus is received at the laboratory of microbiology. (20 marks)