Ex/MSBT2/1/58/2018

M. Sc. Bio-Technology Part II Examination, 2018

DNA Technology

MSBT - 2/1

Time : Four hours Full Marks : 100

(50 marks for each part)

Use a separate Answer-Script for each Group

GROUP - A

Answer ***any six*** questions. 10 marks each

1. a) You recently have made an interesting finding that two cell surface proteins namely β -integrin and ICAM interact together from co-immunopreciptation experiments, which belong to cell surface adherent proteins. You want to further verify interaction in vivo. What probable method you will choose to verify this ? Briefly illustrate the rationale and strategy involved in the technique and just outline the steps involved in this. 4

   b) What are the three types of DNA ends that can be generated after cutting DNA with restriction enzymes ? What reaction in catalyzed by DNA ligase and how ? 4

   c) Bacterial strains also harbor methylases. Why are they not desirable as cloning hosts. 2

2. a) You are given a vector with AOX1 promoter.

      i) What is the protein product of this AOXA gene ? 1

ii) In which organism can this promoter be used and for what purpose ? 2

iii) What is the function of this protein product ? 1

iv) How is the AOX 1 gene regulated ? 2

v) What kind of vector would carry this promoter ? 1

b) How will you go about expressing protein in a large scale within 4 weeks in mammalian system ? Mention the type of transfection, etc to be used. 3

3. a) What is a buffy coat ? How is it prepared and for what purpose ? 1+2+1

b) How is Bacillus thuringienesis useful to the scientists ? 2

c) Why would you want partial cutting of the DNA while preparing genomic DNA libraries ? 1

d) What is a cosmid ? What are the advantages of using them over plasmids ? 1+2

4. a) The genomic DNA extraction follows the CTAB (cetyltrimethylammonium bromide) protocol.

i) What is the working principle of this protocol ? 2

ii) Describe how it is carried about. 2

b) i) How is the lac promoter regulated ? 2

ii) What are the shortcomings of using this promoter ? How is it overcomed ? 1+1

c) How is chemical mutagenesis done ? 2

6. a) Explain briefly the principle of pyrosequencing ? 2

b) What is the full form of CRISPR ? Briefly explain the principle of CRISPR Cas system to edit genome of an organism. 1+3=4

c) You need to make DNA probe for Sorthern blot hybridization experiment. Breiflyy explain methods to make probe by isotropic and non-isotropic labelling (one method for each type of labelling). 2+2=4

with PstI and inserted a 4.0kb fragment into the site. From the data below, determine the restriction map of the resulting plasmid.

PstI : 7.0, 4.0

EcoRI : 6.0, 5.0

BamHI : 8.9, 2.1

PstI + ECoRI : 4.3, 3.3, 2.7, 0.7

PstI + BamHI : 6.1, 2.8, 1.2, 0.9

EcoRI + BamHI : 5.0, 2.1, 2.1, 1.8 6

GROUP - B

Answer ***any four*** questions (each carries 10 marks) 4×10

1. a) What is antisense Oligonucleotides (ASOs) and briefly explain the mechanism by which we can use ASOSs to regulate gene espression ? What are the limitations of using ASO_s to regulate gene expression ? (2+2)=4

   b) Suppose you are a research scholar working in a tumor biology lab. You have identified a novel gene "X" which is upregulated in tomor cells as compared to normal cells. How will you experimentally prove that gene "X" necessary and suffucuent to cause tumor in an in vitro culture system ? 4

   c) How will you locate putative miRNA sequence in the genome of an organism by comparative genomics approach ? 2

2. a) What changes woruld you make in a PCR reaction if you do not get any amplification of the desired sequence ? 2

   b) What are the advantages of using touch-down PCR ? 2

   c) Let's assume that you have 250000 copies of amplified DNA product at the end of 21st cycles in a PCR reaction. How many copies of DNA would be present after the end of 23rd cycle ? Let's imagine that your friend had started with 8 times less DNA as you do. Can you predict at which PCR cycle your friend will have 100000 copies of DNA ? 1+1=2

   d) Explain the 5`-RACE method to obtain the full length sequence of an RNA transcript. (4)

3. a) You are working with very large molecule of DNA fragments (which ranges between 450kb-50kbs). How will you separate these large DNA molecules by using gel electrophoresis ? 2

   b) Explain the terminologies that are used in realtime PCR technique with a diagram. 2 × 2=4

      i) Baseline and threshold

      ii) Ct and Δ Rn

c) You have identified a novel tumor suppressor gene. How would you quantify the expression of that gene in a liver cell by SYBR green method ? Explain the $\Delta\Delta CT$ method to calculate the fold change of that tumor suppressor gene between the normal and the cancerous liver cell ? $2 \times 2=4$

4. a) Write briefly any four different kinds of stem cells that are found in human body ? What do you understand by totipotent, pluripotent and multipotent stem cells ? 2+2=4

b) What are iPS cells and how are they generated ? 2

c) Explain how would you use iPS cells therepeutically to treat type I diabetes ? 4

5. a) Restriction fragment length polymorphism (RFLP) is formerly used for DNA fingerprinting. Briefly explain RFLP (2) 2

b) You would like to make a transgenic mouse where gene "A" will be specifically deleted from brain. Explain the procedure to make the transgenic mouse in detail. 5

c) Explain temporal regulation of gene expression in mouse by Tet-on and Tet-off system. 3

5. a) What is vertical and horizontal transmission ? 2

b) Describe "RACE" with diagrams. 3

c) Mention the function of chvA, chvB, pscA, vir and occ genes. 2.5

d) How are fusion proteins made ? What is required for secretion of protein of interest ? 2.5

6. a) The Lambda EMBL3 system takes advantage of spi selection.

i) What is spi selection ? 1

ii) How does it work ? 3

b) An x domain of protein A is swapped with Y domain of protein B. Explain with diagrams how is this done. 3

c) Distinguish between Yip, Yep and YCp. 3

7. a) What are terminator genes ? How do they work ? 5

b) What are the rationales which justify the early attempt of expressing a foreign gene in heterologous system. 2

c) Mention two problems that arise when eukaryotic proteins are expressed in prokaryotic cells. 1

d) What are the advantages of using *Pichia pastoris* over *saccharomyces* in expression of hetegologous peotein 2

8. a) How is the glycosylation of heterologous proteins in mammalian system controlled ? 2

b) How is Lacz gene important in cloning? 2

c) Plasmid pRIT450 is 7.0 kb in length and has *single* PstI, EcoRI, and BamHI sites. You have cut the palsmid