M. Sc. Bio-Technology Part II Examination, 2017

DNA TECHNOLOGY

Paper - 2/1

Time : Four hours | Full Marks : 100

GROUP - A

Answer ***any Six*** questions.

1. I) You are at a crime scene to identify the criminal. You have gathered some clothes containing blood stains. Do you think this sample collection will help in the identification? If so,
   a) How will you process the sample? State the major steps. (1+4)
   b) How that will help to identify the criminal? (1)
   II) Explain in detail the Agrobacterium model of DNA transmission to plant cells. (4)

2. a) Distinguish between blunt- and sticky-end ligation and explain how the efficiency of blunt-end ligation can be increased. (4)
   b) What is the purpose of having the LacZ gene in a plasmid cloning vector ? (2)
   c) Using the usual blue/white colony screening method, you are looking for a clone. To your dismay, none of the white colonies has the recombinant plasmid. As a desperate effort, you go ahead to test for the blue colonies and find that your desired insert is indeed present in them. Can you explain what might have happened. (4)

3. I)Give reasons for the following.: (5)
   a)Methanol metabolism takes place within the peroxisome.
   b)Expressing heterologous proteins as secreted protein in *P.pastoris* is by far the best system.
   c)Hyperphosphorylation of proteins expressed in insect cells poses a big problem.
   d)Hyperglycosylation of proteins expressed in *S.cerevisiae* is a big disadvantage of this system.
   e)Only correctly folded proteins are secreted
   II) Two proteins X and Y are known to interact with each other. Design an experiment to demonstrate their interaction /association using proper host etc. (5)

4. Translational modification is an essential processing step in the production of eukaryotic proteins. In respect to the above statement answer the following questions.
   a) What steps or measures need to be taken for expressing a heterologous protein in mammalian cells which is to be used for crystallization studies.? (2)
   b) Tetracycline can be used both as a promoter activating and repressing system. Justify the statement. (3+3)
   c) What is gp64 and gp41? Where and when they are found (2)

5. a)Discuss the principle on which pET vectors regulate gene expression. (3)
   b)What do you understand by biolistic gene delivery system ? (2)
   c)How is it that a bacterium that produces a restriction enzyme does not cut its own DNA.(2)
   d) DNA transformation is extremely efficient in yeast. Mention three factors that make it efficient. (3)

6. a) Which promoter is usually used in expression vectors of prokaryotic origin and why? (2)
   b)Describe a robust expression vector which has provision for easy purification of the protein.(4)
   c) How can you regulate the expression of the protein? (2)
   d)Explain the problem of inclusion body and how is it resolved ? (2)

7. a).A linear fragment of DNA (7.5 kb) is cleaved with the individual restriction enzymes *Hind*III and *Sma*I and then with a combination of the two enzymes. The fragments obtained are:
HindIII : 2.5 kb, 5 kb
Sma1: 2.0 kb, 5.5 kbs
HindIII and *SmaI*: 2.5 kb, 3.0 kb, 2.0 kb.
i) Draw the restriction map.
ii) The mixture of fragments produced by the combined enzymes is cleaved with the enzyme *Eco*RI, resulting in the loss of the 3-kb fragment (band stained with ethidium bromide on an agarose gel) and the appearance of a band stained with ethidium bromide representing a 1.5-kb fragment. Mark the *Eco*RI cleavage site on the restriction map. (2+2)
b).Explain how a genomic DNA library differs from a cDNA library. (4)
c) Why is Sodium Hydroxide used in making cDNA? (2)

8. a). Dr.Donald L.Jarvis engineered a special type of insect cell. Answer the following questions with reference to the above statement.
i) What is the special feature of these cells? (1)
ii) What advantages/ disadvantages do these cells pose? (1)
iii) What are the cells named as? (1)
iv) When are these cells used or employed for? (1)
v) How are these special insect cells different from the normal insect cells? (1)
b) Bacterial glucuronidase converts a colourless substance called X-Gluc into a bright blue indigo pigment. The gene for glucuronidase also works in plants if given a plant promoter region. How would you use this gene as a reporter gene to find out in which tissues a plant gene that you have just cloned is normally active? (Assume that X-Glue is easily taken up by the plant tissues) (5)

GROUP- B

Answer any four questions.

9. (a) What are the similarities and differences between siRNA and miRNA? (4)

   (b) How do Dicer and Argonaute proteins participate in gene silencing? (3)

   (c) Suppose you have identified a novel gene "X" and found perfect homology in *C.elegans* and in mammalian cells. How can you induce RNAi-mediated gene silencing of identified gene "X" in both *C.elegans* and cultured mammalian cells? (3)

10. (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 therapeutically to treat type I diabetes? (4)

11. (a). "Dolly is not a transgenic lamb"- Justify the statement. (2)

    (b). You would like to make a transgenic mouse where gene "A" will be specifically deleted from heart. 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).

12. (a) What would you do to reduce non-specific PCR product in a PCR reaction? (2)

    (b) "*DNA Methylation can* change the activity of a DNA segment without changing the sequence." How would you determine the methylated specific DNA segment by using PCR technique? (2)

    (c) Let's assume that you have 250000 copies of amplified DNA product at the end of 23rd cycle in a PCR reaction. How many copies of DNA would be present after the end of 25th

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 1000000 copies of DNA? (1+1 = 2)

(d) Describe AFLP and RFLP based DNA fingerprinting. (4)

13. (a)What are the advantages of using real time PCR over regular PCR? (2)

(b) Explain the terminologies that are used in realtime PCR technique with a diagram. (2x2=4)
i) Baseline and threshold
ii) Ct and ΔRn

(c)You have identified a novel tumor suppressor gene. How would you quantify the expression of that gene in a pancreatic tumor by SYBR green method? Explain the ΔΔCt method to calculate the fold change of that tumor suppressor gene between the pancreatic tumor cell and normal pancreatic cell?(2x2=4)

14. (a). What is the function of dideoxynucleotides in a chain termination sequencing reaction? (2)

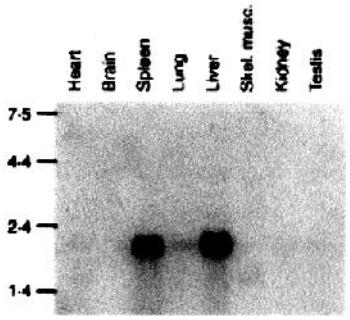
(b). What pattern of peaks do you expect to see on a electropherogram if you carry out a dideoxynucleotides chain termination sequencing reaction after the primer 5' GGTAGC 3' is annealed to the following single stranded DNA fragment ? (2)

3' CCATCGAATGTCGAGC 5'

(c) Explain briefly the principle of pyrosequencing? (2)

(d) What is the full form of CRISPR? Briefly explain the principle of CRISPR-Cas system to regulate gene expression. (2)

(e) You have identified a gene called "TEST"and demonstrated that the gene "TEST" is expressed only in spleen and liver by Northern blot analysis (as shown below). Can you definitely be certain that the gene X is not expressed in any other tissues apart from spleen and liver? (2)



15. a) What are the major advantages of chloroplast transformation over nuclear transformation? 6

b) Which technique is generally used to introduce genes into dicots? Describe the role of Virulence genes in *Agrobacterium* mediated transformation of plants. 1+3