C-official
All-stream

M.TECH/BT/1ST SEM /BIOT 5101/2015
2015

29

Advanced Genetic Engineering
(BIOT 5101)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternatives for the following: **10 x 1=10**

(i) To clone a 100-300 kb DNA, which vector will be the best?
(a) Plasmid (b) Cosmid
(c) PAC (d) Lambda based vector.

(ii) A mRNA coding for a secretory protein, when translated using free ribosome under *in vitro* conditions, resulted in a 40 kDa protein. The same mRNA when translated using the rough endoplasmic reticulum resulted in a 36 kDa protein. The difference in the molecular weight of the two polypeptides is due to the loss of a
(a) 2 kDa peptide from N-terminus and a 2 kDa peptide from the C-terminus
(b) 1 kDa peptide from N-terminus and a 3 kDa peptide from the C-terminus
(c) 4 kDa peptide from the N-terminus
(d) 4 kDa peptide from the C-terminus.

(iii) Why are gene libraries constructed?
(a) to find new gene (b) to create a "bank" of all genes in an organism
(c) to compare genes to other organisms (d) to sequence whole genomes.

(iv) Which of the following terms describe when gene regulation occurs by short dsRNA molecules triggering an enzymatic reaction that degrades the mRNA of a target gene?
(a) Post Transcriptional Gene Silencing (b) RNAi
(c) co-suppression (d) all of the above.

(v) A scientist spread bacteria on a nutrient agar plate and after some time transferred the resulting bacterial colonies to three other plates (replica plating) such that the relative positions of the bacterial colonies was the same in all four plates. The fresh plates all contained the same antibiotic. The scientist notes that on each of the antibiotic-containing plates, a few colonies survive the antibiotic. Which of the following is most likely to be observed?
(a) The number of resistant colonies varies from plate to plate
(b) The number of resistant colonies is about the same in all three
(c) Both the number and position of resistant colonies are the same on all three plates
(d) Insufficient information is provided to make a prediction.

(vi) The 50 mL of competent *E.co/i* cells (10^9 CFU/mL) were transformed using 0.5ng of a 5kb plasmid DNA to which 950 mL of SOC medium was added. Only 50 µL of this was plated on a selective agar plate. After 12h incubation at 37°C, 90 colonies were observed. The efficiency of this transformation in *CFU/llg* of DNA is

(a) 3.6×10^5 (b) 3.6×10^6
(c) 1.8×10^5 (d) 1.8×10^6.

(vii) How are restriction enzyme (RE) and T4DNA ligase used in genetic engineering?

(a) Restriction enzyme cut the DNA at specific site, producing ends that can be ligated back together with ligase

(b) Only restriction enzyme that produces blunt ends after cutting DNA can be ligated with ligase.

(c) Only restriction enzyme that produces sticky ends on the DNA can be ligated with ligase

(d) Restriction enzyme randomly cut DNA and the cut fragment can be ligated back together with ligase.

(viii) In which application fluorescent antibodies are used?

(a) Immunohistochemistry (b) flowcytometry
(c) fluorescence activated cell shorting (d) all of the above.

(ix) Which of the following is an application of PCR?

(a) site directed mutagenesis (b) amplification of specific segments of DNA
(c) for cloning into vectors (d) all of the above.

(x) Which of the following techniques is not involved in the identification of DNA at crime scenes against possible suspects?

(a) PCR (b) western blot (c) DNA Sequencing (d) DNA fingerprinting.

Group – B

2.(a) What is probe in genetic engineering (GE)? Describe a method of preparation of random, 5'-end and 3'-end labelled radioactive probe only by diagram

(b) What is blotting? Describe western blotting hybridization techniques with labelled diagram only.

(c) Starting with 500 template DNA molecules after 30 cycles of PCR, how many molecules of DNA will be produced?

(1+4) + (1+4) +2 = 12

3.(a) What are the parameter one must take into account when designing the primer for PCR amplification?

(b) PCR typically used to amplify DNA that lies between two known sequences, then how you will amplify the end sequences of DNA whose internal sequences is known to you?

(c) An aliquot of template DNA containing 3 X 10^4 copies of target gene is placed into PCR reaction. The reaction has a mean efficiency of 85%. How many cycles are required to produce 2 X 10^{10}?

(d) What is the difference between normal PCR and QPCR? Explain the real-time fluorescent PCR with TaqMan^R probe.

2+3+2+ (1+4) = 12

Group – C

4.(a) Using labelled diagram describe a method of cloning without the use of restriction enzyme and DNA ligase.

(b) A genomic library of a prokaryotic organism is often constructed by cloning the products of a Sau3AI partial digest of the genomic DNA into a BamHI site of the vector.

(i) Why are two different enzymes used in this experiment?

(ii) What is partial digestion and how it is performed?

(iii) What is the significance of use of partial digestion during making of genomic libraries?

The human genome contains about 3X10^9 bp of DNA. How many 200-kb fragments would you have to clone into BAC library to have 90% probability of including a particular sequence?

5+(1+2+2)+2 = 12

5.(a) Describe the mechanism of control of over expression of cloned gene in pET vector system with diagram.

(b) A pure protein X of one bacteria is available, describe the plan to clone the gene of protein X with a labelled diagram.

(c) A researcher desires to clone a gene (1 kb) of a microorganism. Its genome size is 1.5x 10^4 kb. The average size of its library fragment is 5 kb. The genomic library was created in vectors that were transformed into bacterial cells. If there is a 95% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene?

6 + 4 + 2 =12

Group – D

6.(a) What are naturally found stem cells in animals? Describe the transfection method to them.

(b) Describe the steps of developing transgenic mice in detail.

(1 + 4) + 7 =12

7.(a) What is T-DNA? Describe its structure in different strains of *Agrobacterium*.

(b) Mention the steps of generating a transgenic plant by one of the following methods:
i) Gene-gun method
ii) Agro-mediated gene transfer

(c) Compare the advantages and disadvantages of these two methods.

(2+2)+5+3= 12

Group – E

8.(a) Describe a flow chart for cloning of human interferon gene to produce recombinant human interferon.

(b) Describe two detection methods for identifying pathogenic virus or bacteria.

(c) Describe the steps to cure SCID by gene therapy.

4+(2+2)+ 4= 12

9.(a) Write notes on **any three** of the following:
(i) RNAi technology
(ii) AFLP
(iii) DNA micro array
(iv) Yeast two hybrid
(v) Human Genome Project

(4 x 3) = 12