RAMAKRISHNA MISSION VIDYAMANDIRA

(Residential Autonomous College affiliated to University of Calcutta)

B.A./B.Sc. FOURTH SEMESTER EXAMINATION, AUGUST 2021

SECOND YEAR (BATCH 2019-22)

Date : 11/08/2021 **MICROBIOLOGY (Honours)**

Time : 11.00 am – 1.00 pm **Paper : X [CC 10]** Full Marks : 50

Answer **any five** questions of the following : [5×10]

1. a) When cloning a foreign DNA fragment into a plasmid, it is often useful to insert the fragment at a site that interrupts a selectable marker (such as the tetracycline-resistance gene of pBR322). The loss of function of the interrupted gene can be used to identify clones containing recombinant plasmids with foreign DNA. With a bacteriophage vector it is not necessary to do this, yet one can easily distinguish vectors that incorporate large foreign DNA fragments from those that do not. How are these recombinant vectors identified? [4]

   b) Theoretically, gene expression is controlled by promoter. But when transgenes are expressed in unrelated or distantly related organisms, their coding sequences may markedly affect the level of their expression. Explain this fact. [3]

   c) How triparental mating does useful to make co-integrate vector from Ti plasmid? [3]

2. **Briefly justify whether the following statements are true or false:** [5×2]

   a) Blunt end ligation is easier than sticky end ligation.

   b) Colony hybridization technique allows for the hybridization of two bacterial colonies.

   c) More efficient cloning is achieved by using a double restriction enzyme digest than a single restriction enzyme digested DNA,

   d) Human insulin expressed in recombinant bacteria is biologically inactive.

   e) Polynucleotide kinase has 3' phosphatase activity.

3. a) What unique property possessed by terminal nucleotidy l transferase with respect to its polymerizing activity ? [2]

   b) i) " When a cloned hGH cDNA is expressed with in an *E.coli* cell, the purification of the hormone is painstaking".--- Illustrate a suitable alternative to circumvent this problem.

   ii) State the main structural difference of such hGH with regular proteins. [2+1]

   c) How can you purify mRNA from the total RNA pool? [2]

   d) Comment on the nomenclature method of Type II restriction endonuclease with example. [3]

4. a) What are auxotrophic mutant? How LEU2 gene does useful as selectable marker in yeast cloning experiment? [2+3]

   b) Write down the function of i) EDTA and ii) SDS in DNA extraction buffer. [3]

   c) Calculate Tm of the given primer sequence: AGACTCAGAGAGAACC. [2]

5. a) Explain the chemistry behind the acrylamide gel polymerization. [3]

   b) For detecting the proteins in blotting membrane what are the use of primary and secondary antibodies? [2]

   c) What is blue-white screening process? [3]

   d) What are Bicistronic IRES vector? Explain. [2]

6. a) What is gutless vector? Explain. [2]

   b) For obtaining glycosylated recombinant protein of less than 50 Kd molecula,what could be the choice of expression system? [2]

   c) Theoretically, gene expression is controlled by promoter. But when transgenes are expressed in unrelated or distantly related organisms, their coding sequences may markedly affect the level of their expression. Explain this fact. [3]

   d) How does Spi phenotype is useful for selecting recombinant? [3]

7. a) How CAD protein is useful as a dominant marker? [2]

   b) Explain the working principle of qPCR. [3]

   c) How does cryoprotectants are useful in chemical mediated gene transfer process? [2]

   d) Explain in detail what are the modifications required for the generation of replication-defective viral vectors from wild type virus for in vivo gene transfer. [3]

8. a) λEMBL3 is used as a vector to clone 20000-bp fragments generated from a partial Sau3A1 digest of human genome ($3x10^9$ bp). We wish to isolate a gene contained completely on a 20000-bp fragment. To have a 99% chance of isolating this gene in the λEMBL3 recombinant genomic library, how many independent clones must be examined? [3]

   b) Number of restriction target site and produced fragment are same for a specific restriction enzyme of a circular DNA—True or False, Justify. [2]

   c) A good host should lack restriction modification system—Justify. [2]

   d) State the function of RNase H in RDT. [2]

   e) What is nick translation? [1]

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