RAMAKRISHNA MISSION VIDYAMANDIRA

(Residential Autonomous College under University of Calcutta)

B.A./B.SC. SIXTH SEMESTER EXAMINATION, MAY-JUNE 2013

THIRD YEAR

Date : 24/05/2013 **Microbiology (Honours)**

Time : 11am – 1pm Paper : VII Full Marks : 50

Group-A

1. Answer **any two** questions from the following:

(a) (i) What is conditional lethal mutation? 2

(ii) Wild–type *Salmonella typhimurium* cells are killed by phage FO. A Luria-Delbrück fluctuation test was done to determine the rate of mutation to resistance to phage FO. Twenty tubes of medium were inoculated with S. *typhimurium* cells and the cultures were grown to 10^8 cells/ ml. A 0.1 ml sample of each culture was then spread on plates with phage FO. Out of 20 plates, 10 plates exhibited FO resistant (FO^R) colonies where the number of FO^R resistant colonies varied from 1 to 128 per plate. Determine the rate of mutation to FO^R. 3

(iii) Briefly mention the roles of DNA photolyase and N-glycosylase in repair mechanism of damaged DNA. 4

(iv) Cite an example of a biological mutagen. 1

b) i) Differentiate between prototrophs and auxotrophs. 2

ii) You are supplied with an *E.coli* culture in which both wild type and auxotrophic mutant for biotin are present. How will you isolate the bio^- auxotrophs from the mixed culture? 4

iii) Schematically show the action of 5-BU leading to both substrate and template transition. 3

iv) Cite an example of a human disease caused by the defects in DNA repair system? 1

c) i) How does UV-irradiation cause mutation? 3

ii) How can this type of mutation be repaired? 3

iii) Define transition and transversion. Why are reversion studies considered to test the mutagenicity of chemicals? 2+2

d) i) How are temperature-sensitive (T_s) mutants used in detecting the function of specific genes? 4

ii) Briefly mention the processes by which a protooncogene can be converted to an oncogene? 3

iii) What is meant by suppressor mutation? Briefly describe the processes of intragenic and intergenic suppression. 3

2. Answer **any three** questions of the following:

a) i) Give one example each of a restriction enzyme producing DNA fragments with sticky ends and blunt ends, showing the nucleotide sequences recognized and resultant fragments. 4

ii) Bacteria do not cleave its own DNA despite having restriction enzymes- Justify. 2

iii) What are the uses of Klenow fragment in RDT? 2

iv) Describe any one method of preparation of probe for the detection of a particular gene of interest. 2

b) (i) A native gene is usually longer than its c-DNA- Justify 3

(ii) What are the difficulties of expressing eukaryotic genes in bacteria? 4

(iii) Mention the differences between cloning vector and expression vector. 3

c) (i) What is MCS? State its function in cloning. 3

(ii) Describe schematically the cloning of a DNA fragment in a suitable vector both of which has been digested with a restriction enzyme that makes the blunt cut. 3

(iii) You are given a purified protein whose amino acid sequence is known to you. How can you clone the gene that codes the protein? 4

d) i) What are the advantages of using low molecular weight plasmids as a vector?

ii) Describe the characteristic features of type II restriction endonuclease. 2

iii) What is nested PCR? Why is it advantageous from conventional PCR? 1+1

iv) The optimum temperature for ligation is 37^0C, but still ligation of cohesive ends is carried out at $4^0C - 15^0C$ – Why? 2

v) What is star activity? Give examples. 2

e) i) What enzymatic reaction is performed to enhance the cloning efficiency by reducing the frequency of self ligation of the vector? 2

ii) What are cosmids? Why are they better than plasmid? 2

iii) How can you minimize the non specific amplification in a PCR reaction? 2

iv) A 5.5 kb linear DNA fragment gives two bands: 2 kb and 3.5 kb on digestion with EcoRI, three bands: 0.5 kb, 1 kb and 4kb with Hind-III digestion, and four bands: 0.5 kb, 1 kb, 1.5 kb and 2.5 kb on double digestion with EcoRI and Hind III together. Draw a restriction map of the DNA fragment. 3

v) Name two alternative thermostable enzymes that can be used in place of Taq DNA polymerase in PCR. 1

f) i) Draw the basic structure of YAC, indicating its important elements. 2

ii) What is the role of sodium acetate during isolation of plasmids by alkali lysis method? 2

iii) Name two recombinant drugs licensed for human use. State briefly how do you develop any one of those drugs using *E.coli* as host. (with the help of R. D. T.). 3

iv) The restriction enzyme EcoRI cuts the DNA at the sequence GAATTC, and restriction enzyme HaeII cuts DNA at the sequence GCGC. Predict how frequently will each enzyme cut double stranded DNA? 2

v) What is Ti plasmid? 1

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