KUMARAGURU
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Register Number:……….……

M.TECH DEGREE EXAMINATIONS: JUNE 2015

(Regulation 2014)

Second Semester

BIOTECHNOLOGY

P14BTT202: Recombinant DNA Technology

Time: Three Hours   **Maximum Marks: 100**

Answer all the Questions:-

PART A (10 x 1 = 10 Marks)

1. The steps involved in setting up a *Pichia pastoris* expression system are listed below. Work   [K_5] out and list the correct sequence of steps

    1)   Ligate the gene of interest with P.Pastoris expression vector.

    2)   Linearize the expression vector by restriction digestion

    3)   Screen for Jackpot clones

    4)   Transform competent Pichia pastoris

    a)   4, 2, 3, 1   b)   1, 3, 4, 2

    c)   2, 1, 4, 3   d)   3, 4, 1, 2

2. Which of the following is the best host *E. coli* strain for expressing a protein that must contain   [K_1] disulphide bonds for it to function properly?

    a)   BL21(DE3) Origami   b)   BL21(DE3) pLysS

    c)   BL21(DE3) Rosetta   d)   BLR.

3. A novel eukaryotic organism that glows in the dark was discovered. It is believed that this   [K_4] trait is due to a single gene, and in order to clone the gene which of the following strategies is most likely to be successful?

    a)   Isolate the genomic DNA from the organism, digest with a restriction endonuclease, insert into a plasmid vector and transform into bacteria. Screen colonies for the ability to glow in the dark.

    b)   Isolate the genomic DNA from the organism, digest with a restriction endonuclease, insert into a plasmid vector and transform into eukaryotic cells such as yeast. Screen colonies for the ability to glow in the dark.

c) Isolate mRNA from the organism, reverse transcribe and generate cDNA, insert into a plasmid vector and transform into bacteria. Screen colonies for the ability to glow in the dark.

d) Isolate mRNA from the organism, reverse transcribe and generate cDNA, insert into a plasmid vector and transform into eukaryotic cells such as yeast. Screen colonies for the ability to glow in the dark.

4. An Hfr strain of E. coli contains   [K₁]

   a) a vector of yeast or bacterial origin which is used to make many copies of a particular DNA sequence

   b) a bacterial chromosome with a human gene inserted

   c) a bacterial chromosome with the F factor inserted

   d) a human chromosome with a transposable element inserted

5. On average, how many fragments would a restriction enzyme which recognizes a specific 4   [K₄] base sequence in DNA be expected to cleave a double-stranded bacteriophage with a genome size of 5,000 bp into?

   a) about 2   b) about 4

   c) about 20   d) about 50

6. For an application where the sample of the target protein at high purity is required. What   [K₅] would be a good purification strategy if the target protein if fused with an affinity tag is over-expressed?

   a) Affinity chromatography (AC) followed by size exclusion chromatography (SEC).   b) AC only.

   c) AC followed by ion-exchange (IEX) followed by SEC.   d) AC followed by (IEX) followed by Hydrophobic interaction and followed by SEC.

7. Pyrosequencing is especially useful for   [K₂]

   a) Sequencing repetitive DNA in multiple individuals.   b) Sequencing highly condensed DNA regions.

   c) Sequencing short DNA regions in multiple individuals.   d) Sequencing short DNA regions with high AT content

8. Assertion (A): Insect resistant transgenic cotton has been produced by inserting Bt gene.   [K₄] Reason (R) : The Bt gene is derived from a bacterium.

a) Both A and R are true and R is the correct explanation of A. b) Both A and R are true and R is not the correct explanation of A.

c) A is true but R is false. d) A is false but R is true.

9. Match the following [K₃]

GENETICALLY MODIFIED FOOD		DESCRIPTION
(1)	Flavr Savr	(a)	a form of squash grown in the US was genetically modified to resist three viruses.
(2)	Amflora	(b)	Genetically modified to reduce the expression of polyphenol oxidase thus preventing from fruit ripening.
(3)	Artic apples	(c)	Is a genetically modified potato cultivar developed to produce pure amylopectin starch.
(4)	Zucchini	(d)	Made for a longer shelf life and to prevent a substance that causes tomatoes to rot and degrade.

a) 1-d, 2-c, 3-b, 4-a b) 1-d, 2-b, 3-c, 4-a

c) 1-d, 2-a, 3-b, 4-c d) 1-d, 2-c, 3-a, 4-b

10. Assertion (A): Transgenic organisms are genetically altered organisms. [K₄]

Reason (R): Transgenic organisms do not cause any environmental hazards.

a) Both A and R are true and R is the correct explanation of A b) Both A and R are true but R is not the correct explanation of A

c) A is true but R is false d) A is false but R is n true

PART B (10 x 2 = 20 Marks)

11. Differentiate Binary and co-integrate vectors. [K₂]

12. List the salient features of *Bacillus subtilis* expression system. [K₁]

13. Calculate the number of clones required to construct a genomic library for Arabidopsis of genome size 70 Mb using BAC vector with insert size of 0.1 Mb. [K₄]

14. Explain Colony Blot Hybridisation method to screen the genomic library. [K₃]

15. What are recombinant fusion proteins? Give examples. [K₁]

16. List out the factor that influences the choice of expression system. [K₂]

17. What is next generation sequencing? [K₁]

18. Write about quantitative Real time PCR. [K₂]

19. What are edible vaccines? Give example. [K₂]

20. Name four recombinant therapeutic proteins. [K₁]

PART C (6 x 5 = 30 Marks)

21. Elaborate the problems encountered during heterologous protein production in *E.coli* and give the possible solutions for it. [K₄]

22. Describe a flow chart for heterologous gene expression. [K₆]

23. Explain the various steps involved in transposable elements based gene isolation. [K₂]

24. List the different types of IPTG inducible promoters in *E.coli* expression vectors**.** [K₂]

25. Illustrate with a neat diagram the principle and methodology involved in 454 pyrosequencing. Add a note on its advantages. [K₃]

26. Discuss in detail the ABC model for flower development. [K₂]

PART D (4 x 10 = 40 Marks)

27. Create a simple flowchart to produce maximum amount of protein of interest using pET expression vector. Explain the strategy to regulate the gene expression in these vectors. [K₆]

28. Compare and contrast the key features of various strains of bacteria for protein expression. Why not just use DH5alpha cells to express and make protein? [K₅]

29. What are reporter genes? Explain the application of GUS genes to quantify gene expression. [K₄]

30. Discuss in detail the application of rDNA technology in recombinant vaccines**.** [K₃]

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