



INDIAN SCHOOL AL WADI AL KABIR



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WORKSHEET NO:9	TOPIC: UNIT-BIOTECHNOLOGY	NOTE: A4 FILE FORMAT
CLASS & SEC:	NAME OF THE STUDENT:	ROLL NO.

CASE STUDY

Case 1: Designing an RNAi strategy against root-knot nematode

A research team wants to protect tobacco roots from the root-knot nematode *Meloidogyne incognita* by expressing double-stranded RNA (dsRNA) in tobacco that targets an essential nematode gene required for parasitism. They plan to transform tobacco so that roots produce the dsRNA, which will be ingested by nematodes during feeding.

- State the principal of RNA interference and how plant-expressed dsRNA can silence a nematode gene.
- Give two criteria for choosing a nematode target gene for RNAi.
- Briefly describe the procedure of this method.

OR

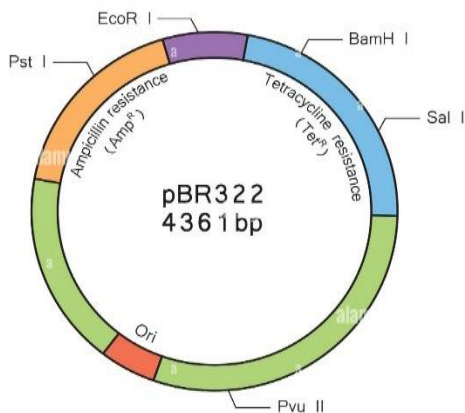
- RNAi takes place in whose body and how?

Case 2: Genetically Engineered Plasmid.

pBR322 is a plasmid and was one of the first widely used *E. coli* cloning vectors. Created in 1977 in the laboratory at the University of California, in pBR322, **p** stands for plasmid, **B** for Bolivar and **R** for Rodriguez. The number 322 in pBR322 denotes the order of synthesis that distinguishes it from the other plasmids synthesized in the same laboratory.

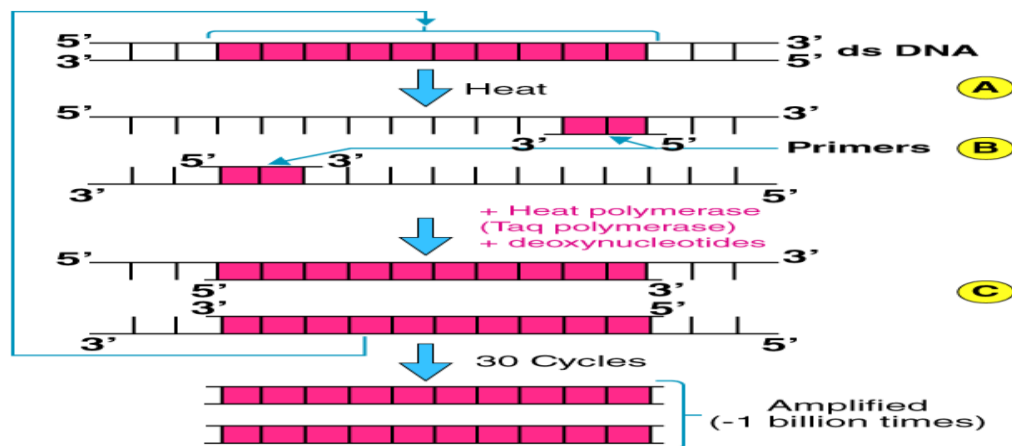
Answer the questions given below in relation to the figure given below

- Identify the restriction sites for the antibiotic and the selectable markers from the above fig.
- What will you observe if an alien DNA is inserted the site Pst 1 of the plasmid and how can you check it by plating method.
- What is insertional inactivation and why is insertional inactivation carried at the Z-gene of the Lac operon.



Case 3: Polymerase Chain Reaction

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest is synthesised *in vitro*. using two sets of primers. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. The figure given below shows the steps of this technique.



- Name the steps as marked A, B, C in the figure.
 - What are the substrates used in this process.
 - What are primers and what is their function in this process.
- Or
- What is Taq polymerase and what is its function in this process.

Case 4: Restriction Enzymes

The foundations of recombinant DNA (rDNA) were laid by the discovery of restriction enzymes. These enzymes are present in many bacteria where they function as a part of their defense mechanism called the Restriction Modification system (RM system). Molecular basis of this system was explained first by Werner Arber in 1962. A restriction enzyme (called restriction endonuclease) identifies the introduced foreign DNA and cuts it into pieces.

The second component is a modification enzyme (methylase) that adds a methyl group to DNA at specific site to protect it from the restriction enzyme cleavage.

- a) Which of the following is the first discovered restriction endonuclease?
- Sal I
 - EcoRI
 - Hind II
 - EcoRII
- b) The restriction enzymes play an important role in rDNA technology-give reason.
- c) With an example illustrate the nomenclature of naming the restriction enzyme.
- Or
- What is palindrome sequence, give an example?

MULTIPLE CHOICE QUESTIONS

- C-peptide of human insulin is:
 - A part of mature insulin molecule
 - Responsible for formation of disulfide bridges
 - Removed during maturation of pro-insulin to insulin
 - Responsible for its biological activity.
- GEAC stands for:
 - Genome Engineering Action Committee
 - Ground Environment Action Committee
 - Genetic Engineering Approval Committee
 - Genetic and Environment Approval committee
- α -1 antitrypsin is:
 - An antacid
 - An enzyme
 - Used to treat arthritis
 - Used to treat emphysema
- Choose the correct option regarding Retrovirus:
 - An RNA virus that can synthesize DNA during infection
 - A DNA virus that can synthesize RNA during infection
 - A ssDNA virus
 - A dsRNA virus
- The site of production of ADA in the body is:
 - Bone marrow
 - Lymphocytes
 - Blood plasma
 - Monocytes
- The trigger for activation of toxin of *Bacillus thuringiensis* is:
 - Acidic pH of stomach
 - High temperature
 - Alkaline pH of gut
 - Mechanical action in the insect gut
- In RNAi, genes are silenced using:
 - ss DNA

- (b) ds DNA
 - (c) ds RNA
 - (d) ss RNA
8. Bt cotton is not:
- (a) A GM plant
 - (b) Insect resistant
 - (c) A bacterial gene expressing system
 - (d) Resistant to all pesticides
9. An enzyme catalyzing the removal of nucleotides from the ends of DNA is:
- (a) Endonuclease
 - (b) Exonuclease
 - (c) DNA ligase
 - (d) Hind – II
10. Which of the given statement is correct in the context of observing DNA separated by agarose gel electrophoresis?
- (a) DNA can be seen in visible light
 - (b) DNA can be seen without staining in visible light
 - (c) Ethidium bromide stained DNA can be seen in visible light
 - (d) Ethidium bromide stained DNA can be seen under exposure to UV light
11. The most important feature in a plasmid to be used as a vector is:
- (a) Origin of replication (ori)
 - (b) Presence of a selectable marker
 - (c) Presence of sites for restriction endonuclease
 - (d) Its size
12. Bacteria protect themselves from viruses by fragmenting viral DNA with
- (a) Ligase
 - (b) Endonuclease
 - (c) Exonuclease
 - (d) Gyrase
13. Southern blotting is
- (a) Attachment of probes to DNA fragments
 - (b) Transfer of DNA fragments from electrophoretic gel to nitrocellulose sheet
 - (c) Comparison of DNA fragments
 - (d) Transfer of DNA fragments to electrophoretic gel from cellulose membrane
14. Plasmids are used as cloning vectors for which of the following reasons?
- (a) Can be multiplied in culture
 - (b) Self-replication in bacterial cells
 - (c) Can be multiplied in laboratories with the help of enzymes
 - (d) Replicate freely outside bacterial cells
15. RNA interference helps in
- (a) Cell proliferation
 - (b) Micropropagation
 - (c) Cell defense
 - (d) Cell differentiation

TWO MARKS QUESTIONS

1. Why is a thermostable DNA polymerase needed in amplification in genetic engineering?

2. Name the method in which foreign DNA is directly introduced into host cell.
3. In bacterial culture some of the colonies produce blue colour in the presence of a chromogenic substrate and some did not due to the presence or absence of an insert (rDNA) in the coding sequence of the beta- galactosidase.
 - a) Mention the mechanism
 - b) How is it better than the technique of plating on two plates having different antibiotics?
4. Why are engineered vectors preferred by biotechnologists for transferring the desired genes into another organism?
5. Dr. Arun developed a vitamin A rich potato through his research on genetics.
 - a) What do you call such potato plants?
 - b) Who can approve the validity and safety of introducing potato for public uses?

THREE MARKS QUESTIONS

1. Draw the diagram of pBR322 vector showing restriction sites
2. Give diagrammatic representation of rDNA technology
3. How is the gene z (for B-galactosidase) used as marker?
4. State the principle underlying gel electrophoresis and mention two applications of this technique in Biotechnology.
5. Explain the work carried out by Cohen and Boyer that contributed immensely to biotechnology.

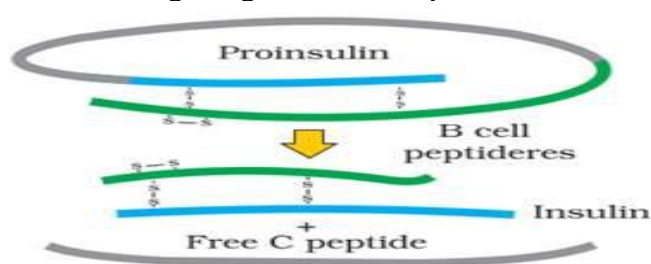
FIVE MARKS QUESTIONS

1. Any recombinant DNA with a desired gene is required in billion copies for commercial use. How is the amplification done? Explain.
2. Giving suitable examples describe the roles of recombinant technology in agriculture
3. Give a brief description about the large-scale production of recombinant protein. What is the role of bioreactor in the production? Draw neat labelled diagrams of any two types of bioreactors.

PREVIOUS BOARD QUESTIONS

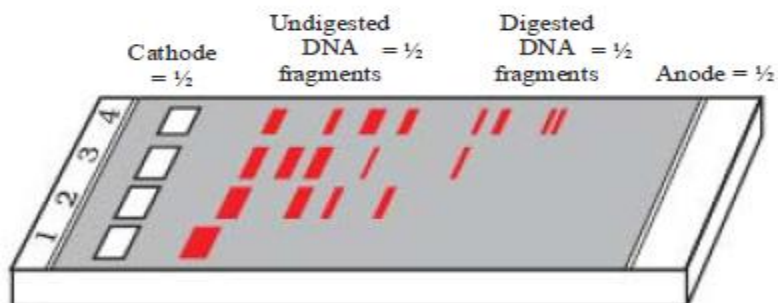
1. (a) Why are restriction endonucleases so called?
(b) What is palindromic nucleotide sequence? How do restriction endonucleases act on palindromic sites, to create sticky ends?
2. (a) Name the technique used for the separation of DNA fragments.
(b) Write the type of matrix used in this technique.
(c) How is the separated DNA visualized and extracted for use in rDNA technology.
3. Some cotton plants grown by farmers are known as 'Bt cotton'.
a) What does Bt stand for?

- b) What is the advantage of this cotton plant?
 c) How did scientists achieve this?
4. A method to prevent infestation of a nematode *Meloidogyne incognita* on roots of tobacco is silencing the specific mRNA. What is the scientific name of the technique? How is this performed by dsRNA?
5. Describe briefly the production of humulin.
6. Identify the following image. Give its importance in rDNA technology.



What you mean by humulin? Give its uses.

7. Give the different roles played by transgenic animals. What is the importance of GEAC in the production of transgenic organisms?
8. Observe the given figure and answer the questions



- (a) Identify the process and give its principle
 (b) Why DNA is moving to anode?
 (c) Identify the smallest and largest DNA fragments.

SECTION A (1 mark each)					MKS
1-C	2-C	3-D	4-A	5-B	
6-C	7-C	8-D	9-B	10-D	
11-A	12-B	13-B	14-B	15-B	
SECTION B					
1. (Hints: Mention the high temperature used in PCR and the name of the enzyme)					2
2. (Hints: Mention the process involved in Microinjection)					2
3.(Hints: (a) – Insertional inactivation; (b) The second method is a cumbersome process as it requires simultaneous plating on two plates having two different antibiotics)					2

4. (Hints: They help easy linking of foreign DNA and selection of recombinants from non-recombinants)	2
5. (Hints: (a) Transgenic plant (b) GEAC)	2
SECTION D	
1. (Hints: PCR – Explanation, steps, importance, figure)	3
2. (Hints: Mention the importance and steps in the production of Bt plants and pest resistant tobacco plants)	3
3. (Hints: Largescale production by bioreactors, importance of bioreactors, types of bioreactors)	3
PREVIOUS BOARD QUESTIONS	
1. (Hints: (a) restricts the growth of bacteriophage and mention the endonuclease activity (b) Action of RE - EcoRI))	5
2. (Hints: (a) Electrophoresis, (b) Agarose gel (c) staining by ethidium bromide and exposure under UV rays)	5
3. (Hints: (a) <i>Bacillus thuringiensis</i> (b) insect resistant (c) steps in the production of Bt plants)	5
4. (Hints: RNA interference, steps in RNAi)	5
5. (Hints: Explanation of Insertion of ‘A’ and ‘B’ genes into separate <i>E.coli</i>, Isolation of ‘A’ and ‘B’ polypeptides, joining with Disulfide Bridge)	5
6. (Hints: Maturation of Insulin, importance, insulin produced by transgenic <i>E.coli</i>, uses of Humulin)	5
7. (Hints: Examples and the functions of different transgenic animals, Roles of GEAC)	5
8. (Hints: (a) Electrophoresis and its principle, (b) DNA is negatively charged, (c) smallest – the one which is close to anode)	3

<i>Prepared by:</i> <i>Ms Agnes Aranha</i>	<i>Checked by:</i> <i>HOD Science</i>
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