## ISOLATION OF A DESIRED DNA FRAGMENT

**1. A wine maker and a molecular biologist who has developed a recombinant vaccine, both claim themselves to be biotechnologists. Who, in your opinion, is right?**

**2. Consider you are appointed as biotechnologist in a National Institute. What are the basic steps to be designed to produce a genetically modified organism?**

**(2) (SAY 2014)**

**3. Explain the merits of genetic engineering when compared to traditional hybridisation procedures.**

**4. A group of students came to know about rDNA technology. They want to know how scientists can produce a new desired product using technology. Can you give them an idea about the important steps that are involved in this process?**

**(4) (SAY 2012, March 2010)**

**5. Genetic engineering leads to the production of desired products, which can be accomplished with the help of certain tools. Name the 5 important tools of genetic engineering?**

**6. Given below are the different steps in rDNA technology. Arrange them according to the sequence of occurrence.**

a. Transferring of rDNA into the host.
b. Extraction of the product.
c. Fragmentation of DNA by restriction *endonuclease*
d. Ligation of DNA fragment into a vector
e. Isolation of DNA
f. Culturing the host cell in a medium at large scale
g. Isolation of desired DNA fragment.

**7. Isolation of DNA from plant cell involves many steps. Explain the different steps.** (2)(Model 2018)

**8. Which enzyme is used to digest the walls of bacteria and fungi in genetic engineering?**

**9. DNA is usually interwined with histone proteins and RNA. But in genetic engineering experiments, DNA must be isolated in a very pure form. How is this possible?**

**10. rDNA technology can be accomplished only if we have the following key tools i.e., restriction enzymes, polymerase enzyme, ligases and vector. State the functions of-**

a. Ligases
b. Restriction enzymes **(2) (March 2015)**

**11. Genetic engineering include creation of recombinant DNA with the restriction enzymes.**

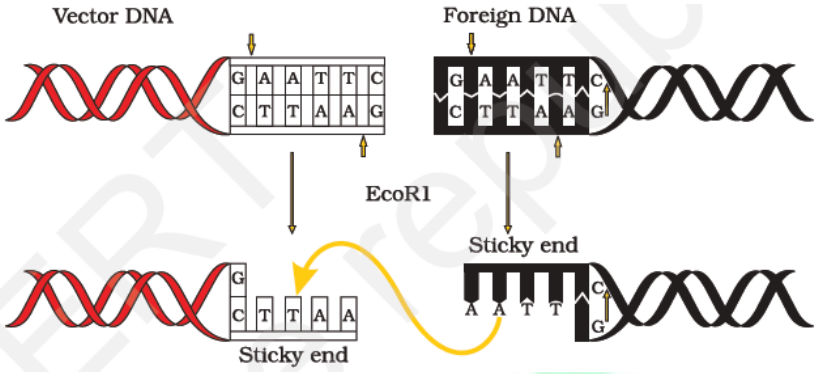
a. Explain rDNA technology.
b. What are restriction enzymes? Name a restriction enzyme

(2) (March 2013)

**12. ..... are the enzymes used for cutting the DNA molecule into fragments.**
**An example for this type of enzyme is *EcoRI*. What does *Eco*, *R* and *I* stand for?** (2) (March 2014)

**13. Sequence of base pairs in DNA that reads the same on both the strands when the orientation of reading is kept the same are called ..... sequences** (1) (March 2017)

**14. While studying nucleotide sequence, Raj found the following sequence which can be recognised by some enzymes:**




a) Give salient features of this sequence
b) Write the class of enzyme which recognise such sequences
c) Elaborate the importance of this enzyme in Genetic engineering

(4) (Model 2019, SAY 2012)

**15. Figure given below is a nucleotide sequence coding for a particular protein**

5′ ——————————— 3′
CGATGAATTCATGCAATCCGATC
GCTACTTAAGTACGTTAGGCTAG
3′ ——————————— 5′

a) Identify the restriction site.
b) Name the enzyme used to cut this site
c) Can you join these fragments of DNA? If so, how?

**16. Identify palindrome sequence from the following.**

**i.** 5'-GAATTC- 3'
3'-CTTAAG- 5'

ii. 5'-ATGC- 3'
3'-TACG- 5'

iii. 5'-AAAAA- 3'
3'-TTTTTT- 5'

iv. 5'-CCCCC- 3'
3'-GGGGG- 5' (1) (SAY 2013)

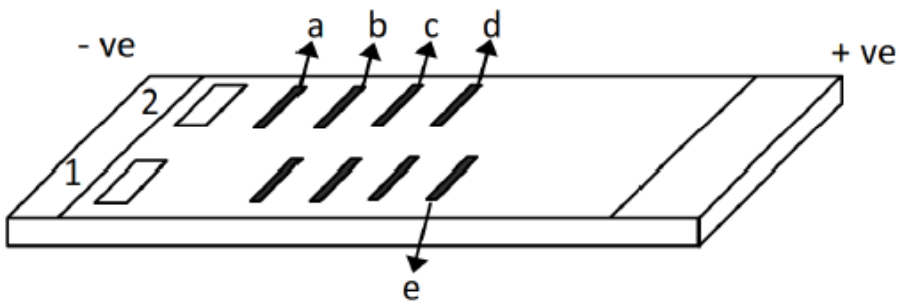
**17. Rinku with a circular DNA contains sequences**
**5'-GGAATTCC- 3'**
**3'-CCTTAAGG- 5'**

**She wishes to add a new segment of DNA into it.**

a. Identify the technology she planned
b. Suggest the specific enzyme to make a cut in the DNA with above sequence.
c. Name the category of enzyme you suggested.
d. How this enzyme identifies the sequence?
e. Draw the cut ends of the DNA with sequence.

(4) (March 2010)

**18. Diagram shows a typical agarose gel showing migration of DNA fragments.**



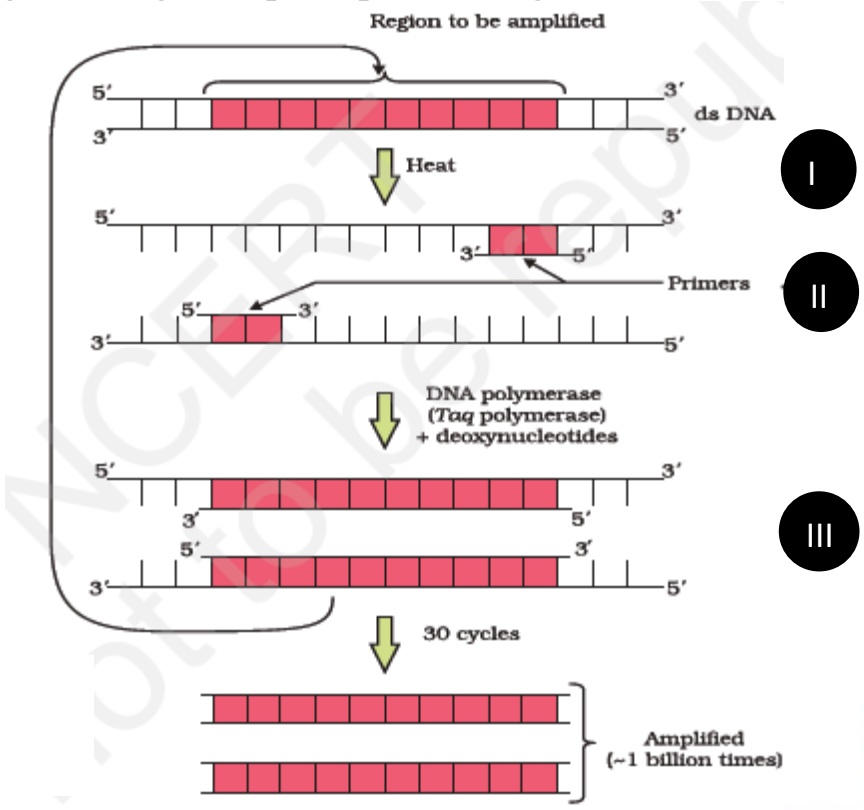

a) Which of the bands has the largest and smallest DNA fragments?
b) How can you make fragments of DNA for electrophoresis?

c) Explain separation of DNA fragments using electrophoresis.
d) Point out a method to visualise the separated DNA fragments after electrophoresis. (4) (SAY 2010)

**19. The DNA fragments can be separated using gel electrophoresis.**
(a) Name the gel used in this technique.
(b) Write the name of technique used to remove the DNA from the gel. (2)(March 2018)

**20. Gel electrophoresis is a technique to separate fragments of DNA from a mixture. Some of the events of electrophoresis are given below. Arrange the events in order:**
i. Cut out DNA bands
ii. Expose to UV
iii. Force DNA to move through gel
iv. Stain DNA with ethidium bromide
**(1) (SAY 2013)**

**21. The picture given below shows the technique used for generating multiple copies of the gene of interest.**




a) What is the technique called?
b) Name the reaction at step I, II & III
c) Explain the principle underlying this technique of DNA amplification. **(4) (March 2011, 2015)**

**22. Manipulating with nucleic acid is a trend in biotechnology.**
(a) Name any one organism used as vector.
(b) What are DNA polymerase?
(2) (March 2016)

**23. PCR is just like a photocopying document.**
(a) Name the commonly used *DNA polymerase* in PCR
(b) What are denaturation and primer extention
(3) (Model 2017)

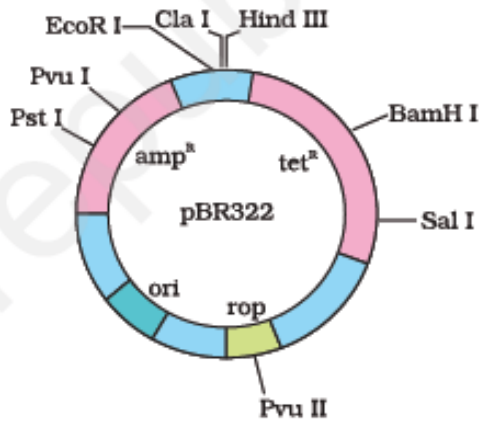
**24. Use of a thermostable DNA polymerase from bacterium, *Thermus aquaticus*, made it possible to generate billion copies of DNA in a very short time using a process.**
a. Name the process
b. Name the 3 important steps involved in this process
(2) (March 2014)

## LIGATION OF THE DNA FRAGMENT INTO A VECTOR

**25. There are many features required to facilitate successful cloning into a vector. Write shortly any 2 such features required by a vector**
**(2) (SAY 2014)**

**26. You have chosen a plasmid as vector for cloning your gene. However this vector plasmid lacks a selectable marker. How would it affect your experiment?**

**27. Observe the following figure:**




a. Identify the Cloning vector.
b. Write down three characteristic features of the above cloning vector (2)(Model 2019)
c. What does (i) $tet^R$ (ii) rop (iii) ori denote? (2)(March 2018)
d. What is Ori? Give its importance.
e. How does the insertion of foreign DNA at BamHI site selected? What is $amp^R$?
c. How many cloning sites are depicted in this vector as shown in the figure? **(4) (March 2011)**

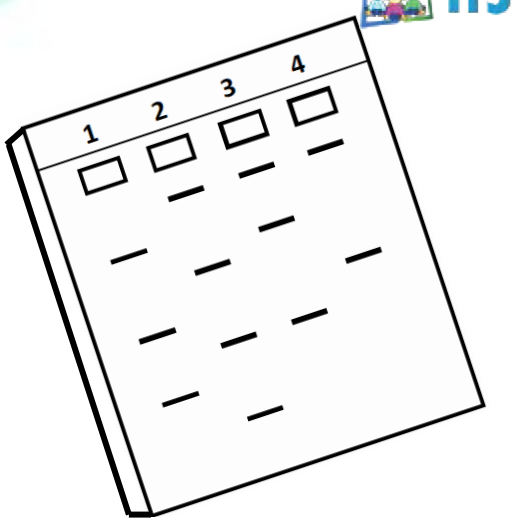
**28. Why is *'Agrobacterium-mediated'* genetic engineering transformation in plants considered as natural genetic engineering of plants?** (2) (Model 2017)

## TRANSFERRING THE rDNA INTO THE HOST

**29. Give reasons for the following.**
(a) DNA move towards the anode
(b) DNA cannot pass through the cell membrane

**30. (A) The following photograph shows the result of a technique showing the separation of DNA**






(a) Name the technique
(b) How the separated DNA is visualised?
(c) DNA fragments of size 500bp, 1600bp and 2000 bp are separated by this process. Which will migrate fast. Why?

**OR**

**(B) Different methods have been suggested to introduce alien DNA into host cells. Give and explain any 3 methods adopted for this purpose.**
**(3) (March 2017)**

**31. Rashid isolated a natural plasmid from a bacterium and planning to facilitate cloning.**
a. What are the minimum requirements for considering the isolated plasmid as a vector?
b. How he identifies whether a foreign DNA is inserted or not after cloning? (March 2010)

**32. A selectable marker is used in the section of recombinants on the basis of their ability to produce colour in presence of chromogenic substrate.**
(a) Mention the name of mechanism involved
(b) Which enzyme is involved in production of colour?
(2) (Model 2017)

**33. Name any 3 suitable 'selectable markers' for *E. coli.***

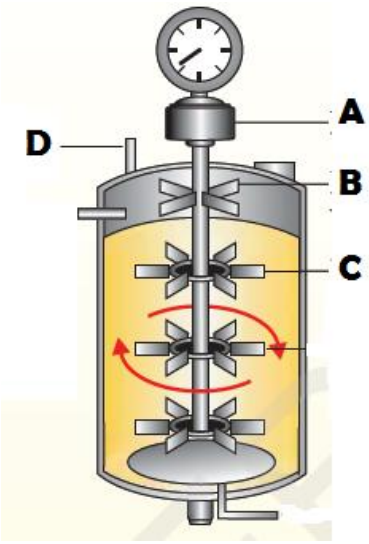
**34. During genetic engineering vector with foreign DNA is transferred into a host bacterium. The next target will be the selection of transformants from non-transformants.**
How antibiotic resistance and insertional inactivation is exploited for this purpose? **(4) (March 2012)**

**35. Restriction endonucleases are the enzymes used to cut the DNA molecules.**
a. Give the general term of the specific sequences where these enzymes cut the DNA.
b. Name the enzyme that joints the foreign DNA and vector DNA
c. Give any 2 procedures to introduce the recombinant DNA into the host cell (4) (March 2012)

**36. Jaya read in a biotechnology book that alien DNA can be introduced into host cell by micro-injection and biolistics. Explain these methods** (2)(March 2013, SAY 2011)

## CULTURING THE HOST CELLS

**37.**




**Observe the sketch of stirred-tank bioreactor and label the parts A, B, C and D.**
(2) (March 2016)

**38. Besides better aeration and mixing properties, what other advantages to stirred tank bioreactors have over shaken flasks?**

## EXTRACTION OF THE DESIRED PRODUCT

**39. Describe briefly the following:**
a) Bioreactors
b) Downstream processing

**40. Match the words in box 'A' with the most accurate answer from box 'B'.**

| A | B |
|---|---|
| i. Lysozyme | a. T-DNA |
| *ii. Agrobacterium tumifaciens* | b. DNA stain |
| iii. Gel electrophoresis | c. Escherichia coli |
| iv. Ethidium bromide | d. Restriction enzymes |
| *v. EcoRI* | e. Bacterial cell breakage |
| vi. Molecular scissors | f. Separation of DNA fragments |