Biotechnology : Principles and Processes

CHAPTER 11

EXAM DRILL

ANSWERS

1. **(a)**

2. **(c) :** *Eco*RI, *Bam* HI and *Sal* I produce sticky ends.

3. **(d) :** Ligase is an enzyme used to join DNA fragments.

4. **(d)**

5. **(b)**

6. Kary Mullis developed the technique Polymerase Chain Reaction (PCR). The objective of this technique is selective amplification of specific region of DNA molecule.

7. Fungal cells are treated with chitinase enzyme and plant cells are treated with cellulase enzyme.

8. When an alien gene is ligated at *Sal* I site of pBR322, the gene tet^R becomes non-functional and plasmid loses its tetracycline resistance. Hence, the cell possessing such recombinant pBR322 will not be able to grow on tetracycline.

9. Three types of restriction endonucleases are type I, type II and type III. Only type II restriction enzymes are used in *r*DNA technology.

10. The first restriction endonuclease isolated was *Hin*d II. Its recognition sequence is

$$5' - G - T - C \downarrow - G - A - C - 3'$$
$$3' - C - A - G - C - T - G - 5'$$

11. **(a) :** The bacterial cell is made competent by treating it with specific concentration of a divalent cation such as calcium to increase the efficiency with which DNA can enter the bacteria through pores of cell wall because DNA is a hydrophilic molecule and it cannot pass through cell membrane so making bacterial cell competent ease the process to take up DNA.

OR

(a) : In recombinant DNA technology, widely used host for replication and amplification of recombinant DNA are prokaryotic *E. coli* and the eukaryotic yeast. They replicate very fast to form a large population which express desired gene. Yeast artificial chromosome (YAC) are important cloning tools for the analysis of complex genome such as that of humans. They allow the maintenance, propagation and analysis of such genome in an experimentally tractable system, the yeast.

12. **(c) :** Selectable markers are the substances which help in identifying recombinants and eliminating non recombinants. Generally the genes encoding the resistance to antibiotics such as tetracycline, ampicillin, kanamycin or chloramphenicol, etc. are useful selectable markers for *E.coli*. The non-recombinant will grow on medium containing both tetracycline and ampicillin resistance antibiotics.

13. **(c) :** Restriction enzyme, a type of endonuclease, functions by "inspecting" the length of a DNA sequence. Once it finds a recognition sequence, it binds and cut each of the two strands of the double helix at specific point. A staggered cut generates two sticky ends and a straight cut generates blunt end. The staggered cut leaves single stranded portions at the ends which results in overhanging stretches called sticky ends. These are named so because they form hydrogen bonds with their complementary counter parts, *i.e.*, they can join similar complementary ends of DNA fragment from some other source with the help of DNA ligase. This stickiness of the ends facilitates the action of the enzyme DNA ligase, not DNA polymerase.

14. **(b)**

15. **(i) (a) :** In pBR322 plasmid, p – denotes that it is a plasmid; BR – stands for Boliver and Rodriguez, who constructed this plasmid; 322 – is a number given to distinguish this plasmid from others developed in the same laboratory.

(ii) (b)

(iii) (c)

(iv) (b) : Plasmid pBR322 has two resistance gene, *i.e.*, ampicillin resistance (amp^R) and tetracycline resistance (tet^R) which are considered useful for selectable markers.

(v) (d)

16. **(i) (a) :** Exonuclease removes nucleotides from the terminal ends (either 5′ or 3′) of DNA in one strand of duplex.

(ii) (b)

(iii) (c)

(iv) (a) : Type I restriction endonuclease consist of three different subunits and requires ATP, Mg^{2+}, S-adenosyl methionine for restriction.

17. Antibiotic resistance gene is a gene which is expressed in a microorganism. It is able to withstand the effect of that

specific antibiotic. In recombinant DNA technology, antibiotic resistant genes are used in the vectors as selectable markers to identify transformed hosts.

18. A bioreactor is a fermentor or natural or artificial (bioreactor vessel) in which raw materials are biologically converted into specific products microbes, plant and animal cell.

19. *Ori* is a sequence of DNA from where replication starts. Any piece of DNA that needs to replicate in the host cell has to be linked to it.
Cloning sites refer to the site sequence of DNA where the alien DNA is linked.

20. The replication of DNA is initiated from the specific DNA sequence called origin of replication. For multiplication of alien DNA in the host, it has to be integrated to the origin of replication (*ori*) site of plasmid.

21. The natural source of agarose is sea weed.
Role of agarose in biotechnology is as follows :
(i) It is used to develop the matrix for gel electrophoresis.
(ii) It helps in the separation of fragments on the basis of their size.

22. (a) A – Vector or plasmid DNA
B – Foreign DNA
(b) *Eco*RI restriction enzyme recognises the given palindrome.
(c) DNA ligase can link vector or plasmid DNA (A) and foreign DNA (B).

23. **(a)** If an alien gene is ligated at *Bam*HI site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid will lose its tetracycline resistance property.
(b) Uses of cloning vector in biotechnology is as follows :
(i) helps in linking the foreign/ alien DNA with that of host's DNA.
(ii) helps in the selection of recombinants from the non-recombinants due to presence of selectable markers.

24. **(a)** Exonucleases remove nucleotides from the terminal ends of the DNA whereas, endonucleases make cuts at specific positions within the DNA.
(b) Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbone. Each restriction endonuclease recognises a specific palindromic nucleotide sequences in the DNA.

25. **(a)** A bioreactor is a vessel in which raw materials are biologically converted into specific products by microbes, plant and animal cells and their enzymes. It provides optimal growth conditions (temperature, pH, substrate, salt, vitamins, oxygen) to the microbes for the synthesis of desired product. Advantage of bioreactor is that it is well suited for large-scale production of microorganisms under aseptic conditions for a number of days. A major drawback or disadvantage of bioreactor is that it is relatively expensive to run it.
(b) The most commonly used bioreactors are of stirring type and these are of two types :
(i) Simple stirred - tank bioreactor
(ii) Sparged stirred - tank bioreactor

OR

If a cloning vector does not have a selectable marker, then it would not be possible to distinguish between transformants (host bacterium having rDNA) and non-transformants. Therefore, an ideal cloning vector should have selectable markers for the selection of transformants.

26. **(a)** P-Origin of replication
Q-Ampicillin resistance gene (amp^R)
R-Tetracycline resistance gene (tet^R)
S-Cleavage site
(b) If foreign DNA is incorporated into Q *i.e.*, ampicillin resistance gene, then ampicillin resistance gene becomes non-functional and bacterial cell is unable to grow in presence of ampicillin.
(c) P represents origin of replication (*ori*). It is a specific sequence of DNA bases which is responsible for initiating replication.

OR

(a) X - Denaturation
Y - Annealing
Z - Extension
(b) Optimum temperature for X, *i.e.*, denaturation is 94°C to 96° C, Y, *i.e.*, annealing is 40°C to 60°C and Z, *i.e*, extension is 72°C.
(c) *Taq* polymerase, obtained from thermophilic bacteria *Thermus aquaticus* carries out the process Z, *i.e*, extension.

27. **(a)** 1-Exonuclease 2-Endonuclease, 3- Endonuclease
(b) 1. Exonuclease removes the nucleotide from the terminal ends of the DNA.
2. In 2, DNA is treated with endonuclease that makes cut at the specific site within the DNA and produce sticky ends.
3. In 3, DNA is treated with endonuclease that makes cut at the specific site within the DNA and produce blunt ends.

28. The polymerase chain reaction (PCR) was invented by Kary Mullis in 1985.
The working procedure of PCR is given as :
A single PCR amplification cycle involves three basic steps; denaturation, annealing and extension (polymerisation).
(i) Denaturation : In this step, the target DNA is heated to

a high temperature (usually 94° to 96°C), resulting in the separation of the two strands. Each single strand of the target DNA then acts as a template for DNA synthesis.
(ii) Annealing : In this step, the two oligonucleotide primers hybridise to each of the single stranded template DNA since the sequence of the primers is complementary to the 3' ends of the template DNA. This step is carried out at a lower temperature (usually 40° to 60°C) depending on the length and sequence of the primers.
(iii) Extension : The final step is extension, wherein *Taq* DNA polymerase (obtained from *Thermus aquaticus*) synthesises the DNA region between the primers, using dNTPs (deoxynucleoside triphosphate) and Mg^{2+}. It means the primers are extended towards each other so that the DNA segment lying between the two primers is copied. The optimum temperature for this polymerisation step is 72°C.

29. **(a)** Cloning vectors are DNA molecules which can multiply independently of the host DNA and increase their copy number along with the foreign DNA attached to them. Cloning vectors may be plasmids, bacteriophages, cosmids, viruses, etc.
Functions :
(i) They help in linking the foreign DNA with that of the host.
(ii) They also help in the selection of recombinants from the non-recombinants.
(b) DNA ligase joins two individual fragments of DNA by the formation of phosphodiester bond between them.

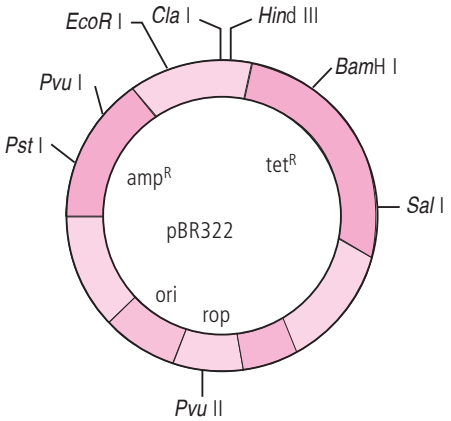
30. Both endonuclease and exonuclease are cleaving enzymes. Endonuclease cleaves DNA at any point except the terminal ends and can make cut on one strand or on both strands of double stranded DNA whereas exonuclease cleaves base pairs of DNA at their terminal ends and act on single strand of DNA or gaps in double stranded DNA.
Sal I and *Hin*d II are two examples of endonucleases.

31. (i) pBR322 and pUC19 both are plasmid vectors.
pBR322 - It contains two antibiotic resistant genes
pUC19 - It contains lac Z gene
(ii) M13 : It is circular and single stranded DNA. Lambda phage : It is linear, double stranded DNA.
(iii) Cosmid : It has features of plasmid and cos sites of phage lambda. It has insert size upto 45 Kb.
Plasmid : It is small circular, extra-chromosomal self replicating naturally present in bacteria. It has insert size 0.5-8 Kb.
(iv) Transformation : Recombinant DNA introduced after treating competent bacterial cell with cold $CaCl_2$.
Transfection : Transfer of rDNA into host cell by mixing foreign DNA with charged substances like calcium phosphate.
(v) BAC or Bacterial artificial chromosome vector : Insert size in BAC is – 300 – 350 Kb.
YAC or yeast artificial chromosome vector : Insert size in YAC is more than 1000 Kb.

OR

(a) Restriction endonuclease producing blunt and sticky ends are :
(i) Blunt ends : Restriction enzyme cuts the DNA in a way that fragments cannot bind together, *e.g.*, *Alu* I
(ii) Sticky ends : Cohesive or sticky ends are annealing and easier to ligate in making recombinant vector, *e.g.*, *Eco*RI
(b) (i) Insulin production is 100 mg/L, so fermentor volume needed for 1 Kg of insulin in 1 Kg/mg = 10000 L; So we need 10,000 litre, fermentor to produce 1 kilogram of insulin in one batch.
(ii) In this case the cell concentration is increased to 50g/L ; so insulin production per litre will be 50 × 100 = 5000 mg = 5-g/L
Thus to produce 1 Kilogram of insulin we need 1 Kilogram/5g = 1000g/5g = 200g. So if the concentration is increased 50 times, we need 200 litre reactor to produce 1 Kilogram of insulin.

32. **(a)** The host cells which have taken up rDNA and express it, are called transformants and those that have not taken up the rDNA are called non-transformants. The selection of transformants depends on the expression or non-expression of certain traits. Insertional inactivation is a method of identification of recombinants or transformants. It is the method in which the insertion of rDNA into the coding sequence of an enzyme, disrupts the coding sequence and leads to inactivation of the enzyme. Insertional inactivation of an antibiotic resistance gene can be seen in *E.coli* cloning vector pBR322. It has two antibiotic resistance genes *i.e.*, for tetracycline and ampicillin. If the rDNA is inserted at the *Bam*HI site of tetracycline resistance gene, the recombinant plasmid will lose the tetracycline resistance due to insertional inactivation and do not grow in tetracycline medium.
(b) Diagram showing parts of plasmid pBR322



Nomenclature of restriction enzymes.
(i) Type II restriction enzymes are named for the bacterium from which they have been isolated.
(ii) The first letter used for the enzyme is the first letter of the bacterium's genus name (in italics).
(iii) Then comes the first two letters of its species (also in italics).
(iv) The fourth letter of the name of enzyme is first letter of the strain. It is written in capital.
(v) The end of the name indicates the order in which the enzyme was isolated. It is written in Roman number. For example, the enzyme *Eco*RI was isolated from the bacterium *Escherichia coli* RY13. Enzyme *Eco*RI is named as follows. The capital letter E comes from the genus *Escherichia*. The letters co are from the species *coli*. The letter R is from RY13 (strain). The Roman number 1 indicates that it was the first enzyme isolated from the bacterium *E.coli RY13*. The discovery of restriction endonuclease enzymes led to Nobel Prize for W. Arber, H. Smith and D. Nathan in 1978.

33. **(a)** *E.coli* has been most widely used host cell for gene cloning because
(i) It is easy to culture and handle under laboratory conditions.
(ii) It can accept wide variety of vectors.
(iii) It exists in a variety of genetically defined strains.
(iv) Under ideal growth conditions, its generation time is only 20 minutes, *i.e.*, the cells double in every twenty minutes and within hours, millions of cells containing the rDNA are produced.
(b) Bacteriophages, because they have very high copy number of their genome within the bacterial cells whereas some plasmids may have only one or two copies per cell and other may have 15-100 copies per cell.
(c) *Bam*HI enzyme should be used, as restriction site for this enzyme is present in tet^R region. *Pvu*I will not be used, as restriction site for this enzyme is present in amp^R region. *Eco*RI will not be used, as restriction site for this enzyme is not present in selectable marker.

OR

Direct or vectorless gene transfer is the process of gene transfer into the host cell without using a vector. This may be done by either of the following four methods :
(i) Microinjection : In this method, foreign DNA is directly injected into the nucleus of animal or plant cell by using microneedles or micropipettes. It is used to transfer gene in oocytes, eggs and embryos.
(ii) Electroporation : In this method, electrical impulses induce formation of transient (temporary) pores in the plasma membrane of host cells by using calcium chloride. These pores of the membrane are the areas through which the DNA molecules are incorporated into the host cells.
(iii) Chemical mediated gene transfer : In this method, chemicals such as polyethylene glycol helps foreign DNA to enter the host cell.
(iv) Biolistic or gene gun method : DNA is coated onto microscopic gold or tungsten pellets which are bombarded with high velocity into the target cells as microprojectiles. Although this technique is suitable for plants, but it is also used to insert genes into animal cells, that promote tissue repair.

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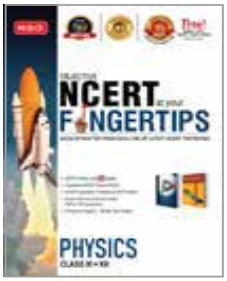

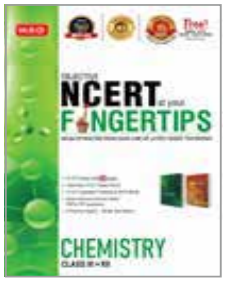

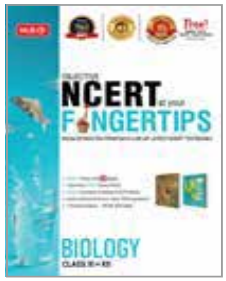

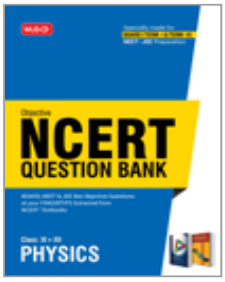

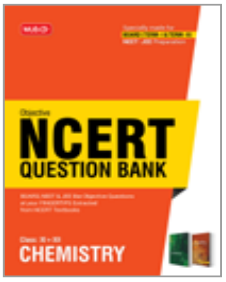

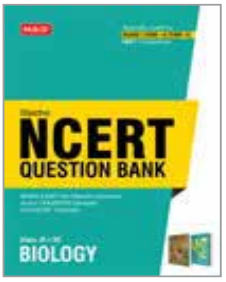

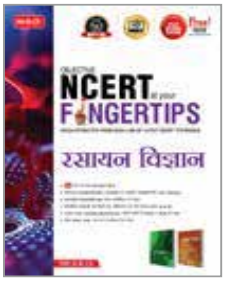

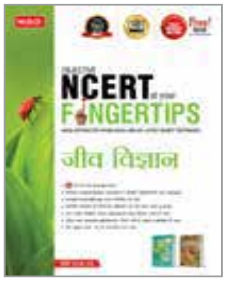

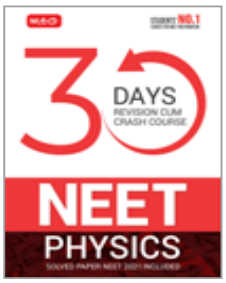

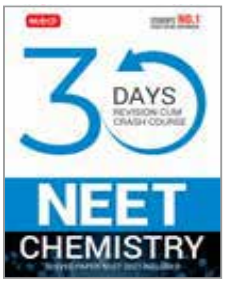

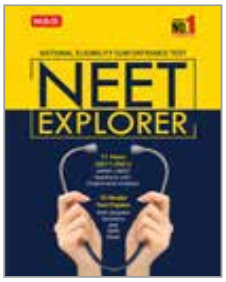

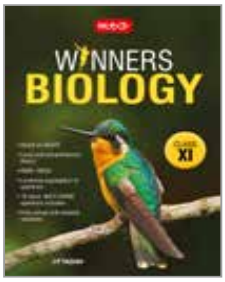

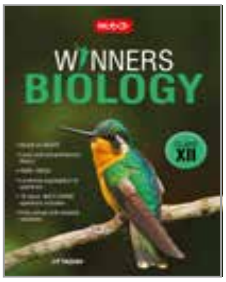

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