11

Biotechnology Principles and Processes

Q. 1 Rising of dough is due to

(a) multiplication of yeast (b) production of CO_2
(c) emulsification (d) hydrolysis of wheat flour starch into sugars

Thinking Process

Dough is formed by fermentation by bacteria.

Ans. ***(b)*** Inoculation of kneaded flour with baker yeast; *Saccharomyces cerevisiae* produce CO_2 during the process of fermentation, causes puffing up of the dough and make it soft and spongy. It is used to make foods like idli, dosa, bread, etc.

Q. 2 An enzyme catalysing the removal of nucleotides from the ends of DNA is

(a) endonuclease (b) exonuclease (c) DNA ligase (d *Hind* II

Ans. ***(b)*** *Restriction enzymes belongs to a class of enzymes called nucleases and are of two types*

(i) **Exonucleases** remove nucleotides from the ends of the DNA.
(ii) **Endonucleases** make cuts at specific positions within the DNA.

DNA ligase is a sealing enzymes (also called as genetic gum), which is responsible for joining of two individual fragments of DNA, whereas *Hind* II is first discovered restriction endonuclease enzyme.

Q. 3 The transfer of genetic material from one bacterium to another through the mediation of a vector like virus is termed as

(a) transduction (b) conjugation (c) transformation (d) translation

Ans. ***(a)*** Transduction is the process by which genetic material (DNA) is transferred from one bacterium to another through the mediation of a vector, like virus

Other option are incorrect because bacterial conjugation is the transfer of genetic material (plasmid) between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells.

Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membranes.

Translation is the process in which cellular ribosomes create proteins. It is a part of the process of gene expression.

Q. 4 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.

Thinking Process

Gel electrophoresis is a technique for separating DNA fragments based on their size.

Ans. ***(d)*** The separated DNA fragments (by the process of gel electrophoresis) are visualised after staining the DNA with ethidium bromide followed by exposure to UV-radiation. These fragments are seen as orange coloured bands.

Q. 5 'Restriction' in restriction enzyme refers to

(a) cleaving of phosphodiester bond in DNA by the enzyme
(b) cutting of DNA at specific position only
(c) prevention of the multiplication of bacteriophage in bacteria
(d) All of the above

Ans. ***(b)*** The restriction enzymes are called 'molecular scissors' and are responsible for cutting DNA. Restriction enzymes belong to a class of enzymes called nucleases.

They are of two types

(i) **Exonucleases** Cut DNA at the ends

(ii) **Endonucleases** Make cuts at specific positions within the DNA.

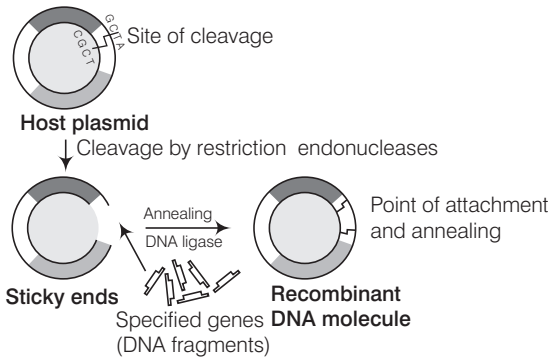
These enzymes are present in bacteria to provide a type of defense mechanism called the 'restriction modification system'.

This system consist of two components, restriction enzyme and modification enzyme. The term 'restriction' refer to the function of these enzyme in restricting the propagation of foreign DNA of bacteriophage in host bacterium, *i.e.*, cutting of DNA, at specific position only.

Q. 6 Which of the following is not required in the preparation of a recombinant DNA molecules?

(a) Restriction endonucleases (b) DNA ligase
(c) DNA fragments (d) *E. coli*

Ans. ***(d)*** Restriction enzymes and DNA ligases can be used to make a stable recombinant DNA molecule, with DNA fragments that has been spliced together from two different organisms.



Q. 7 In agarose gel electrophoresis, DNA molecules are separated on the basis of their

(a) charge only (b) size only (c) charge to size ratio (d) All of these

Thinking Process

*Gel electrophoresis, developed by **A Tiselws** is used in separation of molecule like protein, DNA and RNA. Agarose is commonly used matrix (polysaccharides) in this technique (polysaccharides).*

Ans. ***(b)*** In agarose gel electrophoresis, the DNA fragments separate out (resolve) according to their size or length because of the sieving property of agarose gel. It means, the smaller the fragment size, the farther it will move.

Q. 8 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

Ans. ***(a)*** All of the given features are important to facilitate cloning into a vector but out of them Origin of replication (*ori*) is the most important one.

This is due to the following reasons

(i) *Ori* is a DNA sequence that is responsible for initiating replication. Any piece of DNA when linked to this sequence can replicate within the host cells.

(ii) *Ori* also controls the copy numbers of the linked DNA.

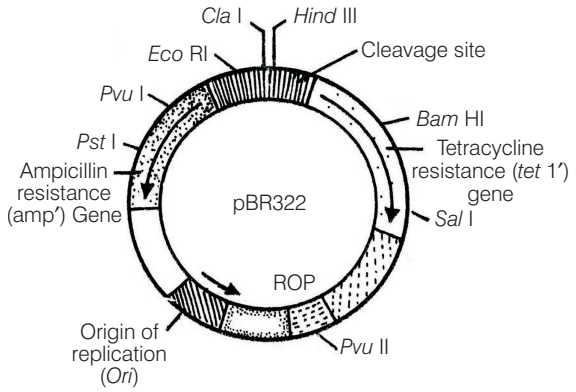


Diagram showing essential features of plasmid pBR322

Q. 9 While isolating DNA from bacteria, which of the following enzymes is not used?

(a) Lysozyme (b) Ribonuclease
(c) Deoxyribonuclease (d) Protease

Ans ***(c)*** In the process of 'recombinant DNA technology' the first step is isolation of DNA.

Since, the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids.

This can be achieved by treating the bacterial cells/plant of animal tissue with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells) and **chitinase** (fungus).

As we know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease, whereas proteins can be removed by treatment with protease.

Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. Deoxyribonuclease is not used in this process as this enzyme causes the lysis of DNA molecules.

Q. 10 Which of the following has popularised the PCR (Polymerase Chain Reaction)?

(a) Easy availability of DNA template
(b) Availability of synthetic primers
(c) Availability of cheap deoxyribonucleotides
(d) Availability of 'Thermostable' DNA polymerase

Ans. ***(d)*** The Polymerase Chain Reaction (PCR) is a reaction in which amplification of specific DNA sequences is carried out *in vitro*. Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus*), which remain active and stable during the high temperature and induced denaturation of double-standard DNA.

Q. 11 An antibiotic resistance gene in a vector usually helps in the selection of

(a) competent cells
(b) transformed cells
(c) recombinant cells
(d) None of these

Thinking Process

The genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered as useful selectable markers for E. coli.

Ans. ***(b)*** Selectable markers help in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. The normal *E. coli* cells do not carry resistance against any of these antibiotics. Competant bacterial cells are made capable to take foreign DNA with chemical treatment, *e.g.,* calcium chloride.

Note *In process of transformation, a piece of DNA is introduced in a host bacterium.*

Q. 12 Significance of heat shock method in bacterial transformation is to facilitate.

(a) Binding of DNA to the cell wall
(b) Uptake of DNA through membrane transport proteins
(c) Uptake of DNA through transient pores in the bacterial cell wall
(d) Expression of antibiotic resistance gene

Thinking Process

DNA being a hydrophilic molecule cannot pass through cell membranes. Therefore, the bacteria should be made competent to accept the DNA molecules by chemical and physical methods.

Ans. ***(c)*** In chemical method, the cell is treated with specific concentration of a divalent cation such as calcium to increase pore size in cell wall. The cells are incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice. This is called heat shock method. The bacteria now takes up the recombinant DNA.

Q. 13 The role of DNA ligase in the construction of a recombinant DNA molecule is

(a) formation of phosphodiester bond between two DNA fragments
(b) formation of hydrogen bonds between sticky ends of DNA fragments
(c) ligation of all purine and pyrimidine bases
(d) None of the above

Ans. ***(a)*** DNA ligase (joining or sealing enzymes) are also called genetic gum. They join two individual fragments of double-stranded DNA by forming phosphodiester bonds between them. Thus they help in sealing gaps in DNA fragments. Therefore, they act as a molecular glue.

Q. 14 Which of the following is not a source of restriction endonuclease?

(a) *Haemophilus influenzae* (b) *Escherichia coli*
(c) *Agrobacterium tumefaciens* (d) *Bacilius amyloli*

Ans. ***(c)*** *Agrobacterium tumefaciens* is a pathogen of several dicot plants. It delivers a piece of DNA known as 'T-DNA' in the Ti plasmid which transforms normal plant cells into tumour cells to produce chemicals against pathogens.

The restriction enzyme *Eco* RI, is isolated from *Escherichia coli* RY13.

The first restriction enzymes *Hind* II was isolated from bacterium *Haemophilus influenza*e. The restriction enzyme *Bam* HI is isolated from *Bacillus amyloli.*

Q. 15 Which of the following steps are catalysed by *Taq* polymerase in a PCR reaction?

(a) Denaturation of template DNA
(b) Annealing of primers to template DNA
(c) Extension of primer end on the template DNA
(d) All of the above

Ans. ***(c)*** In polymerase chain reaction polymerisation or extension step is catalysed by *Taq* polymerase enzyme. *PCR is carried out in the following three steps*

(i) **Denaturation** The double-stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated single stranded strand now acts as template for DNA synthesis.

(ii) **Annealing** Two sets of primers are added which anneal to the 3 end of each separated strand. Primers act as initiators of replication.

(iii) **Extension** DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction.

A thermostable DNA polymerase (*Taq* DNA polymerase) is used in the reaction which can tolerate the high temperature of the reaction.

All these steps are repeated many times to obtain several copies of desired DNA.

Q. 16 A bacterial cell was transformed with a recombinant DNA that was generated using a human gene. However, the transformed cells did not produce the desired protein. Reasons could be

(a) human gene may have intron which bacteria cannot process
(b) amino acid codons for humans and bacteria are different
(c) human protein is formed but degraded by bacteria
(d) All of the above

Thinking Process

Introns are parts of genes that do not directly code for proteins. There are commonly found in multicellular eukaryotes, such as humans and rare in bacteria.

Ans. ***(a)*** The process of making recombinant DNA molecule involves the introduction of a desired gene into the DNA of a host that will produce the desired protein.

Inducing a cloned eukaryotic gene to function in a prokaryotic host can be difficult sometime. The presence of long non-coding introns in eukaryotic genes may prevent correct expression of these genes in prokaryotes, which lack RNA-splicing machinery.

Q. 17 Which of the following should be choosen for best yield if one were to produce a recombinant protein in large amounts?

(a) Laboratory flask of largest capacity
(b) A stirred-tank bioreactor without in-lets and out-lets
(c) A continuous culture system
(d) Any of the above

Ans. ***(c)*** If any protein encoding gene is expressed in a heterologous host. it is called a recombinant protein. The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory.

The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

The cells can also be multiplied in a continuous culture system where in the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase. This type of culturing method produces a larger biomass leading to higher yields of desired protein.

Q. 18 Who among the following was awarded the Nobel Prize for the development of PCR technique?

(a) Herbert Boyer
(b) Hargovind Khurana
(c) Kary Mullis
(d) Arthur Kornberg

Ans. ***(c)*** PCR (Polymerase Chain Reaction) technique was developed by **Kary Mullis** in 1985, and for this he received Nobel Prize for chemistry in 1993. **HG Khurana** discovered DNA ligase enzyme in to phage in 1969.

White DNA polymerase was discovered by **Arthur Kornberg** and **Herbert Boyer** generated first recombinant DNA molecule by combining a gene from a bacterium with plasmid of *E. coli* in 1972.

PCR (Polymerase Chain Reaction) technique is a reaction in which amplification of specific DNA sequences is carried out *in vitro.*

Q. 19 Which of the following statements does not hold true for restriction enzyme?

(a) It recognises a palindromic nucleotide sequence
(b) It is an endonuclease
(c) It is isolated from viruses
(d) It produces the same kind of sticky ends in different DNA molecules

Ans. ***(c)*** The restriction enzymes are called 'molecular scissors' and are responsible for cutting DNA on specific sites. These are not found in viruses.

They are present in bacteria to provide a type of defense mechanism called the 'restriction modification system' and the so called system consist of two component; restriction enzymes and modification enzyme.

The first component include restriction endonuclease, which identify the introduced foreign DNA and cut it into process. Same kind of sticky end in different individual molecule of DNA are also produced by these molecular scissors special sequence in the DNA recognised by restriction endonuclease is called palindromic nucleotide sequence.

Very Short Answer Type Questions

Q. 1 How is copy number of the plasmid vector related to yield of recombinant protein?

Ans. The recombinant DNA can multiply as many times as the copy number of the vector plasmid thereby determining the yield of recombinant protein. So, higher the copy number of plasmid vector, higher the copy number of gene and consequently, protein coded by the gene is produced in high amount.

Q. 2 Would you choose an exonuclease, while producing a recombinant DNA molecule?

Ans. No, as exonuclease acts on the free ends of linear DNA molecule. Therefore, instead of producing DNA fragments with sticky ends, it will shorten or completely degrade the DNA fragment containing the gene of interest and the circular plasmid (vector) will not get cut as it lacks free ends.

Q. 3 What does *H* in '*d*' and III refer to the enzyme *Hind* III?

Ans. **(i)** The first letter '*H*' indicates the genus of the organism from which the enzyme was isolated, *H* = genus *Haemophilus*.

(ii) The fourth letter d indicates the particular strain used to produce the enzyme, *d* = strain Rd.

(iii) The Roman numerals denoted the sequence in which the restriction endonuclease enzyme from that particular genus, species and strain of bacteria have been isolated-III, *i.e.,* third restriction endonuclease to be isolated from this species.

Q. 4 Restriction enzymes should not have more than one site of action in the cloning site of a vector. Comment.

Ans. If the restriction enzymes have more than one recognition site in a vector, then the vector itself will get fragmented on treatment with the restriction enzymes.

Q. 5 What does 'competent' refer to in competent cells used in transformation experiments?

Ans. DNA being a hydrophilic molecule can not pass through cell membranes. Therefore, the bacteria should be made competent to accept the DNA molecules.

Competent means bacterial cells, on treatment with chemicals like $CaCl_2$, are made capable of taking up foreign DNA.

Q. 6 What is the significance of adding proteases at the time of isolation of genetic material (DNA)?

Ans. Proteases degrade the proteins present inside a cell (from which DNA is being isolated). If the proteins are not removed from DNA preparation then they could interfere with any downstream treatment of DNA.

Q. 7 While doing a PCR, 'denaturation' step is missed. What will be its effect on the process?

Ans. If denaturation of double-stranded DNA does not take place then primers will not be able to anneal (joining) to the template. Hence, no extension will take place and after are there will be no amplification.

Q. 8 Name a recombinant vaccine that is currently being used in vaccination program.

Ans. Hepatitis-B recombinant vaccine (engerix) is used for vaccination of hapatitis virus.

Q. 9 Do biomolecules (DNA and protein) exhibit biological activity in anhydrous conditions?

Thinking Process

Water is critical not only for the correct folding of proteins but also for the maintenance of the structure of DNA and protein.

Ans. Biomolecules (DNA, and protein) exhibit change in biological activity in anhydrous conditions, In non-aqueous or anhydrous conditions the rigidity of protein and DNA increases due to the weakening of hydrogen bond strength.

It results into the change in overall free energy, which is the combined effects of the exposure of the interior polar and non-polar groups and their interaction with water. In absence of aqueous condition, the free energy change is negative, which is responsible for the denaturation of biomolecules.

Increasing strength of hydrogen-bond causes water to primarily bond with itself and not to be available for the hydrating structure of proteins or DNA, or for dissolving ions.

On the other hand, if the water-water hydrogen bond strength reduces then the exchange mechanisms operating within the cell, such as hydrogen bonded water chains within and between proteins and DNA, will become non-operational. It will further leads to the denaturation.

Q. 10 What modification is done on the Ti-plasmid of *Agrobacterium tumefaciens* to convert it into a cloning vector?

Thinking Process

T-DNA is the only essential part required to make Ti-plasmid a cloning vector.

Ans. The plasmid is disarmed by deleting the tumour inducing genes in the plasmid. So, that it become an effective cloning vector. The modified tumour inducing (Ti) plasmid of *Agrobacterium tumefaciens* will no longer remain pathogenic to the plants but still deliver genes of interest into a variety of plants.

Short Answer Type Questions

Q. 1 What is meant by gene cloning?

Ans. Gene cloning refers to a process in which a gene of interest is ligated to a vector. The recombinant DNA thus produced is introduced in a host cell by transformation.

Each cell gets one DNA molecule and when the transformed cell grows to a bacterial colony, each cell in the colony has a copy of the gene. This is gene cloning.

Q. 2 Both a wine maker and a molecular biologist who had developed a recombinant vaccine claim to be biotechnologists. Who in your opinion is correct?

Ans. In my opinion both of them are correct. As biotechnology is a very wide area which deals with techniques of using a 'natural' organism (or its parts) as well as genetically modified organism to produce and processes useful for mankind.

A wine maker employs a strain of yeast to produce wine by fermentation (a natural phenomenon), while the molecular biologist has cloned gene for the antigen (that is used as vaccine) in an organism which allows the production of the antigen in large amount.

Q. 3 A recombinant DNA molecule was created by ligating a gene to a plasmid vector. By mistake, an exonuclease was added to the tube containing the recombinant DNA. How does this affect the next step in the experiment, *i.e.,* bacterial transformation?

Thinking Process

Bacterial transformation is the process by which bacterial cells take up naked DNA molecules (exogenous or foreign DNA).

Ans. The experiment will not likely to be affected as recombinant DNA molecule is circular and closed, with no free ends. Hence, it will not be a substrate for exonuclease enzyme which removes nucleotides from the free ends of DNA.

Q. 4 Restriction enzymes that are used in the construction of recombinant DNA are endonucleases which cut the DNA at 'specific'-recognition sequence? What would be the disadvantage if they do not cut the DNA at specific-recognition sequence?

Ans. If the restriction enzymes would cut DNA at random sites instead of at specific sites, then the DNA fragments obtained will not have 'sticky ends'. In the absence of sticky ends, construction of recombinant DNA molecule would not be possible.

Q. 5 A plasmid DNA and a linear DNA (both are of the same size) have one site for a restriction endonuclease. When cut and separated on agarose gel electrophoresis, plasmid shows one DNA b and, while linear DNA shows two fragments. Explain.

Ans. When a plasmid DNA and a linear DNA having one site for a restriction endonuclease are cut and separated, plasmid shows one DNA band, while linear DNA shows two band because of difference in their basic structure.

Plasmid is a circular DNA molecule and when cut with these enzyme, it becomes linear but does not get fragmented due to presence of only one restriction site, whereas a linear DNA molecule gets cut into two fragment.

Q. 6 How does one visualise DNA on an agarose gel?

Ans. A compound called ethidium bromide stains DNA, which on exposure with ultra-violet, (uv) radiation gives orange light band of DNA. Hence, DNA fragments appear as orange band in the presence of ethidium bromide and UV light.

Q. 7 A plasmid without a selectable marker was choosen as vector for cloning a gene. How does this affect the experiment?

Ans. In a gene cloning experiment, first a recombinant DNA molecule is constructed, where the gene of interest is ligated to the vector (the step would not be affected) and introduced inside the host cell (transformation).

Since, not all the cells get transformed with the recombinant/plasmid DNA, in the absence of selectable marker, it will be difficult to distinguish between transformants and non-transformant, because role of selectable marker is in the selection of transformants.

Q. 8 A mixture of fragmented DNA was electrophoresed in an agarose gel. After staining the gel with ethidium bromide, no DNA bands were observed. What could be the reason?

Ans. *The reasons are as follows*

(i) DNA sample that was loaded on the gel may have got contaminated with nuclease (exo or endo both) and completely degraded.

(ii) Electrodes were put in opposite orientation in the gel assembly that is anode towards the wells (where DNA sample is loaded). Since, DNA molecules are negatively charged, they move towards anode and hence, move out of the gel instead of moving into the matrix of gel.

(iii) Ethidium bromide was not added at all or was not added in sufficient concentration and so DNA was not visible.

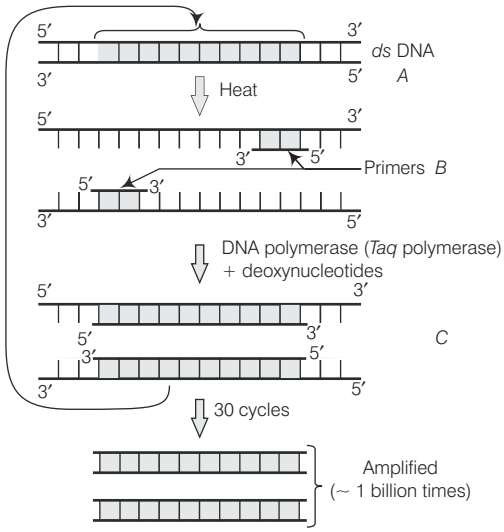
Q. 9 Describe the role of $CaCl_2$ in the preparation of competent cells?

Ans. $CaCl_2$ is known to increase the efficiency of DNA uptake to produce transformed bacterial cells. The divalent Ca^{+2} ions create transient pores on the bacterial cell wall by which the entry of foreign DNA is facilitated into the bacterial cells.

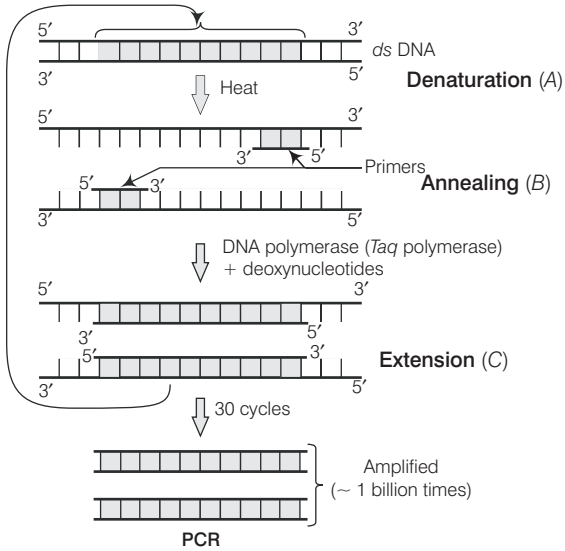
Q. 10 What would happen when one grows a recombinant bacterium in the bioreactor but forget to add antibiotic to the medium in which the recombinant is growing?

Ans. In the absence of antibiotic, there will be no pressure on recombinants to retain the plasmid (containing the gene of our interest). Since, maintaining a high copy number of plasmids is a metabolic burden to the microbial cells, it will thus tend to loose the plasmid.

Q. 11 Identify and explain *A, B* and C in the PCR diagram given below.



Ans. Region to be amplified



In PCR, each cycle has three steps

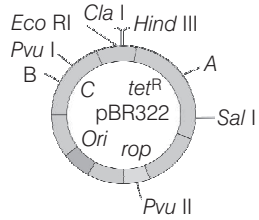
(i) **Denaturation of DNA Sample** Unwinding of two strand of DNA by heating the sample at 92-94°C.

(ii) **Primer Annealing** Primers get positioned on the exposed nucleotides as per base pairing rules.

(iii) **Extension of Primers** DNA polymerase recognises primers as 'start' tags and begins to extend the primers using the free nucleotides provided in the reaction and the genomic DNA as template.

With each round of reactions, the DNA doubles.

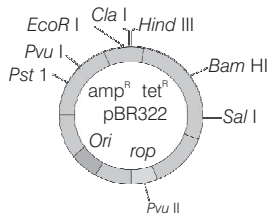
Q. 12 Name the regions marked *A, B* and *C*.



Ans. **Region** A *Bam* HI
Region *B Pot* I
Region C amp^{R}.

E. coli cloning vector pBR322 showing restriction sites (*Hind* III, *Eco* RI, *Bam* HI, *Sal* I, *Pvu* II, *Pst* I, *Cla* I), *Ori* and antibiotic resistance genes (amp^{R} and tet^{R}).

Rop codes for the proteins involved in the replication of the plasmid.



Long Answer Type Questions

Q. 1 For selection of recombinants, insertional inactivation of antibiotic marker has been supercoded by insertional inactivation of a marker gene coding for a chromogenic substrate. Give reasons.

Ans. In selection of recombinants due to inactivation of antibiotics, the transformed cells are first plated on the antibiotic plate which has not been insertionally inactivated (*i.e.,* ampicillin) and incubated overnight for growth of transformants.

For selection of recombinants, these transformants are replica-plated on second antibiotic (*say*, tetracycline) plate (which got inactivated due to insertion of gene).

Non-recombinants grow on both the plates (one carrying ampicillin and the other carrying tetracycline) while recombinants will grow only on ampicillin plate.This entire exercise is labourious and takes more time (two overnight incubation) as well.

However, if we choose insertional inactivation of a marker that produces colour in the presence of a chromogenic compound, we can distinguish between the recombinants and non-recombinants on a single medium plate (containing one antibiotic and the chromogenic compound) after overnight growth.

Q. 2 Describe the role of *Agrobacterium tumefaciens* in transforming a plant cell.

Ans. A soil-inhabiting, plant pathogenic bacterium, *Agrobacterium tumefaciens*, infects broad-leaved crops including tomato, soyabean, sunflower and cotton, but not the cereals. It causes tumours called **crown galls**.

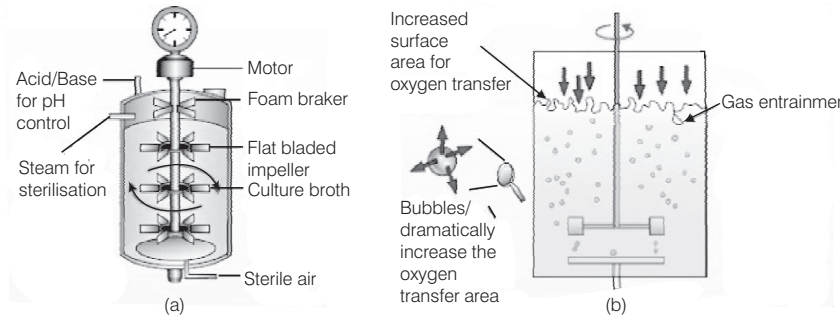
Tumour formation is induced by its plasmid, which is, therefore called **Ti-plasmid** (Ti for tumour inducing). The Ti-plasmid integrates a segment of its DNA, termed T-DNA, into the chromosomal DNA of its host plant cells. The T-DNA causes tumours. As gene transfer occurs without human effort, the bacterium is known as **natural genetic engineer** of plants.

Plant molecular biologists have started using Ti-plasmids as vectors to transfer foreign genes of interest into the target plant cells. They use a version of the plasmid from which tumour forming gene has been eliminated. The transformed bacteria do not cause disease, but still deliver genes of interest into a variety of plants.

Q. 3 Illustrate the design of a bioreactor. Highlight the difference between a flask in your laboratory and a bioreactor which allows cells to grow in a continuous culture system.

Ans. Bioreactors are vessels of large volumes (100-1000 L) in which raw materials are biologically converted into specific products.

The most commonly used bioreactors are of stirring type, which are shown in figure.



(a) Simple stirred-tank bioreactor

(b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor.

If you look at the figure closely you will see that the bioreactor has an agitator system, an oxygen delivery system and a form control system, a temperature control system, pH control system and sampling ports so, that small volumes of the culture can be withdrawn periodically.

Small volume cultures are usually employed in laboratories in a flask for research and production of less quantities of products. However, large scale production of the products is carried out in bioreactors.