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Biotechnology: Principles and Processes

TOPIC 1
Tools of Recombinant DNA Technology

01 Plasmid pBR322 has Pst I restriction enzyme site within gene *amp*^{R} that confers ampicillin resistance. If this enzyme is used for inserting a gene for β-galactoside production and the recombinant plasmid is inserted in an *E.coli* strain **[NEET 2021]**

(a) it will not be able to confer ampicillin resistance to the host cell
(b) the transformed cells will have the ability to resist ampicillin as well as produce β-galactoside
(c) it will lead to lysis of host cell
(d) it will be able to produce a novel protein with dual ability

Ans. ***(a)***

In plasmid vector pBR322, two unique restriction sites PstI arid PvuI are located within the amp^R gene and *Bam*HI, *Sal*I, etc., are located within the tet^R gene. The presence of restriction sites within the marker genes tet^R and amp^R permits an easy selection for cells transformed with the I recombinant pBR322. When restriction enzyme *Bam*HI or *Sal*I is used, the DNA insert is placed within the gene tet^R making it non-functional.

If this enzyme is used for inserting a gene for β-galactoside production and the recombinant plasmid is inserted in an *E.coli* strain, it will not be able to confer ampicillin resistance to the host cell.

02 A specific recognition sequence identified by endonucleases to make cuts at specific positions within the DNA is **[NEET 2021]**

(a) degenerate primer sequence
(b) Okazaki sequences
(c) palindromic nucleotide sequence
(d) poly(A) tail sequence

Ans. ***(c)***

Palindromic nucleotide sequence is a specific recognition sequence in a double-standard DNA and RNA molecules that is identified by endonucleases to make cuts at specific positions. The sequence is the same when one strand is read from left to right and the other strand is read from right to left.

03 Match the following techniques or instruments with their usage. **[NEET (Oct.) 2020]**

	Column I		Column II
A.	Bioreactor	(i)	Separation of DNA fragments
B.	Electroph-oresis	(ii)	Production of large quantities of products
C.	PCR	(iii)	Detection of pathogen, based on antigen-antibody reaction
D.	ELISA	(iv)	Amplification of nucleic acids

Select the correct option.

	A	B	C	D
(a)	(iii)	(ii)	(iv)	(i)
(b)	(ii)	(i)	(iv)	(iii)
(c)	(iv)	(iii)	(ii)	(i)
(d)	(ii)	(i)	(iii)	(iv)

Ans. ***(b)***

Option (b) is correct match which is as follows

Bioreactors are used for the industrial scale production of products.
Electrophoresis helps in the separation of DNA fragments based on their size.
PCR helps to amplify or generate large number of copies of nucleic acids.
ELISA helps in the detection of pathogens based on the principle of antigen-antibody interaction.

04 First discovered restriction endonuclease that always cuts DNA molecule at a particular point by recognising a specific sequence of six base pairs is **[NEET (Oct.) 2020]**

(a) *Eco* RI
(b) Adenosine deaminase
(c) Thermostable DNA polymerase
(d) *Hind* II

Ans. ***(d)***

Hind II was the first discovered endonuclease. It was isolated by Smith Wilcox and Kelley (1968) from *Haemophilus influenzae* bacterium. It always cuts bacterium. DNA at particular point by recognising a specific sequence of six base pairs. It is known as the recognition sequence for *Hind* II and reads as 5′-GTC GAC-3′, 3′-CAG CTG-5′.

05 Choose the correct pair from the following. **[NEET (Sep.) 2020]**

(a) Polymerases	Break the DNA into fragments
(b) Nucleases	Separate the two strands of DNA
(c) Exonucleases	Make cuts at specific positions within DNA
(d) Ligases	Join the two DNA molecules

Ans. *(d)*

The correct pair is option (d). Rest option can be corrected as

The main function of DNA polymerase is to synthesise DNA from deoxyribonucleotides, the building blocks of DNA.

Nucleases hydrolyse the phosphodiester bonds of DNA and RNA.

Exonucleases are a broad class of enzymes that cleave off nucleotides one at a time from the 3′ or 5′ ends of DNA and RNA chains.

06 Identify the wrong statement with regard to restriction enzymes. **[NEET (Sep.) 2020]**

(a) They cut the strand of DNA at palindromic sites
(b) They are useful in genetic engineering
(c) Sticky ends can be joined by using DNA ligases
(d) Each restriction enzyme functions by inspecting the length of a DNA sequence

Ans. *(c)*

Statement in option (c) is incorrect. It can be explained as follows.

Restriction endonucleases make cuts at specific positions within the DNA known as palindromic sites. They function by inspecting the length of a DNA sequence. They are used in genetic engineering to form recombinant molecules of DNA.

DNA ligases join the DNA fragments.

07 A selectable marker is used to **[NEET (Odisha) 2019]**

(a) help in eliminating the non-transformants, so that the transformants can be regenerated
(b) identify the gene for a desired trait in an alien organism
(c) select a suitable vector for transformation in a specific crop
(d) mark a gene on a chromosome for isolation using restriction enzyme

Ans. *(a)*

To facilitate cloning into a vector, the vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

08 The two antibiotic resistant genes on vector pBR322 are for **[NEET (Odisha) 2019]**

(a) Ampicillin and Tetracycline
(b) Ampicillin and Chloramphenicol
(c) Chloramphenicol and Tetracycline
(d) Tetracycline and Kanamycin

Ans. *(a)*

The two antibiotic resistance gene on *E.coli* cloning vector pBR322 are for ampicillin and tetracycline. Cloning vectors are DNA molecules that carry a foreign DNA segment and replicate inside host cell. Plasmid in *E.coli* is a cloning vector.

09 Match the following enzymes with their functions

	Column I		Column II
1.	Restriction endonuclease	i.	Joins the DNA fragments
2.	Restriction exonuclease	ii.	Extends primers on genomic DNA template
3.	DNA ligase	iii.	Cuts DNA at specific position
4.	*Taq* polymerase	iv.	Removes nucleotides from the ends of DNA

Select the correct option from the following. **[NEET (Odisha) 2019]**

	1	2	3	4
(a)	(iii)	(i)	(iv)	(ii)
(b)	(iii)	(iv)	(i)	(ii)
(c)	(iv)	(iii)	(i)	(ii)
(d)	(ii)	(iv)	(i)	(iii)

Ans. *(b)*

The correct matches are

1. Restriction endonuclease	(iii) Cuts DNA at specific site
2. Restriction exonuclease	(iv) Removes nucleotides from the ends of DNA
3. DNA ligase	(i) Joins the DNA fragments
4. *Taq* polymerase	(ii) Extends primars on genomic DNA template.

10 Following statements describe the characteristics of the enzyme Restriction Endonuclease. Identify the incorrect statement. **[NEET (National) 2019]**

(a) The enzyme binds DNA at specific sites and cuts only one of the two strands
(b) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand
(c) The enzyme recognises a specific palindromic nucleotide sequence in the DNA
(d) The enzyme cuts DNA molecule at identified position within the DNA

Ans. *(a)*

The statement about restriction enzymes that the enzyme binds DNA at specific sites and cuts only one of the two strands is incorrect.

These enzymes cut both the strands of DNA helix at specific sites in their sugar phosphate backbone. The sequences being recognised by restriction enzymes are called palindromic sequences which have same reading frame in both 5′→ 3′ and 3′→ 5′ directions. Rest statements are correct.

11 Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes? **[NEET 2018]**

(a) λ phage (b) *Ti*-plasmid
(c) Retrovirus (d) pBR 322

Ans. *(c)*

Usually a **retrovirus** is used as a vector for introducing a DNA fragment in human cells. They are used as vector in gene therapy to introduce the desired gene so as to replace the functioning of a defected gene,. e.g. Severe Combined Immune Deficiency (SCID) is caused due to defect in the gene for the enzyme adenosine deaminase.

In gene therapy against it, lymphocytes are extracted from the bone marrow of the patient. These are grown in a culture outside the body. A functional ADA *c*DNA, using a retroviral vector, is then introduced into these lymphocytes. These are reinjected into the patient's bone marrow.

λ-phage allows cloning of DNA fragments upto 23 Kb lengths. ***Ti*-plasmid** is usually used for plants. **pBR-322** is an artificial cloning vector, usually used for bacteria.

12 A gene, whose expression helps to identify transformed cells is known as **[NEET 2017]**

(a) selectable marker
(b) vector
(c) plasmid
(d) structural gene

Ans. ***(a)***

A gene whose expression helps to identify transformed cell is known as selectable marker. Usually, the genes encoding resistance to antibiotics, such as tetracycline, amphicillin, etc. are useful selectable markers for, e.g. *E. coli*. Concept Enhancer Plasmid pBR^{322} has two resistance genes; ampicillin resistance (amp^R) and tetracyclin resistance (tet^R). These are considered as useful selectable markers.

13 The DNA fragments separated on an agarose gel can be visualised after staining with **[NEET 2017]**

(a) bromophenol blue
(b) acetocarmine
(c) aniline blue
(d) ethidium bromide

Ans. ***(d)***

The DNA fragments separated on an agarose gel can be visualised after staining with ethidium bromide. It is intercalating agent and a fluorescent agent. The stained DNA fragments are seen as bright orange coloured bands under UV-light.

Thinking process Intercalation is the insertion of molecules between the planar bases of DNA. This process is used as a method for analysing DNA. Intercalation occurs, when ligands of an appropriate size and chemical nature fit themselves in between base pairs of DNA. These ligands are mostly polycyclic, aromatic and planar and therefore often make good nucleic acid stains. Intensively studied DNA intercalator include ethidium bromide, proflavine, etc.

14 Which of the following is not a feature of the plasmids? **[NEET 2016, Phase I]**

(a) Circular structure
(b) Transferable
(c) Single-stranded
(d) Independent replication

Ans. ***(c)***

Plasmid is extrachromosomal, double stranded, circular DNA, found in bacterial cells and some yeasts. Discovery of plasmid has led to the revolution in biotechnological research.

15 Which of the following is a restriction endonuclease? **[NEET 2016, Phase I]**

(a) Protease (b) DNase I
(c) RNase (d) *Hin*d II

Ans. ***(d)***

*Hin*d II is a restriction endonuclease. Restriction endonucleases are enzymes used for cutting of DNA at specific locations.

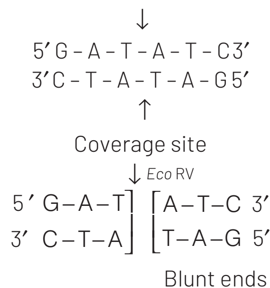
*Hin*dII was the first restriction endonuclease isolated by Smith Wilcox and Kelley in 1968. It was found that it always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs.

16 Which of the following restriction enzymes produces blunt ends? **[NEET 2016, Phase II]**

(a) *Sal* I (b) *Eco* RV
(c) *Xho* (d) *Hin*d III

Ans. ***(b)***

Eco RV is a type II restriction endonuclease isolated from strains of *E. coli*. It creates blunt ends. The enzyme recognises the palindromic 6-base DNA sequence and makes a cut at vertical line. The blunt ends are formed as



17 A foreign DNA and plasmid cut by the same restriction endonuclease can be joined to form a recombinant plasmid using **[NEET 2016, Phase II]**

(a) *Eco* RI (b) *Taq* polymerase
(c) polymerase-III (d) ligase

Ans. ***(d)***

DNA ligases (genetic gum) are used in recombinant DNA technology to join two individual fragments of double-stranded DNA by forming phosphodiester bonds between them to produce a recombinant DNA (plasmid).

18 The cutting of DNA at specific locations became possible with the discovery of **[CBSE AIPMT 2015]**

(a) restriction enzymes
(b) probes
(c) selectable markers
(d) ligases

Ans. ***(a)***

Restriction enzymes are DNA cutting enzymes found in bacteria. A restriction enzyme recognises and cuts DNA only at a particular sequence of nucleotides. For example, the bacterium *Haemophilus aegyptius* produces an enzyme named Hae III that cuts DNA wherever, it encounters the sequence.

19 The DNA molecule to which the gene of interest is integrated for cloning is called **[CBSE AIPMT 2015]**

(a) transformer (b) vector
(c) template (d) carrier

Ans. ***(b)***

The DNA molecule to which the gene of interest is integrated for cloning is called vector. It is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/for expressed. A vector containing foreign DNA is termed as recombinant DNA.

20 Which vector can clone only a small fragment of DNA? **[CBSE AIPMT 2014]**

(a) Bacterial artificial chromosome
(b) Yeast artificial chromosome
(c) Plasmid
(d) Cosmid

Ans. ***(c)***

Plasmid is a small fragment of DNA (about 10 Kbp size) that is physically separate from and can replicate freely of chromosomal DNA within a cell. It can clone small DNA fragments.

Cosmid—45 Kbp

BAC—300-350 Kbp

YAC—1 Mbp/1,000 Kbp-2,500 Kbp)

21 Commonly used vectors for human genome sequencing are **[CBSE AIPMT 2014]**

(a) T-DNA
(b) BAC and YAC
(c) Expression vectors
(d) T/A cloning vectors

Ans. *(b)*

Commonly used vector for human genome sequencing are BAC (Bacterial Artificial Chromosome) and YAC. BAC is a DNA construct, based on a functional fertility plasmid (F plasmid) used for transforming and cloning in bacteria (*E. coli*) and YAC are genetically engineered chromosomes derived from the DNA of the yeast, (*Saccharomyces cerevisiae*) which is then ligated into a bacterial plasma.

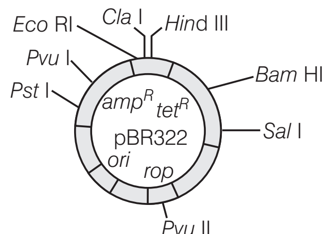
22 The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of **[NEET 2013]**

(a) Non-recombinant bacteria containing β-galactosidase
(b) Insertional inactivation of α-galactosidase in non-recombinant bacteria
(c) Insertional inactivation of α-galactosidase in recombinant bacteria
(d) Inactivation of glycosidase enzyme in recombinant bacteria

Ans. *(c)*

The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of insertional inactivation of alpha galactosidase in recombinant bacteria. Alpha galactosidase is a glycoside hydrolase enzyme that hydrolyse the terminal alpha galactosyl moieties from glycolipids and glycoprotein. It is encoded by the GLA gene.
β-galactosidase is an exoglycosidase, which hydrolyses the β-glycosidic bond formed between a galactose and its organic moiety.

23 The given figure is the diagrammatic representation of the *E. coli* vector pBR322. Which one of the given options correctly identifies its certain component(s)? **[CBSE AIPMT 2012]**



(a) *ori*–original restriction enzyme
(b) rop–reduced osmotic pressure
(c) *Hind* III, *Eco* RI–selectable markers
(d) *amp*^{R}, *tet*^{R}–antibiotic resistance genes

Ans. *(d)*

amp^R and *tet*^{R} are the antibiotic resistant genes. *Ori* represents the site of origin of replication, *rop* represents the proteins that take part in the replication of plasmid. *Hind* III, *Eco* RI are the recognition sites of restriction endonucleases.

24 A single strand of nucleic acid tagged with a radioactive molecule is called **[CBSE AIPMT 2012]**

(a) vector
(b) selectable marker
(c) plasmid
(d) probe

Ans. *(d)*

Probes are 15-30 bases long radioactive labelled oligonucleotides (RNA or DNA) used to detect complementary nucleotide sequences, used for disease diagnosis, etc.

25 For transformation, microparticles coated with DNA to be bombarded with gene gun are made up of **[CBSE AIPMT 2012]**

(a) silver or platinum
(b) platinum or zinc
(c) silicon or platinum
(d) gold or tungsten

Ans. *(d)*

Biolistics or gene gun is a direct or vectorless way used to introduce alien DNA into host cells. In this method of gene transfer, high velocity micro-particles of gold or tungsten, coated with DNA are bombarded on the plant cells.

26 Which one of the following is a case of wrong matching? **[CBSE AIPMT 2012]**

(a) Somatic hybridisation–Fusion of two diverse cells
(b) Vector DNA – Site for *t*RNA synthesis
(c) Micropropagation – *In vitro* production of plants in large numbers
(d) Callus – Unorganised mass of cells produced in tissue culture

Ans. *(b)*

Out of the following the statement (b) is wrong because a vector is a DNA molecule used as a vehicle to transfer foreign genetic material into desired cell.

The *t*RNA is synthesised in the nucleus on a DNA tempelate. Only 0.025% of total DNA content codes for *t*RNA.

27 Given below is a sample of portion of DNA strand giving the base sequence on the opposite strands. What is so, special shown in it? **[CBSE AIPMT 2011]**

5′— GAATTC — 3′
3′ — CTTAAG — 5′

(a) Replication completed
(b) Deletion mutation
(c) Start codon at the 5′ end
(d) Palindromic sequence of base pairs

Ans. *(d)*

Palindromic DNA is a base sequence of DNA, which reads the same forward or backward. It has similar sequence in both the strands. Different types of palindromic sequences are recognised by restriction endonucleases.

28 There is a restriction endonuclease called *Eco* RI. What does 'co' part in it stand for? **[CBSE AIPMT 2011]**

(a) Colon
(b) Coelom
(c) Coenzyme
(d) *Coli*

Ans. *(d)*

Restriction endonuclease recognises a particular palindromic sequence and degrades the same. It was so, called because it restricted the growth of bacteriophage in the bacterium (e.g. *E. coli*). The convention for naming these enzymes is the first letter of the name comes from the bacterial genus; the second two letters come from the species, and the fourth letter from strain, e.g. *Eco* RI comes from *Escherichia coli* RY13. Roman numbers following the names indicate the order in which the enzymes were isolated.

29 Which one of the following is used as vector for cloning genes into higher organisms? **[CBSE AIPMT 2010]**

(a) Baculovirus
(b) *Salmonella typhimurium*
(c) *Rhizopus nigricans*
(d) Retrovirus

Ans. *(d)*

Retroviruses are RNA containing animal viruses that replicate through a DNA intermediate. Retroviruses in animals have the ability to transform normal cells into cancerous cells. A better

understanding of the act of delivering genes by pathogen in these eukaryotic hosts has generated knowledge to transform these tools of pathogen into useful vectors for delivering genes of interest of humans. Retroviruses have been disarmed and are now used to deliver desirable genes into animal cells.

30 Restriction endonucleases are enzymes which
[CBSE AIPMT 2010, 06, 02, 01, 98, 95]

(a) make cuts at specific positions within the DNA molecule
(b) recognise a specific nucleotide sequence for binding of DNA ligase
(c) restrict the action of the enzyme DNA polymerase
(d) remove nucleotides from the ends of the DNA molecule

Ans. *(a)*

Restriction endonuclease recognises a specific DNA base sequence (recognition sequence or recognition site, restriction sequence or restriction site) and cleaves both the strands of DNA at or near that site. The enzyme cuts the DNA, generating restriction fragments with overhanging ends or blunt ends.

31 The linking of antibiotic resistance gene with the plasmid vector became possible with
[CBSE AIPMT 2008]

(a) DNA polymerase
(b) exonucleases
(c) DNA ligase
(d) endonucleases

Ans. *(c)*

The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme DNA ligase, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular autonomously replication DNA created *in vitro* and is known as recombinant DNA.

32 Which one of the following is commonly used in transfer of foreign DNA into crop plants?
[CBSE AIPMT 2009]

(a) *Trichoderma harzianum*
(b) *Meloidogyne incognita*
(c) *Agrobacterium tumefaciens*
(d) *Penicillium expansum*

Ans. *(c)*

The uptake of foreign DNA or transgenes by plant cells is called transformation.

A variety of techniques have been used to introduce transgenes into plant cells, these can be grouped into the following two categories (i) *Agrobacterium*-mediated and (ii) direct gene transfers. *Agrobacterium tumefaciens* mediated transformation eliminates the need for regeneration from tissue explants.

33 Restriction endonucleases
[CBSE AIPMT 2004]

(a) are present in mammalian cells for degradation of DNA when the cell dies
(b) are used in genetic engineering for ligating two DNA molecules
(c) are used for *in vitro* DNA synthesis
(d) are synthesised by bacteria as part of their defense mechanism

Ans. *(d)*

Restriction endonucleases are found in bacteria and are synthesised by bacteria as a part of their defense mechanism. They function in restricting the multiplication of viruses in bacterial cells by cutting up the genetic material of invading virus.

34 Manipulation of DNA in genetic engineering became possible due to the discovery of
[CBSE AIPMT 2003]

(a) restriction endonuclease
(b) DNA ligase
(c) transcriptase
(d) primase

Ans. *(a)*

Manipulation of DNA in genetic engineering became possible due to the discovery of restriction endonuclease. These are isolated from bacterial cells, and are tools for molecular biologists.

Several hundred restriction enzymes are now known, each with a specific sequence requirement dictating where it will cut DNA. Therefore, digesting DNA with a restriction enzyme creates a characteristic set of fragments, which can be isolated by electrophoresis and subsequently analysed.

35 A mutant strain of T_4-bacteriophage R-II, fails to lyse the *E. coli* but when two strains R-II^x and R-II^y are mixed then they lyse the *E. coli*. What may be the possible reason?
[CBSE AIPMT 2002]

(a) Bacteriophage transforms in wild
(b) It is not mutated
(c) Both strains have similar cistrons
(d) Both strains have different cistrons

Ans. *(d)*

The possible reason is that both strains have different cistron because the enzymes required for lysing *E. coli* could not be synthesised by the mutant strain. Two different strains had cistrons for synthesising different enzymes which acted together.

36 In bacteria, plasmid is
[CBSE AIPMT 2002]

(a) extrachromosomal material
(b) main DNA
(c) non-functional DNA
(d) repetitive gene

Ans. *(a)*

Plasmid is a piece of circular DNA molecule (mostly in bacteria but in yeast also) which is not part of the normal chromosomal DNA of a cell, and is capable of replicating independently.

37 Plasmid is **[CBSE AIPMT 2000, 01]**

(a) fragment of DNA which acts as vector
(b) fragment which joins two genes
(c) *m*RNA which acts as carrier
(d) autotrophic fragment

Ans. *(a)*

A plasmid is a piece of DNA, mostly in bacteria (but also in yeast) not forming a part of normal chromosomal DNA of a cell, but capable of replicating independently of it. These often act as vehicles for gene transfer.

38 Plasmids are suitable vectors for gene cloning becauseu
[CBSE AIPMT 2000]

(a) these are small circular DNA molecules which can integrate with host chromosomal DNA
(b) these are small circular DNA molecules with their own replication origin site
(c) these can shuttle between prokaryotic and eukaryotic cells
(d) these often carry antibiotic resistance genes

Ans. *(b)*

Plasmids replicate autonomously. These carry a signal situated at their replication origin which determines how many copies are to be made and this number can be artificially increased for cloning a given gene.

39 The process of replication in plasmid DNA, other than initiation, is controlled by **[CBSE AIPMT 1999]**

(a) mitochondrial gene
(b) bacterial gene
(c) plasmid gene
(d) None of the above

Ans. ***(b)***

The process of replication in plasmid DNA, other than initiation, is controlled by bacterial gene.

40 Which of the following is related to genetic engineering? **[CBSE AIPMT 1999]**

(a) Mutation (b) Plasmid
(c) Plastid (d) Heterosis

Ans. ***(b)***

Plasmids are used as vectors in genetic engineering.

41 Recombinant DNA is obtained by cleaving the pro-DNA by **[CBSE AIPMT 1998]**

(a) primase
(b) exonucleases
(c) ligase
(d) restriction endonuclease

Ans. ***(d)***

Recombinant DNA is obtained by cleaving the pro-DNA by restriction endonucleases. They can cleave DNA at specific base sequences called restriction sites.

42 Genetic engineering is possible, because **[CBSE AIPMT 1998]**

(a) the phenomenon of transduction in bacteria is well understood
(b) we can see DNA by electron microscope
(c) We can cut DNA at specific sites by endonucleases like DNAs-I
(d) restriction endonucleases purified from bacteria can be used *in vitro*

Ans. ***(d)***

Genetic engineering is the manipulation of genetic material of an organism using enzyme restriction endonuclease. Nathans and Smith (1970) isolated the first restriction endonuclease. Jackson, Symons and Paul Berg (1972) successfully generated recombinant DNA molecules *in vitro*.

43 Two bacteria found to be very useful in genetic engineering experiments are **[CBSE AIPMT 1998]**

(a) *Nitrosomonas* and *Klebsiella*
(b) *Escherichia* and *Agrobacterium*
(c) *Nitrobacter* and *Azotobacter*
(d) *Rhizobium* and *Diplococcus*

Ans. ***(b)***

The most important tool in genetic engineering of plants has been the Ti plasmid of soil bacterium, *Agrobacterium tumefaciens*. *E. coli* has also been extensively used for genetic engineering in animals, like in production of humulin, somatotropin, etc.

TOPIC 2

Processes of Recombinant DNA Technology

44 During the process of gene amplification using PCR, if very high temperature is not maintained in the beginning, then which of the following steps of PCR will be affected first? **[NEET 2021]**

(a) Annealing (b) Extension
(c) Denaturation (d) Ligation

Ans. ***(c)***

Denaturation is first step of PCR that involves seperation of double-stranded DNA. The DNA is subjected to heating at high temperature (95ºC). This leads to breaking of hydrogen bonds between nucleotides and formation of single-stranded DNA. Thus, if high temperature is not maintained, denaturation will be affected.

Annealing is second step of PCR which involves annealing of primer to DNA strands. In this step, DNA must be cooled to 50ºC.

Extension, is third step in which *taq* polymerase enzyme extends the primers thus, completing replication of rest of DNA. Ligation is the binding of amplified sequence of interest.

45 Which of the following is not an application of PCR (Polymerase Chain Reaction)? **[NEET 2021]**

(a) Molecular diagnosis
(b) Gene amplification
(c) Purification of isolated protein
(d) Detection of gene mutation

Ans. ***(c)***

Polymerase Chain Reaction or PCR, is a technique to make many copies of a specific DNA region *in vitro* (in a test tube rather than an organism).

Following are the applications of PCR

The amplification of gene fragments (Gene amplification).

The modification of DNA fragments.

The sensitive detection of pathogenic microorganisms, if desired followed by an accurate genotyping. (Molecular diagnosis)

DNA analysis of archaeological specimens.

Proof-reading PCR (PR-PCR) is designed to detect known mutations within genomic DNA.

46 During the purification process for recombinant DNA technology, addition of chilled ethanol precipitates out **[NEET 2021]**

(a) RNA (b) DNA
(c) histones (d) polysaccharides

Ans. ***(b)***

The role of chilled ethanol and monovalent cation is to remove the solvation (solvent interface of any chemical compound or biomolecule) shell surrounding the DNA and permitting the precipitation of the DNA in pellet form.

Ethanol has a lower dielectric constant than water, making it to promote ionic bond formation the Na^+ (from the salt) and the PO_3^- (from the DNA backbone), further, causing the DNA to precipitate.

47 Which of the íollowing is a correct sequence of steps in a PCR (Polymerase Chain Reaction)? **[NEET 2021]**

(a) Denaturation, Annealing, Extension
(b) Denaturation, Extension, Annealing
(c) Extension, Denaturation, Annealing
(d) Annealing, Denaturation, Extension

Ans. ***(a)***

PCR stands for Polymerase Chain Reaction. It is a technique in which multiple copies of gene of interest is synthesised using two sets of primers and the enzyme DNA polymerase. The correct sequence of steps in a PCR (Polymerase Chain Reaction) are

Denaturation In which the double-stranded template DNA is heated at 95°C to separate it into two single strands.

Annealing In which the temperature is lowered to 50°C which enables the DNA primers to attach to the template DNA.

Extension/Extending In which the temperature is raised and the new

strand of DNA is made by the *taq* polymerase enzyme.

These three stages are repeated 20-40 times, doubling the number of DNA copies each time.

48 Select the correct statement from the following. **[NEET (Oct.) 2020]**

(a) Gel electrophoresis is used for amplification of a DNA segment
(b) The polymerase enzyme joins the gene of interest and the vector DNA
(c) Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzymes of optimum conditions
(d) PCR is used for isolation and separation of gene of interest

Ans. *(c)*

Statement in option (c) is correct as restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzymes of optimum conditions. Other statements are incorrect and can be corrected as

Gel eletrophoresis is used for separation and isolation of DNA fragments.

The polymerase enzyme uses DNA templates to catalyse polymerisation of deoxynucleotides.

PCR is Polymerase Chain Reaction, it is used for amplification of a DNA segment.

49 In a mixture, DNA fragments are separated by **[NEET (Oct.) 2020]**

(a) bioprocess engineering
(b) restriction digestion
(c) electrophoresis
(d) polymerase chain reaction

Ans. *(c)*

Electrophoresis is a technique used for the separation of substances of different ionic properties. It is used in RDT to separate the DNA fragments that are being cut by restriction endonuclease. In this technique, DNA fragments are loaded on agarose gel and then electric field is applied. Due to negatively charged, DNA fragments move towards anodes (+ ve charge).

The smaller fragments move farther away as compared to larger fragments and thus, these get separated.

50 In recombinant DNA technology antibiotics are used **[NEET Oct.) 2020]**

(a) to keep medium bacteria-free
(b) to detect alien DNA
(c) to impart disease-resistance to the host plant
(d) as selectable markers

Ans. *(d)*

In recombinant DNA technology, antibiotics are used as selectable markers, which help in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. Normally, the genes encoding resistance to antibiotics such as chloramphenicol, ampicillin, tetracycline or kanamycin, etc., are considered useful selectable markers for *E.coli.*

51 Match the following columns and select the correct option. **[NEET (Sep.) 2020]**

Column I	Column II
A. *Bt* cotton	1. Gene therapy
B. Adenosine deaminase deficiency	2. Cellular defence
C. RNAi	3. Detection of HIV infection
D. PCR	4. *Bacillus thuringiensis*

	A	B	C	D
(a)	3	2	1	4
(b)	2	3	4	1
(c)	1	2	3	4
(d)	4	1	2	3

Ans. *(d)*

The correct option is (d). It can be explained as follows.

In *Bt* cotton the specific *Bt* toxin gene was isolated from *Bacillus thuringiensis.*

The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency.

RNAi (RNA interference) takes place in all eukaryotic organisms as a method of cellular defence.

PCR is now routinely used to detect HIV in suspected AIDS patients.

52 In gel electrophoresis, separated DNA fragments can be visualised with the help of **[NEET (Sep.) 2020]**

(a) ethidium bromide in UV radiation
(b) acetocarmine in UV radiation
(c) ethidium bromide in infrared radiation
(d) acetocarmine in bright blue light

Ans. *(a)*

In gel electrophoresis, separated DNA fragments can be visualised with the help of ethidium bromide in UV radiation because DNA fragments cannot be seen in visible light without staining.So, they are stained with ethidium bromide and made observable through UV radiation as bright orange coloured bands.The bands are cut out of agarose gel and extracted. The purified DNA fragments are then used in constructing recombinant DNAs by attaching them to cloning vectors.

53 Given below are four statements pertaining to separation of DNA fragments using gel electrophoresis. Identify the incorrect statements.

1. DNA is negatively charged molecule and so it is loaded on gel towards the anode terminal.
2. DNA fragments travel along the surface of the gel whose concentration does not affect movement of DNA.
3. Smaller the size of DNA fragment larger is the distance it travels through it.
4. Pure DNA can be visualised directly by exposing UV-radiation.

Select the correct option from the following **[NEET Odisha) 2019]**

(a) 1, 3 and 4 (b) 1, 2 and 3
(c) 2, 3 and 4 (d) 1, 2 and 4

Ans. *(d)*

Statements (1), (2) and (4) are incorrect because DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix. The concentration of gel does affect the resolution of DNA separation.

The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation. Only statement 3 is correct. The DNA fragments spearate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.

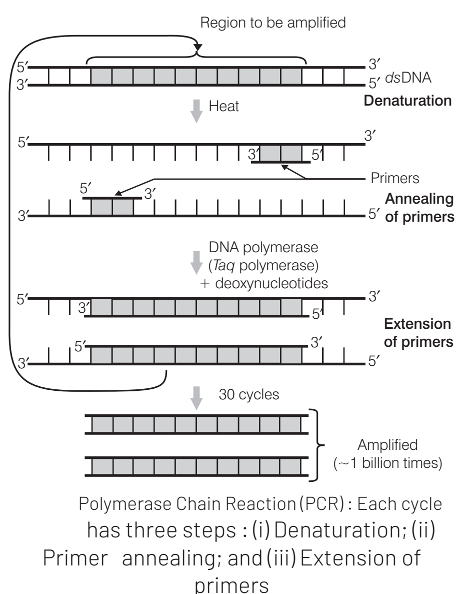
54 The correct order of steps in Polymerase Chain Reaction (PCR) is **[NEET 2018]**

(a) Denaturation, Extension, Annealing
(b) Annealing, Extension, Denaturation
(c) Extension, Denaturation, Annealing
(d) Denaturation, Annealing, Extension

Ans. *(d)*

The Polymerase Chain Reaction (PCR) involves three basic steps; denaturation, annealing and extension. In the **denaturation** step, DNA is heated at high temperature (94° C to 96° C) to separate the two strands. In the next step (**annealing**), the two oligo-nucleotide primers anneal to each single-stranded template DNA.

This step is carried out at a lower temperature (40° C to 60° C). The final step is **extension**, wherein *Taq* DNA polymerase synthesises the DNA region between the primers, using *d*NTPs (deoxynucleoside triphosphates) and Mg^{2+} ions.



Polymerase Chain Reaction (PCR) : Each cycle has three steps : (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

55 The process of separation and purification of expressed protein before marketing is called **[NEET 2017]**

(a) upstream processing
(b) downstream processing
(c) bioprocessing
(d) postproduction processing.

Ans. *(b)*

The process of separation and purification of expressed protein before marketing is called downstream processing.

In this process, a whole range of biochemical separation and purification techniques are used such as drying, chromatography, solvent extraction and distillation. After purification quality control testings are done.

56 What is the criterion for DNA fragments movement on agarose gel during gel electrophoresis? **[NEET 2017]**

(a) The larger the fragment size, the farther it moves
(b) The smaller the fragment size, the farther it moves
(c) Positively charged fragments move to farther end
(d) Negatively charged fragments do not move

Ans. *(b)*

Gel electrophoresis is used for the separation of molecules of similar electric charge on the basis of their size. Hence, smaller the DNA fragment size the farther it moves.

Thinking Process Agarose gel matrix functions as sieve. Smaller DNA fragments easily move and larger fragments take time to move in gel matrix.

57 The *Taq* polymerase enzyme is obtained from **[NEET 2016, Phase I]**

(a) *Thiobacillus ferroxidans*
(b) *Bacillus subtilis*
(c) *Pseudomonas subtilis*
(d) *Thermus aquaticus*

Ans. *(d)*

Taq polymerase is a thermostable DNA polymerase obtained from *Thermus aquaticus. Thermus aquaticus* is a bacterium that lives in hot springs and hydrothermal vents.

58 Which of the following is not a component of downstream processing? **[NEET 2016, Phase II]**

(a) Separation
(b) Purification
(c) Preservation
(d) Expression

Ans. *(d)*

Downstream process is the process of separation and purification of products synthesised in bioreactors. After the completion of biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product.

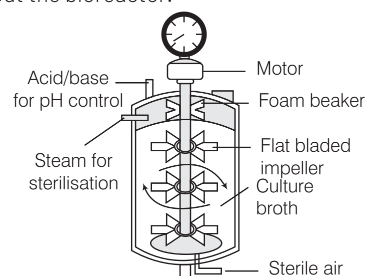
The processes include separation and purification, collectively referred to as downstream processing. The product is then formulated with suitable preservatives. Hence, option (d) is incorrect and all other options are correct.

59 Stirred-tank bioreactors have been designed for **[NEET 2016, Phase II]**

(a) purification of product
(b) addition of preservatives to the product
(c) availability of oxygen throughout the process
(d) ensuring anaerobic conditions in the culture vessel

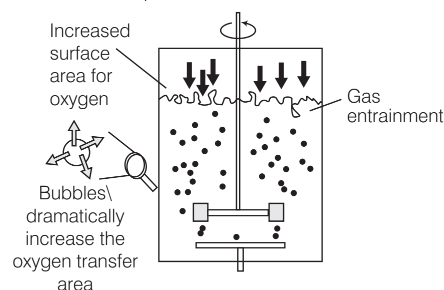
Ans. *(c)*

A stirred tank bioreactor is usually cylindrical, possessing a stirrer which facilitates even mixing of the reactor contents and oxygen availability through out the bioreactor.



(a) Simple stirred-tank bioreactor

The bioreactor has an agitator system, an oxygen delivery system, foam control, pH and temperature control systems. Hence, option (c) is correct.



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

60 An analysis of chromosomal DNA using the Southern hybridisation technique does not use **[CBSE AIPMT 2014]**

(a) electrophoresis (b) blotting
(c) autoradiography (d) PCR

Ans. *(d)*

Southern hybridisation is a technique used in molecular biology for detection of a specific DNA sequence in DNA samples in which except PCR we use all three methods such as electrophoresis, blotting and autoradiography. PCR is the method used for the amplification of DNA sample. *In vitro* clonal propagation is characterised by PCR and RAPD.

61 *In vitro* clonal propagation in plants is characterised by **[CBSE AIPMT 2014]**

(a) PCR and RAPD
(b) Northern blotting
(c) electrophoresis and HPLC
(d) microscopy

Ans. *(a)*

RAPD (Random Amplified Polymorphic DNA) is a type of PCR reaction, but the segments of DNA that are amplified are random. Often, PCR is used to amplify a known DNA sequence like *in vitro* clonal propagation in plants.

62 DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by **[NEET 2013]**

(a) centrifugation
(b) polymerase chain reaction
(c) electrophoresis
(d) restriction mapping

Ans. *(c)*

DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by electrophoresis. The polymerase chain reaction is simply DNA replication in a test-tube. Restriction mapping is the process of obtaining structural information on a piece of DNA by the use of restriction enzymes,
e.g. endonucleases that recognise specific 4-8 base regions of DNA.

63 PCR and restriction fragment length polymorphism are the methods for **[CBSE AIPMT 2012]**

(a) study of enzymes
(b) genetic transformation
(c) DNA sequencing
(d) genetic fingerprinting

Ans. *(d)*

PCR and RFLP are methods used for genetic fingerprinting. As Restriction Fragment Length Polymorphism (RFLP) is the basis of genetic (or DNA) fingerprinting and is useful in identifying individuals from their semen, blood or tissues or any other DNA sample and resolution of parent hood disputes.

Polymerase Chain Reaction (PCR) is also useful in genetic fingerprinting as it can amplify the DNA sample even if available in a very small amount.

64 Which one is a true statement regarding DNA polymerase used in PCR? **[CBSE AIPMT 2012]**

(a) It is used to ligate introduced DNA in recipient cells
(b) It serves as a selectable marker
(c) It is isolated from a virus
(d) It remains active at high temperature

Ans. *(d)*

Polymerase Chain Reaction (PCR) is used to amplify a DNA segment or to synthesise *in vitro* the multiple copies of gene (or DNA) of interest, using two sets of primers and the enzyme DNA polymerase. This enzyme is isolated from a bacterium *Thermus aquaticus* and it remains active during the high temperature but high temperature induced denaturation of double stranded DNA.

65 Agarose extracted from sea weeds is used in **[CBSE AIPMT 2011]**

(a) spectrophotometry
(b) tissue culture
(c) PCR
(d) gel electrophoresis

Ans. *(d)*

For gel electrophoresis the commonly used matrix is agarose which is a natural polymer extracted from seaweeds (e.g. *Gelidium, Gracilaria, Gigartina*, etc.). Gel electrophoresis is a technique to separate fragments of DNA. Since, DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.

66 Stirred-tank bioreactors have been designed for **[CBSE AIPMT 2010]**

(a) addition of preservatives to the product
(b) purification of the product
(c) ensuring anaerobic conditions in the culture vessel
(d) availability of oxygen throughout the process

Ans. *(d)*

The most common type of aerobic bioreactor in use today is the stirred-tank bioreactor, which may feature a specific internal configuration designed to provide a specific circulation pattern. The stirred tank bioreactor have been designed for availability of oxygen through out the processes.

67 Polyethylene glycol method is used for **[CBSE AIPMT 2009]**

(a) biodiesel production
(b) seedless fruit production
(c) energy production from sewage
(d) gene transfer without a vector

Ans. *(d)*

Polyethylene glycol method is used for gene transfer without a vector. Introduction of DNA into plant cells without the involvement of a biological agent and leading to stable transformation is known as direct gene transfer. There are various methods for direct direct gene transfer, one of which is chemical method.

Certain chemicals, e.g. PEG (Polyethylene Glycol), polyvinyl alcohol and calcium phosphate enhance the uptake of DNA by plant protoplast. PEG and calcium phosphate are thought to precipitate the DNA onto the outer surface of plasmalemma and the precipitate is taken up by the endocytosis.

68 Gel electrophoresis is used for **[CBSE AIPMT 2008]**

(a) construction of recombinant DNA by joining with cloning vectors
(b) isolation of DNA molecules
(c) cutting of DNA into fragments
(d) separation of DNA fragments according to their size

Ans. *(d)*

When genomic DNA extracted from any tissue of a plant or animal species is digested with a restriction enzyme, it is cleaved into segments. The segments of different sizes can be separated through gel electrophoresis. Gel electrophoresis involves movement of fragments or molecules from a well created on one edge of the gel.