Biotechnology

EXERCISE [PAGES 291 - 292]

Exercise | Q 1.1 | Page 291

Choose the correct option

The bacterium which causes a plant disease called crown gall is _____.

1. Helicobacter pylori
2. **Agrobacterium tumifaciens**
3. Thermophilus aquaticus
4. Bacillus thuringienesis

Solution: The bacterium which causes a plant disease called crown gall is **Agrobacterium tumifaciens**.

Exercise | Q 1.2 | Page 291

Choose the correct option

The enzyme nuclease hydrolyses _____ of polynucleotide chain of DNA.

1. hydrogen bonds
2. **phosphodiester bonds**
3. glycosidic bonds
4. peptide bonds

Solution: The enzyme nuclease hydrolyses **phosphodiester bonds** of the polynucleotide chain of DNA.

Exercise | Q 1.3 | Page 291

Choose the correct option

In vitro amplification of DNA or RNA segment is known as _____.

1. chromatography
2. Southern blotting
3. **polymerase chain reaction**
4. gel electrophoresis

Solution: In vitro amplification of DNA or RNA segment is known as the **polymerase chain reaction.**

Exercise | Q 1.4 | Page 291

Choose the correct option

Which of the following is the correct recognition sequence of restriction enzyme hind III?

1.
5' ---A-A-G-C-T-T---3'
3' ---T-T-C-G-A-A--- 5'

2.
3' ---C-T-T-A-A-G--- 5'
5' ---C-G-A-T-T-C---3'

3.
3' ---G-C-T-A-A-G--- 5'
5' ---G-G-C-C---3'

4.
3' ---C-C-G-G--- 5'

Solution:

5' ---A-A-G-C-T-T---3'
3' ---T-T-C-G-A-A--- 5'

Exercise | Q 1.5 | Page 291

Choose the correct option

Recombinant protein ..... is used to dissolve blood clots present in the body.

1. insulin
2. **tissue plasminogen activator**
3. relaxin
4. erythropoietin

Solution: Recombinant protein **tissue plasminogen activator** is used to dissolve blood clots present in the body.

Exercise | Q 1.6 | Page 291

Choose the correct option

The recognition sequence of restriction enzymes is generally _____ nucleotide long.

1. 2 to 4
2. **4 to 8**
3. 8 to 10
4. 14 to 18

Solution: The recognition sequence of restriction enzymes is generally **4 to 8** nucleotides long.

Exercise | Q 2.1 | Page 291

Very short answer type question

Name the vector which is used in the production of human insulin through recombinant DNA technology.

Solution: pBR322 vector is used in the production of human insulin through recombinant DNA technology.

Exercise | Q 2.2 | Page 291

Very short answer type question

Which cells from Langerhans of the pancreas do produce a peptide hormone insulin?

Solution: Insulin is a peptide hormone produced by β-cells of islets of Langerhans of the pancreas.

Exercise | Q 2.3 | Page 291

Very short answer type question

Give the role of Ca^{++} ions in the transfer of the recombinant vector into the bacterial host cells.

Solution: Ca^{++} ions assist the transfer of the recombinant vector into a bacterial host cell.

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

YAC

Solution: YAC: Yeast Artificial Chromosome

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

RE

Solution: RE: Restriction Enzyme

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

dNTP

Solution: dNTP: Deoxyribonucleoside triphosphate

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

PCR

Solution: PCR: Polymerase Chain Reaction

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

GMO

Solution: GMO: Genetically Modified Organism

Exercise | Q 2.4 | Page 291

Very short answer type question

Expand the following acronym which is used in the field of protechnology.

MAC

Solution: MAC: Mammalian Artificial Chromosome

Exercise | Q 2.5 | Page 291

Very short answer type question

Fill in the blanks and complete the chart.

GMO	Purpose
i. Bt cotton	_____
ii. _____	Delay the softening of tomato during ripening.
iii. Golden rice	_____
iv. Holstein cow	_____

Solution:

GMO	Purpose
i. Bt cotton	**Insect pest resistance**
ii. **Flavr savr tomato**	Delay the softening of tomato during ripening.
iii. Golden rice	**High vitamin A content**
iv. Holstein cow	**High milk yield capacity**

Exercise | Q 3.1 | Page 291

Short answer type question

Explain the properties of a good or ideal cloning vector for rDNA technology.

Solution:

Following characteristic properties, a cloning vector must possess in order to be used in rDNA technology:

i. A good vector should have the ability of independent replication so that as the vector replicates (through ori gene) and a large number of copies of the DNA insert will be formed.

ii. The vector should be able to easily introduce into host cells.

iii. A vector should have marker genes for antibiotic resistance.

iv. A vector must contain a unique cleavage site in one of the marker genes for the restriction enzyme.

v. It should have at least suitable control elements like a promoter, operator, ribosomal binding sites, etc.

vi. The plasmids obtained naturally do not possess all the characteristics. Hence, they are constructed by inserting a gene for antibiotic resistance.

e.g. pBR322, pBR320, pACYC177 are the constructed plasmids. pBR322 is mostly used in rDNA technology in plants.

Exercise | Q 3.2 | Page 291

Short answer type question

A PCR machine can raise the temperature up to 100°C but after that, it is not able to lower the temperature below 70°C automatically. Which step of PCR will be hampered first in this faulty machine? Explain why?

Solution:

i. If a PCR machine fails to lower the temperature below 70°C then the annealing step (in which primers attach to the respective ends of the DNA template) would be affected.

ii. The annealing step of PCR requires temperature ranging between 40°C to 60°C therefore if a PCR machine fails to lower the temperature below 70°C, primers will not attach to the DNA templates.

Exercise | Q 3.3 | Page 291

Short answer type question

In the process of rDNA technology, if two separate restriction enzymes are used to cut vector and donor DNA then which problem will arise in the formation of rDNA or chimeric DNA? Explain.

Solution:

i. Restriction enzymes cut the DNA at a specific recognition site and produce either sticky ends or blunt ends.

ii. For e.g., if we use the restriction enzyme EcoR I to cut vector DNA then it will produce vector DNA having sticky ends and if we use the Hind II restriction enzyme to cut donor DNA then it will produce donor DNA with blunt ends.

iii. A vector DNA to get ligated to the desired gene, they both must possess similar kind of ends i.e. both should have either blunt ends or sticky ends.

iv. If two separate restriction enzymes are used to cut vector and donor DNA then they will fail to form complementary base pairing and chimeric DNA will not be formed.

Exercise | Q 3.4 | Page 292

Short answer type question

Match and write the pairs.

Recombinant protein	It's used in or for
i. platelet-derived growth factor	a. Anemia

ii. α-antitrypsin	b. cystic fibrosis
iii. Relaxin	c. Haemophilia A
iv. Erythropoietin	d. Diabetes
v. Factor VIII	e. Emphysema
vi. DNase	f. Parturition
	g. Atherosclerosis

Solution:

Recombinant protein	It's used in or for
i. platelet-derived growth factor	g. Atherosclerosis
ii. α-antitrypsin	e. Emphysema
iii. Relaxin	f. Parturition
iv. Erythropoietin	a. Anemia
v. Factor VIII	c. Haemophilia A
vi. DNase	b. cystic fibrosis

Exercise | Q 4.1 | Page 292

Long answer type question

Define and explain the term

Biopiracy

Solution:

Biopiracy is defined as 'theft of various natural products and then selling them by getting a patent without giving any benefits or compensation back to the host country'.

i. For proper and lawful working of biopatent, the nation should be rich in bio-diversity, people residing there should have traditional knowledge and the nation should also have sufficient financial resources.

ii. However, it is generally observed that industrialized nations are rich in financial resources and technology but lack bio-diversity, whereas developing countries are rich in biodiversity and traditional knowledge but are short of financial resources and advanced technology. These situations lead to biopiracy.

iii. Industrialized nations have always been enjoying immense profits by patenting the indigenous biomedical knowledge and bioresources of third world communities without paying any compensation to the indigenous group who originally developed such knowledge.

Example:

i. **Texmati case:** A strain of Basmati rice was patented by Texas-based company Rice Tec Inc with trade name Texmati. This patenting was illegal and unethical as Basmati is a long-grained, aromatic variety of rice indigenous to the Indian subcontinent. India fought a long legal battle after which the patent was canceled.

ii. **Turmeric:** Since ancient times, Indians have been using Haldi (Turmeric powder) as an antiseptic for healing wounds for killing pests and medicinal purposes. However, American companies have patented Turmeric and many medicinal plants of India. After a long legal battle, most of the patents have been revoked.

iii. **Neem (Azadirachta indica):** The patenting of the fungicidal properties of Neem was an example of biopiracy. The USDA and an American MNC W.R. Grace in the early 90s sought a patent from the European Patent Office (EPO) on the “method for controlling on plants by the aid of hydrophobic extracted neem oil.”

Exercise | Q 4.1 | Page 292

Long answer type question
Define and explain the term
Biopatent
Solution:

i. Biopatent is a biological patent awarded for strains of microorganisms, cell lines, genetically modified strains, DNA sequences, biotechnological processes, product processes, products, and product applications.

ii. Biopatents are awarded to recognize real innovative contributions made by the inventor to the cause of human welfare.

iii. The awards are given to inculcate encouragement and values in developing scientific culture and in emphasizing the role of biology in shaping human society.

iv. Indian patent allows ‘process patent’ and not the ‘product patent’. Biopatent allows the patent holder to exclude others from making, using, selling, or importing a protected invention for a limited period of time.

v. The duration of biopatents is five years from the date of the grant or seven years from the date of filing the patent application, whichever is less.

Exercise | Q 4.1 | Page 292

Long answer type question

Define and explain the term

Bioethics

Solution:

i. Bioethics helps to study moral vision, decisions, and policies of human behaviour in relation to biological phenomena or events.

ii. Ethics deals with ‘Life’ e.g. in vitro fertilization, sperm bank, gene therapy, cloning, gene manipulations, euthanasia, death, maintaining those who are in a comatose state, prenatal genetic selection, etc.

iii. The era of biotechnology has brought a wide spectrum on new topics like cloning, transgenic, gene therapy, eugenics, rDNA technology, etc.

iv. The use of all these has drawn a wide range of reactions in the society.

v. Ethical aspects pertaining to the use of biotechnology seems to be more controversial and frightening.

vi. These concerns are broadly summarized below: Use of animals causes great sufferings to them; violation of integration of species caused due to transgenesis; transfer of human genes into animals and vice versa; indiscriminate use of biotechnology poses risk to the environment, health, and biodiversity.

vii. The introduction of Genetically Modified Organisms (GMOs) has led to a wider debate on bioethical concerns affecting social, economic, and environmental spheres.

viii. These include the effects on non-target organisms, insect resistance crops, gene flow, and the loss of diversity as well as the issue of interfering with nature.

ix. Ethics in biotechnology also includes the general subject of what should and should not be done in using recombinant DNA techniques.

Exercise | Q 4.2 | Page 292

Long answer type question

Explain the steps in process of rDNA technology with suitable diagrams.

Solution:

The steps involved in gene cloning are as follows:

i. Isolation of DNA (gene) from the donor organism:

a. The desired gene to be cloned is obtained from the source organism (donor).

b. Initially, the cells of the donor organism are sheared with the blender and treated with a suitable detergent.

c. Genetic material from the donor is isolated and purified using several techniques.

d. Isolated DNA can be spooled on to a glass rod.

ii. Cutting of the desired gene:

a. Isolated purified DNA is then cleaved by using restriction enzymes i.e. restriction endonucleases.

b. These enzymes cleave DNA at restriction sites and break the DNA into fragments.

c. There are several types of restriction endonucleases.

d. Cleaved DNA fragments have cohesive, sticky, staggered ends or blunt ends.

e. From cleaved DNA fragments, a fragment containing the desired gene is isolated and selected for cloning. This is now called foreign DNA or passenger DNA.

f. The desired gene can also be obtained directly from genomic library or cDNA library.

iii. Insertion of a desired foreign gene into a cloning vector (vehicle DNA):

a. The foreign DNA or passenger DNA is now inserted into a cloning vector or vehicle DNA.

b. The most commonly used cloning vectors are plasmids of bacteria and bacteriophage viruses like lambda phage and M13.

c. The most commonly used plasmid is pBR322.

d. Plasmids are isolated from the vector organisms i.e. bacterium.

e. By using the same restriction enzyme (which is used in the isolation of the desired gene from the donor), plasmid i.e. vector DNA is cleaved.

f. Now by using enzyme DNA ligase, foreign DNA is inserted/ integrated into the vector DNA.

g. The combination of vector DNA and foreign DNA is now called Recombinant DNA or Chimeric DNA and the technology is referred to as rDNA technology.

iv. Transfer of rDNA into suitable competent host or cloning organism:

a. Finally, the recombinant DNA is transferred for expression into a competent host cell which is usually a bacterium.

b. The host cell takes up naked rDNA by process of 'transformation' and incorporates into its own chromosomal DNA which finally expresses the trait controlled by passenger DNA.

c. The transfer of rDNA into a bacterial cell is assisted by divalent Ca^{++} .

d. The cloning organisms used in plant biotechnology are E. coli and Agrobacterium tumefaciens.

e. The host/ competent cell which has taken up rDNA is now called a transformed cell.

f. Foreign DNA can also be transferred directly into the naked cell or protoplast of the competent host cell, without using vector.

g. This is done by using techniques like electroporation, microinjection, lipofection, shotgun, ultra-sonification, biolistic method, etc. But in plant biotechnology, the transformation is through Ti plasmids of A. tumefaciens.

v. Selection of the transformed host cell:

a. The transformation process generates a mixed population of transformed (recombinant) and non-transformed (non-recombinant) host cells.

b. For the isolation of recombinant cells from non-recombinant cells, the marker gene of the plasmid vector is employed.

c. For example, the pBR322 plasmid vector contains different marker genes (Ampicillin resistant gene and Tetracycline resistant gene).

d. When the PstI restriction enzyme is used, it knocks out Ampicillin resistant gene from the plasmid, so that the recombinant cell becomes sensitive to Ampicillin.

vi. Multiplication of transformed host cell:

a. Once transformed, host cells are separated by the screening process.

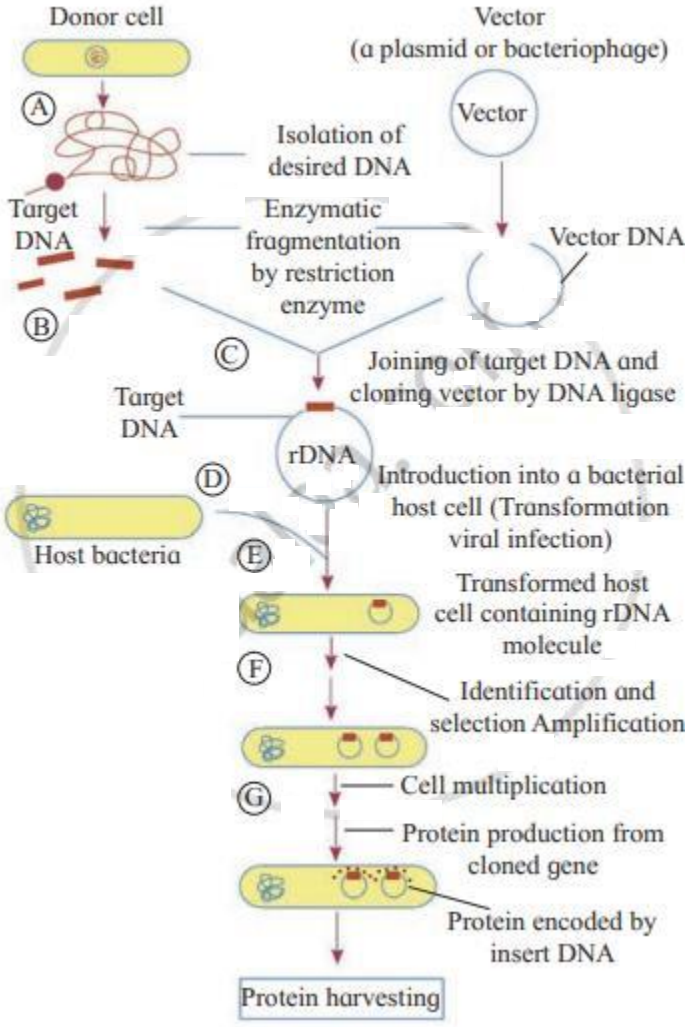
b. In this step, the transformed host cells are introduced into fresh culture media.

c. At this stage, the host cells multiply along with the replication of the recombinant DNA carried by them.

vii. Expression of the gene to obtain the desired product:

a. The next step involves the production of desired products like alcohol, enzymes, antibiotics, etc.

b. Finally, the desired product is separated and purified through downstream processing using a suitable bioreactor.



Outline of the process of recombinant DNA technology

Exercise | Q 4.3 | Page 292

Long answer type question

Explain the gene therapy. Give two types of it.

Solution:

Gene therapy is the treatment of disease by replacing, altering, or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for the disease.

The following are the different ways through which gene therapy is being used for the treatment of a disease or disorder:

i. Replace missing or defective genes;

ii. Deliver genes that speed up the destruction of cancer cells;

iii. Supply genes that cause cancer cells to revert back to normal cells;

iv. Deliver bacterial or viral genes as a form of vaccination;

v. Deliver DNA to antigen expression and generation of immune response;

vi. Supply of gene for impairing viral replication;

vii. Provide genes that promote or impede the growth of new tissue; and

viii. Deliver genes that stimulate the healing of damaged tissue.

There are two forms of gene therapy based on the types of cells in which genes are delivered:

i. Germline gene therapy:

a. In this method, healthy genes can be introduced into germ cells like sperms, eggs, early embryos.

b. It allows the transmission of the modified genetic information to the next generation.

c. Though it is highly effective in counteracting genetic disorders, it is not encouraged for application in human beings due to a variety of technical and ethical reasons.

ii. Somatic cell gene therapy:

a. In this type the gene is introduced only in somatic cells like bone marrow cells, hepatic cells, fibroblasts endothelium, and pulmonary epithelial cells, central nervous system, endocrine cells, and smooth muscle cells of blood vessel walls.

b. Modification of somatic cells only affects the person being treated and the modified chromosomes cannot be passed on the future generations.

c. Somatic cell gene therapy is the only feasible option and the clinical trials have already employed for the treatment of acquired disorders such as cancer and rheumatoid arthritis and blood disorders including SCID, Gaucher's disease, familial hypercholesterolemia, haemophilia, phenylketonuria, cystic fibrosis, sickle cell anaemia, Duchenne muscular dystrophy, emphysema, thalassemia, etc.

Exercise | Q 4.4 | Page 292

Long answer type question

How are the transgenic mice used in cancer research?

Solution:

Transgenic mice:

a. Transgenic mice that have been modified using a particular oncogene (cancer-causing gene) and thus developed a certain type of cancer, is useful to answer questions concerning the relationship between oncogenes and cancer development.

b. Theoretically, such animals can also be used for research into cancer treatment and prevention of malignancy.

c. In the laboratory, one such a transgenic mouse model for the investigation of breast cancer was developed. The oncogenes Myc and ras were analyzed to find out if they lead to breast cancer in mice transformed with these genes.

Exercise | Q 4.5 | Page 292

Long answer type question

Give the steps in PCR or polymerase chain reaction with suitable diagrams.

Solution:

Polymerase Chain Reaction (PCR) is the process of in vitro amplification of the gene of interest using a PCR machine.

i. PCR can generate a billion copies of the desired segment of DNA or RNA, with high accuracy and specificity, in a few hours.

ii. The process of PCR is completely automated and involves automatic thermal cycles for denaturation and renaturation of double-stranded DNA.

iii. The device required for PCR is called a thermal cycler.

iv. **Requirements for polymerase chain reaction:**

a. DNA containing the desired segment to be amplified

b. several molecules of four deoxyribonuclueoside triphosphates (dNTPs)

c. excess of two primer molecules

d. heat-stable DNA polymerase and

e. appropriate quantities of Mg^{++} ions.

Mechanism of PCR:

At the start of PCR, all the requirements are mixed together in ‘eppendorf tube’ and the following operations are performed sequentially:

Step i: Denaturation

The reaction mixture is heated to a temperature (90–98oC) to separate two strands of desired DNA. This is called denaturation.

Step ii: Annealing

The mixture is allowed to cool (40–60oC) that permits the pairing of the primer to the complementary sequences in DNA. This step is called annealing.

Step iii: Primer extension / Polymerization

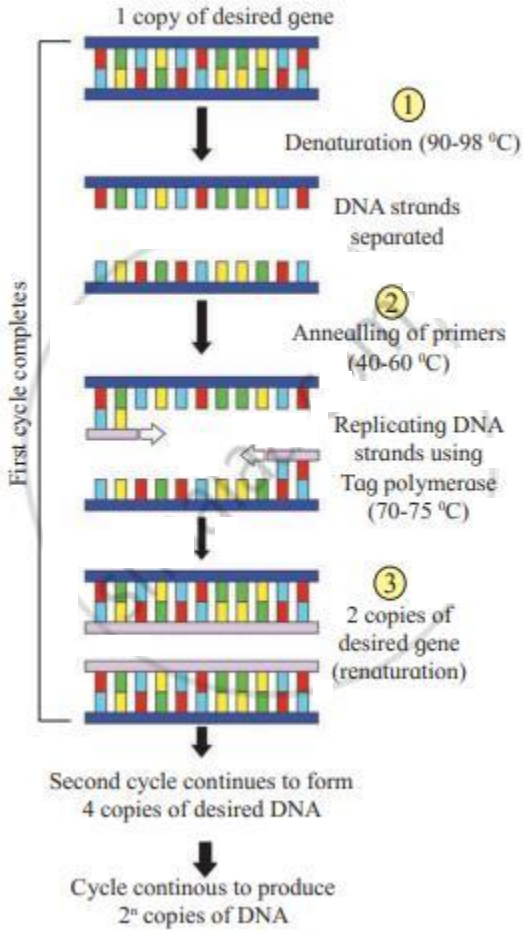
The temperature (70–75oC) allows thermostable Taq DNA polymerase to use single-stranded DNA as a template and adds nucleotides. This is called primer extension. It takes around two minutes duration.

v. One cycle takes around 3 to 4 minutes.

vi. To begin the second cycle, DNA is again heated to convert double-stranded DNA into single strands.

vii. In an automatic thermal cycler, the above three steps are automatically repeated 20-30 times. Thus, at the end of 'n' cycles, 2n copies of DNA segments are produced.

viii. The machine performs the entire operations automatically and precisely.



DNA replication through a polymerase chain reaction.

Exercise | Q 4.6 | Page 292

Long answer type question

What is a vaccine? Give advantages of oral vaccines or edible vaccines.

Solution:

A vaccine is a biological preparation that provides active acquired immunity against a certain disease.

The benefit of such vaccines is the comfort of administration, low cost, and ease of storage.

Exercise | Q 4.7 | Page 292

Long answer type question

Enlist different types of restriction enzymes commonly used in rDNA technology? Write about their role.

Solution:

There are three types of restriction enzyme:

i. Type I – These enzymes function simultaneously as endonuclease and methylase e.g. EcoK.

ii. Type II – These enzymes have separate activities for cleaving and methylation; they are more stable and are used in rDNA technology

e.g. EcoRI, BglII; these enzymes cut DNA at specific sites within the palindrome. There are thousands of type II restriction enzymes that are recognized/ discovered.

iii. Type III – These enzymes cut DNA at specific non-palindromic sequences e.g. HpaI, MboII.

Role of restriction enzymes:

Restriction enzymes either cut straight across the DNA in the region of palindrome to give blunt ends or cuts producing short, single-stranded projections at each end of DNA to produce, cohesive or sticky ends or staggered ends.

Exercise | Q 4.8 | Page 292

Long answer type question

Enlist and write in brief about the different biological tools required in rDNA technology.

Solution:

The different biological tools required in rDNA technology:

i. Instruments: PCR, Agarose Gel Electrophoresis, SDS-PAGE

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DNA replication through a polymerase chain reaction.

ii. Biological tools: Enzymes, Cloning Vectors, Competent host

Different enzymes used in rDNA technology are as follows: Lysozymes, Nucleases such as exonucleases, endonucleases, restriction endonucleases, DNA ligases, DNA polymerases, alkaline phosphatases, reverse transcriptase, etc.

i. Enzymes that cut the phosphodiester bonds of polynucleotide chains are called nuclease.

ii. These are of two types- exonuclease and endonuclease.

iii. Exonucleases cut nucleotides from the ends of DNA strands whereas endonuclease cut DNA from within.

iv. The phosphodiester backbone at highly specific sites on both strands of the duplex is cut by these enzymes called restriction endonucleases or simply restriction enzymes.

v. The restriction enzymes are thus the molecular scissors that are used to recognize and cut DNA at specific sequences.

vi. The sites recognized by them, are called recognition sequences or recognition sites.

vii. Different restriction enzymes found in different organisms recognize different nucleotide sequences and therefore cut DNA at different sites.

The following characteristic properties a cloning vector must possess in order to be used in rDNA technology:

i. A good vector should have the ability of independent replication so that as the vector replicates (through ori gene) and a large number of copies of the DNA insert will be formed.

ii. The vector should be able to easily introduce into host cells.

iii. A vector should have marker genes for antibiotic resistance.

iv. A vector must contain a unique cleavage site in one of the marker genes for the restriction enzyme.

v. It should have at least suitable control elements like a promoter, operator, ribosomal binding sites, etc.

vi. The plasmids obtained naturally do not possess all the characteristics. Hence, they are constructed by inserting a gene for antibiotic resistance.

e.g. pBR322, pBR320, pACYC177 are the constructed plasmids. pBR322 is mostly used in rDNA technology in plants.

Competent hosts (cloning organisms) used are usually bacteria like Bacillus haemophilus, Helicobacter pylori, and E. coli. Mostly E. coli is used for the transformation with recombinant DNA.