First Internal Examination (2019-20)
B.Sc. (BT) II Year
Recombinant DNA Technology
SET-A

Time: 1:30 Hours **Max. Marks-30**

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1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**

i. Give any 2 examples of restriction enzymes that produce blunt ends?

ANS…Sal I & SmaI

ii. Who is known as father of RDT or Genetic Engineering?

ANS… The first person to surmount these obstacles was the American biochemist **Paul Berg**, often referred to as the "father of genetic engineering." In 1973, **Berg** developed a method for joining the DNA from two different organisms: a monkey virus known as SV40 and a virus known as lambda phage.

iii. What are the functions of restriction enzymes?

ANS… A **restriction enzyme** is an **enzyme** that cuts DNA after recognizing a specific sequence of DNA. One can think of **restriction enzymes** as molecular scissors. When it finds its target sequence, a **restriction enzyme** makes a double stranded cut in the DNA molecule. **Restriction enzymes** are found in many different strains of bacteria, where their **biological role** is to participate in cell defense.

iv. What is reverse transcription?

ANS… The synthesis of DNA from an RNA template, via reverse transcription, produces complementary DNA (cDNA). Reverse transcriptases (RTs) use an RNA template and a short primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA.

v. A Vector should have what properties to be used for recombination?

ANS… **Characteristics of a Cloning Vector**

Origin of replication (ORI)

This process marks autonomous replication in vector. ORI is a specific sequence of nucleotide in DNA from where replication starts.

Selectable Marker

Besides ORI, a cloning vector should have selectable marker gene. This gene permits the selection of host cells which bear recombinant DNA (called transformants) from those which do not bear rDNA (non-transformants).

Restriction sites

It should have restriction sites, to allow cleavage of specific sequence by specific Restriction Endonuclease.

vi. Name any 4 enzymes essential for Recombinant DNA Technology?

ANS…Restriction enzymes, DNA Polymerase, DNA Ligase, Nucleases

vii. Which enzyme is used for synthesizing DNA molecules using mRNA as template?

ANS… A **reverse transcriptase** (RT) is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed **reverse transcription**.

viii. What is DNA ligase?

ANS… **DNA ligase** is a specific type of enzyme, a **ligase**, (EC 6.5.1.1) that facilitates the joining of **DNA** strands together by catalyzing the formation of a phosphodiester bond.

2. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. Write short note on steps involved in RDT?

ANS… **Steps in Recombinant DNA Technology:**

Basic steps involved in rec DNA technology (or genetic engineering) are given below (Fig.1):

i. Selection and isolation of DNA insert

ii. Selection of suitable cloning vector

iii. Introduction of DNA-insert into vector to form rec DNA molecule

iv. rec DNA molecule is introduced into a suitable host.

v. Selection of transformed host cells.

vi. Expression and multiplication of DNA-insert in the host.

(i) Selection and isolation of DNA insert:

First step in rec DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed as DNA insert or foreign DNA or target DNA or cloned DNA.

(ii) Selection of suitable cloning vector:

A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids and bacteriophages.

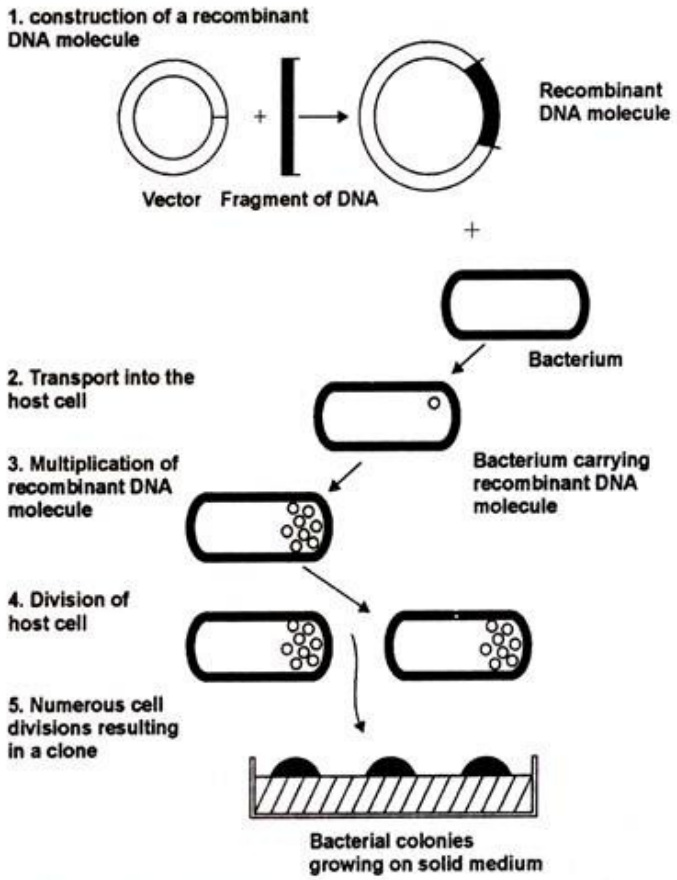


Fig. 1. The basic steps of rec DNA Technology using the bacterial plasmid as cloning vector.

(iii) Introduction of DNA-insert into vector to form recDNA molecule:

The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes [in step (i)] are now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

(iv) rec DNA molecule is introduced into a suitable host:

Suitable host cells are selected and the rec DNA molecule so formed [in step (iii)] is introduced into these host cells. This process of entry of rec DNA into the host cell is called transformation. Usually selected hosts are bacterial cells like E. coli, however yeast, fungi may also be utilized.

(v) Selection of transformed host cells:

Transformed cells (or recombinant cells) are those host cells which have taken up the recDNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

(vi) Expression and Multiplication of DNA insert in the host:

Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

ii. What is the function of alkaline phosphatase and polynucleotide kinase enzymes?

ANS… Functions of alkaline phosphatase: Alkaline Phosphatase is suitable for removal of terminal monoesterified phosphates from deoxyribo-oligonucleotides. Used for the removal of single phosphate groups from 5´-ends of linear vectors to prevent re-circularization during cloning or to dephosphorylate DNA prior to kinase labelling protocols.

Features:
– Free from detectable nonspecific nuclease, endonuclease, RNase and DNase activities
– Very stable enzyme
– Supplied with an optimized reaction buffer containing Zn^{2+} for efficient activity
Applications:
– Dephosphorylation of DNA
– Treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis.
Functions of Polynucleotide kinase: **Polynucleotide** 5'-hydroxyl-**kinase**. ... **Polynucleotide kinase** is a T7 bacteriophage (or T4 bacteriophage) **enzyme** that catalyzes the transfer of a gamma-phosphate from ATP to the free hydroxyl end of the 5' DNA or RNA. The resulting product could be used to end-label DNA or RNA, or in a ligation reaction.

- PNK is a homotetramer with phosphatase activity at 3' end and kinase activity at 5' end with a tunnel like active site. The active site has side chains which interact with NTP donor's beta-phosphate and 3' phosphate of acceptor with an acid which activated 5' –OH. Lys-15 and Ser-16 are important for the kinase activity of the enzyme.

- The basic residues of active site of PNK interact with the negatively charged phosphates of the DNA.

- Polynucleotide kinase (PNK) catalyzes the transfer of a phosphate group (PO_4^{-2}) from γ position of ATP to the 5' end of either DNA or RNA and nucleoside monophosphate .

- PNK can convert 3' PO_4 /5' OH ends into 3' PO_4 /5' PO_4 ends which blocks further ligation by ligase enzyme.

- PNK is used to label the ends of DNA or RNA with radioactive phosphate group.

- T4 polynucleotide kinase is the most widely used PNK in molecular cloning experiments, which was isolated from T4 bacteriophage infected *E.coli*.

3 Long Answer Questions (7 Marks each 2x7= 14 Marks)

i. What are restriction enzymes, explain in detail?

ANS… The breakthrough that made **recombinant DNA technology** possible was the discovery and characterization of **restriction enzymes**. **Restriction enzymes** are produced by bacteria as a defense mechanism against phages. The **enzymes** act like scissors, cutting up the DNA of the phage and thereby inactivating it. Restriction enzymes are found in bacteria (and other prokaryotes). They recognize and bind to specific sequences of DNA, called **restriction sites**. Each restriction enzyme recognizes just one or a few restriction sites. When

it finds its target sequence, a restriction enzyme will make a double-stranded cut in the DNA molecule. Typically, the cut is at or near the restriction site and occurs in a tidy, predictable pattern.

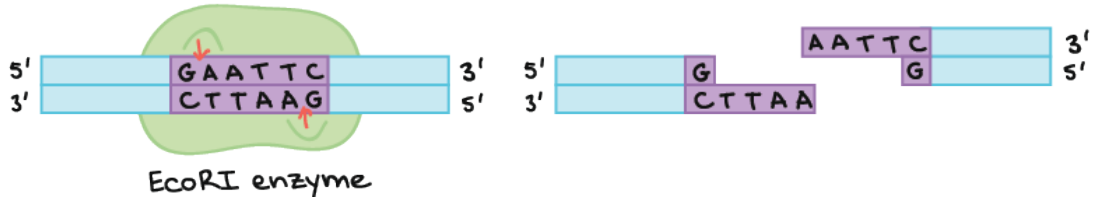
It's thought that restriction enzymes evolved as a defense mechanism, allowing bacteria to chop up potentially harmful foreign DNA (e.g., DNA from bacteria-infecting viruses).

As an example of how a restriction enzyme recognizes and cuts at a DNA sequence, let's consider *Eco*RI, a common restriction enzyme used in labs. *Eco*RI cuts at the following site:

5'-...GAATTC...-3' 3'-...CTTAAG...-5'

EcoRI site

When EcoRI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA "overhangs":



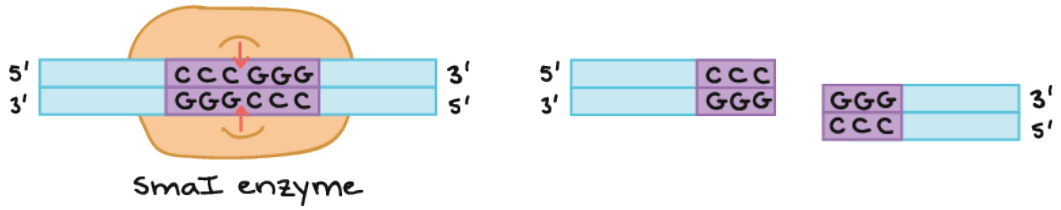
An *Eco*RI enzyme binds to an *Eco*RI site in a piece of DNA and makes a cut on both strands of the DNA. The pattern of the cut is:

5'-...G|AATTC...-3' 3'-...CTTAA|G...-5'

Thus, it produces an overhang of 5'-AATT-3' on each end of the cut DNA.

If another piece of DNA has matching overhangs (for instance, because it has also been cut by EcoRI), the overhangs can stick together by complementary base pairing. For this reason, enzymes that leave single-stranded overhangs are said to produce **sticky ends**. Sticky ends are helpful in cloning because they hold two pieces of DNA together so they can be linked by DNA ligase.

Not all restriction enzymes produce sticky ends. Some are "blunt cutters," which cut straight down the middle of a target sequence and leave no overhang. The restriction enzyme *Sma*I is an example of a blunt cutter:



A *Sma*I enzyme binds to the *Sma*I restriction site, which is:

5'-...CCCGGG...-3' 3'-...GGGCCC...5'

It makes a cut right in the middle of this sequence on both strands, producing blunt ends. The cut sites are:

5'-...CCC|GGG...-3' 3'-...GGG|CCC...5'

Blunt-ended fragments can be joined to each other by DNA ligase. However, blunt-ended fragments are harder to ligate together (the ligation reaction is less efficient and more likely to fail) because there are no single-stranded overhangs to hold the DNA molecules in position.

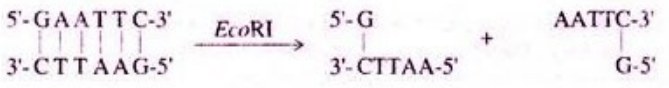
Table 1: Recognition sequences of several Restriction Endonucleases

Enzyme	Source Organism	Recognition Sequence 5′ ..... 3′	Blunt or Sticky ends.
EcoRI	*Escherichia coli*	GAATCC	Sticky
BglII	*Bacillus globigii*	AGATCT	Sticky
HindII	*Haemophilus influenzae*	GTPyPuAC	Blunt
HindIII	*Haemophilus influenzae*	AAGCTT	Sticky
HinfI	*Haemophilus influenzae*	GANTC	Sticky
HpaI	*H. parainfluenzae*	GTTAAC	Blunt
HaeIII	*H. aegyptius*	GGCC	Blunt
Sau 3A	*Staphylococcus aureus*	GATC	Sticky
PvuI	*Proteus vulgaris*	CGATCG	Sticky
BamHI	*Bacillus amyloliquefaciens*	GGATCC	Sticky
TaqI	*Thermus aquaticus*	TCGA	Sticky
SmaI	*Serratia marcescens*	CCCGGG	Blunt
SfiI	*Streptomyces fimbriatus*	GGCCNNNNNGGCC	Sticky
SalI	*Streptomyces albus*	GTCGAC	Sticky

ii. Write short note on milestones of Recombinant DNA Technology?

ANS… The first break through of rDNA technology occurred with the discovery of restriction endonucleases (restriction enzyme) during the late 1960s by Werner, Arber and Hamilton Smith. The restriction enzymes were discovered in microorganisms. These enzymes protect the host cell from the bacteriophage. The restriction enzymes are described in the preceding section.

In 1969, Herbert Boyer isolated restriction enzyme EcoRI from E. coli that cleaves the DNA between G and A in the base sequence GAATTC as below:



In 1970 Howard Temin and Davin Baltimore independently discovered the enzyme reverse transcriptase from retroviruses. Later on this enzyme was used to construct a DNA called complementary DNA (cDNA) from any mRNA.

In, 1972 David Jackson, Robert Symons and Paul Berg successfully generated rDNA molecules. They allowed the stickly ends of complementary DNA by using an enzyme DNA ligase.

In 1973 for the first time S.Cohen and H. Boyer developed a recombinant plasmid (pSC101) which after using as vector replicated well within a bacterial host. In, 1975, Edwin M.Southern developed a method for detection of specific DNA fragments for isolation of a gene from complex mixture of DNA. This method is known as the Southern blotting technique.

Milestones of Recombinant DNA:

Some milestones of recombinant DNA technology have been summarized as below:

1976 – First prenatal diagnosis by using gene specific probe.

1977 – Methods for rapid DNA sequencing, discovery of split genes and somatostanin by rDNA.

1979 – Insulin synthesized by using rDNA; first human viral antigen.

1981 – Foot and mouth disease viral antigen cloned.

1982 – Commercial production of coli of genetically engineered human insulin, Isolation, cloning and characterization of human cancer gene.

1983 – Engineered Ti-plasmid used to transform plants.

1985 – Insertion of cloned gene from Salmonella into tobacco plant to make resistant to herbicide glyphosphate; Development of PCR technique.

1986 – Development of gene gun.

1989 – First field test of genetically engineered virus (baculovirus) that kills cabbage looper caterpillars.

1990 – Production of first transformed com.

1991 – Production of first transgenic pigs and goats, manufacture of human haemoglobin, first test of gene therapy on human cancer patients.

1994 – The Flavr Savr tomato introduced; the first genetically engineered whole food approved for sale. Fully human monoclonal antibodies produced in genetically engineered mice.

1997 – World's first mammalian clone (Dolly) developed from a non-reproductive cell of an adult animal through cloning by nuclear transplantation.

First Internal Examination (2019-20)
B.Sc. (BT) II Year
Recombinant DNA Technology
SET-B

Time: 1:30 Hours **Max. Marks-30**

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1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**
   i. Define Recombinant DNA technology?

ANS… **Recombinant DNA technology**, joining together of **DNA** molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.

ii. How many types of restriction enzymes are found?

ANS… Restriction enzymes are traditionally classified into three types on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements.

There are 3 main categories of restriction endonuclease enzymes:

Type-I Restriction Endonucleases

Type-II Restriction Endonucleases

Type-III Restriction Endonucleases

iii. Who created the first strand of recombinant DNA?

ANS… **Stanley Cohen** and **Herbert Boyer** inserted the recombinant DNA molecule they created into E. coli bacteria by means of a plasmid, thereby inducing the uptake and expression of a foreign DNA sequence known as "transformation."

iv. What is gene cloning?

ANS… Gene cloning is a commonly used molecular biological technique in which a gene of interest is fused into a self-replicating genetic element called a plasmid, which when introduced into a suitable host (usually bacteria), self-replicates and generates a large number of identical copies of the particular gene.

v. A Host cell should have what properties to be used in Recombinant DNA Technology?

ANS… 1. Can allow the easy entry of the recombinant DNA easily into the cell.

2. Should not destroy the recombinant DNA as a foreign DNA and degrade it. The main feature determining transformation efficiency is the presence or absence of endogenous DNA-degrading systems.

3. Can stably maintain the recombinant DNA. Once a recombinant DNA has entered the cell, it is still not guaranteed to replicate indefinitely and stably even if it has a suitable origin of replication.

4. The transformed host must not independently sustain outside the laboratory.

5. Should be easy to maintain and handle.

6. Should be available as a wide variety of genetically defined strains.

7. Should accept a range of vectors.

vi. What is cDNA?

ANS… Complementary DNA (cDNA) is DNA synthesized from a single stranded RNA (e.g., messenger RNA (mRNA) or microRNA) template in a reaction catalyzed by the enzyme reverse transcriptase. *cDNA* is often used to clone eukaryotic genes in prokaryotes.

vii. Who first isolated EcoRI enzyme?

ANS… Restriction enzymes were discovered and characterized in the late 1960s and early 1970s by molecular biologists Werner Arber, Hamilton O. Smith, and Daniel Nathans.

viii. What is Taq Polymerase?

ANS… Taq polymerase is a thermostable DNA polymerase named after the thermophilic bacterium Thermus aquaticus from which it was originally isolated by Chien et al. in 1976. Its name is often abbreviated to Taq Pol or simply Taq. Taq polymerase is an enzyme that copies DNA. It is isolated from a heat-loving bacterium that is naturally found in hot springs, so the enzyme doesn't break down at the high temperatures necessary for copying DNA using a polymerase chain reaction.

2. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. What is restriction modification system?

ANS… The **restriction modification system** (**RM system**) is found in bacteria and other prokaryotic organisms, and provides a defense against foreign DNA, such as that borne by bacteriophages.

Bacteria have restriction enzymes, also called restriction endonucleases, which cleave double stranded DNA at specific points into fragments, which are then degraded further by other endonucleases. This prevents infection by effectively destroying the foreign DNA introduced by an infectious agent (such as a bacteriophage). Approximately one-quarter of known bacteria possess RM systems and of those about one-half have more than one type of system.

As the sequences recognized by the restriction enzymes are very short, the bacterium itself will almost certainly contain some within its genome. In order to prevent destruction of its own DNA by the restriction enzymes, methyl groups are added. These modifications must not interfere with the DNA base-pairing, and therefore, usually only a few specific bases are modified on each strand.

Endonucleases cleave internal/non-terminal phosphodiester bonds. Restriction endonucleases cleave internal phosphodiester bonds only after recognising specific sequences in DNA which are usually 4-6 base pairs long, and often palindromic.

ii. Write short note on DNA polymerases?

ANS… **DNA polymerases:**

These are the enzymes which synthesize a new complementary DNA strand of an existing DNA or RNA template. A few important types of DNA polymerases are used routinely in genetic engineering. One such enzyme is DNA polymerase ! which , prepared from E coli. The Klenow fragment of DNA polymerase-I .s employed to make the protruding ends double-stranded by extension of the shorter strand.

Another type of DNA polymerase used in genetic engineering is Taq DNA polymerase which is used in PCR (Polymerase Chain Reaction).

Reverse transcriptase is also an important type of DNA polymerase enzyme for genetic engineering. It uses RNA as a template for synthesizing a new DNA strand called as cDNA a e complementary DNA). Its main use is in the formation of cDNA libraries. Apart from all these above mentioned enzymes, a few other enzymes also mark their importance in genetic engineering.

3. Long Answer Questions (7 Marks each 2x7= 14 Marks)

i. Write short note on steps & tools involved in Reverse Transcription?

ANS… **Steps in Recombinant DNA Technology:**

Basic steps involved in rec DNA technology (or genetic engineering) are given below (Fig.1):

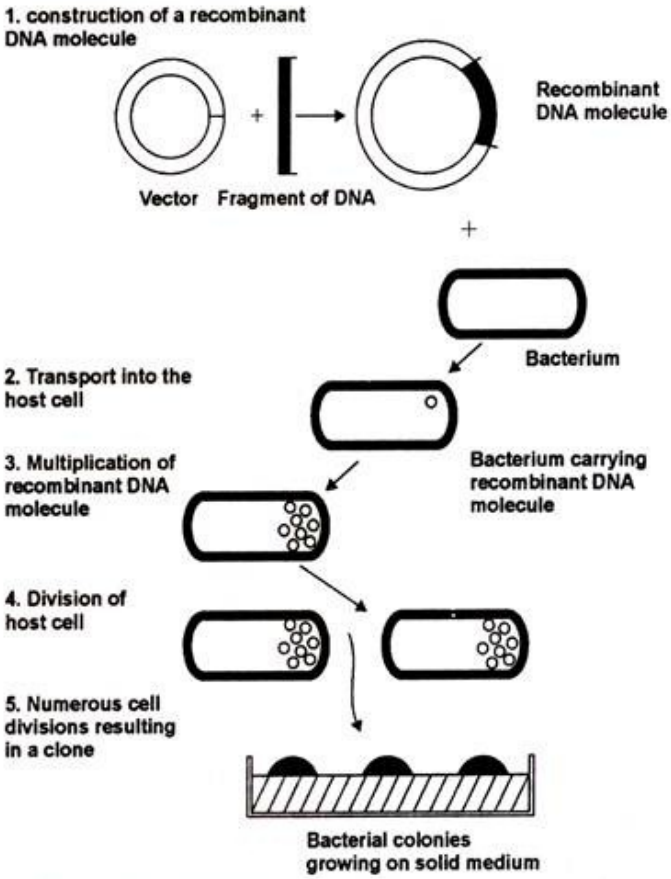

Fig. 1. The basic steps of rec DNA Technology using the bacterial plasmid as cloning vector.

i. Selection and isolation of DNA insert

ii. Selection of suitable cloning vector

iii. Introduction of DNA-insert into vector to form rec DNA molecule

iv. rec DNA molecule is introduced into a suitable host.

v. Selection of transformed host cells.

vi. Expression and multiplication of DNA-insert in the host.

(i) Selection and isolation of DNA insert:

First step in rec DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed as DNA insert or foreign DNA or target DNA or cloned DNA.

(ii) Selection of suitable cloning vector:

A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids and bacteriophages.

(iii) Introduction of DNA-insert into vector to form recDNA molecule:

The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes [in step (i)] are now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

(iv) rec DNA molecule is introduced into a suitable host:

Suitable host cells are selected and the rec DNA molecule so formed [in step (iii)] is introduced into these host cells. This process of entry of rec DNA into the host cell is called

transformation. Usually selected hosts are bacterial cells like E. coli, however yeast, fungi may also be utilized.

(v) Selection of transformed host cells:

Transformed cells (or recombinant cells) are those host cells which have taken up the recDNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

(vi) Expression and Multiplication of DNA insert in the host:

Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

Tools for Recombinant DNA Technology:

Important biological tools for rec DNA technology are:

(A) Enzymes:

a. Restriction Endonucleases

b. Exonucleases

c. DNA ligases

d. DNA polymerase

(B) Cloning Vector

(C) Host organism

(D) DNA insert or foreign DNA

(E) Linker and adaptor sequences.

ii). what are the enzymes involved in recombinant DNA Technology, briefly explain?

ANS… ENZYMES:

A number of specific enzymes are utilized to achieve the objectives of rec DNA technology.

The enzymology of genetic engineering includes the following types of enzymes:

(a) Restriction Endonuclease:

These enzymes serve as important tools to cut DNA molecules at specific sites, which is the basic need for rec DNA technology.

These are the enzymes that produce internal cuts (cleavage) in the strands of DNA, only within or near some specific sites called recognition sites/recognition sequences/ restriction sites 01 target sites. Such recognition sequences are specific for each restriction enzyme. Restriction endonuclease enzymes are the first necessity for rec DNA technology.

The presence of restriction enzymes was first of all reported by W. Arber in the year 1962. He found that when the DNA of a phage was introduced into a host bacterium, it was fragmented into small pieces. This led him to postulate the presence of restriction enzymes. The first true restriction endonuclease was isolated in 1970s from the bacterium E. coli by Meselson and Yuan.

Another important breakthrough was the discovery of restriction enzyme Hind-II in 1970s by Kelly, Smith and Nathans. They isolated it from -the bacterium Haemophilus influenza. In the year 1978, the Nobel Prize for Physiology and Medicine was given to Smith, Arber and Nathans for the discovery of endonucleases.

Types of Restriction Endonucleases:

There are 3 main categories of restriction endonuclease enzymes:

Type-I Restriction Endonucleases

Type-II Restriction Endonucleases

Type-III Restriction Endonucleases

Type-I Restriction Endonucleases:

These are the complex type of endonucleases which cleave only one strand of DNA. These enzymes have the recognition sequences of about 15 bp length (Table 1).

They require Mg++ ions and ATP for their functioning. Such types of restriction endonucleases cleave the DNA about 1000 bp away from the 5′ end of the sequence ‘TCA’ located within the recognition site. Important examples of Type-I restriction endonuclease enzyme are EcoK, EcoB, etc.

Table 1: Recognition sequences of several Restriction Endonucleases

Enzyme	Source Organism	Recognition Sequence 5′ ..... 3′	Blunt or Sticky ends.
EcoRI	*Escherichia coli*	GAATCC	Sticky
BglII	*Bacillus globigii*	AGATCT	Sticky
HindII	*Haemophilus influenzae*	GTPyPuAC	Blunt
HindIII	*Haemophilus influenzae*	AAGCTT	Sticky
HinfI	*Haemophilus influenzae*	GANTC	Sticky
HpaI	*H. parainfluenzae*	GTTAAC	Blunt
HaeIII	*H. aegyptius*	GGCC	Blunt
Sau 3A	*Staphylococcus aureus*	GATC	Sticky
PvuI	*Proteus vulgaris*	CGATCG	Sticky
BamHI	*Bacillus amyloliquefaciens*	GGATCC	Sticky
TaqI	*Thermus aquaticus*	TCGA	Sticky
SmaI	*Serratia marcescens*	CCCGGG	Blunt
SfiI	*Streptomyces fimbriatus*	GGCCNNNNNGGCC	Sticky
SalI	*Streptomyces albus*	GTCGAC	Sticky

Type-II Restriction Endonucleases:

These are most important endonucleases for gene cloning and hence for rec DNA technology. These enzymes are most stable. They show cleavage only at specific sites and therefore they produce the DNA fragments of a defined length. These enzymes show cleavage in both the strands of DNA, immediately outs.de then- recognition sequences. They require Mg++ ions for their functioning.

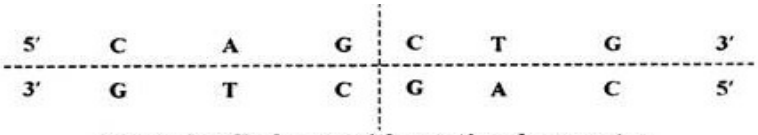

Fig. 2. A palindrome with rotational symmetry.

Such enzymes are advantageous because they don't require ATP for cleavage and they cause cleavage in both strands of DNA. Only Type II Restriction Endonucleases are used tor gene cloning due to their suitability.

The recognition sequences for Type-II Restriction Endonuclease enzymes are in the form of palindromic sequences with rotational symmetry, i.e., the base sequence .n the first half of one strand of DNA is the mirror image of the second half of other strand of that DNA double helix (Fig. 2). Important examples of Type-II Restriction endonucleases include Hinfl, EcoRI, PvuII, Alul, Haelll etc.

Type-III Restriction Endonucleases:

These are not used for gene cloning. They are the intermediate enzymes between Type-I and Type-II restriction endonuclease. They require Mg++ ions and ATP for cleavage and they cleave the DNA at well-defined sites in the immediate vicinity of recognition sequences, e.g. Hinf III, etc.

Nature of cleavage by Restriction Endonucleases:

The nature of cleavage produced by a restriction endonuclease is of considerable importance.

They cut the DNA molecule in two ways:

i. Many restriction endonucleases cleave both strands of DNA simply at the same point within the recognition sequence. As a result of this type of cleavage, the DNA fragments with blunt ends are generated. PvuII, Haelll, Alul are the examples of restriction endonucleases producing blunt ends. Blunt ends may also be referred to as flush ends.

ii. In the other style of cleavage by the restriction endonucleases, the two strands of DNA are cut at two different points. Such cuts are termed as staggered cuts and this results into the generation of protruding ends i.e., one strand of the double helix extends a few bases beyond the other strand. Such ends are, called cohesive or sticky ends.

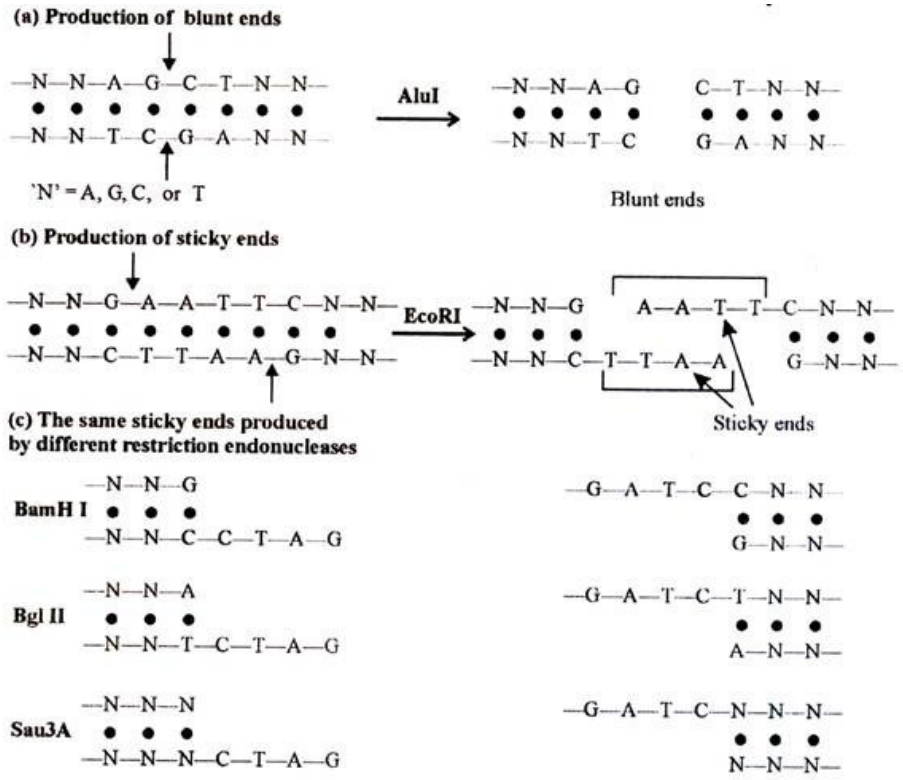

Fig. 3. Mode of Action of Restriction Endonucleases.

Such ends have the property to pair readily with each other when pairing conditions are provided. Another feature of the restriction endonucleases producing such sticky ends is that two or more of such enzymes with different recognition sequences may generate the same sticky ends.

(b) Exonucleases:

Exonuclease is an enzyme that removes nucleotides from the ends of a nucleic acid molecule. An exonuclease removes nucleotide from the 5′ or 3′ end of a DNA molecule. An exonuclease never produces internal cuts in DNA.

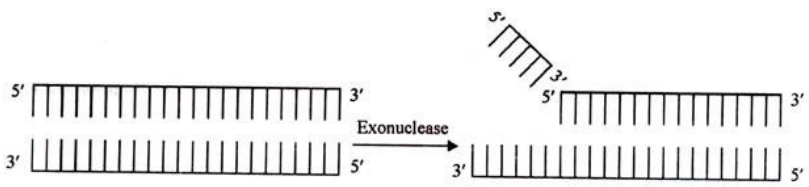

Fig. 4. An Exonuclease activity : Nucleotides are removed from the end of DNA

In rec DNA technology, various types of exonucleases are employed like Exonuclease Bal31, E. coli exonuclease III, Lambda exonuclease, etc.

Exonculease Bal31 are employed for making the DNA fragment with blunt ends shorter from both its ends.

E coli Exonuclease III is utilized for 3'end modifications because it has the capability to remove nucleotides from 3′-OH end of DNA.

Lambda exonuclease is used to modify 5′ ends of DNA as it removes the nucleotides from 5′ terminus of a linear DNA molecule.

(c) DNA ligase:

The function of these enzymes is to join two fragments of DNA by synthesizing the phosphodiester bond. They function to repair the single stranded nicks in DNA double helix and in rec DNA technology they are employed for sealing the nicks between adjacent nucleotides. This enzyme is also termed as molecular glue.

(d) DNA polymerases:

These are the enzymes which synthesize a new complementary DNA strand of an existing DNA or RNA template. A few important types of DNA polymerases are used routinely in genetic engineering. One such enzyme is DNA polymerase ! which , prepared from E coli. The Klenow fragment of DNA polymerase-I .s employed to make the protruding ends double-stranded by extension of the shorter strand.

Another type of DNA polymerase used in genetic engineering is Taq DNA polymerase which is used in PCR (Polymerase Chain Reaction).

Reverse transcriptase is also an important type of DNA polymerase enzyme for genetic engineering. It uses RNA as a template for synthesizing a new DNA strand called as cDNA a e complementary DNA). Its main use is in the formation of cDNA libraries. Apart from all these above mentioned enzymes, a few other enzymes also mark their importance in genetic engineering.

A brief description of these is given below:

(a) Terminal deoxynucleotidyl transferase enzyme:

It adds single stranded sequences to 3′-terminus of the DNA molecule. One or more deoxynbonucleotides (dATP, dGTP, dl IP, dCTP) are added onto the 3′-end of the blunt-ended fragments.

(b) Alkaline Phosphatase Enzyme:

It functions to remove the phosphate group from the 5′-end of a DNA molecule.

(c) Polynucleotide Kinase Enzyme:

It has an effect reverse to that of Alkaline Phosphatase, i.e. it functions to add phosphate group to the 5′-terminus of a DNA molecule