

[1] Note on cloning vectors

Note on PBR-822 vector.

→ cloning vectors are DNA molecules that carry a foreign DNA segment & replicate in host cell.

(MSD) A vector must possess following characters :-

(1) It must replicate after introductⁿ to cell.

(2) It must possess marker gene such as "tet^R" for tetracyclin resistance & "amp^R" for ampicillin resistance.

(3) This help in selection of transformed cell from untransformed cell.

(4) It should have specific control system like promoters, terminators, ribosome binding site etc so that cloned DNA can express.

(5) Should be small in size so that can easily integrate into specific host cell.

(5) Needs a restriction site for insertion of target DNA.

⇒ Types of cloning vector :-

① Plasmids :- Small circular extrachromosomal double stranded DNA, self replicating molecule present in Prokaryotic cells.

→ Can carry foreign DNA of size 5-15 kbp to bacteria or plant cells.

② Viral DNAs → Single / double stranded, Circular / linear DNAs.

DNA of phage λ & M13 phage carry gene to bacteria.

DNA of Simian virus 40 carry gene to animal cells.

Viral DNAs can carry 10-25 kbp size segment.

③ Cosmids - Cosmids are type of constructed Plasmids containing complementary single stranded site (cos-site) of λ DNA.

- They can carry DNA segments of 15-45 kbp to bacteria.

PBR-322

- It is an artificial plasmid and a gene cloning vector from E-coli.

- It is constructed from, 2 Plasmids & a transposon
• PSC101 • Tn 3.
• Col E1

- In Plasmid PBR322, P → Plasmid.

BR → Bolívar & Rodriguez,
who created the plasmid.

322 → Specific no., distinguishing it from other plasmid.

- It has 4361 base pairs and unique restriction sites for more than 40 restriction enzymes.

- It has two selectable gene markers Tetacycline (tet^R) & ampicillin resistance (Amp^R) gene.

Advantages → Due to small size (c. 4.4 kbp) enables easy purification & manipulation.

→ Can be amplified upto 1000-3000 copies.

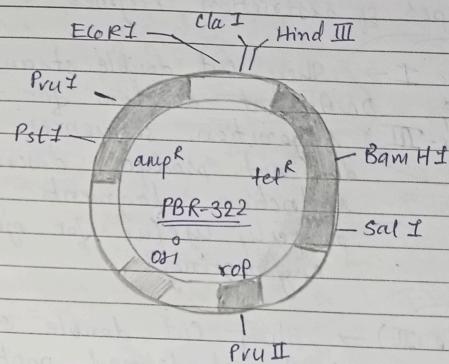
→ Can carry large DNA segments upto 5-10 kbp.

Uses → Widely used cloning vector to introduce desired gene to host cell.

→ Used as a model system to study Prokaryotic transcription & translation.

Disadvantages → There is a limitation of size of gene that can be accommodated.

→ It has very high mobility, so vector may get lost in population of mixed host cell.



c. Define gene cloning & exp gene cloning by restriction endonucleases.

- Note on restriction endonucleases.

"Gene cloning" → Production of multiple copies of particular gene by using techniques of genetic engineering.

→ DNA segment of interest is cleaned from chromosomes using restriction endonucleases.

→ Restriction endonucleases are enzymes that cut DNA at specific recognition sites.

Non-palindromic → sequence is not same in any one direction (e.g. 5' → 3') on both strands

→ Types of restriction enzymes :-

Type I → They cut double stranded DNAs at random far from recognition sequences.

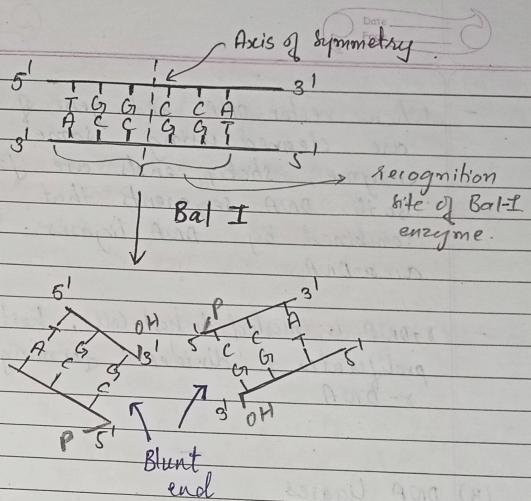
Type III → They produce discrete restriction fragments so not properly useful for genetic engineering.

Type II → They cut double stranded DNAs at defined positions close to or within recognition sequences. They produce discrete restriction fragments so are highly used in genetic engineering.

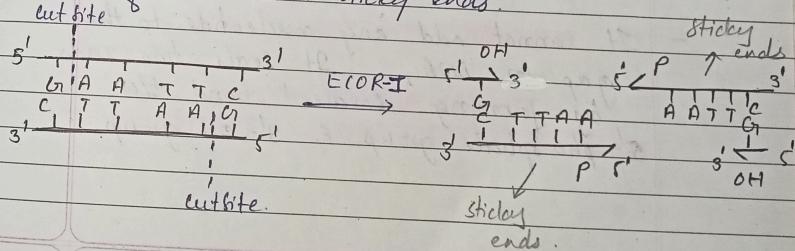
→ Plane of Cutting

- Restriction enzymes hydrolyse covalent phosphodiester bonds of DNA to "blunt" or "sticky" ends.

- If the enzymes cut the DNAs along the axis of symmetry of restriction site, two blunt ends are formed.



→ if the enzyme cut one strand at left side of axis of symmetry and other strand at right side of axis, DNA fragments are formed with sticky ends.



- When vector-DNA and gene of interest are cleaned using same restriction enzyme, sticky ends are formed of both DNA segments that can be combined by DNA ligases. This forms a λ -DNA.
- λ -DNA is inserted to host cell, host cell proliferates & divides forming copies of λ -DNA.

(3) DNA Ligases

- DNA ligase is an enzyme that joins two ends of DNA, this process is called ligation.
- It cannot add any nucleotide to the gap in DNA, it seals two ends by forming covalent bond b/w $5'$ phosphate group & $3'$ -OH group.

- This bond is called phosphodiester bond.
- DNA ligases isolated from *E. coli* requires both ATP & NAD⁺ for enzyme activity & DNA ligases isolated from *lambda T4 phage* requires only ATP for activity.
- Uses → To join vector DNA & Target DNA to form λ -DNA.
- To join DNA fragments of different organisms for making vectors with desired characters.
- To join dinucleotides together for chemical synthesis of DNA.

(4) Recombinant DNA technology

- Recombinant DNA is a hybrid DNA formed by joining desired foreign DNA & vector DNA.
 - r-DNA is also known as "chimeric DNA".
- Recombinant DNAs are constructed for the following reasons :-
- (i) To protect foreign DNA from nucleic acid enzyme of recipient cell.
 - (ii) To make foreign DNA replicate along with vector DNA.
 - (iii) To have genetic markers for selection of recombinants.
 - (iv) To enhance expression of foreign gene in recipient cell.

→ Steps to form r-DNA :-

(given in genetic engineering)

(5) Application of r-DNA technology :-

(i) Genetically modified Products :-

- (i) GM organism
- (ii) " Crops
- (iii) " Animals
- (iv) Artificial Blood

} given in detail
in genetic engineering

(ii) Therapeutic Products

- (i) Recombinant Protein
- (ii) Vaccines
- (iii) Growth hormones
- (iv) Drugs
- (v) antibodies

(iii) Diagnoh's :-

- monitoring devices
- Gene therapy
- CRISPR - clustered regularly interspaced short palindromic repeats .

(iv) Energy application → Bioethanol, Biomethanol

given in detail ← Biohydrogen, Biobutanol,
in genetic engineering Biofuels .

Methods of r-DNA technology:-

- Diff methods involved in r-DNA technology :-

(1) Gene cloning → Isolating & replicating specific gene to produce multiple copies.

(2) PCR → amplifying specific DNA sequences using DNA polymerase allowing prodⁿ of large amount of DNA.

(3) Genomic library → creating collection of construct cloned DNA fragments representing an organism's entire genome.

(4) Transformation → introducing foreign DNA into host cells, often using bacterial cells.

(5) Southern Blotting → Detecting specific DNA sequences using hybridization, useful for studying gene structure & variation.

(6) Northern Blotting → Similar to southern blotting but used to study RNA molecules

(7) Western Blotting → detecting specific proteins using antibodies allowing analysis of protein expression.

Analyzing Any 1 in detail is given ahead - PCR
to, ✓
of protein expression

(6) Production of Hepatitis B vaccine by r-DNA technology. (Draw fig)

- Hepatitis B is irritation of swelling of liver due to infection with hepatitis B virus (HBV)

- Three types of viral coat proteins are recognized to be antigenic

✓ Viral surface antigen (HBsAg)

✓ Viral core antigen (HBcAg)

✓ the e- antigen (HBeAg)

- Recently Hepatitis B virus DNA has been successfully cloned in E.coli & mammalian cells. Synthesis of HBsAg & HBcAg particles has been done in cells.

(Broken)

Hepatitis B antigen Producing gene is isolated from the HB virus by normal isolation method (cell lysis, protein denaturation, precipitation, centrifugation & drying).

Plasmid DNA is extracted from Bacterium E.coli & cut with restriction enzyme forming plasmid vector.

Isolated HB antigen Producing gene is inserted to bacterial plasmid vector forming λ -DNA.

This λ -DNA containing target gene is introduced into yeast cell.

Recombinant yeast cell multiplies in fermentation tank & produces HB antigens

viral surface After (48 hours), yeast cells are ruptured to antigen free HBsAg. The mixture is processed for extraction.

HB antigens are purified, combined with preserving agents & other ingredients & bottled

Ready for vaccination in humans.

(7) Insulin Production by λ -DNA technology (Draw fig.)

- Insulin is a hormone produced by B-cells of Islets of Langerhans in pancreas It is required for blood glucose regulation.

- People suffering from Diabetes mellitus need insulin therapy to remain healthy.

- Insulin is a simple protein with two polypeptide chains, A chain with 21 amino acids & B chain with 30 amino acids. Both chains are linked by disulfide bond.

Genes coding for chain A & chain B of insulin are isolated separately.

↓
Isolation of suitable plasmid DNA & cut with restriction enzymes to form plasmid vector.

Both the genes are incorporated to plasmid vector separately to form λ -DNA molecules.

↓
 λ -DNA molecules introduced to host cells where they express for the chains.

↓
short & long chains are separately isolated & purified from the cultures

Treated with cyanogen bromide to cut B-galactosidase from insulin polypeptide

↓
Short & long polypeptides mixed together to form disulfide bonds.

↓
Biologically active insulin was formed.

Humulin

- Humulin is a brand name insulin used to treat diabetes. It is synthetic form of insulin, produced by λ -DNA technology using non-pathogenic $E. coli$ bacteria.

- Humulin insulin is of 2 types, short acting insulin & intermediate acting insulin.
short acting → controls blood sugar after meal.
long " → maintains blood sugar b/w meals & overnight.

(give method of insulin preparation)

Polymerase chain Reaction

- In 1983 PCR was developed by American Biochemist "Kary Mullis".
- PCR is a laboratory technique to make multiple copies of a segment of DNA.
- In each polymerisation the no. of DNA fragments gets double of original copy.

Components of PCR :-

(1) **DNA template** :- A DNA template containing target DNA sequences to amplify.

(2) **DNA polymerase** :- Tag polymerase is used, it is thermostable & does not denature at (\uparrow) temperatures.

(3) **Oligonucleotide primers** :- short pieces of single stranded DNA complementary to 3' ends of both strands.

(4) Deoxyribonucleotide triphosphate \rightarrow Single units of bases, are building blocks for DNA synthesis and also provide energy for Polymerization.

(5) **Buffer system** \rightarrow Provides optimum condition for DNA denaturation & renaturation.

\rightarrow PCR is carried out in single tube kept in thermal cylinder in which heating & cooling can be adjusted.

\rightarrow DNA, Primers, DNA polymerase, single base units are added into tube. Over this sample a thin layer of mineral oil is poured to prevent evaporation of mixt sample.

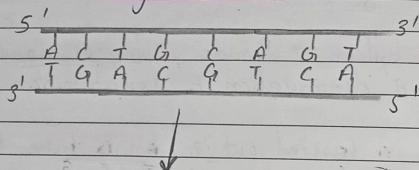
PCR reaction consists of 3 steps;

(1) Denaturation

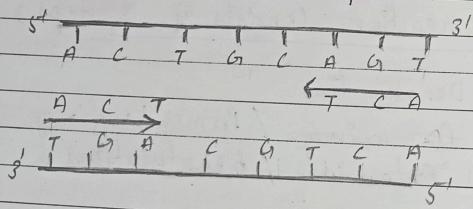
(2) Annealing / Primer binding

(3) Extension / Renaturation

(1) Denaturation :- Mixture is heated at 95°C to denature i.e. Separates double stranded DNA to two single strands

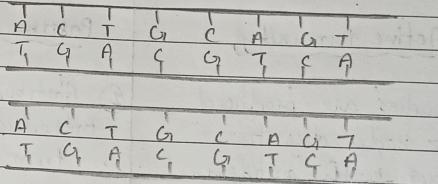


(2) Annealing :- Mixture is cooled to $60-65^{\circ}\text{C}$ so that Primers bind (anneal) to DNA template



(3) Extension :- Mixture is heated to $70-72^{\circ}\text{C}$ that is optimum temperature of DNA polymerase.

- DNA polymerase catalyses synthesis of new strands of DNA starting from primers by adding complementary nucleotide bases.



→ At the end of 1st cycle each ds DNA molecule consist of 1 daughter strand & 1 Parent strand.

→ Repeating the cycle 30 times can give billions of copies of targetted DNA.