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Introduction

Overview

- Biotechnology uses live organisms or enzymes to produce useful products and processes.
- European Federation of Biotechnology defines it as integration of natural science with organisms, cells, parts, and molecular analogues for products and services.
- Includes traditional and modern techniques like in vitro fertilisation, gene synthesis, DNA vaccines, and gene therapy.
- Recombinant DNA technology enables modification of microorganisms and other organisms to create novel capabilities.
- Genetic engineering is the manipulation of genetic material; Berg is the "father of genetic engineering" (Nobel Prize 1980).
- Herbert Boyer and Stanley Cohen pioneered biotechnology by combining restriction enzymes and plasmids to produce proteins in bacteria.

Memory Aids

- Berg = Father of Genetic Engineering (Nobel 1980).
- Boyer + Cohen = Recombinant DNA pioneers.
- Biotechnology = Organisms + Science = Useful products.

Quick Checkpoints

- What is the EFB definition of biotechnology?
- Who are the founders of recombinant DNA technology?
- What is genetic engineering?

Principles of Biotechnology

Core Techniques

- Genetic engineering: Alters DNA/RNA to change host phenotype by gene introduction.

- Chemical engineering: Maintains sterile conditions for microbial or cell growth to produce antibiotics, vaccines, enzymes.
- Genetic engineering involves recombinant DNA creation, gene cloning, and gene transfer.
- First recombinant DNA made by Cohen and Boyer in 1972 using plasmid DNA from *Salmonella typhimurium*.
- Three steps to create GMOs/transgenic organisms:
 - Identify DNA with desired genes.
 - Introduce DNA into host.
 - Maintain DNA in host and transfer to progeny.
- Alien DNA replicates only when integrated into host chromosome with origin of replication (ori).
- Cloning = making multiple identical copies of DNA.
- Restriction enzymes cut DNA at specific sites; plasmids act as vectors to transfer DNA.
- DNA ligase joins cut DNA ends to form recombinant DNA (circular, autonomously replicating DNA).

Memory Aids

- GMO steps: Identify, Introduce, Maintain.
- Restriction enzymes = molecular scissors.
- DNA ligase = molecular glue.
- Plasmid = vector for gene transfer.

Quick Checkpoints

- What are the two core techniques of modern biotechnology?
- What is the role of restriction enzymes?
- How is recombinant DNA formed?
- What are the steps to create a transgenic organism?

Tools of Recombinant DNA Technology

Restriction Enzymes

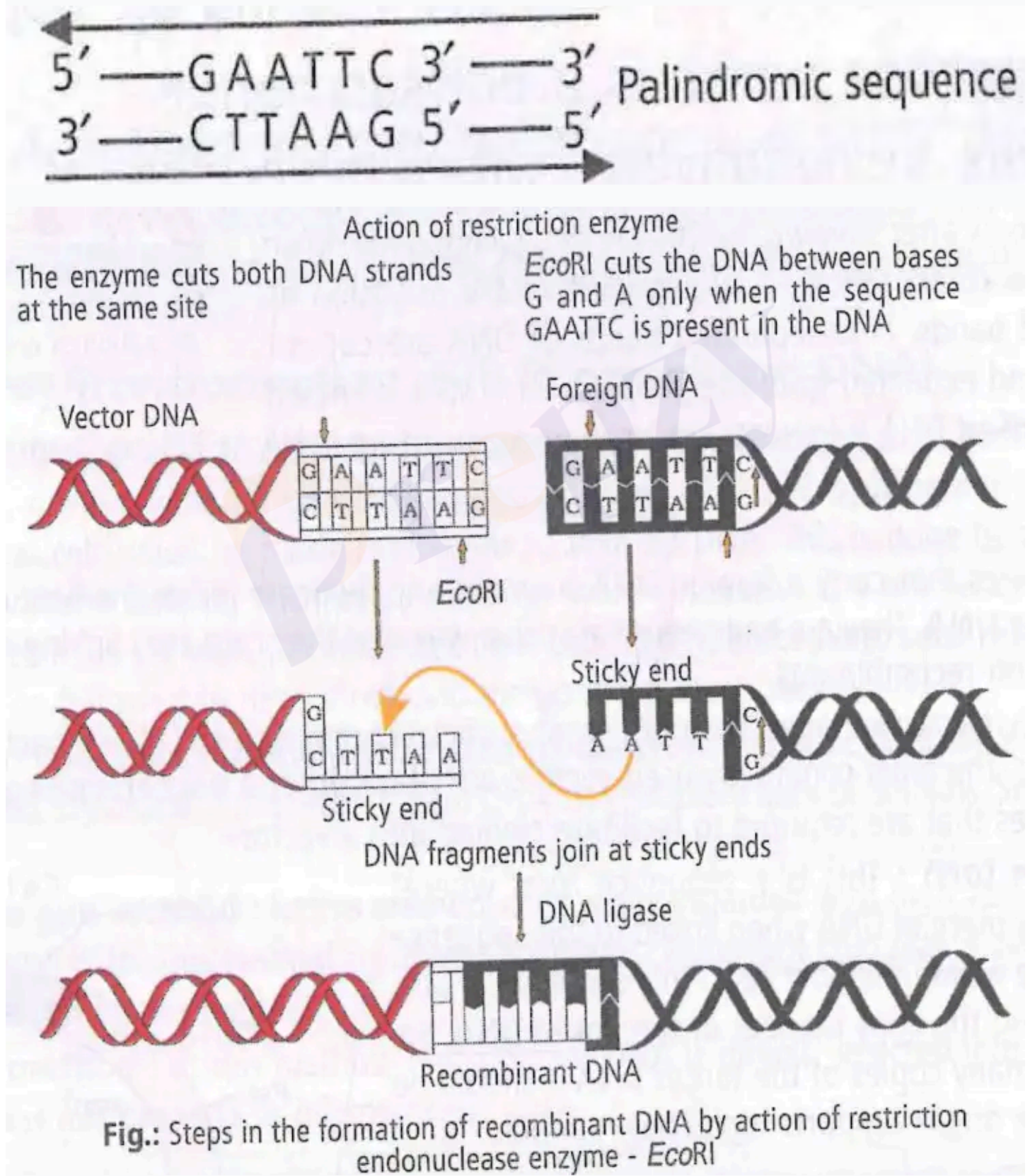
- Isolated first by W. Arber (1963) in bacteria; Nobel Prize awarded to Arber, Smith, Nathan for discovery.
- Hind II was the first restriction endonuclease; cuts DNA at specific 6-base recognition sequence.
- Enzyme names derive from source organism: first letter genus, next two species (italicized), fourth letter strain (capital), Roman numeral order.
- Restriction enzymes are nucleases: exonucleases remove nucleotides from ends; endonucleases cut within DNA.
- Types based on properties:
 - Type I: 3 subunits, require ATP, Mg²⁺, S-adenosyl methionine; not used in rDNA.
 - Type II: require Mg²⁺; used in rDNA.
 - Type III: 2 subunits; not used in rDNA.
- EcoRI and HindIII produce sticky ends; EcoRII and HindII produce blunt ends.
- Other enzymes:
 - Alkaline phosphatase removes 5' phosphate, modifies DNA ends.
 - DNA ligase seals gaps in DNA fragments.
 - Reverse transcriptase synthesizes cDNA from mRNA template.
- Restriction enzymes recognize palindromic sequences (same read 5'→3' on both strands).
- They cut DNA near center of palindrome, generating 3' or 5' protruding ends (sticky ends).
- Sticky ends have complementary sequences, enabling pairing and ligation by DNA ligase.
- Restriction fragments separated by gel electrophoresis using agarose gel and stained with ethidium bromide for visualization under UV light.

Memory Aids

- Restriction enzyme names = Genus (italic) + species (italic) + strain (capital) + order (Roman numeral).
- Sticky ends = EcoRI, HindIII; Blunt ends = EcoRII, HindII.
- Type II enzymes used in rDNA; Type I & III not used.
- Palindromic sequences read same 5'→3' on both strands.

Quick Checkpoints

- What defines a restriction enzyme's recognition site?
- Difference between sticky and blunt ends?
- Role of DNA ligase in recombinant DNA?
- How are DNA fragments separated after restriction digestion?



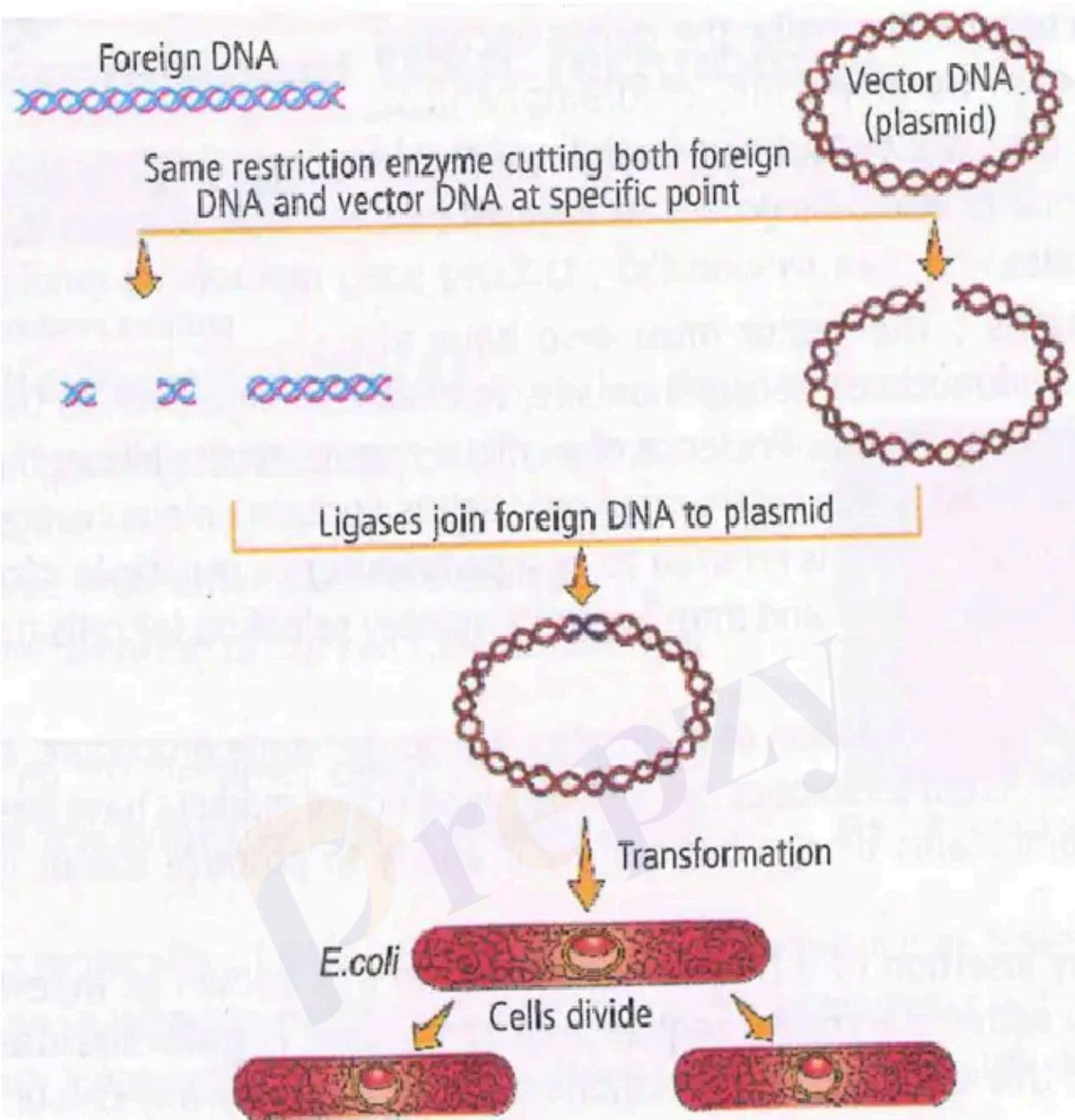


Fig.: Diagrammatic representation of recombinant DNA technology

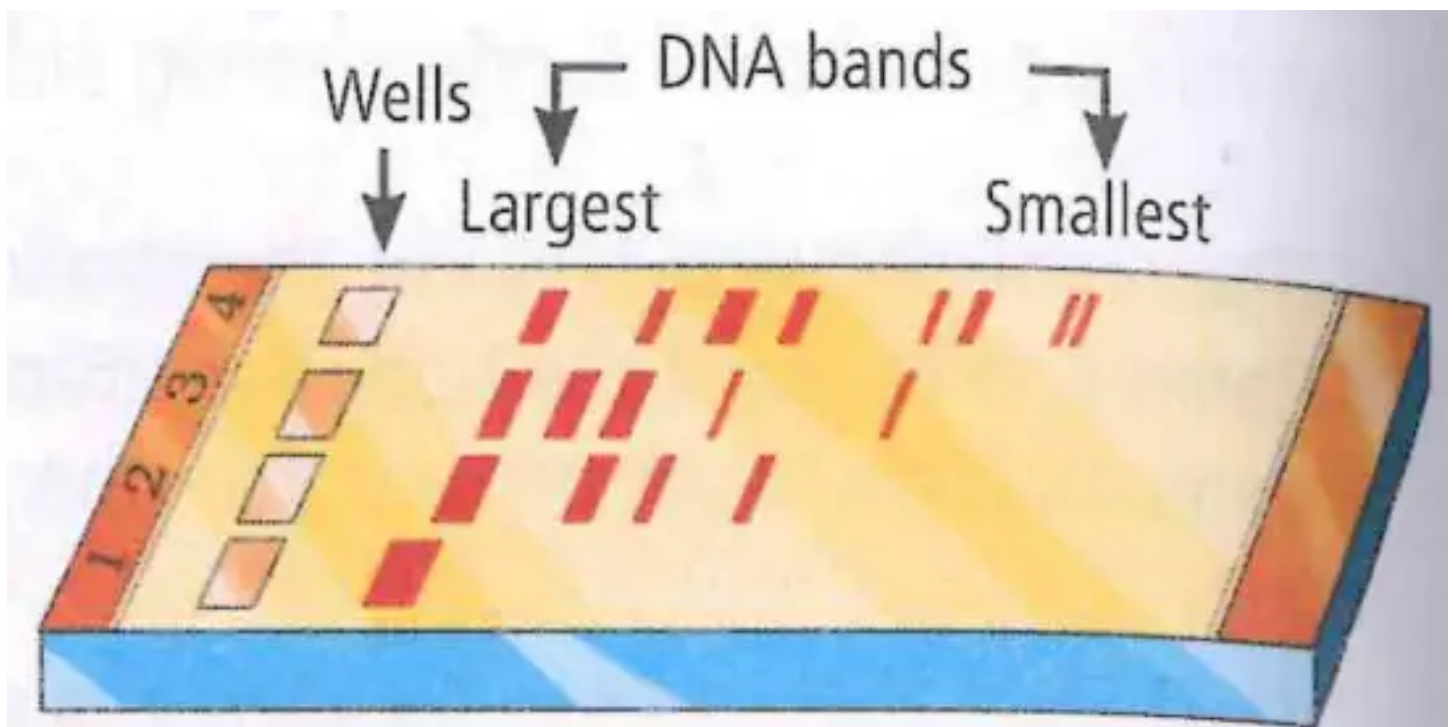


Fig.: A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

Cloning Vectors

Features and Types

- Vectors carry foreign DNA and replicate inside host cells; also called vehicle or carrier DNA.
- Common vectors: plasmids, bacteriophages, cosmids, YACs, BACs, transposons.
- Key features:
 - **Origin of replication (ori):** Start site for replication; controls copy number.
 - **Selectable marker:** Antibiotic resistance genes (ampicillin, tetracycline, kanamycin) to select transformants.
 - **Cloning sites:** Unique restriction sites (polylinker or MCS) for foreign DNA insertion.
- Insertional inactivation: foreign DNA insertion disrupts marker gene (e.g., B-galactosidase), enabling color-based selection of recombinants.
- Vectors for plants and animals:
 - *Agrobacterium tumefaciens* Ti plasmid modified for plant gene delivery.
 - Disarmed retroviruses used for animal gene delivery.
- pBR322: first artificial cloning vector (1977); contains amp^R, tet^R, ori, rop, and multiple restriction sites.

- Shuttle vectors replicate in both eukaryotic and prokaryotic cells.

Memory Aids

- pBR322 = first cloning vector with amp^R and tet^R markers.
- Selectable markers help distinguish transformants by antibiotic resistance or color change.
- Polylinker = multiple cloning sites for easy DNA insertion.
- Agrobacterium Ti plasmid used for plant transformation.

Quick Checkpoints

- What is the role of origin of replication in vectors?
- How do selectable markers aid in recombinant selection?
- What is insertional inactivation?
- Name common vectors used in genetic engineering.

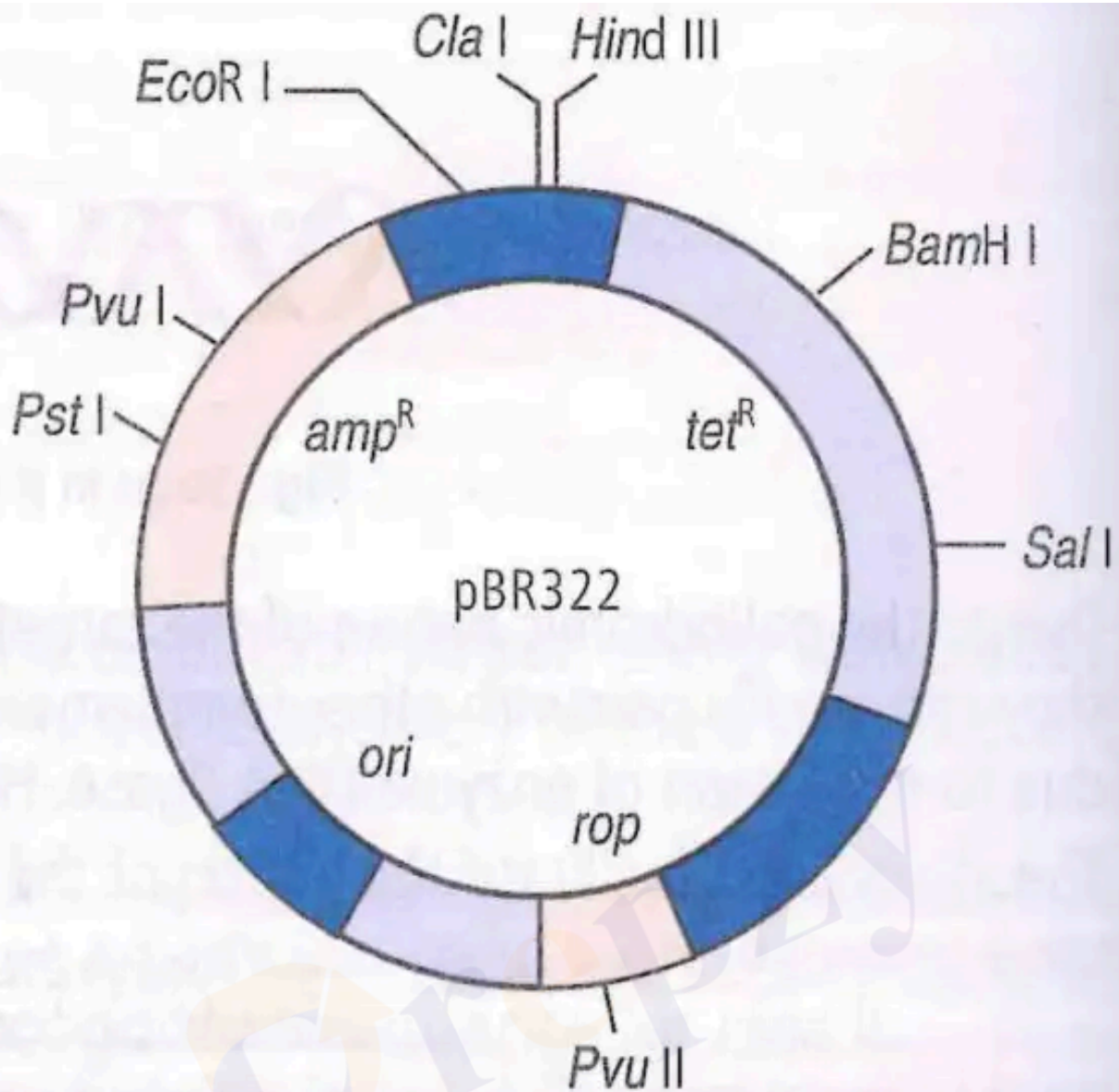


Fig.: *E. coli* cloning vector pBR322 showing restriction sites (*Hind* III, *EcoR* I, *Bam*H I, *Sal* I, *Pvu* II, *Pst* I, *Cla* I), *ori* and antibiotic resistance genes (*amp*^R and *tet*^R). *rop* codes for the proteins involved in the replication of the plasmid.

Competent Host (For Transformation with Recombinant DNA)

Host Preparation and Methods

- Competent hosts (*E. coli*, yeast, animal, plant cells) are required to take up recombinant DNA.
- DNA is hydrophilic; cannot cross membranes without treatment.

- Bacterial cells made competent by treating with divalent cations (e.g., Ca^{2+}) to increase DNA uptake through cell wall pores.
- Transformation steps: incubate cells with DNA on ice, heat shock, then back on ice.
- In eukaryotes, transformation is called transfection.
- Vectorless gene transfer methods:
 - **Biolistic (gene gun):** DNA-coated gold/tungsten particles bombarded into plant cells; used in crops like wheat, rice, maize.
 - **Microinjection:** Direct injection of DNA into animal cell nucleus using micropipette.
- Electroporation creates temporary pores in membranes using lysozyme or calcium chloride.
- Chemicals like polyethylene glycol (PEG) facilitate DNA entry into host cells.

Memory Aids

- Competency increased by Ca^{2+} treatment and heat shock.
- Biolistic method for plants; microinjection for animals.
- Electroporation and PEG aid DNA uptake.

Quick Checkpoints

- Why must bacterial cells be made competent?
- Difference between transformation and transfection?
- Methods to introduce DNA into eukaryotic cells?
- Role of divalent cations in transformation?

Processes of Recombinant DNA Technology

Steps Involved

- Isolation of genetic material (DNA) from cells.
- Cutting DNA at specific sites using restriction enzymes.
- Amplification of gene of interest by PCR.
- Insertion of recombinant DNA into host cells.
- Production of foreign gene product.
- Downstream processing for product purification.

Memory Aids

- Remember the sequence: Isolate, Cut, Amplify, Insert, Produce, Process.

Quick Checkpoints

- What is the first step in rDNA technology?
- Which enzyme cuts DNA at specific locations?
- How is the gene of interest amplified?
- What is the purpose of downstream processing?

Isolation of Genetic Material

Key Points

- Isolation means removing plasmid or genomic DNA from cells in pure form.
- Cell membranes and walls are broken using enzymes like lysozyme (bacteria), cellulase (plants), chitinase (fungi).
- RNA removed by ribonuclease; proteins removed by protease.
- Purified DNA precipitates with chilled ethanol.

Memory Aids

- Use specific enzymes to break cell walls and remove contaminants.
- Precipitate DNA with cold ethanol for purity.

Quick Checkpoints

- Which enzymes help break cell walls during DNA isolation?
- How are proteins and RNA removed from DNA samples?
- What is the role of chilled ethanol in DNA isolation?



Fig.: DNA that separates out can be removed by spooling

Cutting of DNA at Specific Locations

Key Points

- Restriction enzymes cut purified DNA at specific sequences.
- Digestion progress checked by agarose gel electrophoresis.
- Cut gene of interest and vector DNA are joined using DNA ligase to form recombinant DNA.

Memory Aids

- Restriction enzymes act like molecular scissors at specific sites.
- Gel electrophoresis confirms DNA cutting.

Quick Checkpoints

- What enzyme cuts DNA at specific sites?
- How is the success of DNA cutting verified?
- Which enzyme joins DNA fragments to form recombinant DNA?

Amplification of Gene of Interest using PCR

Key Points

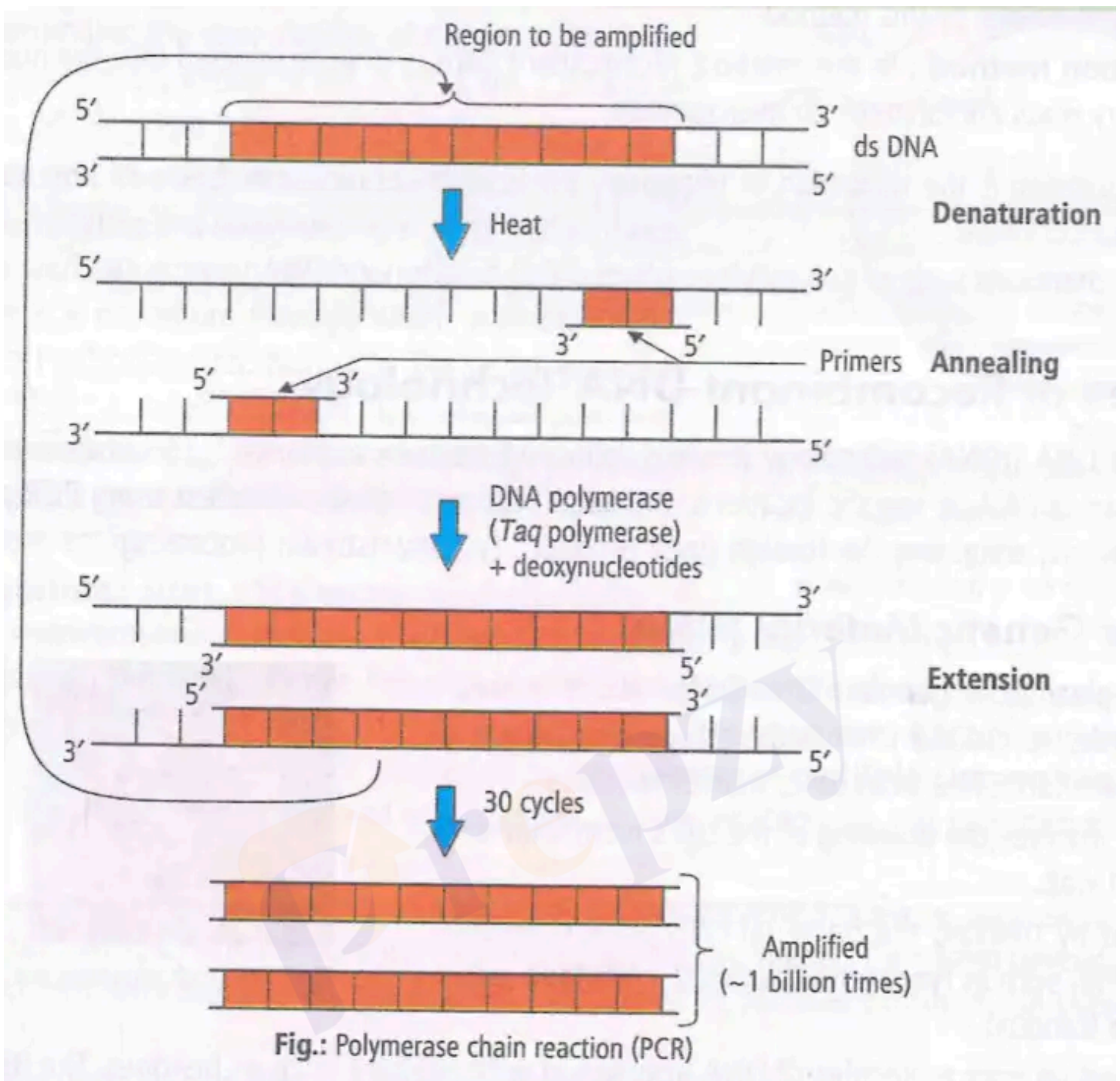
- PCR replicates DNA in vitro using primers and DNA polymerase.
- Thermostable Taq polymerase allows repeated cycles at high temperatures.
- Three steps per cycle: denaturation (90°-98°C), primer annealing (40°-60°C), extension (72°C).
- Amplifies DNA up to a billion times.
- Applications: pathogen diagnosis, DNA fingerprinting, prenatal diagnosis, gene therapy.

Memory Aids

- Denature, Anneal, Extend = PCR cycle steps.
- Taq polymerase works at high heat without denaturing.

Quick Checkpoints

- What enzyme is used in PCR?
- What are the three steps of a PCR cycle?
- How many times can PCR amplify DNA?



Insertion of Recombinant DNA into Host Cell

Key Points

- Recipient cells are made competent to take up recombinant DNA.
- Vector and alien DNA cut by same restriction enzyme to produce complementary sticky ends.
- DNA ligase joins sticky ends forming recombinant DNA.
- Selectable markers like antibiotic resistance genes help identify transformed cells.

- Example: Ampicillin resistance gene allows selection of transformed *E. coli* on ampicillin plates.

Memory Aids

- Competent cells take up DNA from surroundings.
- Selectable markers identify successful transformants.

Quick Checkpoints

- What makes cells competent?
- How are recombinant DNA fragments joined?
- What is a selectable marker?

Obtaining the Foreign Gene Product

Key Points

- Foreign gene expresses protein called recombinant protein in host cells.
- Cells grown in continuous culture to maintain active growth phase for high protein yield.
- Bioreactors provide optimal conditions: temperature, pH, oxygen, nutrients.
- Two types of stirred-tank bioreactors: simple and sparged (with air bubbles).
- Bioreactors have systems for mixing, oxygen delivery, foam control, temperature and pH control, and sampling.

Memory Aids

- Bioreactors optimize growth and protein production.
- Sparged bioreactors improve oxygen supply with air bubbles.

Quick Checkpoints

- What is a recombinant protein?
- Why use continuous culture systems?
- What conditions do bioreactors control?

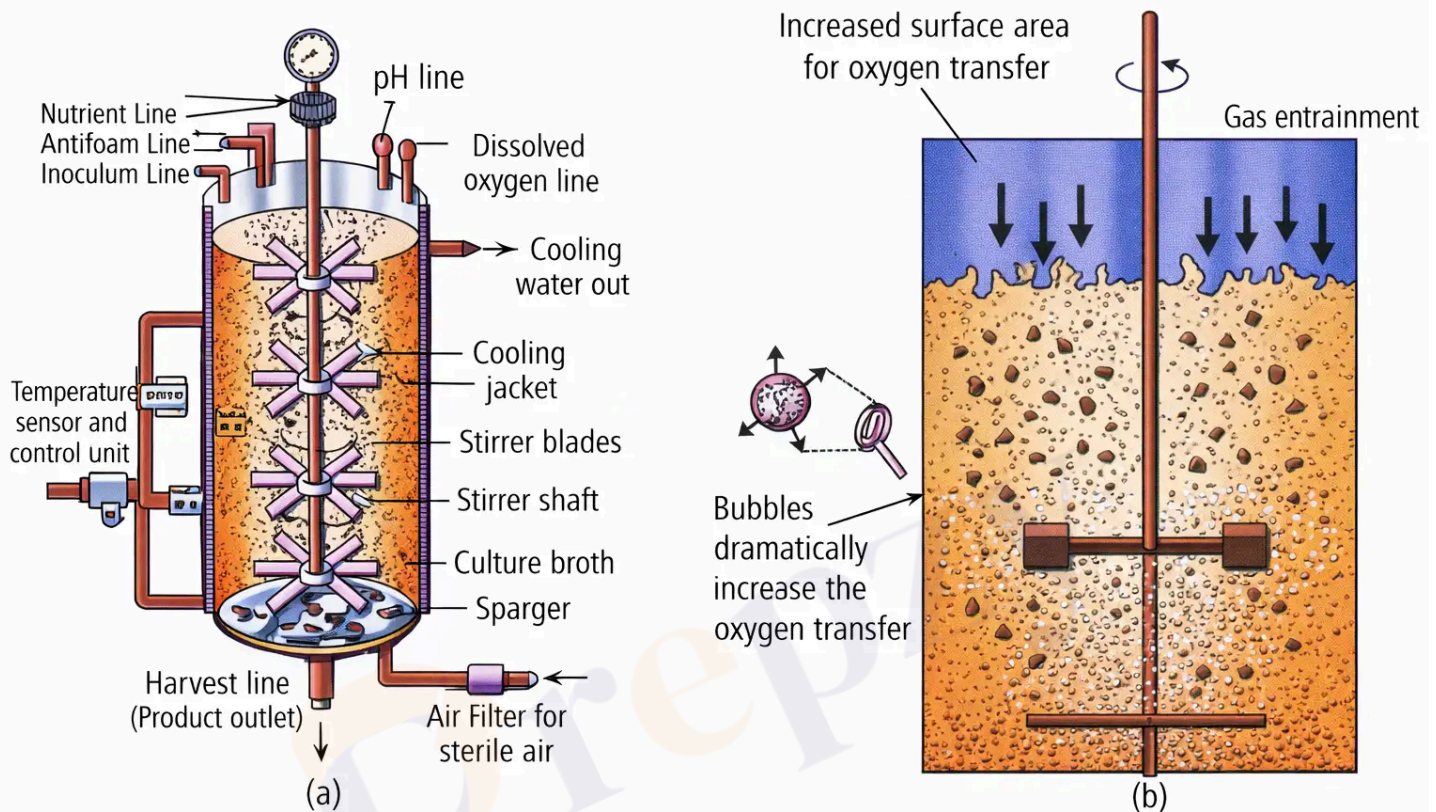


Fig.: (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which air bubbles

Downstream Processing

Key Points

- Involves separation and purification of the product after biosynthesis.
- Product formulation with preservatives and clinical trials for drugs.
- Strict quality control testing is essential.
- Processes vary depending on the product.

Memory Aids

- Downstream = purification and quality control.
- Formulation and testing ensure product safety.

Quick Checkpoints

- What is the purpose of downstream processing?
- Why is quality control important?
- Does downstream processing vary by product?

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