STUDY MATERIAL

VIVEKANANDA COLLEGE THAKURPUKUR

NAAC ACCREDITED GRADE—'A'

Subject: Molecular biology
Topic: The basic concept of Recombinant DNA technology

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Introduction

We know that Genetic mutation and recombination provide a diversity of organisms and the process of natural selection allows the growth of those best adapted to a given environment.

DNA recombination is the process by which microorganism can exchange genes. So, genetic modification occurs.

An Overview of Recombinant DNA Technologies:

Step 1: Gene of interest (DNA) is isolated

Step 2: A desired gene is inserted into a DNA molecule – **vector**

Step 3: The vector inserts the DNA into **a new cell,** which is grown to form a **clone**.

Step 4: Large quantities of the **gene product** can be harvested from **the clone**.

Tools for Genetic engineering

1. Restriction Enzymes: Restriction endonucleases that naturally produced by bacteria. Endonuclease destroy bacteriophage DNA in bacterial cells but can not digest host DNA because of their methylated cytosine.

sticky ends **blunt ends**

DNA is the substrate of a restriction enzyme, recognizes one particular nucleotide sequence in DNA and **cuts** the DNA molecule (breaks down the bond between two nucleotides)

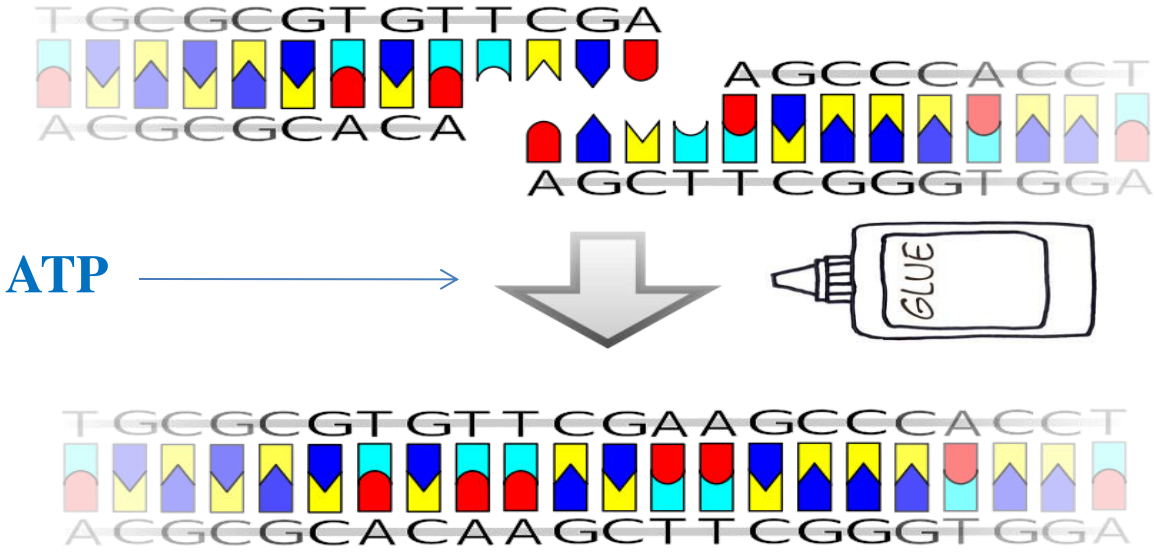
Known RE used in RDT and their cut site:

Enzyme	Bacterial Source	Recognition Sequence
*Bam*HI	*Bacillus amyloliquefaciens*	G↓G A T C C G C T A G↑G
*Eco*RI	*Escherichia coli*	G↓A A T T C C T T A A↑G
*Hae*III	*Haemophilus aegyptius*	G G↓C C C C↑G G
*Hind*III	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A

Tools for Genetic engineering

2. Ligase:

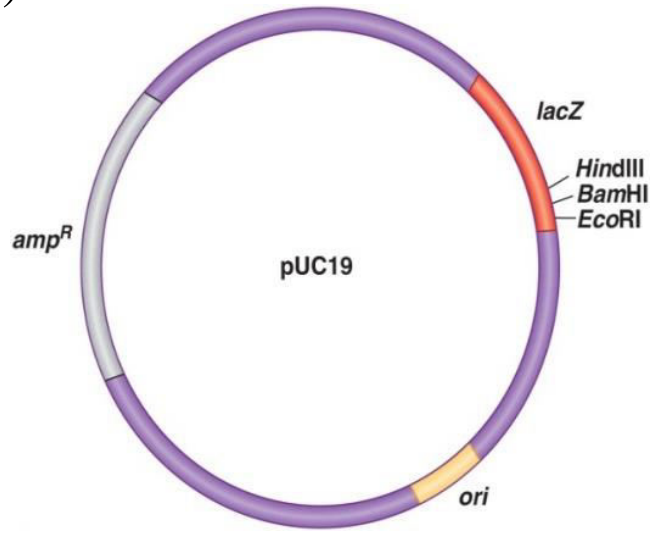
- **DNA ligase** is an enzyme that can link together DNA strands that have double-strand breaks (a break in both complementary strands of DNA).
  - Naturally DNA ligase has applications in both **DNA replication** and **DNA repair .**
  - Needs ATP
- DNA ligase has extensive use in molecular biology laboratories for **genetic recombination experiments**



Vectors

1. Plasmid vectors:

- Plasmids are **self-replicating circular** molecules of DNA
- Encode antibiotic resistance (selection marker)



2. Viral vectors – **retroviruses, adenoviruses and herpes viruses**

- Accept much larger pieces of DNA
- Mammalian host

Known hosts for RDT

1. **Bacteria :** ***E. coli*** - used because is easily grown and its genomics are well understood.

- Gene product is purified from host cells.

2. Yeasts: ***Saccharomyces cerevisiae*****-**Used because it is easily grown and its genomics are known.

❖May express eukaryotic genes easily.
❖Continuously secrete the gene product.
❖Easily collected and purified.

3. Plant cells and whole plants

- May express eukaryotic genes easily
- Plants are easily grown - produce plants with new properties.

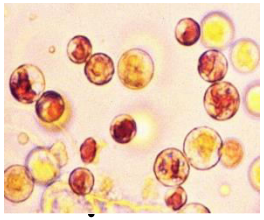
4. Mammalian cells

- May express eukaryotic genes easily
- Harder to grow
- Medical use.

Insertion of the naked DNA into a host cells

1.Transformation

* treatment make cells competent to accept foreign DNA ($CaCl_2$ make pores in cell membrane)



2. Electroporation

*use electrical current to form microscopic pores in the membranes of cell

3. Protoplast fusion

– yeast, plants and algal cells

4. Microinjection

5. Gene gun

Cloning

A process of producing genetically modified organisms.

A multi-step process……

1. **Isolating** and **copying** the genetic material of interest (DNA fragment).

2. **Building a construct** (recombinant DNA - vector and desired gene) containing all the genetic elements for correct expression.

3. **Inserting** the vector into the **host organism**, directly through injection or transformation.

4. **Selecting** the cells expressing that gene by growing under positive selection (of an antibiotic or chemical) – **clone** .

5. Growing successfully the clone (transformed organisms).

Blue-white screening system

1. Plasmid vector contains the genes for: Ampicillin resistance gene and the cloning site (restriction enzymes site) is inserted into the β-galactosidase gene. Cloning the desired gene at that site destroys β-galactosidase gene.

2. The vector is then transformed into host competent cell (bacteria).

- Host is **sensitive to ampicillin**
- Host is **β-galactosidase negative** (do not carry LacZ gene)

3. The transformed cells are grown in the presence of:

- **ampicillin.**
- **X-gal** – substrate for β-galactosidase
  - a colourless modified galactose sugar
  - When metabolized by β-galactosidase form an insoluble product (5-bromo-4 chloroindole) which is bright **blue,** and thus functions as an indicator

4. Results

- Clones lacking the vector will not grow.
- Clones containing the vector without the new gene will be **resistant to ampicillin, able to metabolized X-gal and** will **be blue.**
- Clones containing the recombinant vector will be **resistant to ampicillin** and **unable to hydrolyze X-gal** (white colonies).

- If the ligation was successful, the bacterial colony will be white; if not, the colony will be blue.

Applications of recombinant DNA technology

1. Shotgun sequencing - Recombinant DNA techniques were used to map the human genome through the **Human Genome Project.**

2. Diagnose genetic disease

RFLP analysis (Restriction fragment length polymorphism): DNA profiling involved restriction enzyme digestion, followed by Southern blot analysis.

Southern blotting: is used for detection of a specific DNA sequence in DNA sample.

DNA probes: can be used to quickly identify a pathogen in body tissue or food.

PCR analysis with specific primers

3. Genetic fingerprinting identification

- **Forensic microbiology -** use of **DNA fingerprinting** to identify the source of bacterial or viral pathogens.
  - bioterrorism attacks (Anthrax in U.S. Mail)
  - medical negligence (Tracing HIV to a physician who injected it)
  - outbreaks of foodborne diseases

Applications of recombinant DNA technology

4. Agricultural Applications

- Cells from plants with desirable characteristics can be cloned to produce many identical cells, then can be used to produce whole plants from which seeds can be harvested.
- Some bacteria can transfer genes to unrelated species
  - *Agrobacterium tumefaciens* - a plant pathogen
  - Cause tumors in plants
  - **Natural genetic engineer**
  - **Genetic engineering manipulation**

✓ Genes for resistance to herbicide glyphosate, Bt toxin, and pectinase suppression have been engineered into crop plants.

✓ Genetically modified *Rhizobium* has enhanced nitrogen fixation.

✓ Genetically modified *Pseudomonas* is a biological insecticide that produces *Bacillus thuringiensis* toxin.

Applications of recombinant DNA technology

5. Nanotechnology:

•Bacteria can make molecule-sized particles

•*Bacillus* cells growing on selenium form chains of elemental selenium

6. Therapeutic Applications : Produce human proteins – hormones and enzymes

i) Insulin ii) hGH iii) INFα, INFβ and INFγ

- **Vaccines**
  - **Cells** and **viruses** can be modified to produce a pathogen's surface protein
    - Influenza
    - Hepatitis B
    - Cervical cancer vaccine
  - Nonpathogenic viruses carrying genes for pathogen's antigens as **DNA vaccines**
  - DNA vaccines consist of circular rDNA
- **Gene therapy** can be used to cure genetic diseases by **replacing the defective or missing gene**.
- **Gene silencing** – RNA interference - siRNA or microRNA