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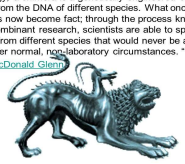
Definition:

- **Single chimeric DNA formed by combining two or more different fragments of DNA from diverse organisms is generally called as recombinant DNA and the method applied to create recombinant DNA is called recombinant DNA technology.**

"In Greek mythology, the chimera was part lion, part goat, part serpent, which was slain by the hero Bellerephon. In modern day biology, a chimera is a genetically engineered creature created from the DNA of different species. What once was fiction has now become fact; through the process known as DNA recombinant research, scientists are able to splice genes together from different species that would never be able to mate under normal, non-laboratory circumstances. "

- Linda MacDonald Glenn



- **Recombinant DNA technology Encompasses a number of experimental protocols leading to the transfer of genetic information (DNA) from one organism to another. Involves the manipulation of genetic material (DNA) to achieve the desired goal in a pre-determined way.**
- **The organism, from which the candidate DNA is isolated, is called Donor organism. The organism which will accept the foreign gene is called Host organism.**
- **The present day rDNA technology has its roots in the experiments performed by Boyer & Cohen.**
- **A hybrid of the *SV40* mammalian DNA virus genome and phage λ was one of the recombinant DNA molecules to be first engineered.**

Discovery of Recombinant DNA technology

- **1953:-Discovery of DNA structure by Watson & Crick.**
- **1960:- Ist breakthrough of rDNA**
- **1967:-Isolation of DNA ligase enzyme.**
- **1969:-Herbert Boyer isolated Restriction Enzyme -E.coRI**
- **1970:-Howard Temin and David Baltimore independently studied Reverse transcriptase and retro Viruses which are important for the development of cDNA.**
- **1970:-Isolation of Restriction enzyme**
- **1972:- Paul Berg, David Jackson and Robert Symons generated rDNA technology.**

- **1973:-S. Cohen & H. Boyer produced first plasmid (pSC 101) vector capable of being replicated within a bacterial host.**
- **1975:-Edwin Southern developed a method known as Southern Blotting technique.**
- **1979:-Insulin synthesized by rDNA.**
- **1983:-Engineered Ti-Plasmid used to transform.**
- **1983:-Insertion of cloned gene from Salmonella into tobacco plant to make resistance to herbicide glyphosphate.**

- **1986:-Development of Gene Gun**
- **1989:-First field test of Genetically engineered Virus.**

- **1990:-Production of transformed Corn.**

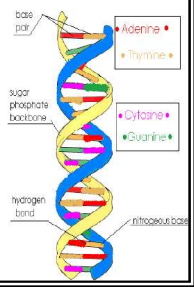
- **1991:- Production of transformed Pigs & goats.**

  Ist test of gene theropy on Human Cancer Patient.
- **1994:-Human monoclonal antibody produced in engineered mice.**
- **1997:-Ist monoclonal clone (Dolly) developed from non reproductive cell.**

WHAT IS DNA?

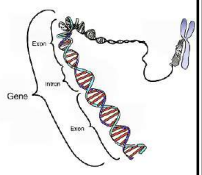
DNA= Deoxyribu-Nucelic Acid

- DNA is a very large molecule, made up of smaller units called nucleotides
- Each nucleotide has three parts: a sugar (ribose), a phosphate molecule, and a nitrogenous base.
- The nitrogenous base is the part of the nucleotide that carries genetic information
- The bases found in DNA are four: adenine, cytosine, guanine, and thymine (ATP, CTP, GTP, and TTP)



What is gene?

- A gene is a stretch of DNA that codes for a type of protein that has a function in the organism.
- It is a unit of heredity in a living organism.. All living things depend on genes
- Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring.

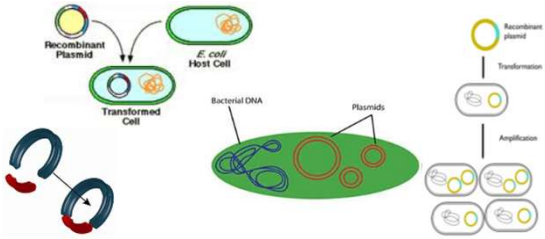


Basic principles of rDNA technology:

Gene cloning:- It can be defined as the isolation and amplification of an individual gene sequence by insertion of that individual gene sequence into a bacterium where it can be replicated.

Step1:-
A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a chimera or recombinant DNA (rDNA) molecule.
Step 2
The vector acts as a vehicle that transports the gene into a host cell, which is usually a bacterium although other types of living cell can be used. This process is called transformation.
Step 3
Within the host cell the vector multiplies producing numerous identical copies not only of itself but also of the gene that it carries.

Step 4 When the host cell divides, copies of rDNA molecule are passed to the progeny and further vector replication takes place.
Step 5 After large no: of cell divisions a colony or clone of identical host cells is produced. Each cell in the clone contains one or more copies of the rDNA molecule
Step 6 Then, the host cells are then lysed and rDNA can be separated.

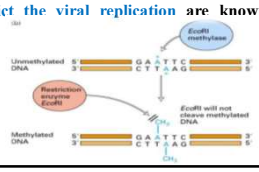


Key tool for RDT

- **Restriction Enzyme**
- **Ligases**
- **Polymerase enzyme**
- **Host**
- **Vector**
- **Gene library**

Restriction Enzyme

- **A restriction enzyme is a nuclease enzyme functions by 'inspecting' the length of a DNA sequence that cleaves DNA sequence at a random or specific recognition sites known as restriction sites.**
- **These were first discovered in *E.coli* restricting the replication of bacteriophages, b cutting the viral DNA(The host *E.coli* DNA is protected from cleavage by addition o methyl groups).Thus, the enzymes that restrict the viral replication are known a restriction enzymes or restriction endonucleases.**



There are two different kinds of restriction enzymes:

(1) Exonucleases catalyses hydrolysis of terminal nucleotides from the end of DNA or RNA molecule either 5'to 3' direction or 3' to 5' direction. Example: exonuclease I, exonuclease II etc.

(2) Endonucleases can recognize specific base sequence (restriction site) within DNA or RNA molecule and cleave internal phosphodiester bonds within a DNA molecule. Example: EcoRI, Hind III, BamHI etc.

- Each restriction endonuclease recognises a specific palindromic nucleotide sequences in the DNA.
- The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences read the same on the two strands in 5′ *à* 3′ *direction. This is also true if* read in the 3′ *à* 5′ *direction.*

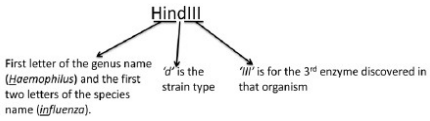
5′ —— *GAATTC* —— 3′
3′ —— *CTTAAG* —— 5′

History:

In 1970 the first restriction endonuclease enzyme *Hind*II was isolated. For the subsequent discovery and characterization of numerous restriction endonucleases, in 1978 Daniel Nathans, Werner Arber, and Hamilton O. Smith awarded for Nobel Prize for Physiology or Medicine. Since then, restriction enzymes have been used as an essential tool in recombinant DNA technology.

Restriction Endonuclease Nomenclature:

Restriction endonucleases are named according to the organism in which they were discovered, using a system of letters and numbers. For example, *HindIII* (pronounced *"hindee-three"*) was discovered in *Haemophilus influenza* (strain d). The Roman numerals are used to identify specific enzymes from bacteria that contain multiple restriction enzymes indicating the order in which restriction enzymes were discovered in a particular strain.



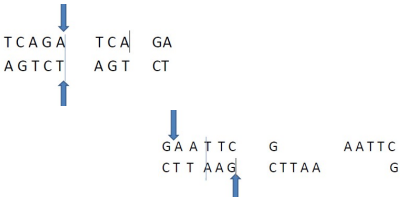
Classification of Restriction Endonucleases:

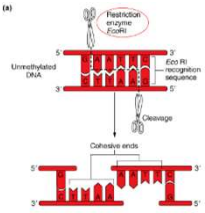
There are three major classes of restriction endonucleases based on the types of sequences recognized, the nature of the cut made in the DNA, and the enzyme structure:

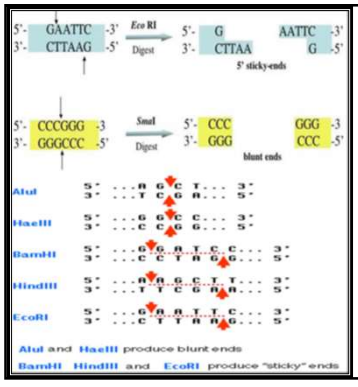
Property	Type I restriction enzymes	Type II restriction enzymes	Type III restriction enzymes
Abundance	Less common than Type II	Most common	Rare
Recognition site	Cut both strands at a non-specific location > 1000 bp away from recognition site	Cut both strands at a specific, usually palindromic recognition site (4-8 bp)	Cleavage of one strand, only 24-26 bp downstream of the 3′ recognition site
Restriction and modification	Single multifunctional enzyme	Separate nuclease and methylase	Separate enzymes sharing a common subunit
Nuclease subunit structure	Heterotrimer	Homodimer	Heterodimer
Cofactors	ATP, Mg2+, SAM	Mg2+	Mg2+ (SAM)
DNA cleavage requirements	Two recognition sites in any orientation	Single recognition site	Two recognition sites in a head-to-head orientation
Site of methylation	At recognition site	At recognition site	At recognition site

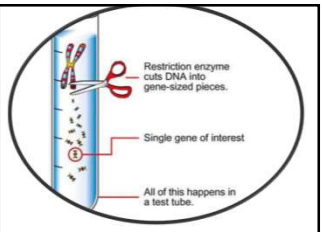
Cleavage patterns:

- The cut DNA fragments by restriction endonucleases may have mostly sticky ends or blunt ends.
- DNA fragments with sticky ends are particularly useful for rDNA experiments, since single stranded sticky DNA ends can easily pair with any other DNA fragment having complementary









Microorganism	Restriction Enzyme Name	Restriction Site
Bacillus amyloliquefaciens H	BamH1	G|G A T C C C C T A G|G
Brevibacterium albidum	Ball	T G G|C C A A C C|G G T
Escherichia coli RY13	EcoRI	G|A A T T C C T T A A|G
Haemophilus aegyptius	HaeII	Pu G C G C|Py Py|C G C G Pu
Haemophilus aegyptius	HaeIII	G G|C C C C|G G
Haemophilus influenzae R_d	HindII	G T Py|Pu A C C A Pu|Py T G
Haemophilus influenzae R_d	HindIII	A|A G C T T T T C G A|A
Haemophilus parainfluenzae	HpaI	G T T|A A C G A A|T T G
Haemophilus parinfluenzae	HpaII	C|C G G G G C|C
Providencia stuartii 164	PstI	C T G C A|G G|A C G T C
Streptomyces albus G	SalI	G|T C G A C C A G C T|G

Table 2.1: *Restriction enzymes*

Star activity

Star activity is defined as the alteration in the digestion specificity that occurs under sub-optimal enzyme conditions. Star activity results in cleavage of DNA at non-specific sites. Some of the sub-optimal conditions that result in star activity are as follows:

- **pH >8.0**
- **glycerol concentration of >5%**
- **enzyme concentration >100 units/mg of DNA**
- **increased incubation time with the enzyme**
- **presence of organic solvents in the reaction mixture**
- **incorrect cofactor or buffer**

Restriction Enzyme Recognition Sequences

The length of restriction recognition sites varies: The enzymes EcoRI, SacI and SstI each recognize a 6 base-pair (bp) sequence of DNA, whereas NotI recognizes a sequence 8 bp in length, and the recognition site for Sau3AI is only 4 bp in length. Length of the recognition sequence dictates how frequently the enzyme will cut in a random sequence of DNA. Enzymes with a 6 bp recognition site will cut, on average, every 4^6 or 4096 bp; a 4 bp recognition site will occur roughly every 256 bp.

Enzyme	Recognition Sequence
BamH I	GGATCC CCTAGG
Not I	GCGGCCGC CGCCGGCG
Sau3A I	GATC CTAG
Sac I	GAGCTC CTCGAG
Sst I	GAGCTC CTCGAG
Hinf I	GANTC CTNAG
Xho II	PuGATCPy PyCTAGPu

Isoschizomer:

Isoschizomers are pairs of restriction enzymes specific to the same recognition sequence. For example, SphI (CGTAC/G) and BbuI (CGTAC/G) are isoschizomers of each other. The first enzyme discovered which recognizes a given sequence is known as the prototype; all subsequently identified enzymes that recognize that sequence are isoschizomers.

Neoschizomers:

An enzyme that recognizes the same sequence but cuts it differently e.g. SmaI (CCC/GGG) and XmaI (C/CCGGG) are known as neoschizomers of each other. AatII (recognition sequence: GACGT↓C and ZraI (recognition sequence: GAC↓GTC are are neoschizomers of one another.

SmaI CUTS ---------C C C | G G G--------
XmaI CUTS ---------C | C C G G G--------

Isocaudomers:

An enzyme that recognizes a slightly different sequence but produces the same ends. i.e. blunt ends
e.g. Bam HI G | GATCC BclI T | GATCA

Applications:

In various applications related to genetic engineering DNA is cleaved by using these restriction enzymes.

• They are used in the process of insertion of genes into plasmid vectors during gene cloning and protein expression experiments.

• Restriction enzymes can also be used to distinguish gene alleles by specifically recognizing single base changes in DNA known as single nucleotide polymorphisms (SNPs). This is only possible if a mutation alters the restriction site present in the allele.

Restriction enzymes are used for Restriction Fragment Length Polymorphism (RFLP) analysis for identifying individuals or strains of a particular species.

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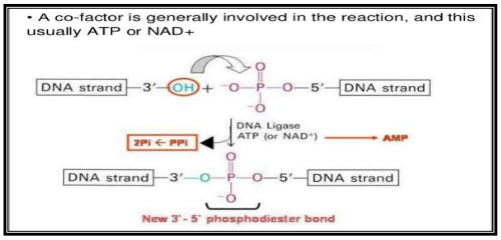
- **Reece R. J. 2000. *Analysis of Genes and Genomes;* John Wiley & Sons, U.K.**
- **Lehninger'sPrinciples of Biochemistry**
- **http://www.biology-pages.info/R/RestrictionEnzymes**

DNA ligases

- **These were originally isolated from viruses, also occur in *E.coli* & eukaryotic cells.**
- **The cut DNA fragments are covalently joined together by DNA ligases.**
- **DNA ligase joins the DNA fragments by forming a phosphodiester bond between the phosphate group of 5'-carbon of one deoxyribose with the hydroxyl group of 3'-carbon of another deoxyribose.**



Host cells

- **The hosts are the living systems or cells in which the carrier of rDNA molecule or vector can be propagated.**
- **Host cells can be prokaryotic or eukaryotic.**
- **Microorganisms are preferred as host cells, since they multiply faster compared to cells of higher organisms. E.g. *E.coli***
- **This was the first organism used in the DNA technology experiments.**
- **The major drawback is that it cannot perform post translational modifications.**
- **Eukaryotic Hosts are preferred to produce human proteins, since these have complex structure suitable to synthesise complex proteins.**

  E.g., Tissue plasminogen activator
- **Mammalian cells possess the machinery to modify the protein to the active form. (translational modifications)**

VECTOR

- Are the DNA molecules, which can carry a foreign DNA fragment to be cloned.
- These are **self replicating** in an appropriate host cell.
- A **cloning vector** is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, that can best ably maintained in an organism, and into which a **foreign DNA fragment can be inserted for cloning purposes.**
- Expression vector is used for **expression of foreign gene into a protein**.
- If a vector is designed to perform equally in two different hosts, it is called a **shuttle vector**.
- Most important Cloning vectors are plasmids, bacteriophages, Cosmids & artificial chromosome vectors.

Features that are required to facilitate cloning into a vector

- **Origin of replication.**
- **Cloning site.**
- **Selectable marker.**
- **Reporter gene.**
- **Origin of replication (ori) *:* This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is also responsible for controlling the copy number of the linked DNA.**
- **Selectable marker *:* In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.**
- **If a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into *E. coli* cells, the host cells become transformed into ampicillin-resistant cells.**

- **If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed recipient cells will die. Since, due to ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a selectable marker.**

Cloning sites: In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.

Competent Host (For Transformation with Recombinant DNA) Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA.

The selection of a suitable vector system depends mainly on the size limit of insert DNA and the type of host intended for cloning or expression of foreign DNA.

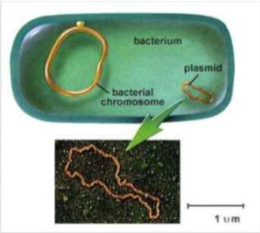
PLASMID VECTOR

- Are extra chromosomal, double stranded, circular, **self-replicating** DNA molecules. Plasmids range in size from about 1.0 kb to over 250 kb.
- Usually plasmids contribute to about 0.5%-5.0% of the total DNA of bacteria.
- A few bacteria contain linear plasmids. **E.g., streptomyces sp, Borelia burgdorferi**.
- The plasmids carries genes resistance for ampicillin & tetracycline that serve as markers for the identification of clones carrying plasmids. **E.g., pBR322,pUC**
- Contains a Multiple Cloning Site (MCS).
- Easy to be isolated from the host cell.
- Plasmids encode only few proteins required for their own replication (replication proteins) and these proteins encoding genes are located very close to the ***ori***. All the other proteins required for replication, e.g. DNA polymerases, DNA ligase, helicase, etc., are provided by the **host cell.**

- Thus, only a small region surrounding the ***ori* site is required for replication**. Other parts of the plasmid **can be deleted** and foreign sequences can be added to the plasmid without compromising replication.
- The host range of a plasmid is determined by its *ori* region. Plasmids whose *ori* region is derived from plasmid **Col E1 have a restricted host range.** They only replicate in enteric bacteria, such as *E. coli, Salmonella,* etc.
- Plasmids of the **RP4 type will replicate in most gram negative bacteria**, to which they are readily transmitted by conjugation.
- Plasmids like **RSF1010 are not conjugative but can be transformed into a wide range of gram -ve bacteria.**
- Plasmids isolated from ***Staphylococcus aureus* have a broad host range** and can replicate in many other gram-positive bacteria.

Some of the phenotypes which the naturally occurring plasmids confer on their host cells:

- Antibiotic resistance
- Antibiotic production
- Degradation of aromatic compounds
- Haemolysin production
- Sugar fermentation
- Enterotoxin production
- Heavy metal resistance
- Bacteriocin production
- Induction of plant tumors
- Hydrogen sulphide production



Types of Plasmids

The plasmids are divided into 6 major classes as described below depending on the phenotype:

i) **Resistance or R plasmids** carry genes which give resistance to the bacteria from one or more chemical agents, such as antibacterial agents. R plasmids are very important in clinical microbiology as they can have profound consequences in the treatment of bacterial infections. Eg: RP4 plasmid, which is commonly found in *Pseudomonas* and in many other bacteria.

ii) **Fertility or F plasmids** are conjugative plasmid found in F+ bacterium with higher frequency of conjugation. F plasmid carries transfer gene (*tra)* and has the ability to form Conjugation Bridge (F pilus) with F- bacterium. Eg: F plasmid of *E. coli*.

iii) **Col plasmids** have genes that code for colicins, proteins that kill other bacteria. Eg: ColE1 of *E. coli*.

iv) **Degradative plasmids** allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid. Eg TOL of *Pseudomonas putida*.

v) **Virulence plasmids** confer pathogenicity on the host bacterium. Eg: Ti plasmids of *Agrobacterium tumefaciens*, which induce crown gall disease on dicotyledonous plants.

vi) **Cryptic Plasmids** do not have any apparent effect on the phenotype of the cell harboring them. They just code for enzymes required for their replication and maintenance in the host cell.

Based on the origin or source of plasmids, they have been divided into two major classes:

i) **Natural plasmids**: They occur naturally in prokaryotes or eukaryotes. Example*:* **ColE1.**

ii) **Artificial plasmids**: They are constructed *in-vitro* by re-combining selected segments of two or more other plasmids (natural or artificial). Example: **pBR322.**

Plasmid	Size (kb)	Origin	Host range	Antibiotic resistance	Additional marker genes showing insertional inactivation
RSF1010	8.6	*E.coli* (strain K-12)	Broad host range	Streptomycin and sulfonamides.	None
ColE1	6.6	*E.coli*	Narrow host range	None	Immunity to colicin E1
R100	94.2	*E.coli*	*E.coli* K-12, *Shigella flexneri* 2b	Streptomycin, chloramphenycol, tetracycline	Mercuric (ion) reductase, putative ethidium bromide (EtBr) resistant protein.

Artificial Plasmids:

Artificial plasmids vectors are classified into two broad types based on their use:

1. Cloning vector
2. Expression vector

Cloning vectors are designed for efficient **transfer of foreign DNA into the host**. Expression vectors have efficient machinery for **cloning and expression** of foreign gene in the host system. Selection of a vector depends upon various criteria decided by the experimental goal.

CloningVector:

A cloning vector is defined as a vector used for **replication of a cloned DNA fragment** in a host cell. These vectors are frequently engineered to contain **"ori" – origin of replication sites** particular to the host organism. Examples of commonly used cloning vectors are: pUC18, pUC19, pBluescript vectors etc.

Important features of a cloning vector used to carry DNA molecules are as follows:-

- **Stability in host cell:** Vectors should be stabile in host cell after introduction and should not get lost in subsequent generations. This permits replication of vectors producing large copies of gene of interest.
- **Ability to control their own replication:** This property enables them to multiply and exist in high copy number.
- **Small size:** Ideal vector should be less than or equal to 10kb. The small size is essential for easy introduction in cell by transformation, transduction and electroporation.
- **Multiple cloning sites:** This property permits the insertion of gene of interest and plasmid re-circularization.
- **Should not be transferred by conjugation:** This property of vector molecule prevents recombinant DNA to escape to natural population of bacteria.
- **Selectable make gene:** Vector molecules should have some detectable traits. These traits enable the transformed cells to be identified among the non-transformed ones. **eg. antibiotic resistance gene.**

Types of Cloning Vectors:

- Cloning vectors extensively used in molecular cloning experiments can be considered under following types: **plasmid**, **phage vector** and **cosmid.**
- Different vectors have different insert size and also vary in mode of replication inside the host.
- Mammalian genes are usually too large (~100 kb) and thus suffer from restrictions in complete inclusion with the conventional cloning vectors having limited insert size.
- Vectors engineered more recently, known as artificial chromosomes, have overcome this problem by mimicking the properties of host cell chromosomes. They have much larger insert size than other vectors.

Vector	Insert size	Source	Application
Plasmid	≤ 15 kb	Bacteria	Subcloning and downstream manipulation, cDNA cloning and expression assays
Phage	5-20 kb	Bacteriophage λ	Genomic DNA cloning, cDNA cloning and expression library
Cosmid	35-45 kb	Plasmid containing a bacteriophage λ *cos* site	Genomic library construction
BAC (bacterial artificial chromosome)	75-300 kb	Plasmid ocntaining *ori* from *E.coli* F- plasmid	Analysis of large genomes
YAC (yeast artificial chromosome)	100-1000 kb (1 Mb)	*Saccharomyces cerevisiae* centromere, telomere and autonomously replicating sequence	Analysis of large genome, YAC transgenic mice
MAC (mammalian artificial chromosome)	100 kb to > 1 Mb	Mammalian centromere, telomere and origin of replication	Under development for use in animal biotechnology and human gene therapy

Table 1-3.6: Different type of cloning vectors

Examples of Cloning Vector:

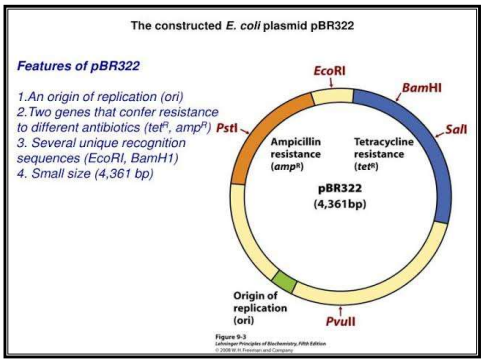
pBR322

- pBR322 is a widely-used *E. coli* cloning vector. It was created in 1977 in the laboratory of **Herbert Boyer** at the University of California San Francisco.
- The *p* stands for "**plasmid**" and *BR* for "**Bolivar**" and "**Rodriguez**", researchers who constructed it.

• pBR322 is **4361** base pairs in length.

• pBR322 plasmid has the following elements:

- **"*rep*" replicon** from plasmid **pMB1** which is responsible for replication of the plasmid.
- **"*rop*" gene** encoding Rop protein. Rop proteins are associated with stability of RNAI-RNAII complex and also decrease copy number. The source of "*rop*" gene is pMB1plasmid.
- **"*tet*" gene** encoding tetracycline resistance derived from **pSC101** plasmid.
- **"*bla*" gene** encoding **β lactamase** which provide ampicillin resistance (source: transposon Tn3).



pUC plasmids:

• pUC plasmids are small, high copy number plasmids of size **2686bp**.

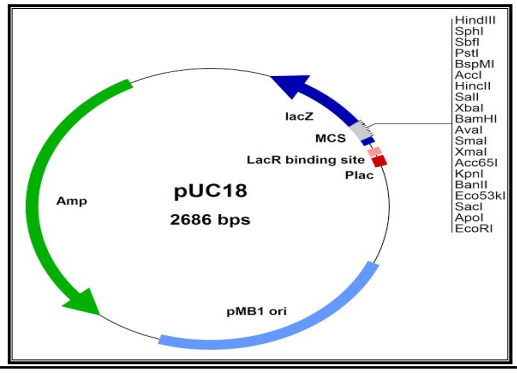
• This series of cloning vectors were developed by Messing and co-workers in the **University of California**. The p in its name stands for plasmid and UC represents the University of California.

• pUC vectors contain a ***lacZ* sequence** and **multiple cloning site (MCS)** within *lacZ*. This helps in use of broad spectrum of restriction endonucleases and permits rapid visual detection of an insert.

• pUC18 and pUC19 vectors are identical apart from the fact that the MCS is arranged in opposite orientation.

• pUC vectors consists of following elements:

- **pMB1 "*rep*" replicon region** derived from **plasmid pBR322 with single point mutation** (to increase copy number).
- **"*bla*" gene encoding β lactamase which provide ampicillin resistance** which is derived from pBR322. **This site is different from pBR322 by two point mutations.**
- ***E.coli* lac operon system.** • **"*rop*" gene** is **removed from** this vector which leads to an increase in copy number.



An MCS is a short DNA sequence consisting of restriction sites for many different restriction endonucleases. MCS escalates the number of potential cloning strategies available by extending the range of enzymes that can be used to generate a restriction fragment suitable for cloning. By combining them within a MCS, the sites are made contiguous, so that any two sites within it can be cleaved simultaneously without excising vector sequences. The MCS is inserted into the *lacZ* sequence, which encodes the promoter and the α-peptide of β-galactosidase. Insertion of the MCS into the *lacZ* fragment does not affect the ability of the α-peptide to mediate complementation, while cloning DNA fragments into the MCS does. Therefore, recombinants can be detected by blue/white screening on growth medium containing X gal in presence of IPTG as an inducer.

Bacteriophages

- **Are the viruses that replicate within the bacteria.**
- **In case of certain phages, their DNA gets incorporated into the bacterial chromosome & remains there permanently.**
- **Can take up larger DNA segments than plasmids, hence preferred for working with**
- **genomes of human cells. E.g., phage λ, phageM13.**

Cosmids

- **Are the vectors possessing the characteristics of both plasmid & bacteriophage λ**
- **These carry larger fragments of foreign DNA compared to plasmids.**

Artificial chromosome vectors

E.g. Human artificial chromosome, Yeast artificial chromosomes, Bacterial artificial chromosome

- **These can accept large fragments of foreign DNA.**

THANK YOU