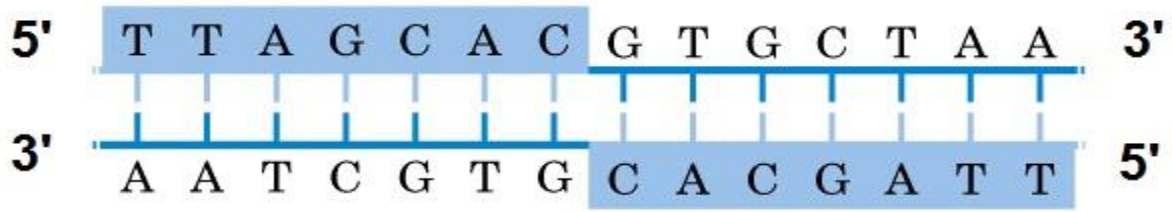
Restriction endonucleases

Restriction endonucleases (Restriction Enzymes)

- They are bacterial enzymes that cut dsDNA into fragments after recognizing specific nucleotide sequence known as restriction sites, and have evolved by the bacteria to provide a defense mechanism against invading viruses (They are also known as molecular scissor).

- By scanning the DNA sequence, a short and a specific set of nucleotides known as restriction sites (palindromic sequences) in the DNA can be recognized where they cut within those specific palindromic sequences (usually a 4- or 6 base pair sequence).

- **A palindrome sequence** is the sequence that can be read the same in both directions, (the sequence in DNA is one in which the 5' to 3' base pair sequence is identical on both strands)



Palindromic sequence

Discovering Restriction Enzymes

- *Hind*II - first restriction enzyme – was discovered accidentally in 1970 while studying how the bacterium *Haemophilus influenzae* takes up DNA from the virus recognizes and cuts DNA at particular sequence.

Recognition sites of restriction enzymes

Enzyme	Organism from which derived	Target sequence (cut at *) 5' -->3'
Bam HI	***Bacillus amyloliquefaciens***	**G* G A T C C**
Eco RI	***Escherichia coli RY 13***	**G* A A T T C**
Hind III	***Haemophilus inflenzae Rd***	**A* A G C T T**
Mbo I	***Moraxella bovis***	***G A T C**
Pst I	***Providencia stuartii***	**C T G C A * G**
Sma I	***Serratia marcescens***	**C C C * G G G**
Taq I	***Thermophilus aquaticus***	**T * C G A**
Xma I	***Xanthamonas malvacearum***	**C * C C G G G**

Nomenclature of restriction enzymes

- Smith and Nathans (1973) proposed enzyme naming scheme three-letter acronym for each enzyme derived from the source organism
- First letter from genus, next two letters are from the first two letters of its species, they are printed in italics. Followed by the strain number letter or number represent the strain or serotypes, such as the enzyme, these are identified by Roman numerals I, II, III, etc.
- For example

    Eco R1 was isolated from the bacterium Escherichia (E), coli (co), strain RY13 (R), it was the first endonuclease (1)

Classification

- Synonymous to Restriction Endonuclease
- Endonuclease: Cut DNA from inside
- Highly heterogeneous
- Evolved independently rather than diverging form a common ancestor
- Broadly classified into four Types, categorized based on their composition, co-factor requirement, the nature of their target sequence position of their DNA cleavage site relative to the target sequence.

Type I

- Multi-subunit proteins
- Function as a single protein complex
- Contain two R (restriction) subunits, two M (methylation) subunits and one S (specificity) subunit
- Cleave DNA at random length from recognition site

Type II

- Most useful for gene analysis and cloning
- More than 3500 REs
- Recognize 4-8 bp sequences
- Need Mg^{2+} as cofactor
- Cut in close proximity of the recognition site
- Homodimers
- ATP hydrolysis is not required

Type III

- Large enzymes
- Combination restriction-and-modification
- Cleave outside of their recognition sequences
- Require two recognition sequences in opposite orientations within the same DNA molecule
- No commercial use or availability

Type IV

- Cleave only modified DNA (methylated and hydroxymethylated bases).
- Recognition sequences have not been well defined
- Cleavage takes place ~30 bp away from one of the sites.

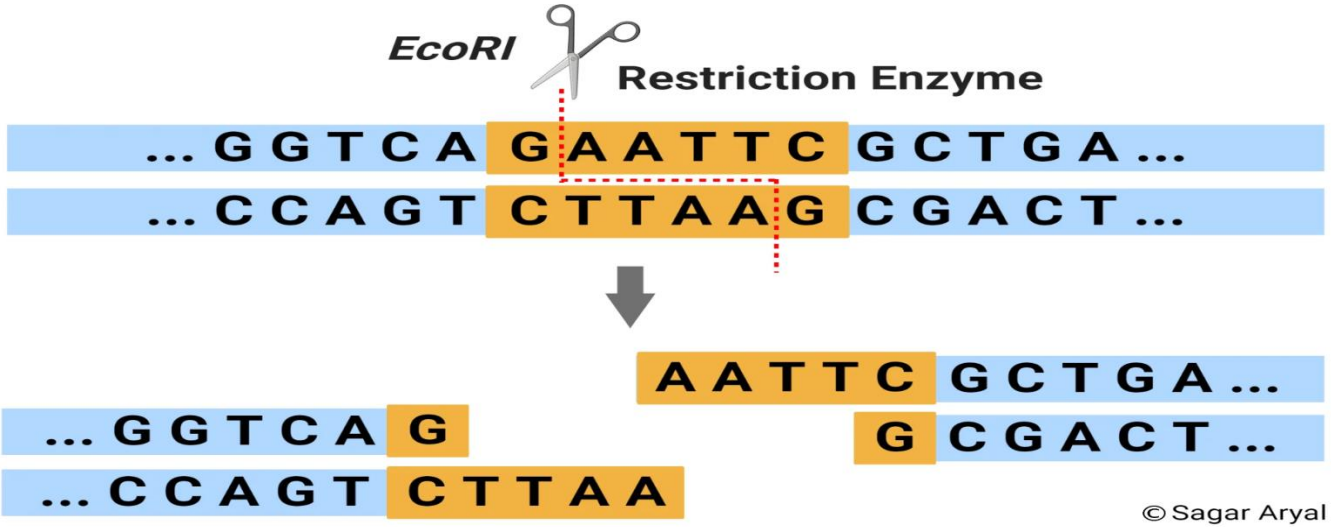
Feature	Type I	Type II	Type III	Type IV
Structural				
Subunits	Three different	Two identical	Two different	Two different
Enzyme activity	Endonuclease, methyltransferase ATPase	Endonuclease or methyltransferase	Endonuclease, methyltransferase ATPase	Endonuclease GTPase
Biochemical				
Cofactors for DNA cleavage	ATP, AdoMet, Mg^{2+}	Mg^{2+}	ATP, Mg^{2+} (AdoMet)	Mg^{2+}, GTP
Methylation	AdoMet, Mg^{2+}	AdoMet	AdoMet, Mg^{2+}	—
Recognition sequence	Asymmetric, bipartite	Usually symmetric	Asymmetric	Bipartite, methylated
Cleavage site	Random, atleast 1000 bp from recognition site	At or near recognition site	25–27 bp from recognition site	Between methylated bases at multiple positions
DNA translocation	Yes	No	Yes	Yes

How restriction endonucleases work?

- Restriction enzymes recognize a specific sequence of nucleotides, and produce a double-stranded cut in the DNA. these cuts are of two types:

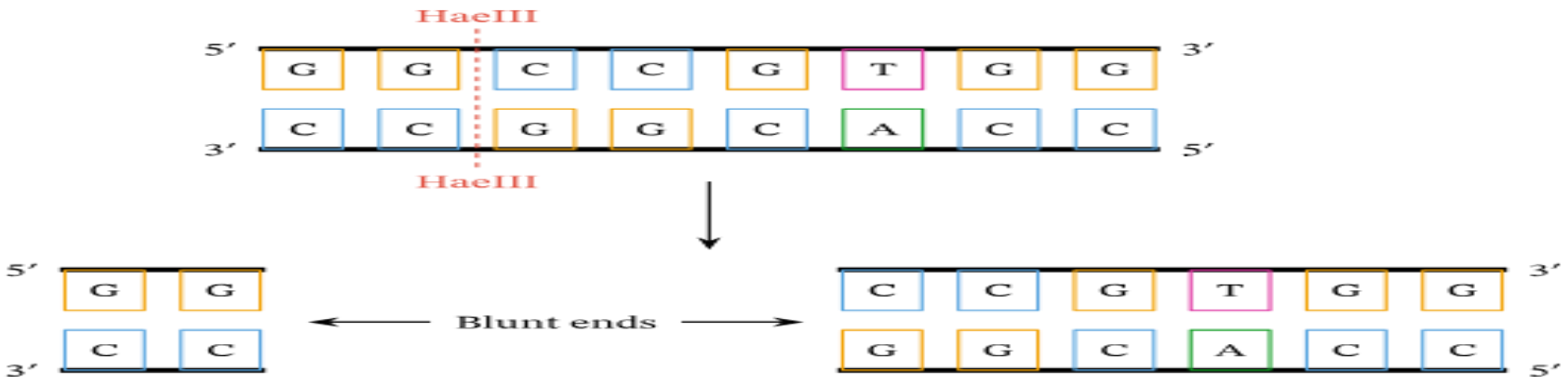
Sticky end cutters

- Most restriction enzymes make staggered cuts
- Staggered cuts produce single stranded “sticky-ends”
- DNA from different sources can be spliced easily because of sticky-end overhangs.
- DNA fragments with complementary sticky ends can be combined to create new molecules which allows the creation and manipulation of DNA sequences from different sources.



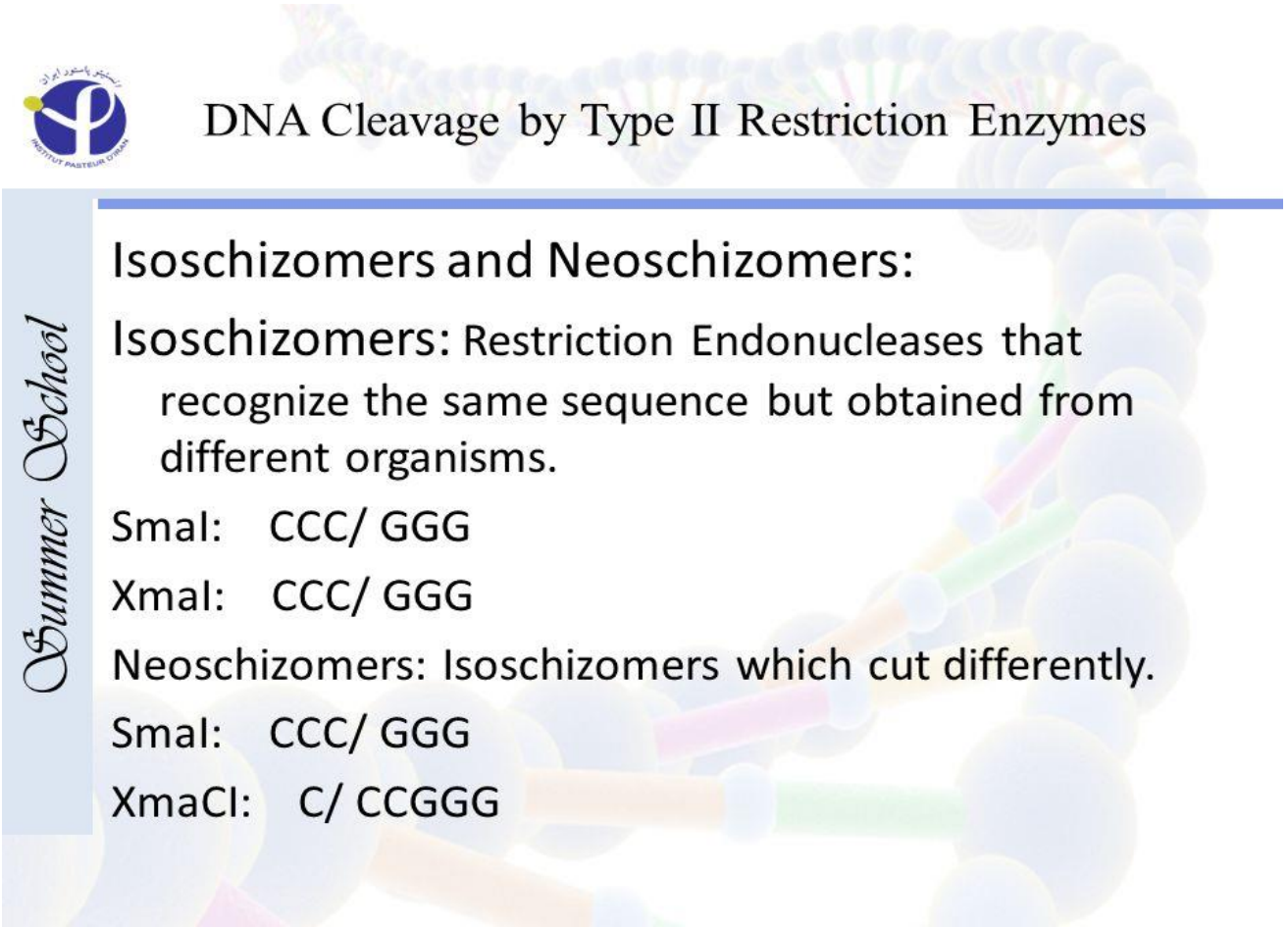
Blunt end cutters

- Some restriction enzymes cut DNA at opposite base
- They leave blunt ended DNA fragments
- These are called blunt end cutters
- These blunt ended fragments can be joined to any other
- Enzymes useful for certain types of DNA cloning experiments



Isoschizomers and neoschizomers

- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are isoschizomers
- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are neoschizomers



Application of restriction enzymes

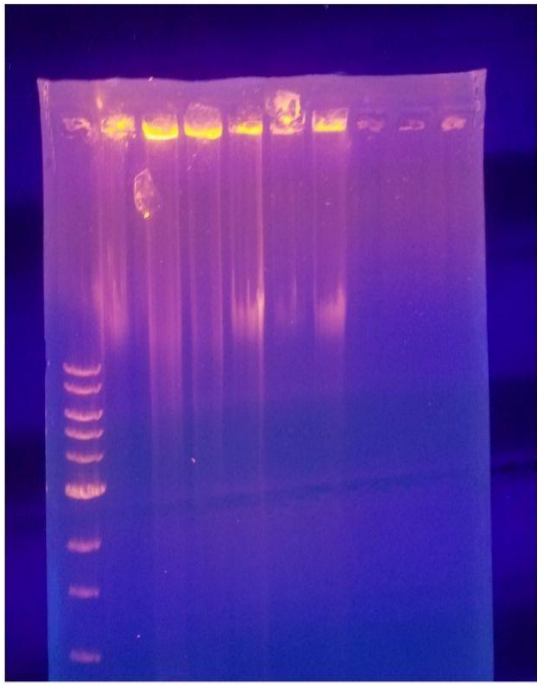
- They are used in gene cloning and protein expression experiments.
- Discovery of enzymes that cut and paste DNA make **genetic engineering** possible, where restriction enzyme cuts DNA and generates fragments, then ligase joins different DNA fragments.

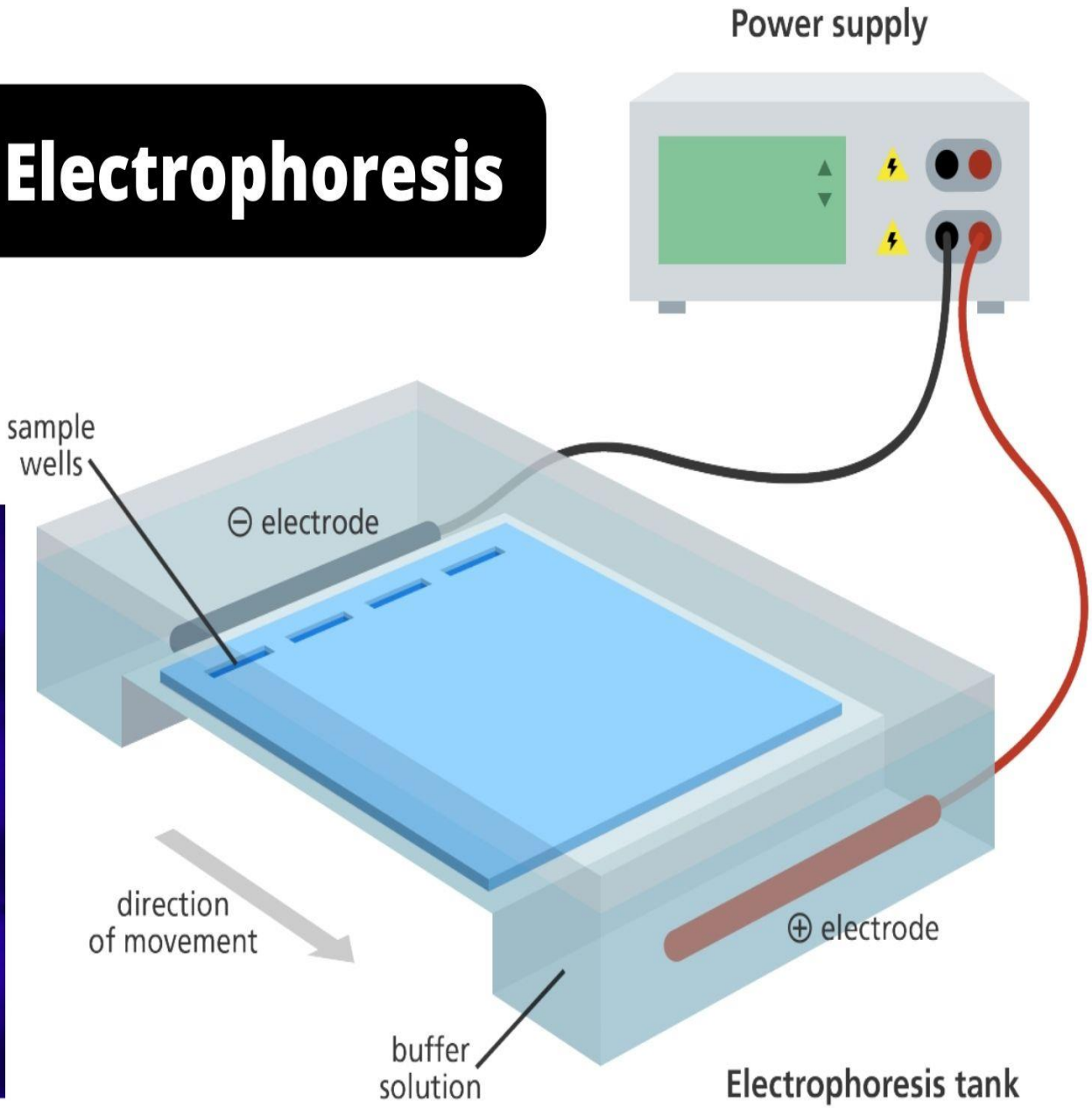
- DNA fragments from different species can be ligated (joined) to create **Recombinant DNA**

- They are used in biotechnology to cut DNA into smaller strands in order to study fragment length differences among individuals (**RFLP**). **Restriction Fragment Length Polymorphism**

- Each of one of these methods depends on the use of agarose gel electrophoresis for separating DNA fragments.

Agarose Gel Electrophoresis





Restriction Fragment Length Polymorphism (RFLP)

- It is a technique in which organisms may be differentiated by the analysis of patterns derived from cleavage of their DNA by exploiting variations in homologous DNA sequences.

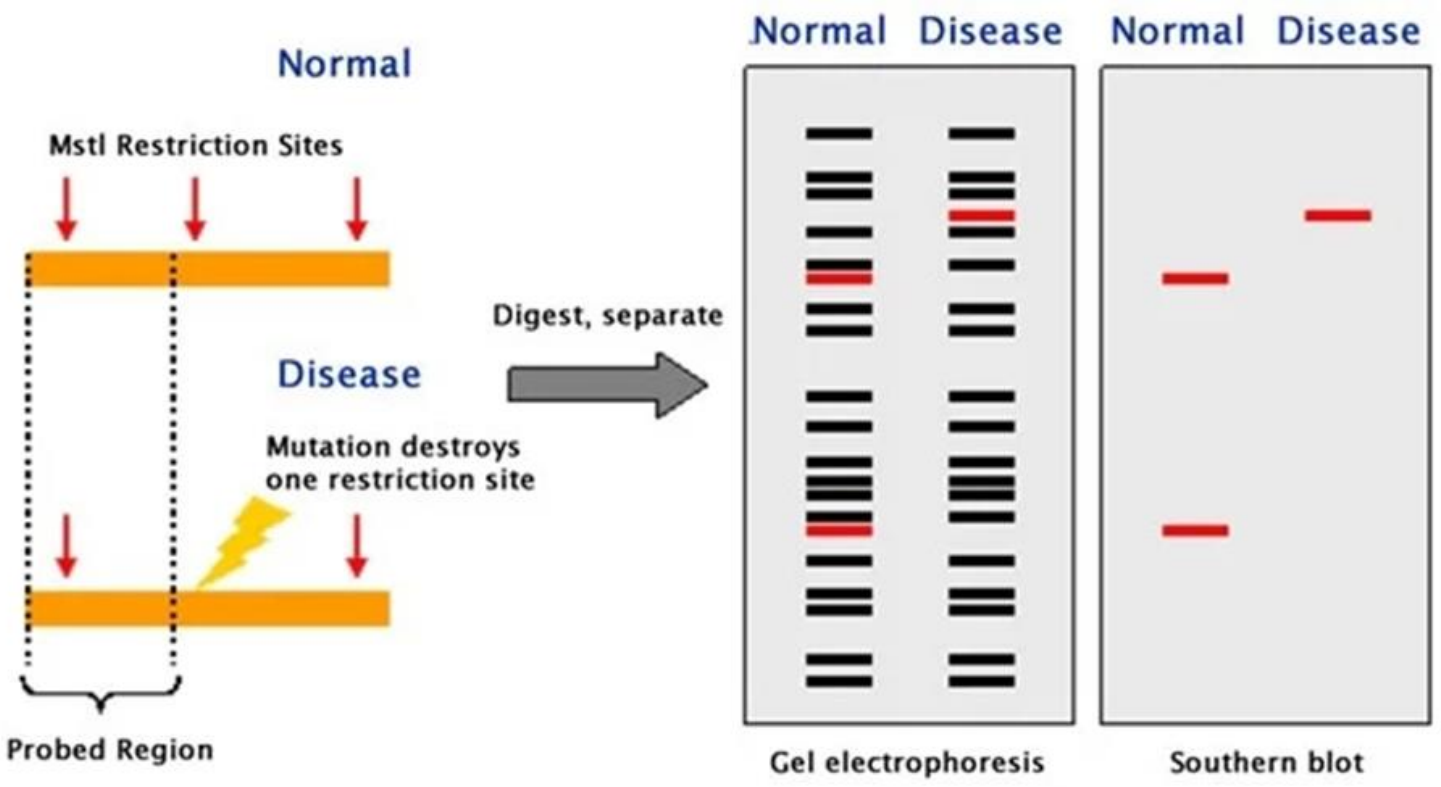
- it is defined by the existence of alternative alleles associated with restriction fragments that differ in size from each other. Simply, it is variations in the restriction DNA fragments length between individuals of a species.

- The basic technique of identifying such restriction fragment length polymorphisms involve fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest.

- The resulting DNA fragments are then separated by length using agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure.

- Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe.

- An RFLP occurs when the length of a detected fragment varies between individuals.



DNA Cloning

- DNA cloning is a technique for reproducing DNA fragments.
- A vector is required to carry the DNA fragment of interest into the host cell.

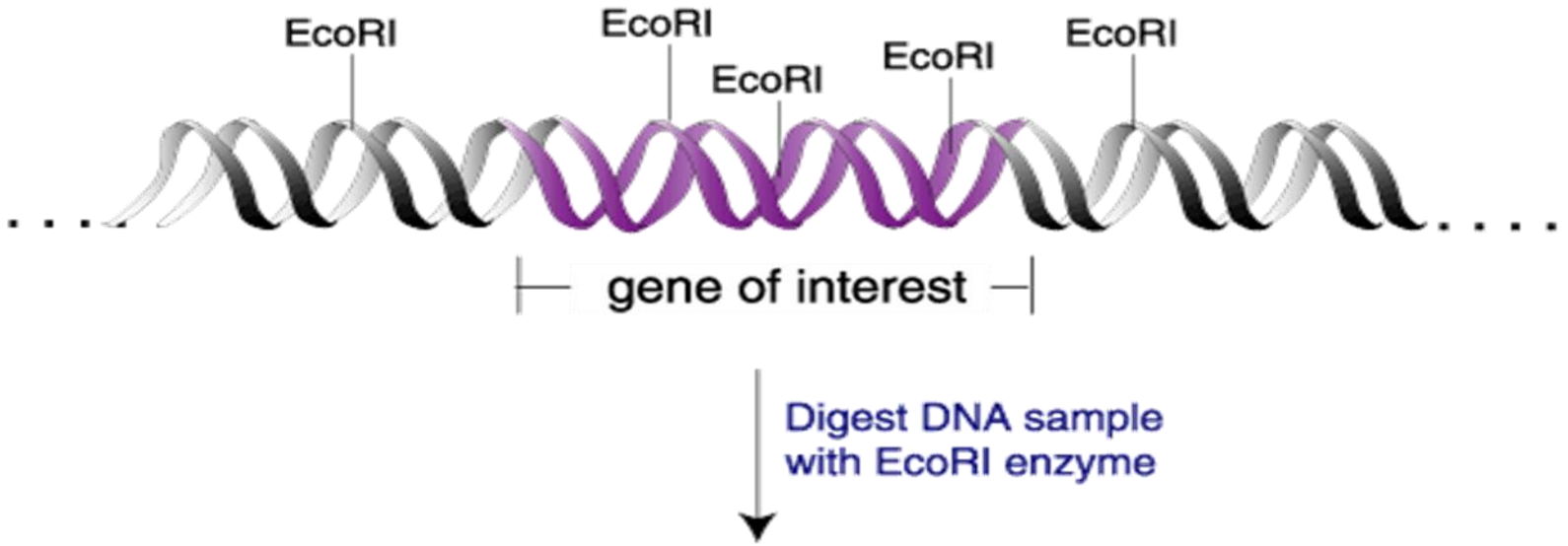
- DNA cloning allows a copy of any specific part of a DNA (or RNA) sequence to be selected among many others and produced in an unlimited amount.

- This technique is the first stage of most of the genetic engineering experiments:
  - Production of DNA libraries
  - PCR with Massive amplification of DNA sequences
  - DNA sequencing

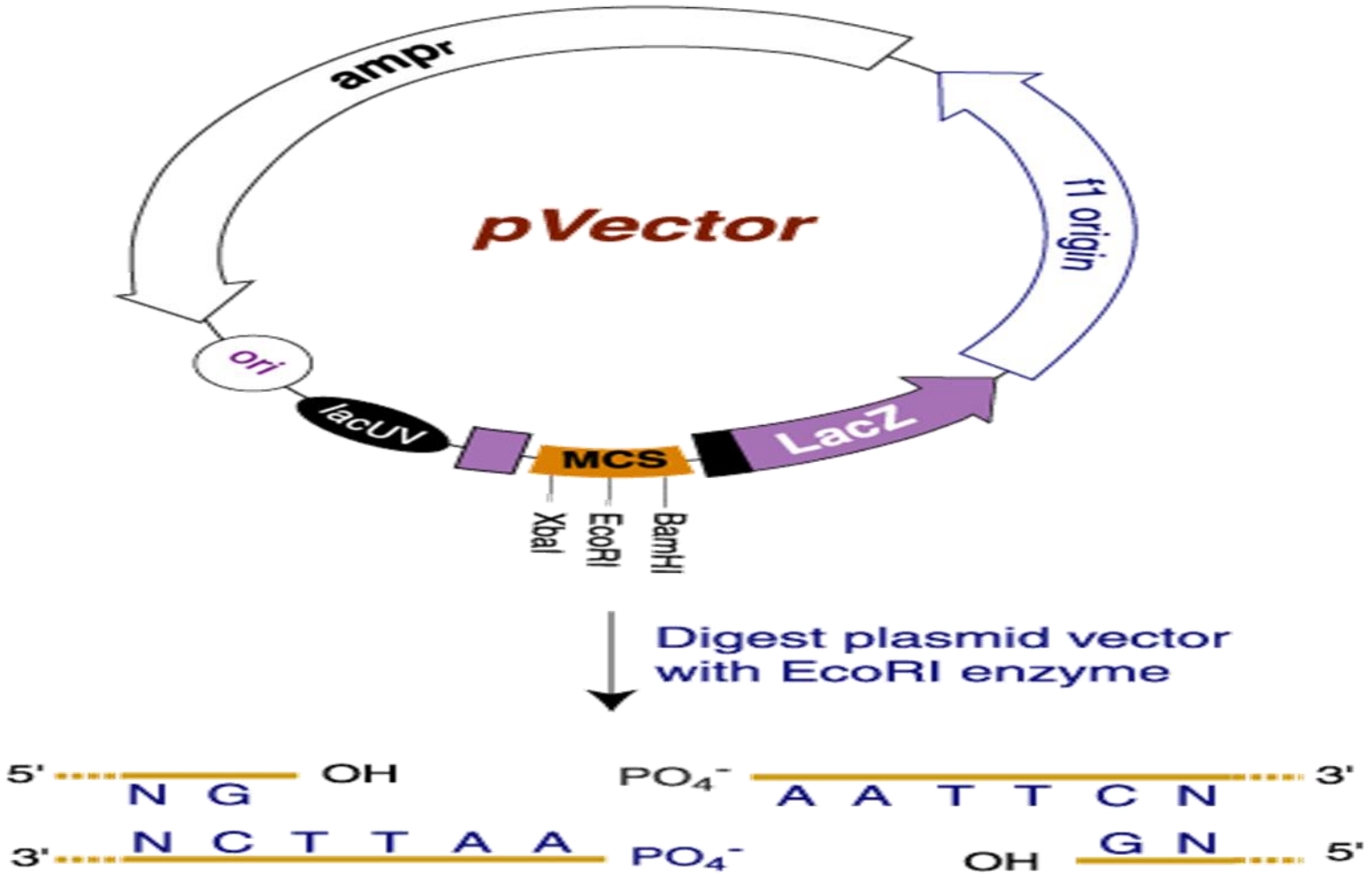
Cloning process

- Gene of interest is cut out with RE
- Host plasmid is cut with same RE
- Gene is inserted into plasmid and ligated with ligase
- New plasmid inserted into bacterium (transform)
- Growth on agar plates with selection for antibiotic resistance.

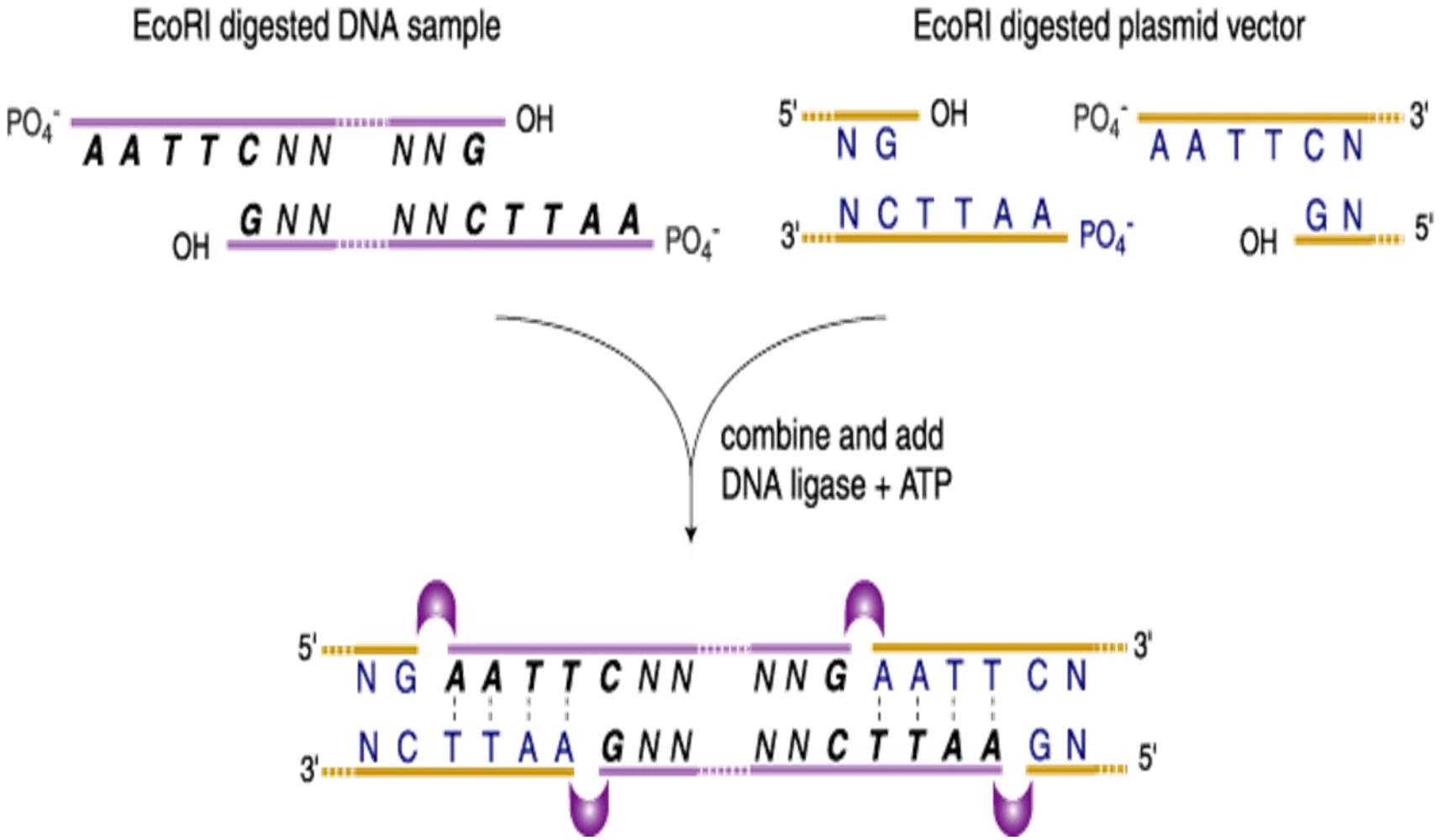
Step 1. RE digestion of DNA sample



Step 2. RE digestion of plasmid DNA

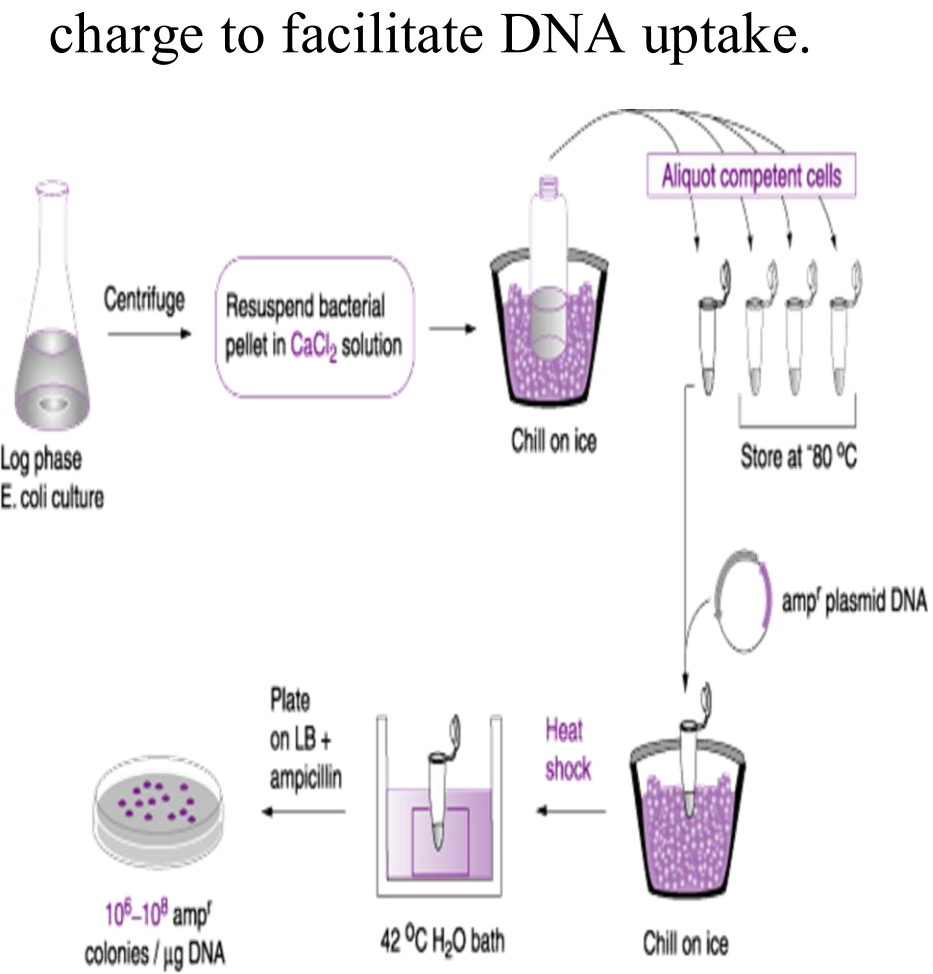


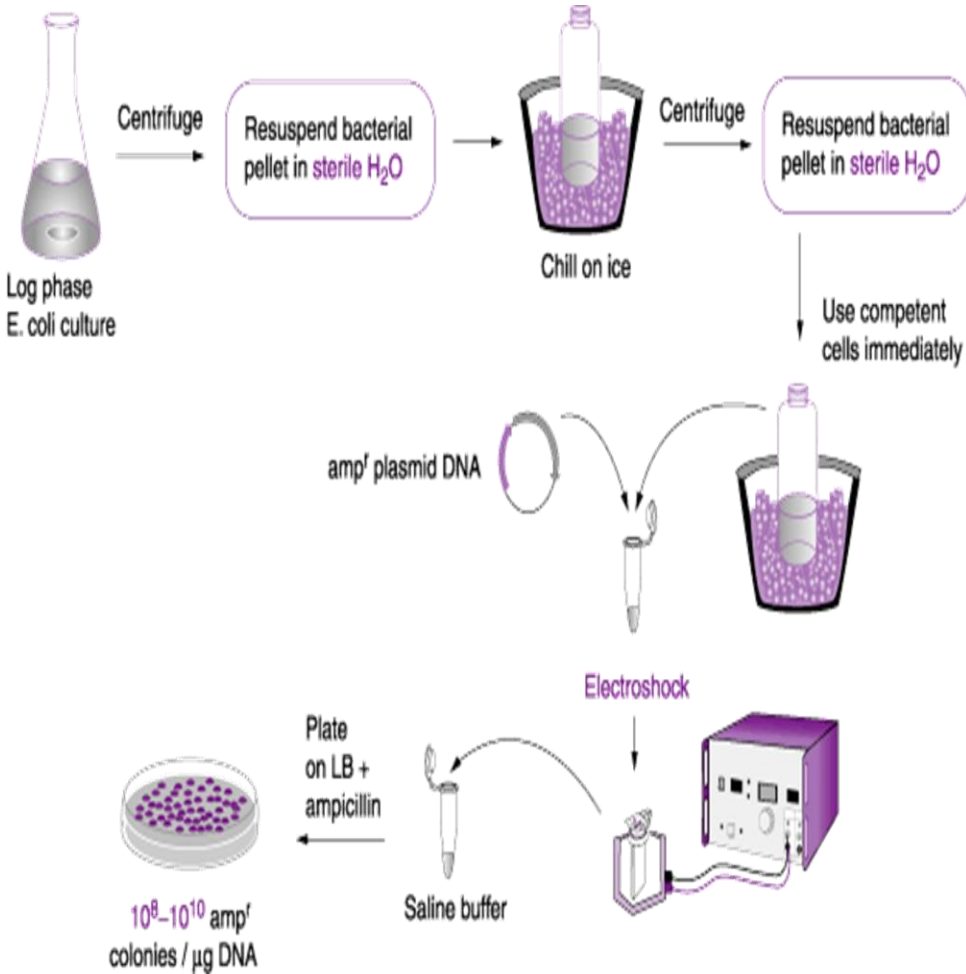
Step 3. ligation of DNA sample and plasmid DNA



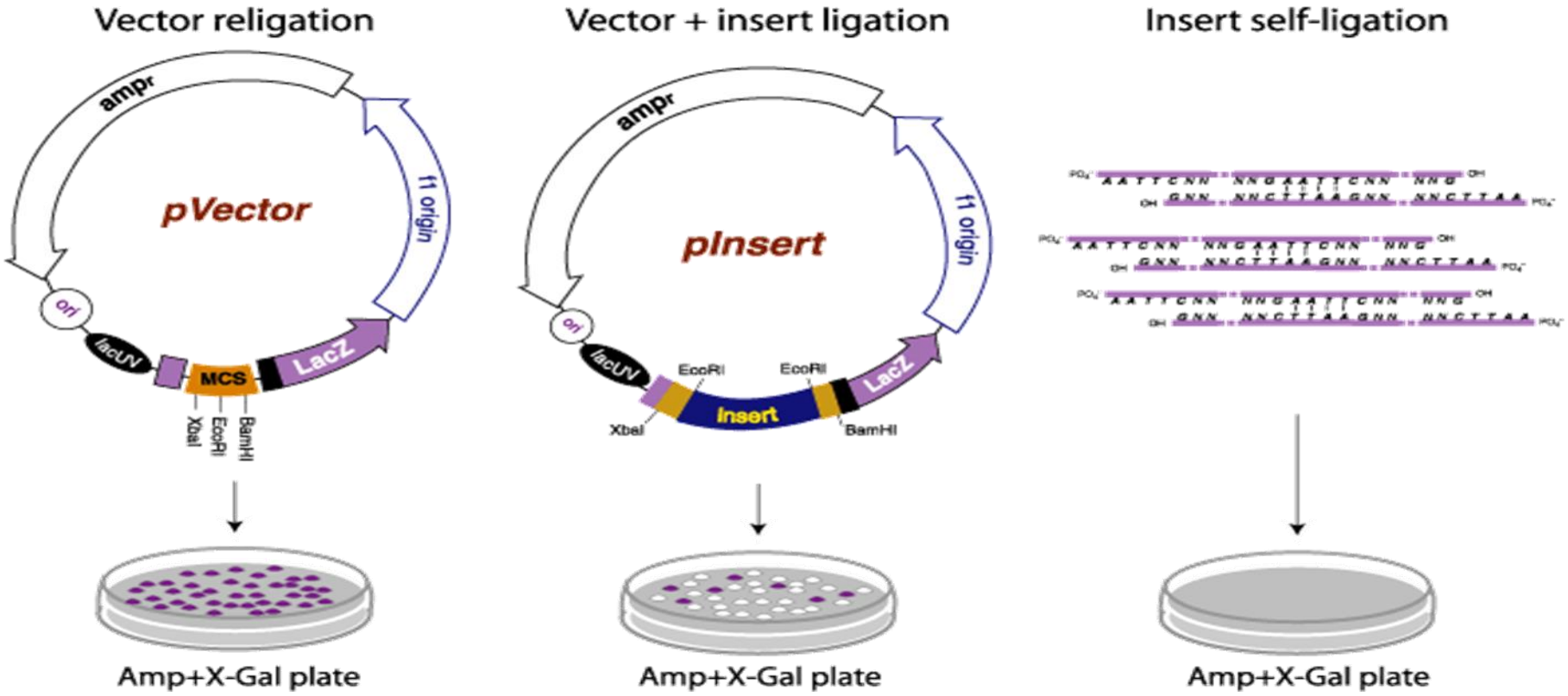
Step 4. transformation of ligation products

- The process of transferring exogenous DNA into cells is “transformation”
- There are basically two general methods for transforming bacteria.
- The first is a chemical method utilizing CaCl2 and heat shock to promote DNA entry into cells.
- A second method is called electroporation based on a short pulse of electric charge to facilitate DNA uptake.





Step 5. growth on agar plates



- Blue colonies represent Ampicillin-resistant bacteria that contain pVector and express a functional alpha fragment from an intact LacZ alpha coding sequence.

- White colonies represent Ampicillin-resistant bacteria that contain pInsert and do not produce LacZ alpha fragment

Types of cloning vectors

I. Plasmid vectors

- Plasmid vectors are used to clone DNA ranging in size from several base pairs to several thousands of base pairs (100bp -10kb), cannot accept large fragments
- Standard methods of transformation are inefficient

II. Bacteriophage Lambda

- Phage lambda is a bacteriophage or phage, i.e. bacterial virus, that uses *E. coli* as host.
- Its structure is that of a typical phage: head, tail, tail fibers.
- Lambda viral genome: 48.5 kb linear DNA with a 12 base ssDNA "sticky end" at both ends; these ends are complementary in sequence and can hybridize to each other (this is the cos site: cohesive ends).
- Infection: lambda tail fibres adsorb to a cell surface receptor, the tail contracts, and the DNA is injected.
- The DNA circularizes at the cos site, and lambda begins its life cycle in the *E. coli* host.

III. Cosmid vector

- It clones large inserts of DNA: size ~ 45 kb
- Cosmids are Plasmids with one or two Lambda Cos sites.
- Presence of the Cos site permits in vitro packaging of cosmid DNA into Lambda particles

IV. Yeast Artificial Chromosomes (YAC)

- They are cloning vehicles that propagate in eukaryotic cell hosts as eukaryotic Chromosomes
- Clone very large inserts of DNA: 100 kb - 10 Mb
- YAC cloning vehicles are plasmids
- Final chimeric DNA is a linear DNA molecule with telomeric ends
- Often have a selection for an insert
- The YAC can use both yeast and bacteria as a host

V. Bacterial Artificial Chromosomes (BAC)

- Can be cloned as a plasmid in a bacterial host, and its natural stability generally permits cloning of large pieces of insert DNA, i.e. up to a few hundred kb of DNA.