

Lab5:

Gene cloning

Cloning include insert a foreign piece DNA into living organism that does not contain this piece to exceed the species barrier and is the main aim in the genetic engineering technique

Steps in cloning

In standard molecular cloning experiments, the cloning of any DNA fragment essentially involves seven steps:

- 1- Choosing the host cell and cloning vector,
- 2- Preparation of DNA to be cloned (interesting gene),
- 3- Preparation of vector DNA,
- 4- Creation of recombinant DNA (insert +vector),
- 5- Transformation of recombinant DNA into host cell,
- 6- Selecting the transformed cell that contains the recombinant DNA,
- 7- Screening for clones with desired DNA inserts and biological properties

Types of cloning

- 1- Gene cloning
- 2- Cell cloning
- 3- Reproductive cloning
- 4- Therapeutic cloning

Characterization of prokaryotic vectors cloning

- 1- It has the ability to self-replicate independently of the bacterial chromosome (containing replication origin (ori))
- 2- Easy to isolate (Small size)
- 3- Non-toxic to the host cell

- 4- It has multiple cloning site (MCS) to insert DNA fragment
- 5- Containing genes, which can be detected all the way to the elected details (antibiotic resistance genes)
- 6- It has a private places by restriction enzyme
- 7- large number of copies

Types of cloning vectors

1- Plasmids:

Plasmid is an autonomously replicating circular extra-chromosomal DNA. They are the standard cloning vectors and the most commonly used. Most general plasmids may be used to clone DNA insert of up to 15 kb in size. Function carrying the genes of (antibiotic resistance genes or the production of toxins).

It is classified into two types:

A- Natural Plasmid ex: COL E1

Three examples of Natural Plasmid

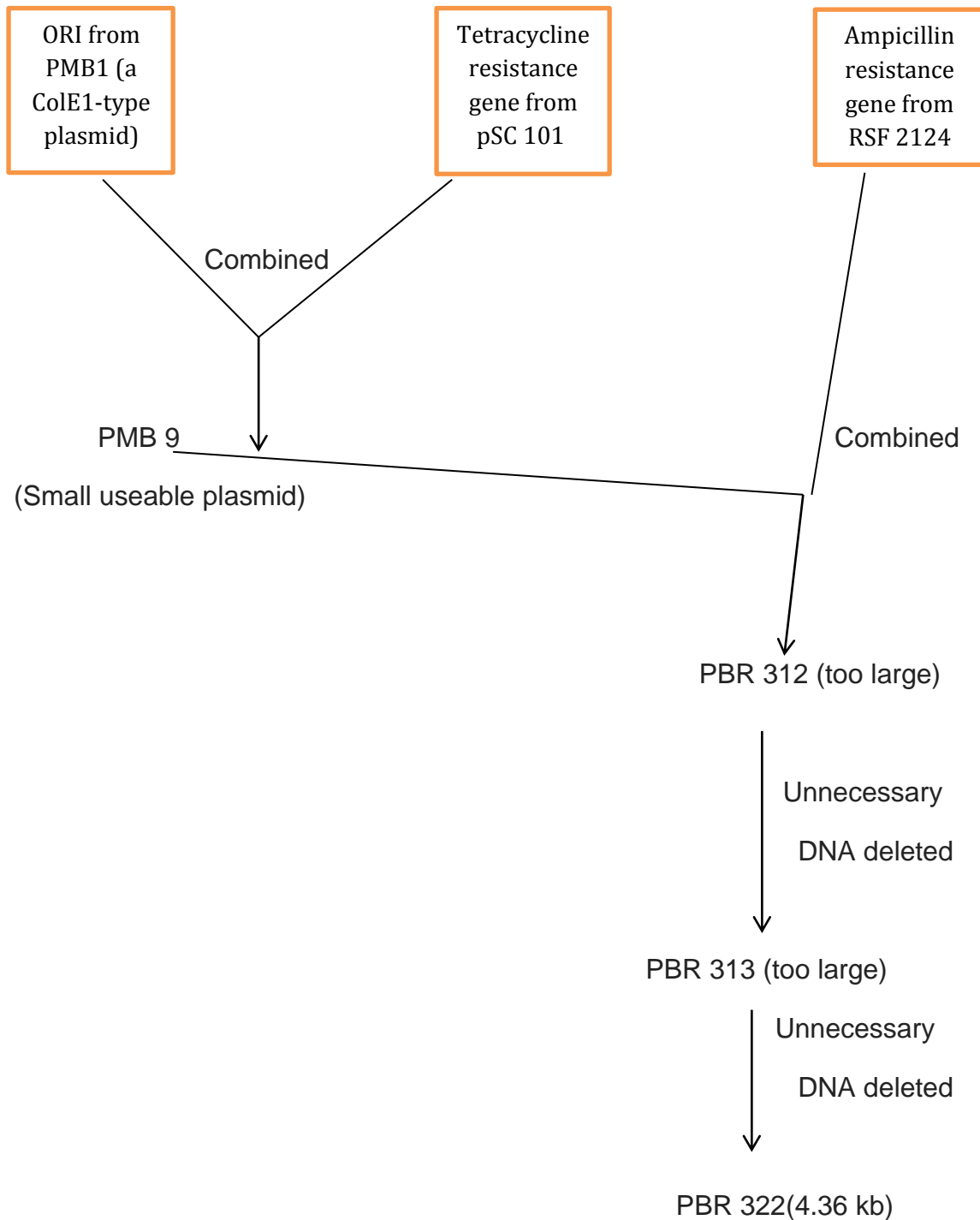
Plasmid	Size (kb)	Relaxed (amplified)	Single sites for restriction enzymes	Marker genes for selecting transformant	Additional marker genes showing insertional inactivation
Psc101	6.5	No	XhoI, EcoR1, PvuII, HincII	Tetracycline resistance	-
			HindIII, BamHI, Sall	-	Tetracycline resistance
ColE1	8		EcoRI	Immunity to colicin E1	colicin E1 production
RSF2124	11		EcoRI, BamHI	Ampicillin resistance	colicin E1 production

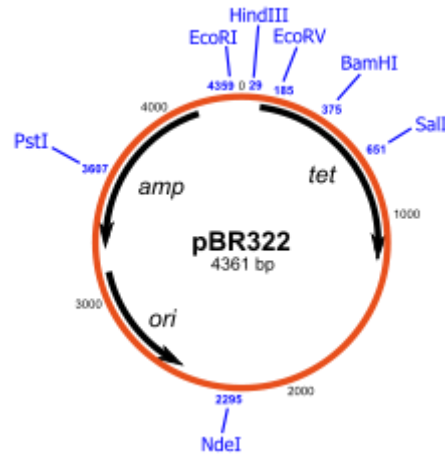
Note: There is no perfect plasmid in nature

B- Artificial Plasmid ex: pBR322

pBR322 is a plasmid and was one of the first widely used *E. coli* cloning vectors. The p stands for "plasmid," and BR for "Bolivar" and "Rodriguez. It is 4361 base pairs

Construction of an artificial cloning plasmid pBR322





2- Bacteriophage

The bacteriophages that used for cloning are the phage λ (linear DsDNA) and M13 phage (circular ssDNA).

phage λ : phag Infects *E.coli*

And a linear DNA containing the ends of the adhesive, when entering the phage into the host cell endings you will be linked with each lens you shall take the form of a circular molecule. Genes in hand, be left off the map for this chromosomal phage proteins coded for the head and tail of phage. The centrist genes responsible for the re-engagement and the right of the map genes are responsible for gene regulation work.

Genome of wild type phage

COS

COS

Head , Tail

Lysogeny

Regulation

It was observed that the genes of the central region is essential for the growth of the phage and so can be removed without affecting the phage and this was an important point in the construction of vectors which are derived from cloning phage .

How is the use of phage λ in experiments :

- 1- Isolated DNA phage λ from molecule of phage λ .
- 2- Cutting DNA phage λ and foreign DNA by restriction enzyme (removed the central region)
- 3- Link foreign DNA with DNA phage λ (Instead of the central region) by DNA ligase .
- 4- Insert recombinant molecule to the head phage λ .
- 5- Infects *E.coli* with phage λ and Begins replicate .
- 6- Isolated the plaques formed which contains cloning phage λ .
- 7- The selection of the container cells on the phage λ , it is Carrier recombinant molecule by DNA hybridization.
- 8- Multiplication the cells .

Cosmid

A recombinant molecule composed of a plasmid containing end site (cos) derived from phage λ and DNA from the plasmid does not cause lysis of the host cell It is normally used to clone large DNA fragments between 28 to 45 Kb , but this vector is not stable .

How is the use of Cosmid in experiments

- 1- Cosmid cutting and open from the ends of the adhesive to phage λ by enzyme .
- 2- foreign DNA cutting by the same enzyme cutter.
- 3- Connecting pieces of foreign DNA with open cosmid by DNA ligase.
- 4- Phage Formation by in –vitro package , Phage formation immature add-head proteins and tail.
- 5- Infection *E.coli*.
- 6- Selection colonies resistance Amp^r as Character appearance.