Fundamentals of Genetic Engineering

What are vectors?

- Vectors are DNA molecules that act as destination for GOI.
- Vectors act as a vehicle to ultimately transfer the gene into host
- Host are like biofactory!

Vectors can be:

Plasmid, Bacteriophage, Cosmid/ phagemid, Transposon, Virus, Artificial chromosome

2

What is Plasmid?

- Plasmids are **extra-chromosomal** DNA molecule.
- Plasmid are replicons which are stably inherited.
- They range from **1kb to 200kb** in size.
- Most plasmids exists as **double-stranded circular DNA molecule**.
- Plasmids are present in prokaryotes.
- Plasmids gives **special phenotypes** to the host.

Table 4.1 Some phenotypic traits exhibited by plasmid-carried genes.

Antibiotic resistance
Antibiotic production
Degradation of aromatic compounds
Haemolysin production
Sugar fermentation
Enterotoxin production
Heavy-metal resistance
Bacteriocin production
Induction of plant tumours
Hydrogen sulphide production
Host-controlled restriction and modification

3

Nomenclature of plasmid cloning vectors

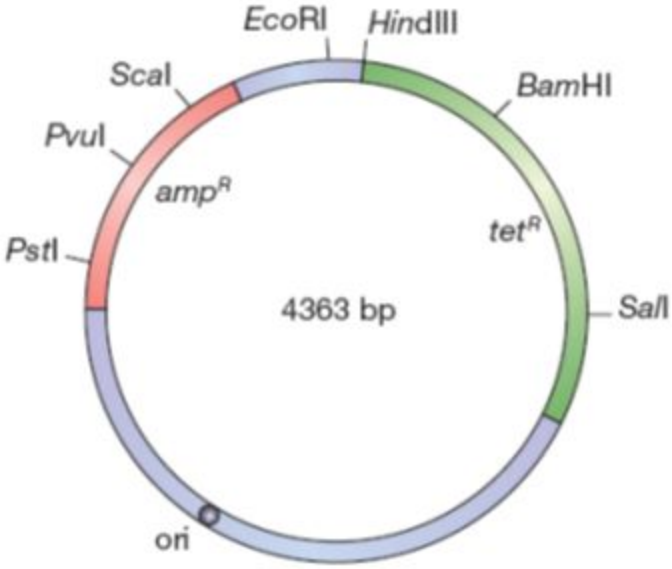
- **pBR322**

- 'p' indicates that this is a plasmid.
- 'BR' indicates the laboratory where the vector was constructed- **B**oliver and **R**odriguez are the researchers who developed the constructs
- '322' is a serial number. Distinguishes it from other plasmids.

Plasmid cloning vectors

- **Useful properties of pBR322**

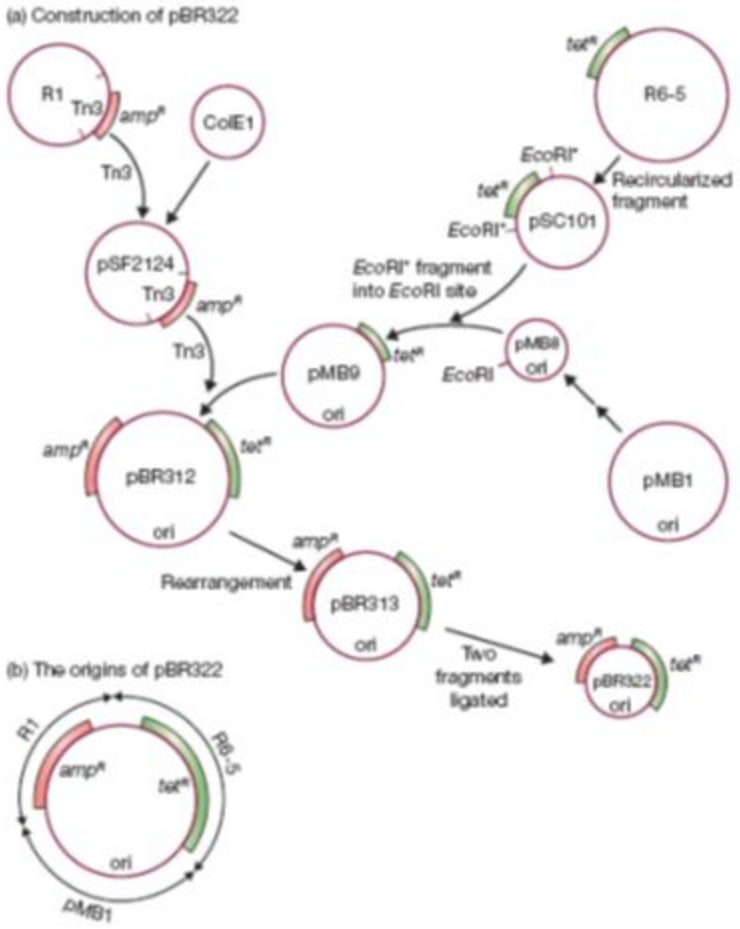
- < 10 kb; 4363bp; can thus be easily purified
- Selectable marker- amp^r and tet^r;
- insertional inactivation is possible as there are multiple cutting sites
- High copy number; generally 15 but can be extended up to 1000-3000 copies
- Conjugative plasmid



Plasmid cloning vectors

- **pBR322**

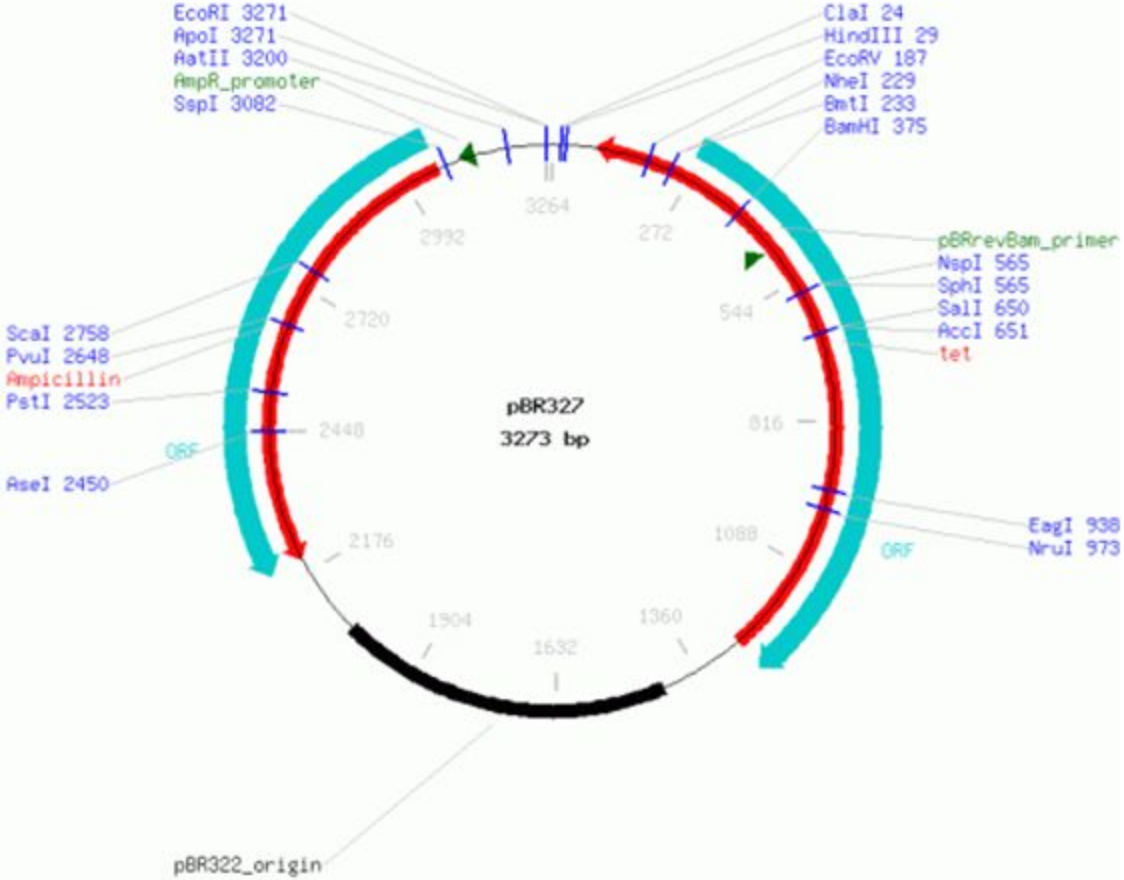
- Pedigree: how from naturally occurring vectors pBR322 was produced
- Amp^r from Plasmid R1
- Tet^r from R6-5
- Ori from pMB81(which is closely linked to ColE1)



Plasmid cloning vectors

- **pBR327**

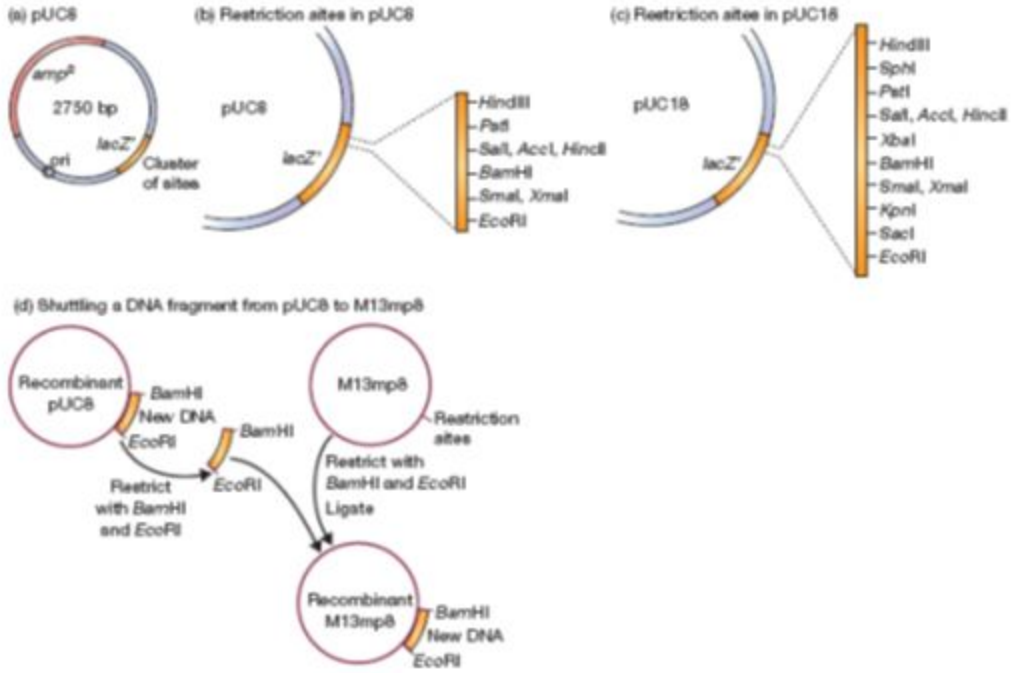
- Higher copy number; generally 30-45
- Better suited for studying the **function** of the clone
- It is **non conjugative**



Plasmid cloning vectors

- **pUC8**

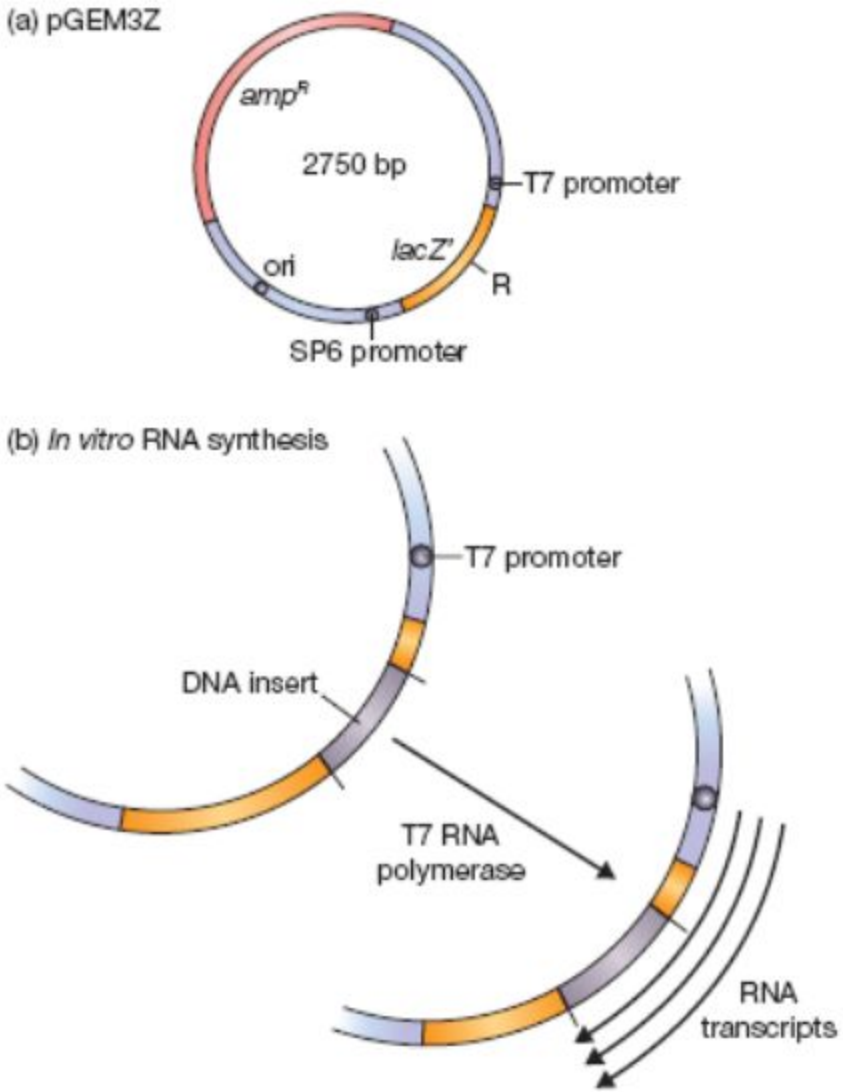
- UC stands for University of California!
- Lac selection plasmid
- 500-700 copy (Due to lucky mutation on ori of pBR322!)
- Insertional inactivation of *lacZ*
- Selection by Amp^r
- Multiple cutting site: **so can be double digested with 2 enzymes** to produce a vector that has a **direction! e.g. one *Eco*R1 sticky end one *Bam*HI sticky end.**



Plasmid cloning vectors

- **pGEM3Z**

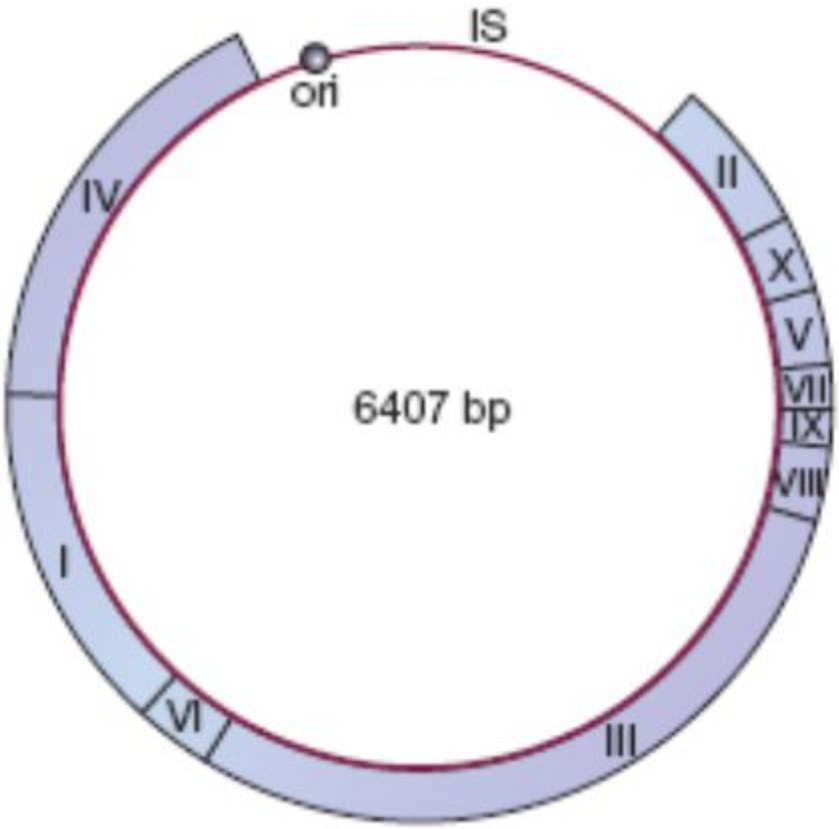
- Similar to pUC
- Speialized for transcription as additional RNA pol binding site has been added
- RNA pol of phages can also be used; just change the recognition sequence as per requirement and infect the bacterial cells with phage!!



Phage based cloning vectors

- **M13**

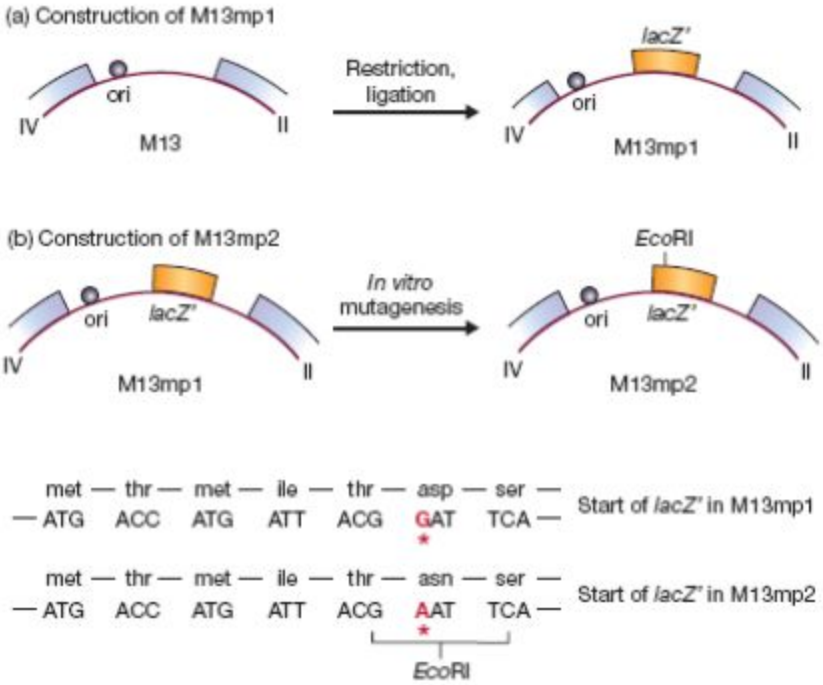
- Viral genome is very small and tightly packed (everything unnecessary has been deleted in the course of evolution)
- So, freedom of engineering original phage vector is tough
- Only 507 nt IS (Intergenic sequence) is manupulable

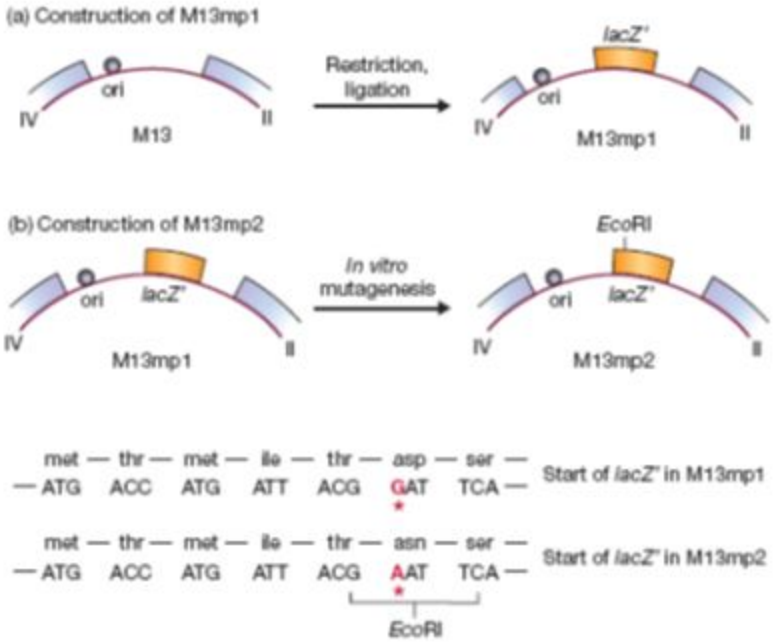


Phage based cloning vectors

- **Development of M13mp2**

- LacZ insertion in IS
- Mutagenesis to introduce an *Eco*R1 site



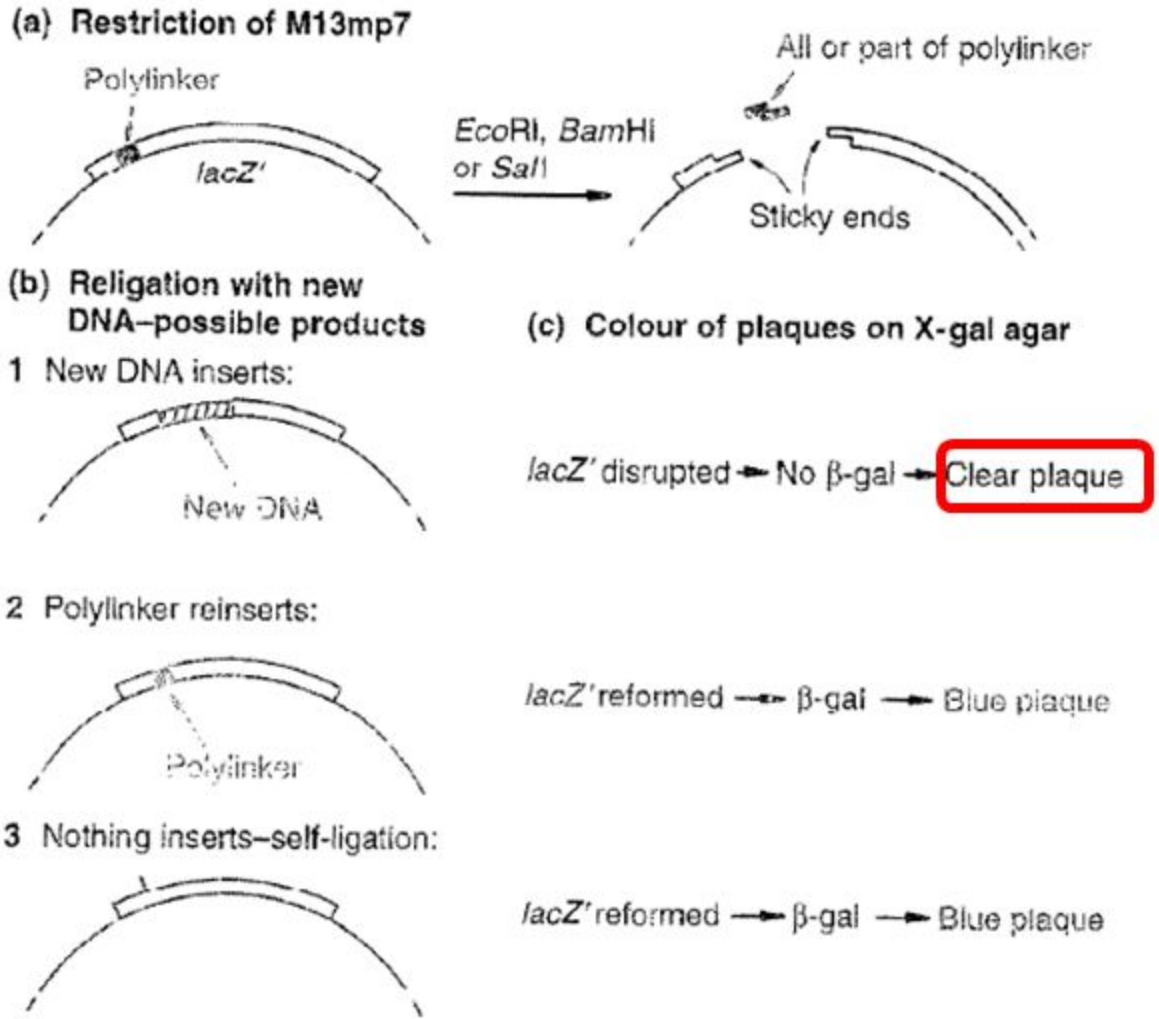


Development of M13mp7

Add a polylinker (to introduce **MCS**)

Phage based cloning vectors

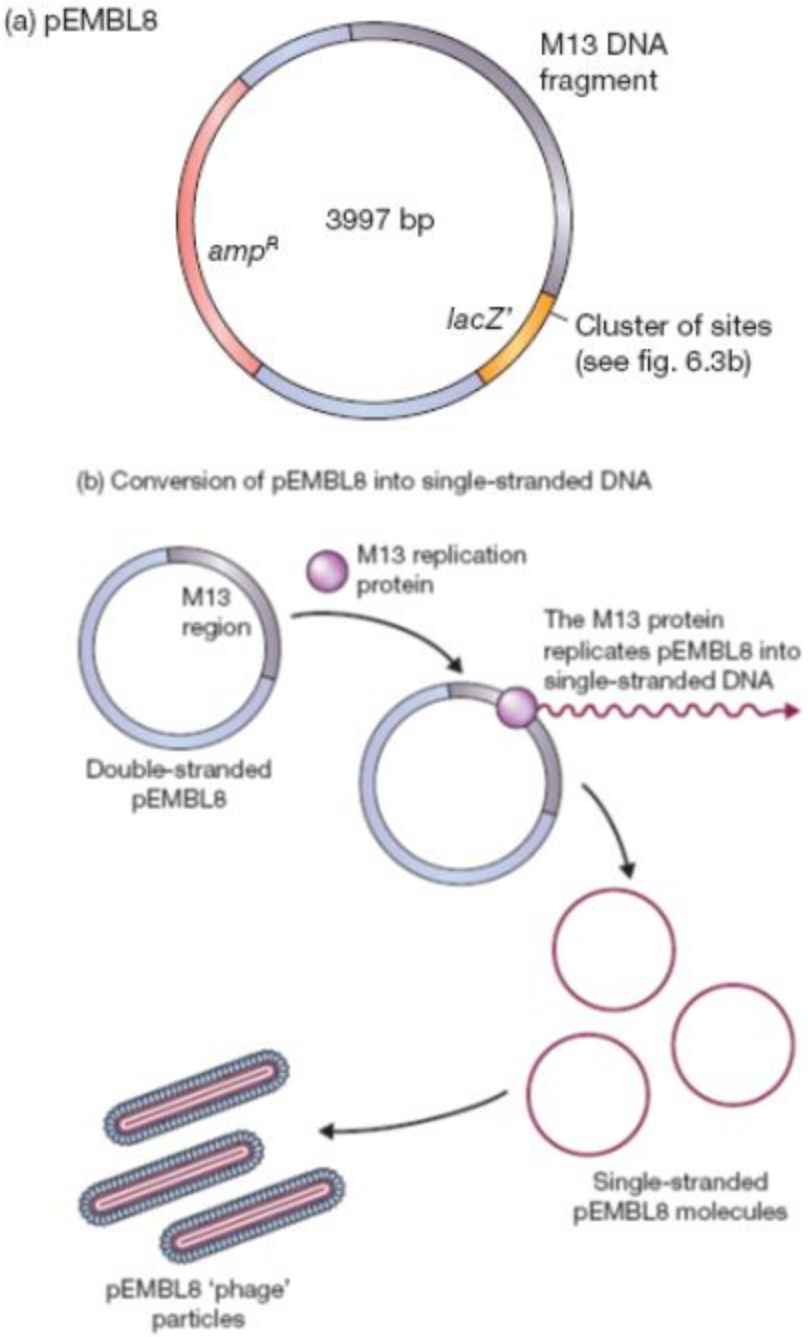
- **What happens restriction digestion happens?**



Hybrid vectors

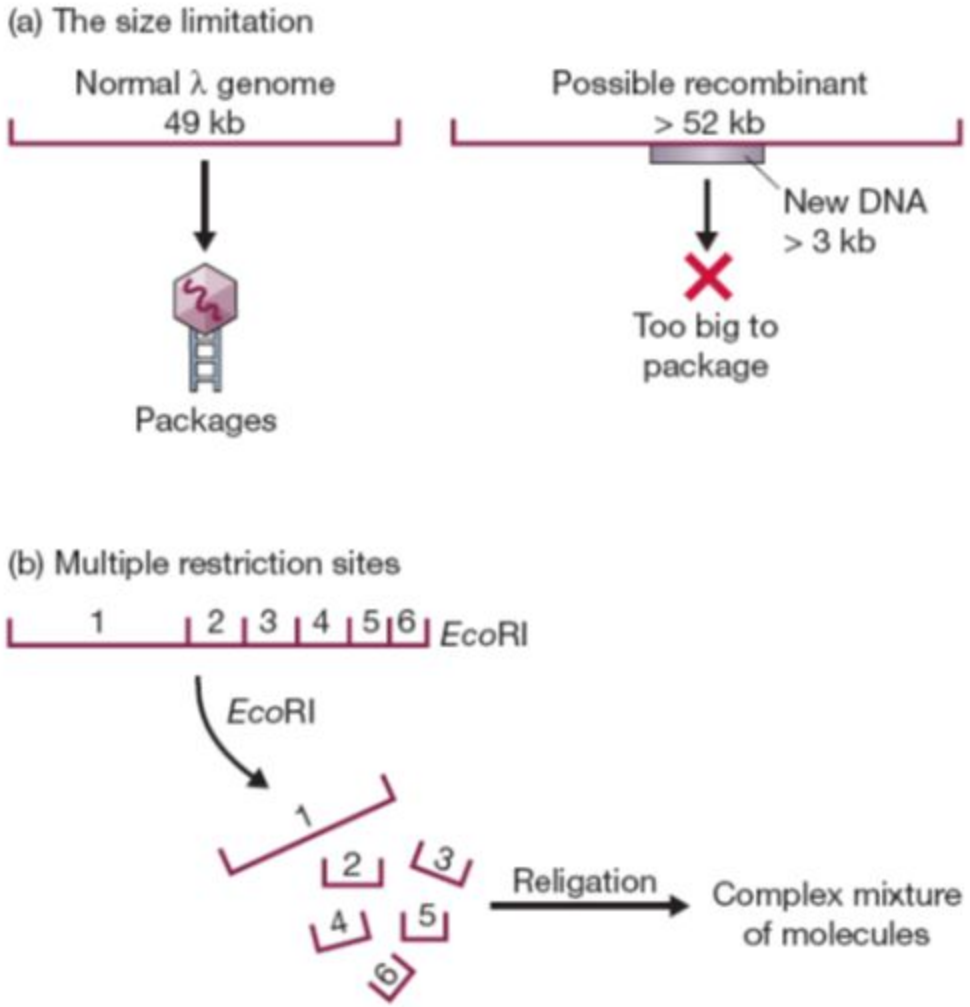
- **Phagemids**

- Plasmid+ phage vectors = Phagemids
- **Why is this useful?**
- There is a size limitation for Phage vectors. Inserts higher than 1500 kb is hard to be accomodated
- **pEMBL8:** pUC skeleton with 1300 bp from M13 genome
- pEMBL8 is able to replicate in phage!



Cloning vectors based on λ phage

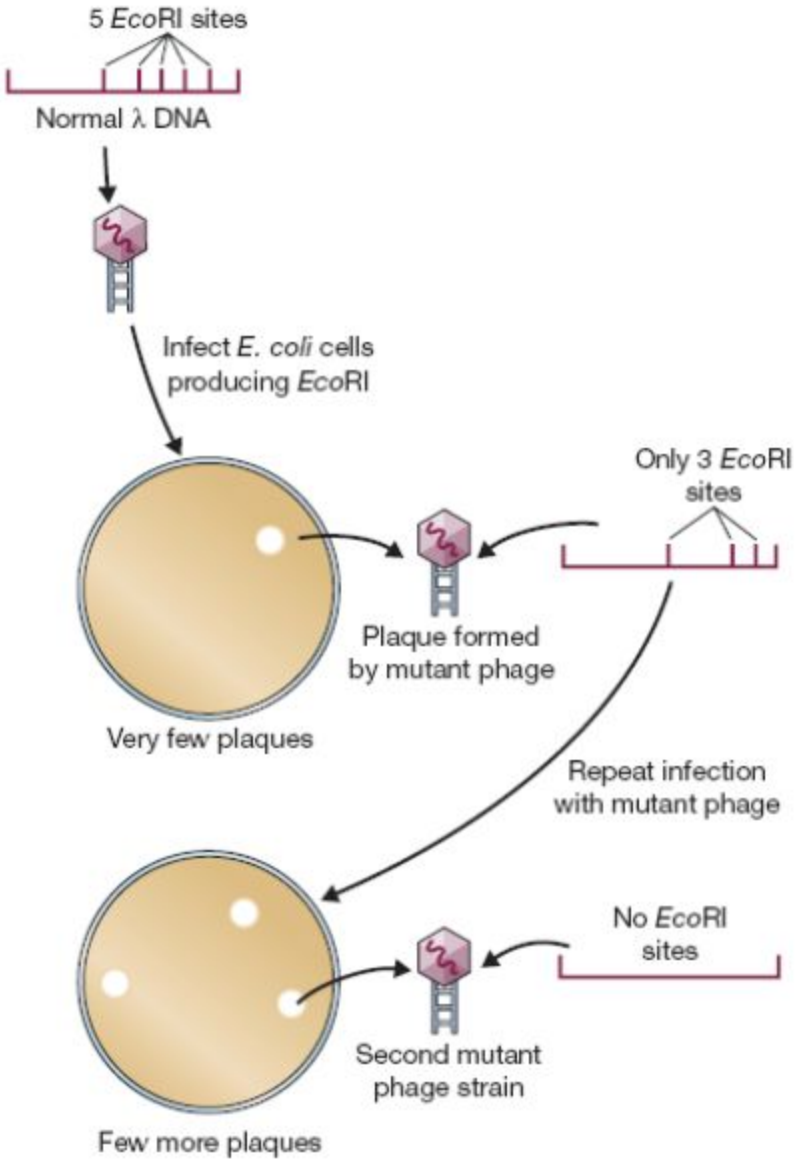
- **Problems with original λ phage as a vector**
- **Size limitation:** Normal genome 49 kb and highest lcapacity of phage head is 52 kb; so only 3 kb insert is possible!
- **Multiple site for all the restriction enzymes** (so no single/one cutter is possible)



Cloning vectors based on λ phage

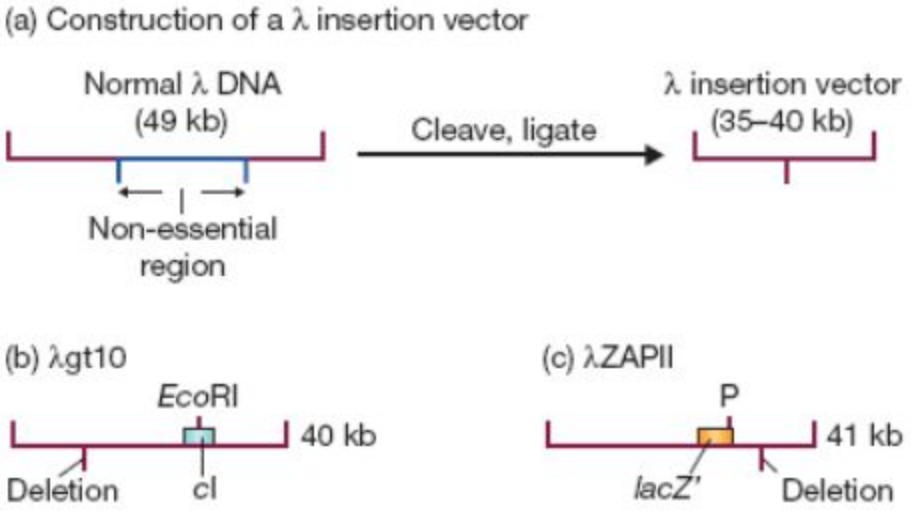
- **Solution to Size limitation:** Delete region without which the phage is viable; 15 kb can be deleted! So 18 kb insert is possible!

- **Solution to lack of single cutter site:** Use mutagenesis, but in nature's way! Natural selection

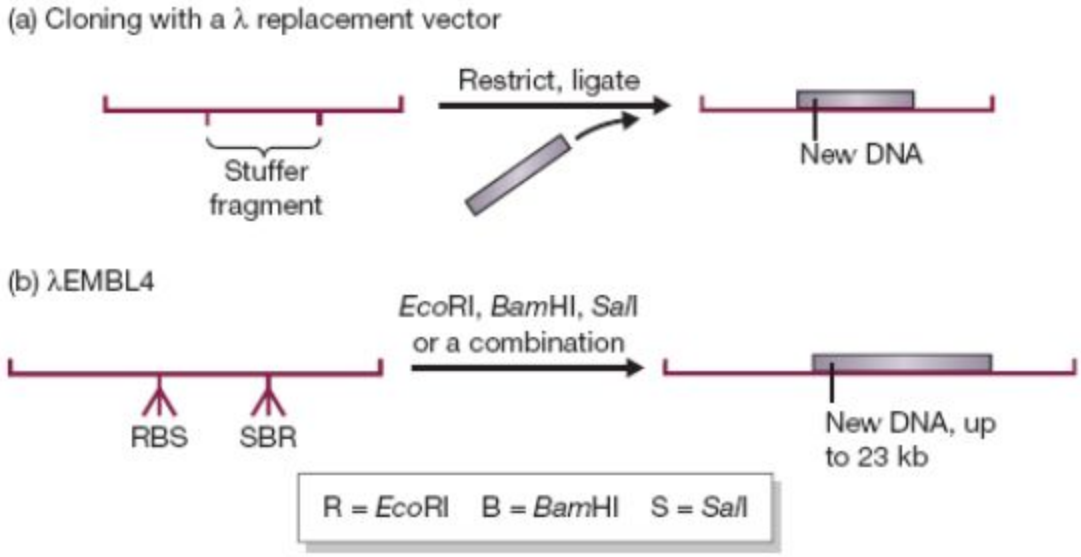


Cloning vectors based on λ phage

- **Insertion vector:**
- **λ gt10** (can carry 8kb into a EcoR1 site in CI gene), **λZAPII** (10 kb insertion possible using any of the six enzymes

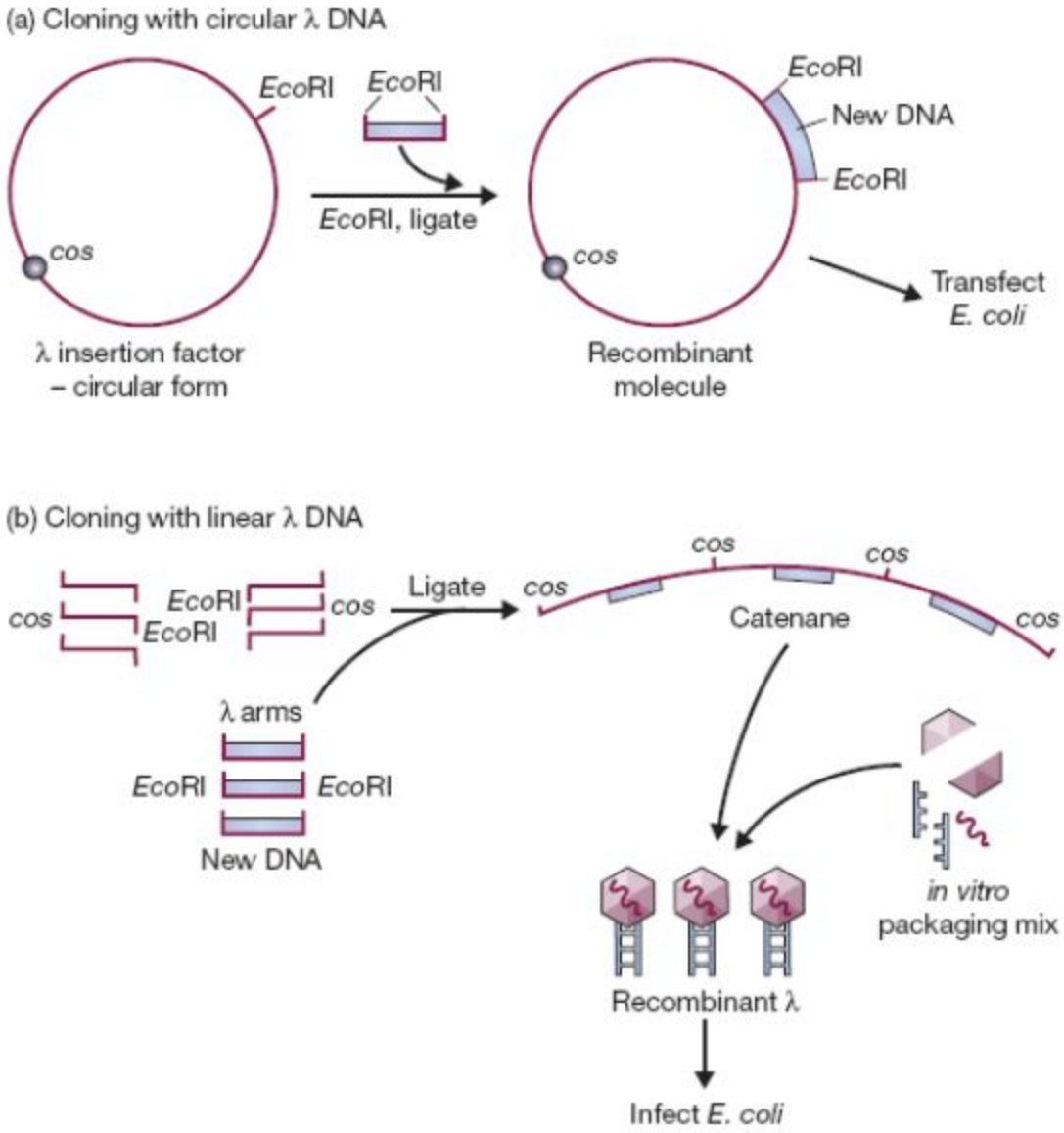


- **Replacement vector:** Do not insert; rather substitute! **λEMBL4, λGEM11, λGEM12**



Cosmids

- **Plasmid + λ Phage DNA = COSMID**
- Specialized for very large DNA fragments
- Special cos (cohesive) site



& BAC, YAC etc.