RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

CLONING VECTORS

➢ Genetic vectors are vehicles to deliver foreign DNA into cells.

➢Vectors have ability to replicate autonomously and characteristically include features to assist the manipulation of DNA and a genetic marker for their selective recognition.

For Example;

❖Plasmids,

❖Bacteriophages,

❖Cosmid

❖Phagmid

❖Phasmid

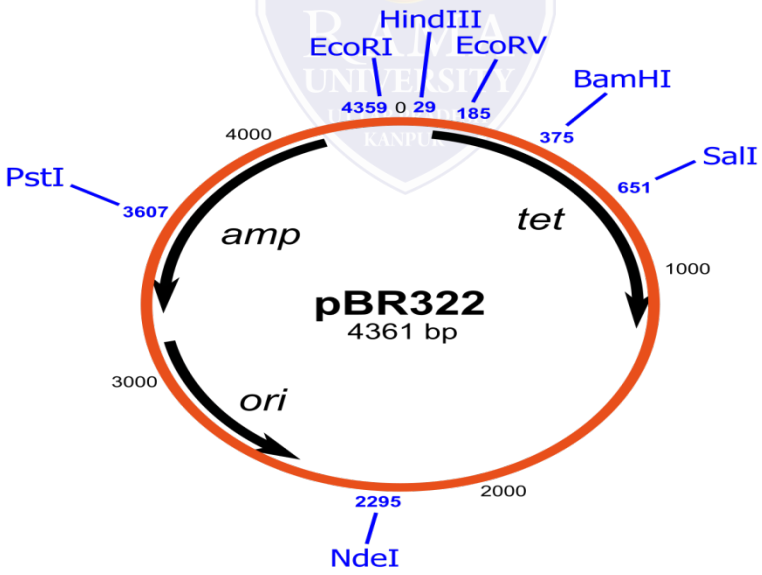
❖Bacterial artificial chromosomes (BACs),

❖Yeast artificial chromosomes (YACs)

❖Mammalian artificial chromosomes (MACs)

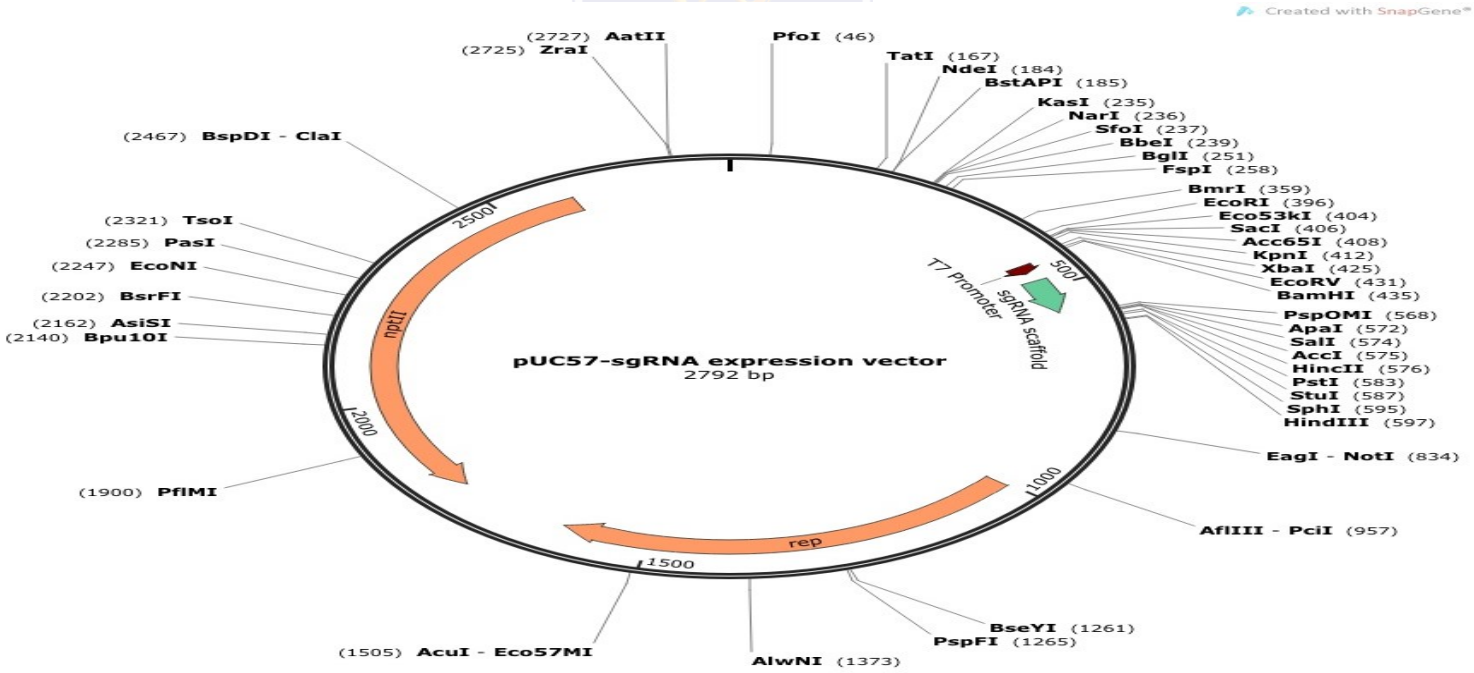
ESSENTIAL CHARACTERSTICS OF CLONING VECTORS

- It should be self-replicating inside the host cell.
- It should possess a multiple cloning site for restriction endonuclease enzymes.
- It should have some marker genes for the identification of recombinant cell.
- It should be easy to isolate from host cell.
- Introduction of donor DNA fragment must not interfere with replication property of the vector.



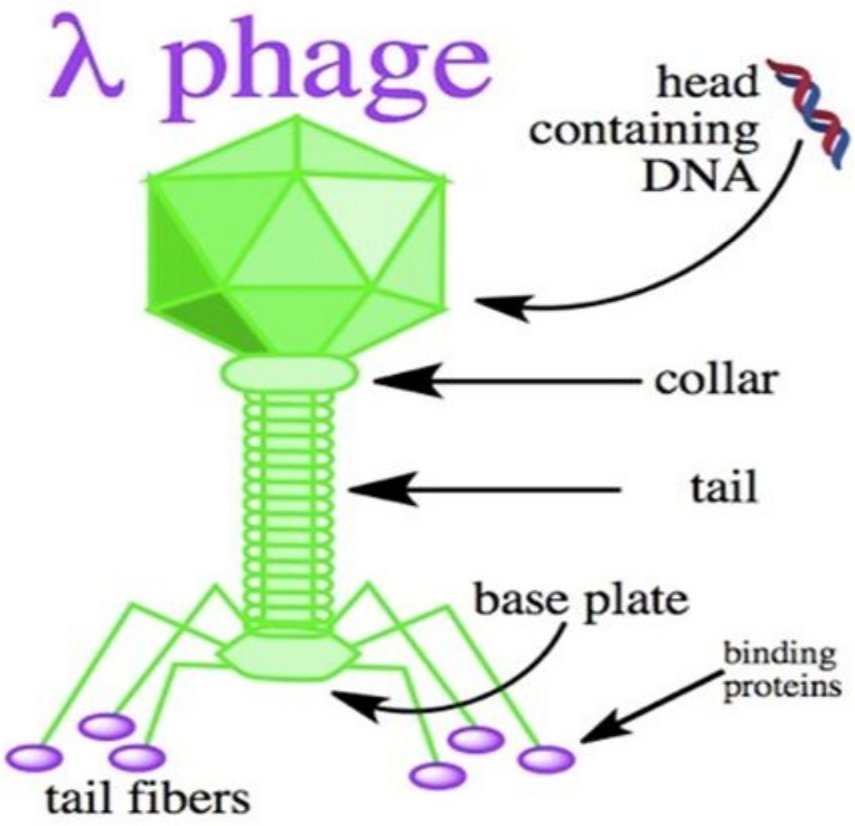
EXPRESSION VECTORS

- Vectors designed specifically for the expression of the transgene in the target cell are called expression vectors.
- Expression vector must have a promoter that drives expression of the transgene.
- Expression vectors produce proteins through the transcription of the vector's insert followed by translation of the mRNA produced.
- Expression Vector should possess taq (His, Glutathione, Streptavidin) for the purification of recombinant proteins.



BACTERIPHAGE VECTORS

Bacteriophage λ vector



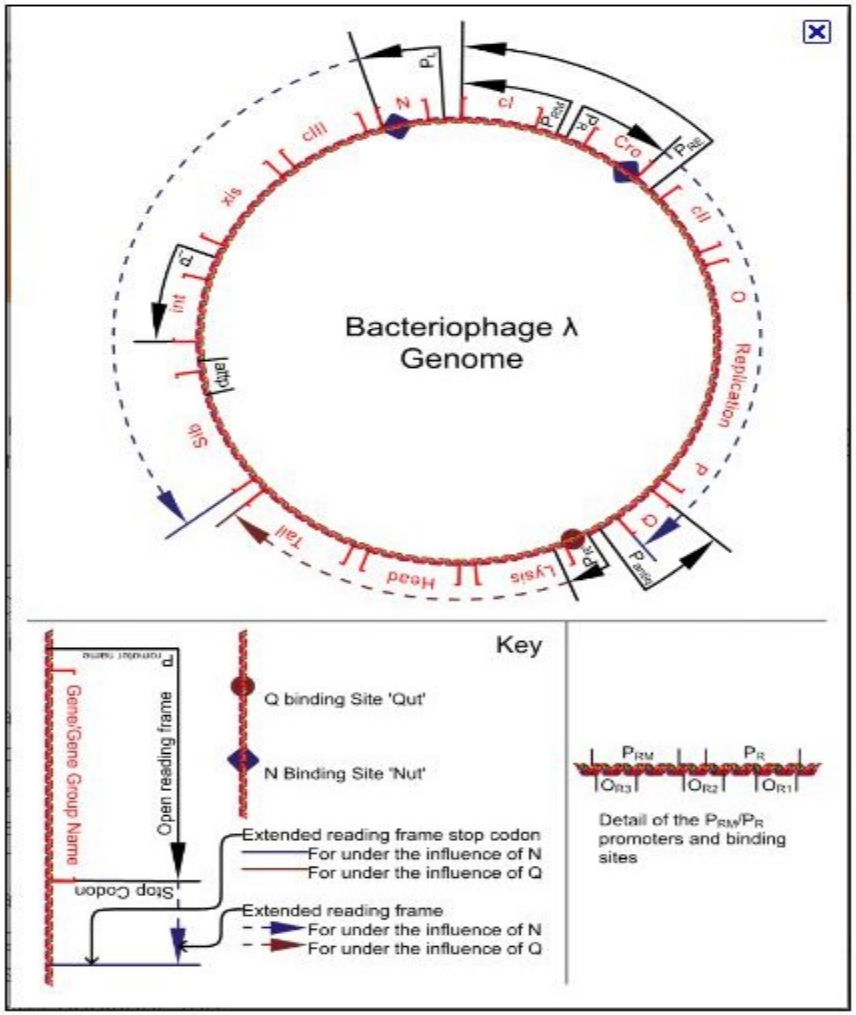
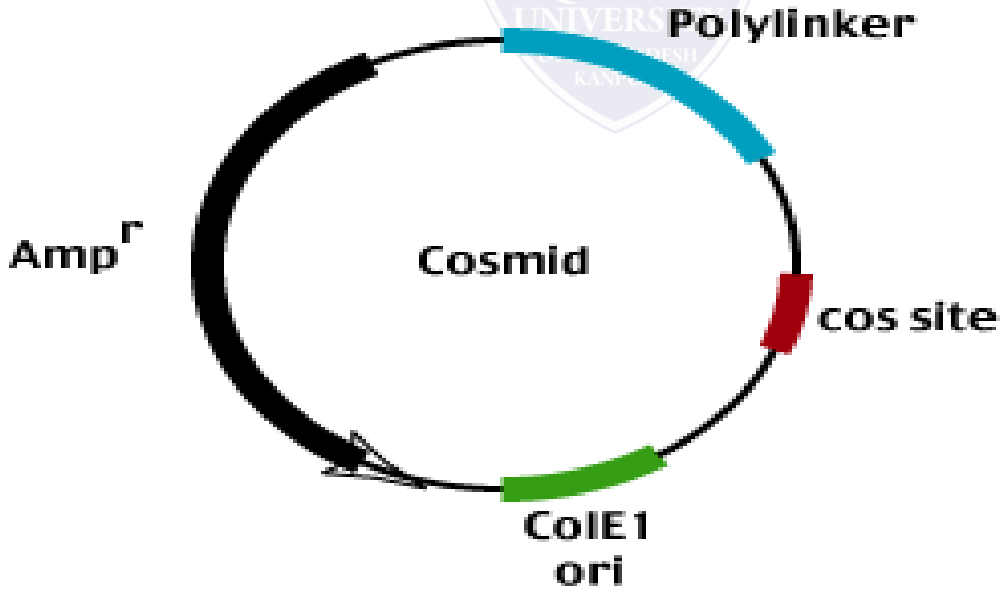


Image taken from Slide player

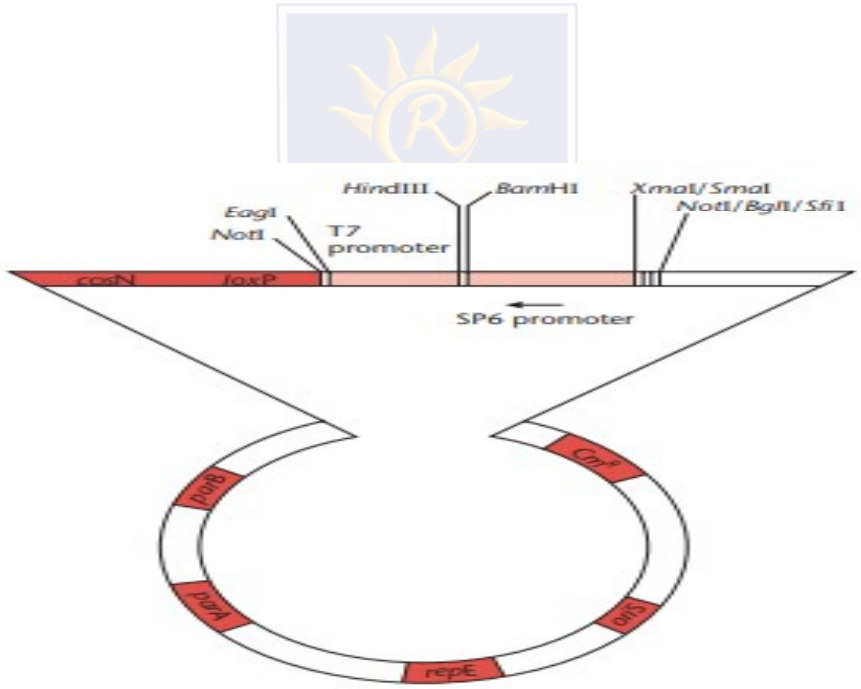
COSMID VECTOR

- Plasmids have been constructed which contain a fragment of λ DNA including the cos site.
- These plasmids have been termed cosmids and can be used as gene-cloning vectors in conjunction with the in vitro packaging system.
- A cosmid vector is a hybrid containing both plasmid and phage vectors, in which the COS site from λ DNA phage DNA is inserted into a plasmid vector about 5 kb long. Up to 45 kb DNA fragments can be cloned into cosmid vectors.
- Cosmids provide an efficient means of cloning large pieces of foreign DNA due to their capacity for large fragments of DNA,
- Cosmids are particularly attractive vectors for constructing libraries of eukaryotic genome fragments.
- Partial digestion with a restriction endonuclease provides suitably large fragments



BAC (Bacterial Artificial Chromosome) VECTOR

BAC vectors are plasmids constructed with the replication origin of *E. coli* F factor, and so can be maintained in a single copy per cell.
These vectors can hold DNA fragments of up to 300 kb.
They are present in low copies, recombination between the high copy plasmids.



YAC (YEAST ARTIFICIAL CHROMOSOME) VECTOR

➢YAC vectors contain all the elements needed to maintain a eukaryotic chromosome in the yeast nucleus: a yeast origin of replication, two selectable markers, and specialized sequences (derived from the telomeres and centromere, regions of the chromosome needed for stability and proper segregation of the chromosomes at cell devisions.

➢Before being used in cloning, the vector is propagated as a circular bacterial plasmid.

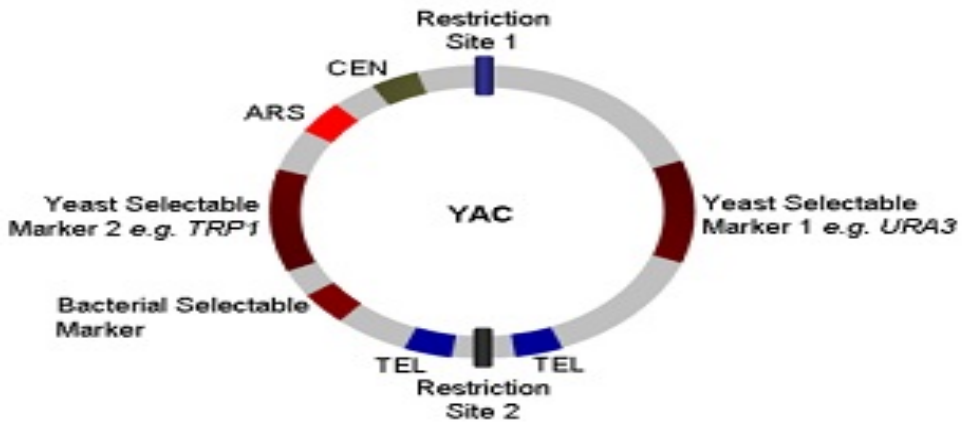
➢Cleavage with a restriction endonuclease (here BamH I) remove a length of DNA between two telomere sequences leaving the telomeres at the ends of the linearized DNA.

➢Cleavage at another internal site (EcoRI) divides the vector into two DNA segments, referred to as vector arms, each with a different selectable marker.

➢The genomic DNA is prepared by partial digestion with restriction endonucleases (EcoRI) to obtain a suitable fragment size.

➢Genomic fragments are then separated by pulsed field gel electrophoresis a variation of gel electrophoresis allows the separation of very large DNA segmei DNA fragments of appropriate size (up to 2 x 106 bp).

➢The fragments are ligated into YACs and transformed into yeast cells.



HOST & DNA INSERT SIZE OF VECTORS

Vector	Host	Insert size
Λ phage	*E. coli*	5–25 kb
Λ cosmids	*E. coli*	35–45 kb
P1 phage	*E. coli*	70–100 kb
PACs	*E. coli*	100–300 kb
BACs	*E. coli*	≤ 300 kb
YACs	*E. coli*	200–2000 kb