

Cloning Vectors

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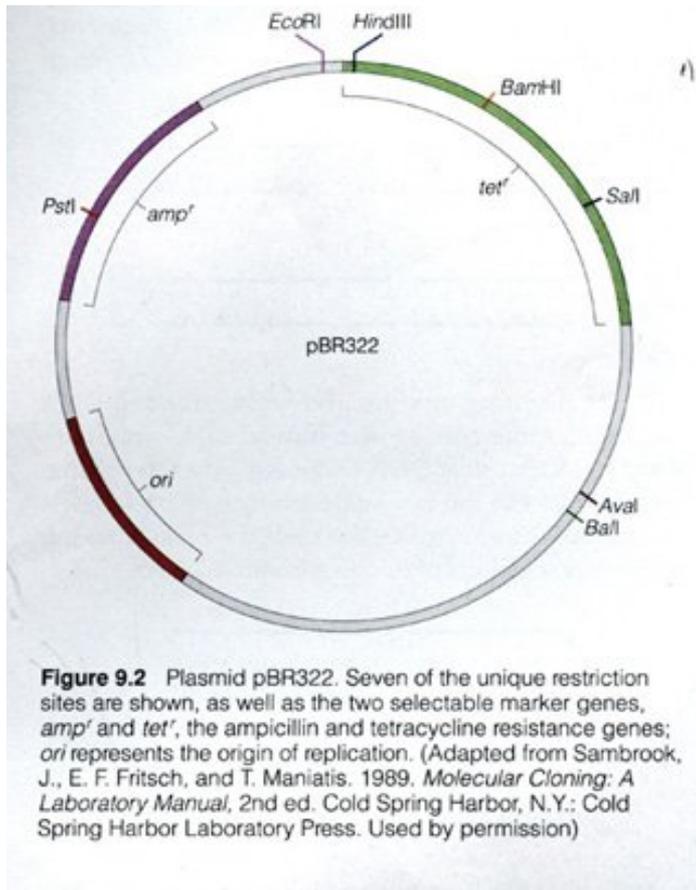
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Cloning Vectors

- ▶ A vector is used to amplify a single molecule of DNA into many copies. A DNA fragment must be inserted into a cloning vector. A cloning vector is a DNA molecule that has an origin of replication and is capable of replicating in a bacterial cell.
- ▶ Most vectors are genetically engineered plasmids or phages. There are also cosmid vectors, bacterial artificial chromosomes, and yeast artificial chromosomes.

Plasmid Cloning Vectors



- ▶ Plasmids are circular, double-stranded DNA molecules that exist in bacteria and in the nuclei of some eukaryotic cells.
- ▶ They can replicate independently of the host cell. The size of plasmids ranges from a few kb to near 100 kb
- ▶ Can hold up to 10 kb fragments
- ▶ Plasmids have an origin of replication, antibiotic resistance genes as markers, and several unique restriction sites.
- ▶ After culture growth, the clone fragment can be recovered easily. The cells are lysed and the DNA is isolated and purified.
- ▶ A DNA fragment can be kept indefinitely if mixed with glycerol in a -70 degrees C freezer.

Plasmid Polylinkers and Marker Genes for Blue-White screening



Figure 9.8 Blue-white screening on medium with ampicillin, X-gal, and IPTG. Blue colonies contain nonrecombinant plasmids. White colonies contain recombinant plasmids and can be isolated directly from this plate.

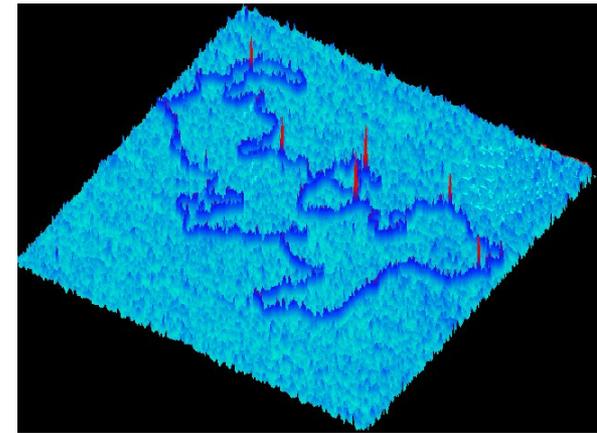
- ▶ A vector usually contains a sequence (polylinker) which can recognize several restriction enzymes so that the vector can be used for cloning a variety of DNA samples.
- ▶ Colonies with recombinant plasmids are white, and colonies with nonrecombinant plasmids are blue.
- ▶ Example: pUC19
- ▶ Resistant to ampicillin, has (amp^r gene)
- ▶ Contains portion of the lac operon which codes for beta-galactosidase.
- ▶ X-gal is a substrate of beta-galactosidase and turns blue in the presence of functional beta-galactosidase is added to the medium.
- ▶ Insertion of foreign DNA into the polylinker disrupts the lac operon, beta-galactosidase becomes non-functional and the colonies fail to turn blue, but appear white.

Phage Cloning Vectors

- ▶ Fragments up to 23 kb can be accommodated by a phage vector
- ▶ Lambda is most common phage
- ▶ 60% of the genome is needed for lytic pathway.
- ▶ Segments of the Lambda DNA is removed and a stuffer fragment is put in.
- ▶ The stuffer fragment keeps the vector at a correct size and carries marker genes that are removed when foreign DNA is inserted into the vector.
- ▶ Example: Charon 4A Lambda
- ▶ When Charon 4A Lambda is intact, beta-galactosidase reacts with X-gal and the colonies turn blue.
- ▶ When the DNA segment replaces the stuffer region, the lac5 gene is missing, which codes for beta-galactosidase, no beta-galactosidase is formed, and the colonies are white.

Cosmid Cloning Vectors

- ▶ Fragments from 30 to 46 kb can be accommodated by a cosmid vector.
- ▶ Cosmids combine essential elements of a plasmid and Lambda systems.
- ▶ Cosmids are extracted from bacteria and mixed with restriction endonucleases.
- ▶ Cleaved cosmids are mixed with foreign DNA that has been cleaved with the same endonuclease.
- ▶ Recombinant cosmids are packaged into lambda capsids
- ▶ Recombinant cosmid is injected into the bacterial cell where the rcosmid arranges into a circle and replicates as a plasmid. It can be maintained and recovered just as plasmids.



Shown above is a 50,000 base-pair long DNA molecule bound with six EcoRI molecules, and imaged using the atomic force microscope. This image clearly indicates the six EcoRI "sites" and allows an accurate restriction enzyme map of the cosmid to be generated.

<http://homer.ornl.gov/cbps/afmimaging.htm>

Bacterial Artificial Chromosomes(BACs) and Yeast Artificial Chromosomes(YACs)

- ▶ BACs can hold up to 300 kbs.
- ▶ The F factor of E.coli is capable of handling large segments of DNA.
- ▶ Recombinant BACs are introduced into E.coli by electroportation (a brief high-voltage current). Once in the cell, the rBAC replicates like an F factor.
- ▶ Example: pBAC108L
- ▶ Has a set of regulatory genes, OriS, and repE which control F-factor replication, and parA and parB which limit the number of copies to one or two.
- ▶ A chloramphenicol resistance gene, and a cloning segment.
- ▶ YACs can hold up to 500 kbs.
- ▶ YACs are designed to replicate as plasmids in bacteria when no foreign DNA is present. Once a fragment is inserted, YACs are transferred to cells, they then replicate as eukaryotic chromosomes.
- ▶ YACs contain: a yeast centromere, two yeast telomeres, a bacterial origin of replication, and bacterial selectable markers.
- ▶ YAC plasmid→Yeast chromosome
- ▶ DNA is inserted to a unique restriction site, and cleaves the plasmid with another restriction endonuclease that removes a fragment of DNA and causes the YAC to become linear. Once in the cell, the rYAC replicates as a chromosome, also replicating the foreign DNA.