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Charon Phage

A part from containing useful target sites for restriction enzymes. λ vector should also satisfy some other requirement.

- Firstly they should allow cloning of DNA molecules of a broad size range.
- Secondly it should be possible to distinguish recombinant and parent phages by plaque morphology or marker inactivation.
- Thirdly recombinant phages should be obtainable with high yields.
- Fourthly such vectors should generate a sufficient level of biological safety.
- A set of Charon phages (λ vectors) meets almost all the requirements mentioned above.

Charon Phages are named for the ferryman of Greek mythology who conveyed the spirits of the dead across the river Styx which separated the realm of the living from Hades, the underworld. The **E. coli** I replacement vector Charon 4 A for example. Contains three E. coli RI sites in its non-essential region cleavage with E. coli RI therefore yields DNA fragments. The central fragments can be easily purified away from the two other fragments at the ends of the molecules by centrifugation.

Since left and right arms are 19.9 and 11.04 kb in length this vector can accommodate insertion between 7 and 20 kb. Charon 4 A is therefore used for cloning large E. coli RI fragments. Separation of the internal fragment also eliminated the two markers lac 52 and co 256.

Another vector Charon 16 A possess only one ECO RI site for the insertion of foreign DNA. The vector itself can be packaged because its size is smaller than the critical 38 Kb which are essential for packaging. Charon phage is not restricted to ECOR. target sites. A second generation of vectors has been developed to allow cloning at a no. of other restriction sites. Charon 30 is a Bam HI replacement vector.

Most vectors contain genetic markers important for their biological safety. Charon 34 and 35 are replacement vectors which differ from each other only in their central fragment and will accept fragment 9-20 kb long.

Shuttle vectors

These are the vectors which can replicate in 2 different species.

They contain two origins of replication, one specific for each host sps.

They contain those genes necessary for their replication not provided by the host cells.

These vectors are created by recombinant technique some can be grown in two different prokaryotic species while others can propagate in a prokaryotic sps usually E.coli and a Eukaryotic sps eg. Yeast plants animals **shuttle vectors** because they can be grown in one host and then moved into another without any extra manipulation.

Some yeast plasmid vectors are often used as shuttle vector provided they contain replication origin that is active in both yeast and other host cell such as **E. coli**.

Example of Shuttle Vector

Shuttle vectors designed to replicate in **E.Coli** and Streptomyces.

Constructions of such a vector can be done as follows:-

- a) Modules for DNA replication in streptomycetes and methylenomycin.
- b) A resistance gene derived from a streptomycetes plasmid.

This shuttle vector allows the initial cloning of streptomycetes. DNA insert in **E.coli** and their subsequent tests in streptomycetes. Shuttle vectors have been specifically designed to satisfy this need. Other examples of shuttle vectors are

E. coli and **Bacillus subtilis**

E. coli and **Agrobacterium tumefaciens**

E. coli and **Corynebacterium**

In case of **E. coli** and cary me bacterium shuttle vectors the E. coli portion of the plasmid could encode resistance to the antibiotic tetracycline chloramphenicol or kanamycin. Because both E.coli and carynebacterium sp. are susceptible to these antibiotics they could be used as selectable marker in both organisms. Most of the Eukaryotic vectors are shuttle vectors.

Most broad host range plasmid vectors replicate only in gram negative organism.

Most of the eukaryotic vectors are shuttle vectors.