Lab. Work 10. Phage lambda as cloning vector

Important considerations in the use of phage lambda as a cloning vehicle include the following:

1. The vector systems are of the gene replacement type. That is, the final recombinant clones remain infectious for E. coli since the genes which are deleted from the phage are non-essential essential for lytic phage growth.
2. Thus, typical lambda phage vectors are in many ways analogous to certain mammalian virus vectors - such as vaccinia virus or herpesvirus vectors in which a foreign gene has replaced some non-essential viral gene (such as the gene encoding thymidine kinase).
3. The phage's genome cleavage and packaging machinery makes specific nucleolytic cleavages at the cohesive ends between concatemeric genomes (so called cos sites).
4. This releases the genome unit-length molecules for packaging. Many mammalian viruses also have specific terminal sequences which are important for genome replication and packaging.
5. The wild-type phage genome (i.e., 38-53 kb or so), since the lambda phage head has a tight constraint on the amount of DNA that it will accommodate.
6. The packaging size limitation of lambda phage vectors is one which is common to most mammalian virus vectors also (i.e., recombinant viral genomes must usually be of wild-type length + 5% or so). Two of the few exceptions are the bacteriophage M13 and mammalian rhabdoviruses (such as vesicular stomatitis virus), both of which can accomodate genomes considerably (>10%) larger than wild-type. This is probably because of the rod-like structure of these viruses (bullet-shape in the case of rhabdoviruses); an increase in the length of the rod allows the particles of these viruses to accomodate an extended genome.

Plasmid for cloning of double-digested restriction fragments.

**Application**

Select for recombinant DNA.

The Pst I and Pvu I cleavage sites in the ampicillin gene, or the Cla I, Hind III, BamH I, and Sal I sites in the tetracycline gene allow the insertion of foreign DNA fragments, and inactivation of one of these genes.

pBR322 DNA has been used in to study artificial metallonuclease activity

#### Sequence

Chain Length 4,361 bp

#### Physical form

Solution, 250 μg/ml, in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, ready-to-use

# General description for PBR322 - PBR322 LOW COPY CLONING VECTOR

This plasmid contains the BR322 (low copy number) origin of replication and provides approximately 20 plasmid copies per bacterial cell. It also contains the mammalian CMV promoter upstream of the MCS and Kan resistance for selection of transfected mammalian cells. Low copy number plasmids are useful for very large transgenes or for transgenes that can be toxic to bacterial cells.

**Promoter Expression Level:**

#### Application

**BR322 Information:**BR322 is a wild type plasmid isolated from E.coli. It contains a low copy origin that allows for plasmid maintenance at a frequency of approximately 10-100 plasmid copies per cell. Typically this number is closer to 30-40 copies per cell. Most common cloning plasmids contain a derivative of the origin of replication in this plasmid called pUC. The pUC origin was created by removing the Rep repressor protein that normally regulates plasmid copy number in E.coli and also making a single point mutation in the origin of replication itself. The point mutation is the most important difference between the origin of replication.This plasmid can be used as a general purpose low copy cloning vector.