Unit 2: Recombinant DNA Technology

Lecture: Cosmid and Shuttle vectors

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Cosmids as Cloning Vectors:

- Plasmid vectors are not suitable for cloning DNA fragments very much larger than their own size, as the transformation frequency fall beyond acceptable limits and cloned fragments or their parts very often get deleted.

- Takagi and co-workers observed as early as 1976 that the presence of the cohesive end site cos λ from the bacteriophage lambda DNA in a plasmid allows it to be packaged in vivo into virus particles.

Refer to the cosmid vector properties schematic diagram provided to you on your Whatsapp/e-mail group.
A cosmid is a plasmid that contains a cos site from the lambda genome.

The vector replicates as a plasmid (it contains a ColE1 origin of replication), uses Amp\(^r\) for positive selection and employs lambda phage packaging to select for recombinant plasmids carrying foreign DNA inserts 45 KB in size.

Refer to the cosmid vector map provided to you on your Whatsapp/e-mail group.
• Cos sequences are ~200 base pairs long and essential for packaging. They contain a cosN site where DNA is nicked at each strand, 12 bp apart, by terminase. This causes linearization of the circular cosmid with two "cohesive" or "sticky ends" of 12bp. (The DNA must be linear to fit into a phage head.) The cosB site holds the terminase while it is nicking and separating the strands. The cosQ site of next cosmid (as rolling circle replication often results in linear concatemers) is held by the terminase after the previous cosmid has been packaged, to prevent degradation by cellular DNases.
• Ligation of cosmid vector and foreign DNA fragments (Sau3A partial digest fragments 45 kb in size) is similar to ligation into a lambda substitution vector.
• The desired ligation product is a concatamer of 45 kb foreign DNA fragment and 5 kb cosmid

Refer to the cosmid vector “cos” site map diagram provided to you on your Whatsapp/e-mail group

• This concatemer is then packaged into viral particles (remember packaging is cos site to cos site) and these are used to infect E. coli where the cosmid vector replicates using the ColE1 origin of replication. Phage packaging serves only to select for recombinant molecules and to transfer these long DNA molecules (50 kb total) into the bacterial host (50 kb fragments transform very inefficiently while phage infection is very efficient).
Refer to the cosmid vector construction and cloning diagram provided to you on your Whatsapp/e-mail group
A great advantage of such a cosmid vector is that:

1. Gene libraries consisting of a smaller number of clone members can span the whole genome of an organism. For example, the genome of Escherichia coli can be accommodated in just 120 cosmids.
2. Other advantages are that large gene can be studied intact and genetic linkage studies can be carried out at the molecular level.
3. An important practical advantage of a cosmid is that background molecules which do not have the intact and genetics linkage studies can be carried out at the molecular level.

Refer to the cosmid vector schematic advantage diagram provided to you on your Whatsapp/e-mail group
### Plasmid vs Cosmid

**Plasmid**
- Definition: Small extrachromosomal DNA present in prokaryotes.
- Composition: Only plasmid DNA
- Nature: Natural vectors
- Length of Inserting Fragment: Up to 25 kb
- Transformation Efficiency: Comparatively less transformation efficiency
- Cos Sites: Absent

**Cosmid**
- Definition: Hybrid vector constructed by joining lambda phage DNA and plasmid DNA
- Composition: cos sequences of bacteriophage lambda and plasmid DNA
- Nature: Constructed vectors
- Length of Inserting Fragment: Up to 45 kb
- Transformation Efficiency: High transformation efficiency
- Cos Sites: Present

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**Why prefer cosmids over plasmids?**
SHUTTLE VECTORS

A shuttle vector is a vector (usually a plasmid) constructed so that it can propagate in two different host species. Therefore, DNA inserted into a shuttle vector can be tested or manipulated in two different cell types. The main advantage of these vectors is they can be manipulated in E. coli, then used in a system which is more difficult or slower to use (e.g. yeast).

Shuttle vectors include plasmids that can propagate in eukaryotes and prokaryotes (e.g. both Saccharomyces cerevisiae and Escherichia coli) or in different species of bacteria (e.g. both E. coli and Rhodococcus erythropolis). There are also adenovirus shuttle vectors, which can propagate in E. coli and mammals.

Shuttle vectors are frequently used to quickly make multiple copies of the gene in E. coli (amplification). They can also be used for in vitro experiments and modifications (e.g. mutagenesis, PCR).

One of the most common types of shuttle vectors is the yeast shuttle vector. Almost all commonly used S. cerevisiae vectors are shuttle vectors. Yeast shuttle vectors have components that allow for replication and selection in both E. coli cells and yeast cells. The E. coli component of a yeast shuttle vector includes an origin of replication and a selectable marker, e.g. antibiotic resistance, beta lactamase, beta galactosidase. The yeast component of a yeast shuttle vector includes an autonomously replicating sequence (ARS), a yeast centromere (CEN), and a yeast selectable marker (e.g. URA3, a gene that encodes an enzyme for uracil synthesis, Lodish et al. 2007).
Refer to the shuttle vector schematic advantage diagram provided to you on your Whatsapp/e-mail group

Why shuttle vectors?

- Prokaryotic vectors cannot exist & work in eukaryotic cells because the system of two groups of organisms varies.
- Prokaryotes lack introns; while eukaryotes consists of introns.
- Therefore, vectors with two origin of replication were constructed which may exist in both eukaryotes and prokaryotes.
Some cloned animal genes may need to be expressed in cultured mammalian cells. Shuttle vectors that can replicate in both mammalian cells and bacteria are used for this. As usual, such vectors contain a bacterial origin of replication and an antibiotic resistance gene allowing selection in bacteria. These vectors must also possess an origin of replication that works in mammalian cells. Usually, this is taken from a virus that infects animal cells, such as SV40 (simian virus 40). Viral promoters are often used because they are strong, producing copious amounts of protein. Alternatively, promoters from mammalian genes that are expressed at high levels (e.g., the genes for metallothionein, somatotropin, or actin) may be used. The multiple cloning sites lie downstream of the strong promoter. Since animal genes are normally cloned as the cDNA, the vector must also provide a polyadenylation signal (i.e., tail signal) at the 3′-end of the inserted gene. Finally, single cells showing high production levels must be isolated.