Vectors for gene cloning

A cloning vector is a small piece of DNA into which a foreign DNA can be inserted for cloning purposes.

A DNA molecule needs to display several features to be able to act as a vector for gene cloning:

1. Most importantly it must be able to replicate within the host cell, so that numerous copies of the recombinant DNA molecule can be produced and passed to the daughter cells.
2. A cloning vector also needs to be relatively small, ideally less than 10 kb in size, as large molecules tend to break down during purification, and are also more difficult to manipulate.
3. It should have a restriction site for the insertion of the target DNA.
4. It should have a selectable marker with an antibiotic resistance gene that facilitates screening of the recombinant organism.
5. It should be capable of inserting a large segment of DNA.
6. It should possess multiple cloning sites (polylinker).
7. It should be capable of working under the prokaryotic and eukaryotic systems.

Two kinds of DNA molecule that satisfy these criteria can be found in bacterial cells:

A. Plasmids

Plasmids are circular molecules of DNA that lead an independent existence in the bacterial cell (Fig.1). Plasmids almost always carry one or more genes, and often these genes are responsible for a useful characteristic displayed by the host bacterium. For example, the ability to survive in normally toxic concentrations of antibiotics such as chloramphenicol or ampicillin is often due to the presence in the bacterium of a plasmid carrying antibiotic resistance genes. In the laboratory,

antibiotic resistance is often used as a **selectable marker** to ensure that bacteria in a culture contain a particular plasmid.

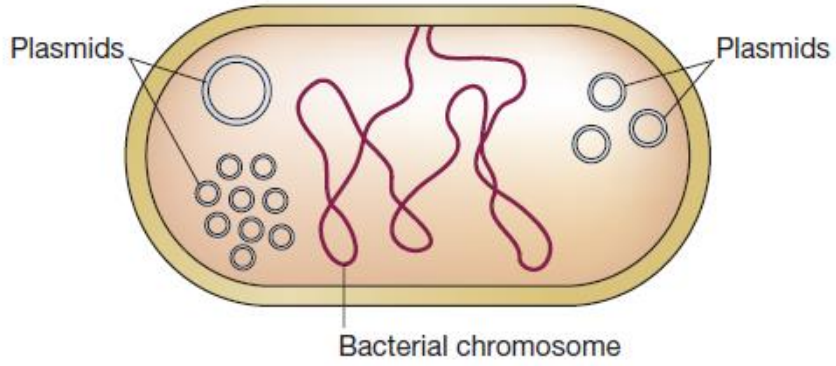

Figure 1. Plasmids: independent genetic elements found in bacterial cells.

Most plasmids possess at least one DNA sequence that can act as an **origin of replication**, so they are able to multiply within the cell independently of the main bacterial chromosome (Figure 2a). The smaller plasmids make use of the host cell's own DNA replicative enzymes in order to make copies of themselves, whereas some of the larger ones carry genes that code for special enzymes that are specific for plasmid replication. A few types of plasmid are also able to replicate by inserting themselves into the bacterial chromosome (Figure 2b). These integrative plasmids or **episomes** may be stably maintained in this form through numerous cell divisions, but always at some stage exist as independent elements.

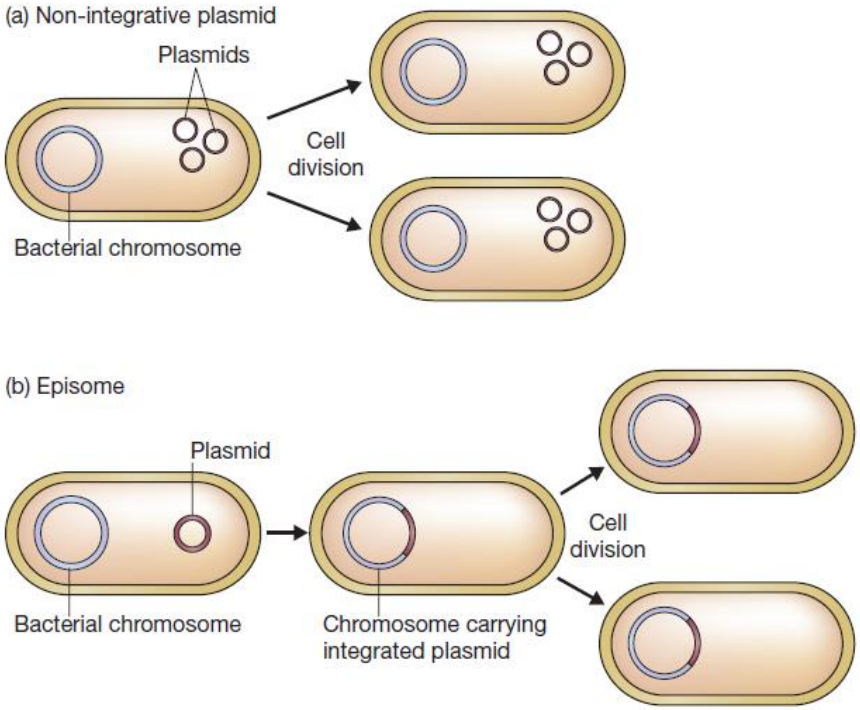

Figure 2. Replication strategies for (a) a non-integrative plasmid, and (b) an episome.

Size and copy number

The size and **copy number** of a plasmid are particularly important as far as cloning is concerned. We have already mentioned the relevance of plasmid size and stated that less than 10 kb is desirable for a cloning vector. Plasmids range from about 1.0 kb for the smallest to over 250 kb for the largest plasmids (Table .1), so only a few are useful for cloning purposes. However, larger plasmids can be adapted for cloning under some circumstances.

Table 1. Sizes of representative plasmids.

	SIZE		
PLASMID	NUCLEOTIDE LENGTH (kb)	MOLECULAR MASS (MDa)	ORGANISM
pUC8	2.1	1.8	*E. coli*
ColE1	6.4	4.2	*E. coli*
RP4	54.0	36.0	*Pseudomonas* and others
F	95.0	63.0	*E. coli*
TOL	117.0	78.0	*Pseudomonas putida*
pTiAch5	213.0	142.0	*Agrobacterium tumefaciens*

The copy number refers to the number of molecules of an individual plasmid that are normally found in a single bacterial cell. The factors that control copy number are not well understood. Some plasmids, especially the larger ones, are **stringent**

and have a low copy number of perhaps just one or two per cell; others, called **relaxed** plasmids, are present in multiple copies of 50 or more per cell. Generally speaking, a useful cloning vector needs to be present in the cell in multiple copies so that large quantities of the recombinant DNA molecule can be obtained.

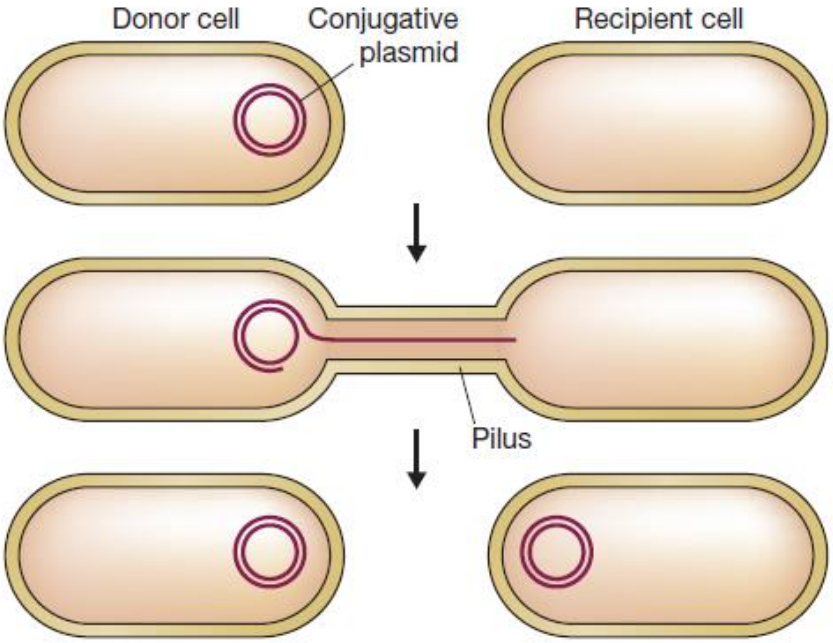

Figure 3. Plasmid transfer by conjugation between bacterial cells. The donor and recipient cells attach to each other by a pilus, a hollow appendage present on the surface of the donor cell. A copy of the plasmid is then passed to the recipient cell. Transfer is thought to occur through the pilus, but this has not been proven and transfer by some other means (e.g. directly across the bacterial cell walls) remains a possibility.

Conjugation and compatibility

Plasmids fall into two groups: conjugative and non-conjugative. Conjugative plasmids are characterized by the ability to promote sexual **conjugation** between bacterial cells (Figure 4), a process that can result in a conjugative plasmid spreading from one cell to all the other cells in a bacterial culture. Conjugation and plasmid transfer are controlled by a set of transfer or *tra* genes, which are

present on conjugative plasmids but absent from the non-conjugative type. However, a non-conjugative plasmid may, under some circumstances, be cotransferred along with a conjugative plasmid when both are present in the same cell. Several different kinds of plasmid may be found in a single cell, including more than one different conjugative plasmid at any one time. In fact, cells of *E. coli* have been known to contain up to seven different plasmids at once. To be able to coexist in the same cell, different plasmids must be **compatible**. If two plasmids are incompatible then one or the other will be rapidly lost from the cell. Different types of plasmid can therefore be assigned to different **incompatibility groups** on the basis of whether or not they can coexist, and plasmids from a single incompatibility group are often related to each other in various ways. The basis of incompatibility is not well understood, but events during plasmid replication are thought to underlie the phenomenon.

Plasmid classification

The most useful classification of naturally occurring plasmids is based on the main characteristic coded by the plasmid genes. The five major types of plasmid according to this classification are as follows:

- **Fertility** or **F plasmids** carry only *tra* genes and have no characteristic beyond the ability to promote conjugal transfer of plasmids. A well-known example is the F plasmid of *E. coli*.
- **Resistance** or **R plasmids** carry genes conferring on the host bacterium resistance to one or more antibacterial agents, such as chloramphenicol, ampicillin, and mercury. R plasmids are very important in clinical microbiology as their spread

through natural populations can have profound consequences in the treatment of bacterial infections. An example is RP4, which is commonly found in *Pseudomonas*, but also occurs in many other bacteria.

- **Col plasmids** code for colicins, proteins that kill other bacteria. An example is

ColE1 of *E. coli*.

l **Degradative plasmids** allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid, an example being TOL of *Pseudomonas putida*.

l **Virulence plasmids** confer pathogenicity on the host bacterium; these include the **Ti plasmids** of ***Agrobacterium tumefaciens***, which induce crown gall disease on dicotyledonous plants.

Plasmids in organisms other than bacteria

Although plasmids are widespread in bacteria they are by no means as common in other organisms. The best characterized eukaryotic plasmid is the **2** F**m circle** that occurs in many strains of the yeast Saccharomyces cerevisiae. The discovery of the 2 fm plasmid was very fortuitous as it allowed the construction of cloning vectors for this very important industrial organism (p. 105). However, the search for plasmids in other eukaryotes (such as filamentous fungi, plants and animals) has proved disappointing, and it is suspected that many higher organisms simply do not harbor plasmids within their cells.

B. Bacteriophage chromosomes.