Plasmid stability

- One of the **characteristic** features of plasmids is their instability. **Plasmid-borne features** are often lost from a population at a higher frequency for the normal processes of mutation.
- The extent of this **instability** varies enormously from one plasmid to another.
- Naturally-occurring plasmids are usually (but not always) reasonably stable: selection will tend to operate in that way, and in **addition to isolating** the strain and looking for the plasmid the more stable plasmids will tend to be selected.
- **Artificially** constructed plasmids on the other hand are often markedly unstable.

There are **three quite distinct phenomena** associated with the **concept of plasmid stability: (1) plasmid integrity, (2) partitioning at cell division and, (3) differential growth rates.**

Plasmid integrity

- **Integrity refers** to the maintenance of the structure of the plasmid. Even naturally occurring plasmids can be '**unstable**' in this respect, showing a tendency to lose **genes due** to the presence of **recombination hot spots**.
- In particular, the presence of repeated sequences, due to the presence of **transposons or insertion** sequences can lead to deletions or inversions due to recombination between the repeats. In many cases, the only evidence (short of sequence data) may be a change in the **restriction map** of the plasmid, since the relative position of some restriction sites will have changed.

Partitioning

- The correct partitioning at **cell division** is essential if the plasmid is to be maintained in the culture.
- Although high copy number plasmids can rely principally on random distribution between the two daughter cells, this can be compromised by a tendency for plasmids to form multimeric structures during replication and by recombination between monomers.
- Furthermore, since a dimer contains two origins of replication, it will be expected to replicate more efficiently than a monomer; multimers would replicate even more efficiently. This could potentially lead to what is known as a 'dimer catastrophe'**.**

- Low copy-number plasmids cannot rely on random partitioning. As well as an active partitioning mechanism, some plasmids supplement their partitioning system with ability to kill any cells that have lost the **plasmid (post-segregational killing)**.

Differential growth rate

- If there is no difference in growth rate, then a failure of partitioning at cell division will lead to only a slow increase in the proportion of plasmid-free cells.
- On the other hand, a substantial difference in growth rate will lead to rapid elimination of the plasmid even if failure of partitioning occurs only rarely.
- Differences in growth rate are expected to arise because of the metabolic load arising from replication of the plasmid and expression of its genes.

Methods for studying plasmids

Associating a plasmid with a phenotype

- Some plasmids it is possible to increase the rate at which the plasmid is lost by various treatments, such as acridine orange or growth at a higher temperature – a procedure known as 'curing' or plasmid elimination.
- It must be stressed that the terms 'elimination' and 'plasmid loss' do not mean the physical removal of the plasmid from a particular cell; the process works by interfering with the replication and/or partitioning of the plasmid so as to increase the rate at which plasmid-free segregants occur.
- This **evidence** should be combined with the detection of plasmid DNA by agarose gel electrophoresis (Figure 1). The chromosomal DNA will show up as a rather diffuse band since it is fragmented randomly by the extraction procedure, while any plasmid DNA will form separate, sharper bands at a position determined primarily by their size. The smaller the molecule, the faster it will run. Small plasmids will be found well ahead of the chromosomal DNA, while larger plasmids actually run slower than the chromosomal DNA.
- The conformation of the plasmid will also affect its mobility. Usually, the intact plasmid will be a **covalently closed, supercoiled circle**, which will migrate differently from a **linear molecule** of the same molecular weight (this must be taken into account when attempting to determine the size of a plasmid) and considerably faster than a **nicked open circular** form. In

addition to these three major forms of the plasmid (**supercoiled, nicked circles, and linear),** there may be dimers and higher multimers which will move more slowly.

- In order to study a plasmid in more depth, it is necessary to purify it. Separating DNA from other cell components is relatively easy to accomplish.

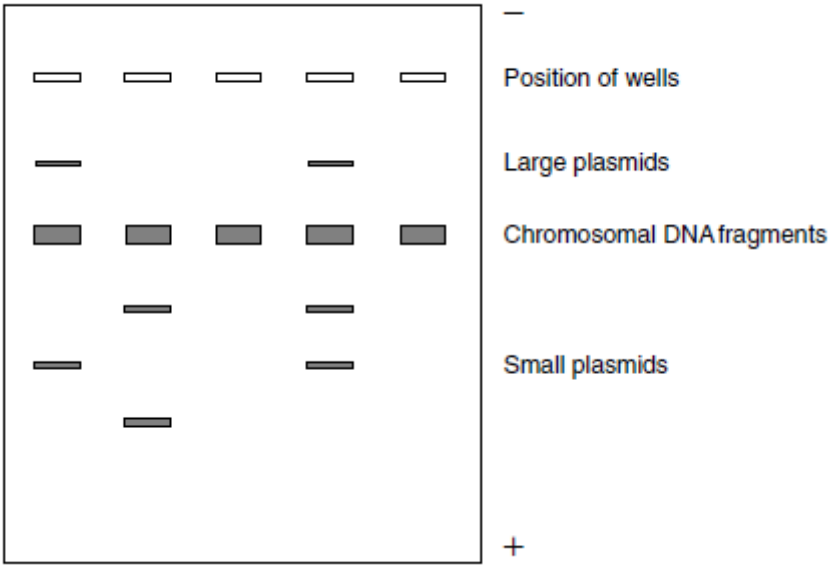

Figure 1. Demonstration of plasmids in cell extracts by agarose gel electrophoresis: Fragments of DNA can be separated by electrophoresis through an agarose gel (or for very small fragments, an acrylamide gel). The samples are applied to slots or wells in the gel and a voltage is applied across the gel to separate the DNA fragments. The gel is then stained with ethidium bromide. The ethidium bromide–DNA complex fluoresces under ultraviolet illumination. Within certain limits, the mobility of a DNA fragment is determined by its size; larger fragments move more slowly through the gel. At the pH used – usually about 8.3 – the DNA is negatively charged and there is essentially no variation in charge between different fragments. Fragment sizes can be estimated by calibrating the gel with a mixture of known DNA fragments and plotting the logarithm of the size against the distance moved. The relationship between size and mobility is only true for molecules with the same conformation. A gel that is calibrated with linear DNA fragments can only be used for sizing linear fragments. Gel electrophoresis can also be used preparatively, by cutting the required band out of the gel and eluting the DNA from it.

- To obtain more conclusive evidence, the best procedure is to introduce the plasmid into a cell that does not have it (by conjugation or transformation, and to determine whether the acquisition of the plasmid leads to a corresponding change in the phenotype. Such experiments are easily carried out with selectable markers like antibiotic resistance.

- If the plasmid carries a resistance gene, the cells that have acquired the plasmid can be detected by simply plating them on a medium containing the antibiotic. In this way extremely rare events can be detected, e.g. one resistant **transconjugant** (or **transformant**) in 10^8 cells.

Classification of plasmids

- It is often useful to be able to compare plasmids identified in different bacterial isolates.
- The division into 'small' and 'large' plasmids is clearly much too crude to cope with the vast number of different plasmids that can be present in such a situation.
- Analysis of the profile of antibiotic resistance genes carried by the plasmid has the virtue of being quick and easy to carry out, but it must be realized that plasmids are very fluid in their make-up; the presence of transposons commonly leads to new resistance genes being acquired by a plasmid, even during the course of a specific outbreak.
- Conversely, plasmids can lose genes by deletions. Excessive reliance on the resistance pattern can therefore be misleading.

Incompatibility groups

- Interactions between the replication control mechanisms of different plasmids may make them incompatible. This forms the basis of a widely used method of classifying plasmids.
- The test is carried out by transferring the unknown plasmid into each of a number of standard plasmid-carrying strains and testing for their incompatibility.
- A large number of incompatibility groups have been defined in this way; F for example belongs to the group **IncFI**, and is incompatible with plasmids such as ColV-K94.
- The R100 plasmid has a similar conjugation and transfer system but is compatible with F; it belongs to the group **IncFII**.

Host range

- Many plasmids, including ColE1 and cloning vectors related to it, are only able to replicate in a limited range of bacterial hosts. However, this is not universally true and some plasmids have a remarkably broad host range.
- Notable amongst these are the P group of plasmids, such as RP4, which are able to replicate in some Gram-positive bacteria, as well as in most

Gram-negatives. Plasmids such as RP4 are also promiscuous in the sense that not only can they replicate in a broad range of host bacteria, but they are also capable of promoting their own transmission by conjugation between widely diverse bacterial species.

- Broad host range plasmids can be useful as genetic tools, as well as their natural role in promoting gene flow between widely diverse bacterial species.

Molecular characterization

- Plasmids can be compared more readily by the use of restriction endonucleases. Since these enzymes cut DNA at specific points, digestion of a plasmid will give rise to a characteristic set of fragments that can be separated on an agarose gel.
- If two plasmids are the same, the restriction pattern will be identical. This procedure is however still subject to the same problem referred to above: plasmids can acquire additional genes by transposition or lose them by deletion.
- A different pattern of restriction fragments may therefore indicate the occurrence of events of this kind rather than the presence of unrelated plasmids.
- Such an analysis can be extended by testing the ability of DNA fragments from one plasmid to hybridize to a second plasmid, which indicates the presence of related sequences. However, since two otherwise dissimilar plasmids may have acquired related antibiotic resistance genes (which will therefore cross-hybridize), these results also need to be interpreted with care.
- Ultimately, of course, the best comparison of two plasmids at the molecular level is to determine the complete sequence of both plasmids.