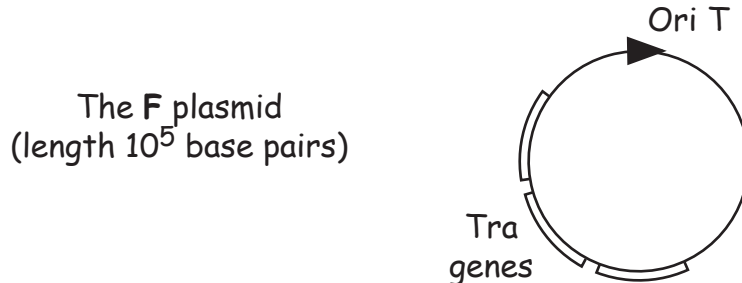


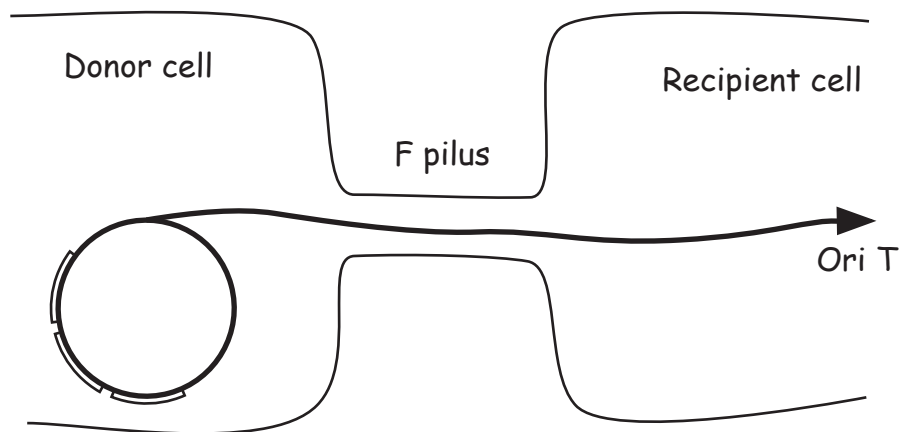
Lecture 15

Plasmid conjugation in Bacteria

In order to perform tests for dominance or for complementation in bacteria we need a way to make the bacteria diploid for part of the chromosome. To do this we need to consider a different extrachromosomal element:

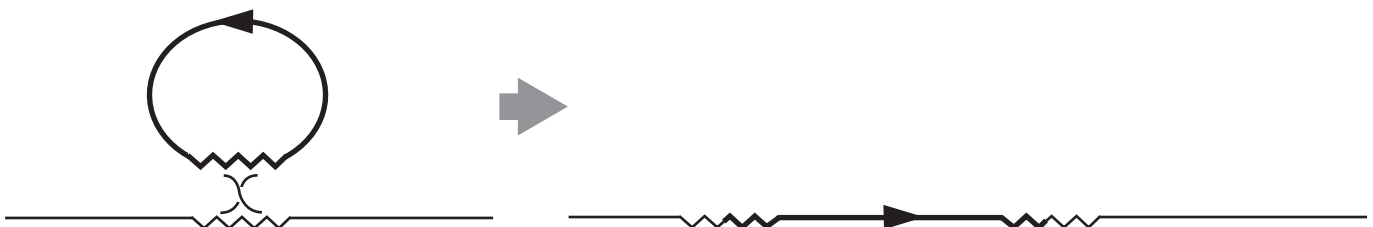


There are some special terms to describe the state of F in a cell: F^- refers to a strain without any form of F, whereas F^+ refers to a strain with an F plasmid.



F is very efficient at transferring itself from an F^+ cell to an F^- cell. After culturing F^+ and F^- cells together about 1/10 of the F^- cells will become F^+ .

The property that makes F useful for genetic manipulation is that at low frequency the plasmid will integrate into chromosome. This occurs because F carries insertion sequences that are also present at multiple locations on the chromosome. Crossing over between insertion sequences on F and on the chromosome gives integration.



Hfr: a strain with F integrated into the chromosome that will give efficient transfer of some chromosomal markers.

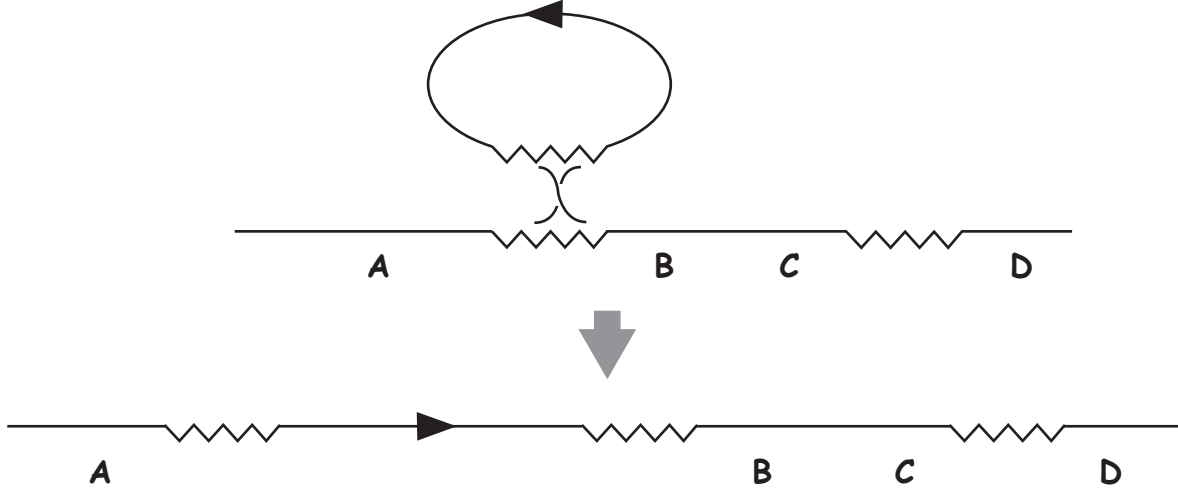
F⁺ plasmid: 1) Transfers itself at a frequency of 0.1

2) Does not transfer chromosomal markers

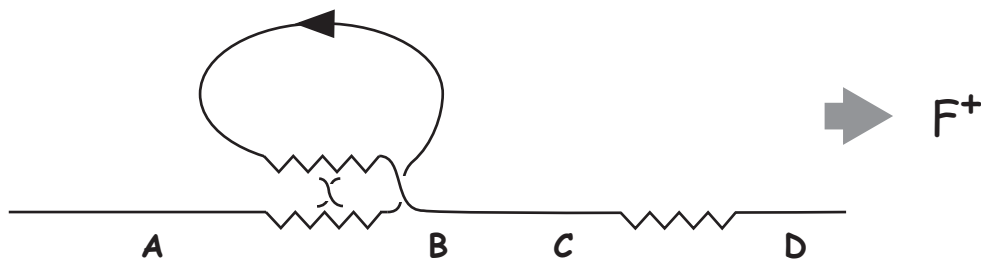
Hfr 1) Transfers some chromosomal markers efficiently

2) Other markers transferred inefficiently - Gradient of transfer
(It takes about 100 minutes to transfer the entire chromosome)

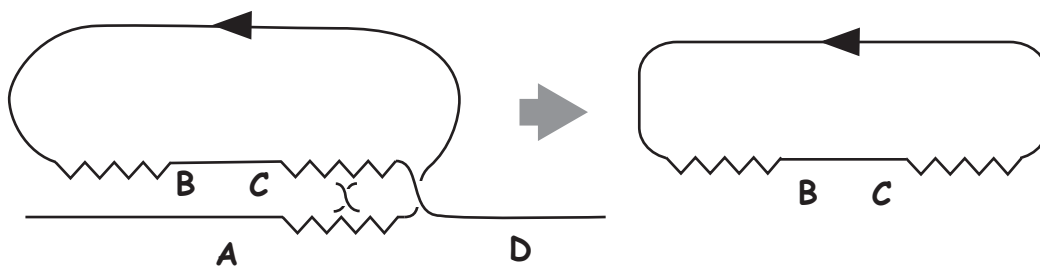
Consider an F⁺ integrating to make an Hfr:



This process can be reversed to go back to the F⁺ state:



The recombination can occur at a different position to give an F plasmid that carries a part of the chromosome. This form of F is called an F'.



F's are usually isolated by selection for early transfer of a marker that is transferred late in the Hfr. In the example above the F' could have been isolated from a population of Hfrs by selecting for early transfer of either B or C.

- F'
- 1) Very efficient transfer of markers carried on F'.
These can be markers that were transferred very late in the Hfr from which the F' was derived.
 - 2) No transfer of chromosomal markers not on F'.

F's can be used to perform genetic tests of function because a cell containing a F' will be diploid for the region of the chromosome carried on F. This is known as a merodiploid. For example, if we isolated a new Lac⁻ mutation we could use an F' Lac⁺ to determine whether the Lac⁻ mutation is dominant or recessive.

Growth on lactose

Lac ⁺	+
Lac ⁻	-
Lac ⁻ / F' Lac ⁺	+ (Lac ⁻ is recessive)

It is also possible to test for functional complementation of two linked mutations. Consider two mutations, A⁻ and B⁻, that are close together and have the same phenotype. We can introduce an F' carrying A⁻ into a strain with a B⁻ mutation. If the merodiploid has a wild type phenotype then we know that the mutations complement and are therefore in different genes.

