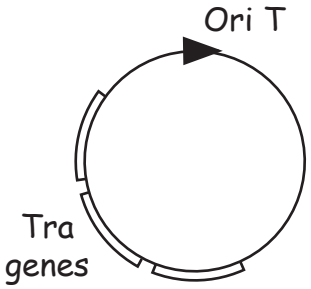
Lecture 17

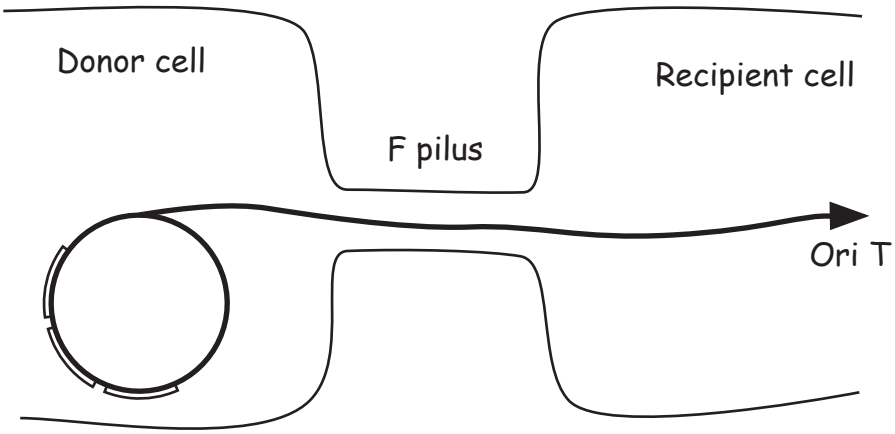
Plasmid conjugation in Bacteria

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:

The **F** plasmid
(length 10^5 base pairs)



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.

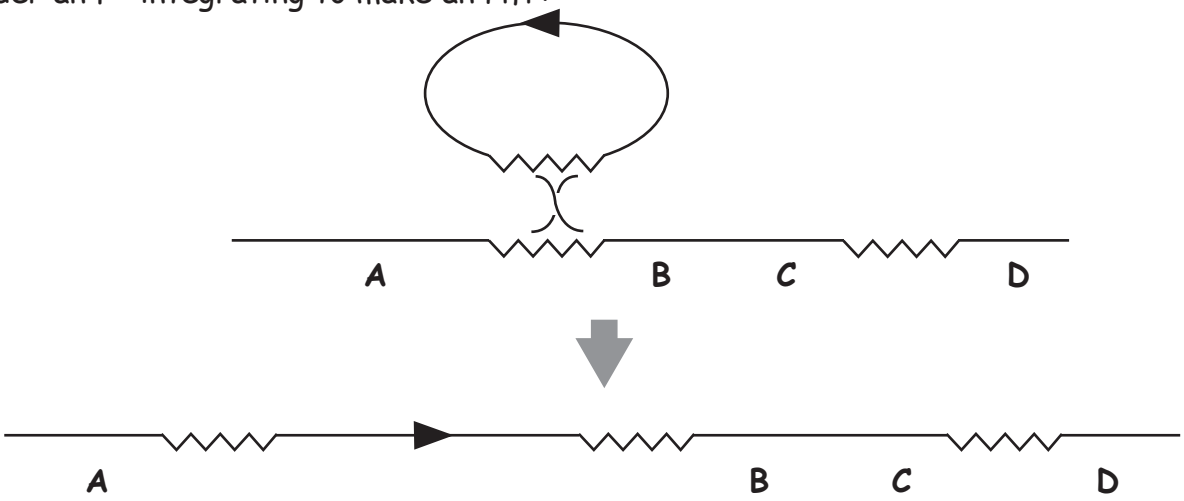
F+ plasmid: 1) Transfers itself at a frequency of 0.1

2) Does not transfer chromosomal markers

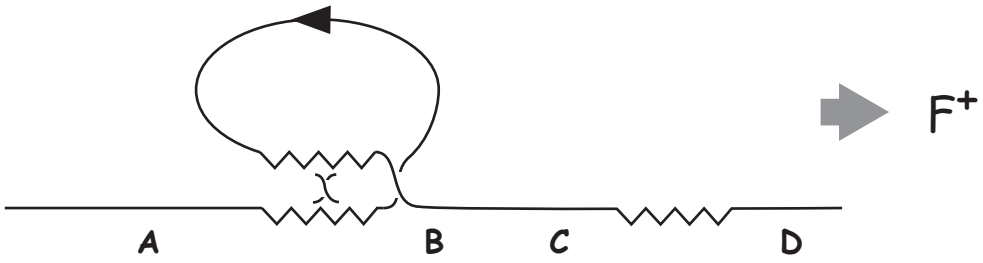
Hfr 1) Transfers some chromosomal markers efficiently

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

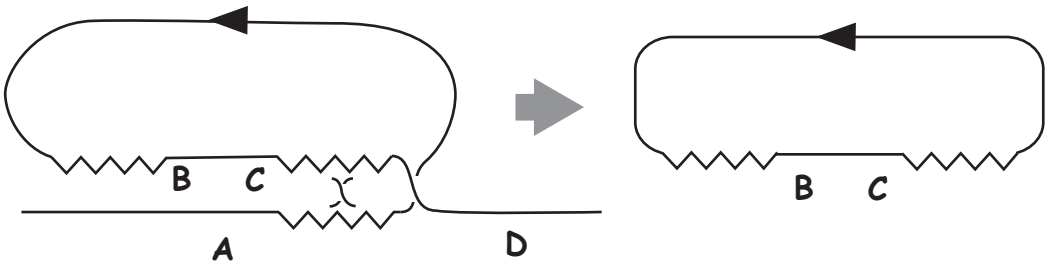
Consider an F^+ integrating to make an Hfr:



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.