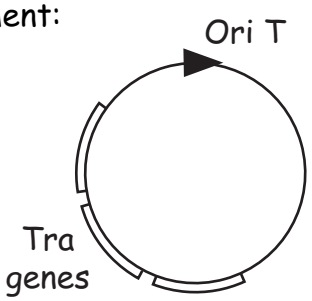
Lecture 14

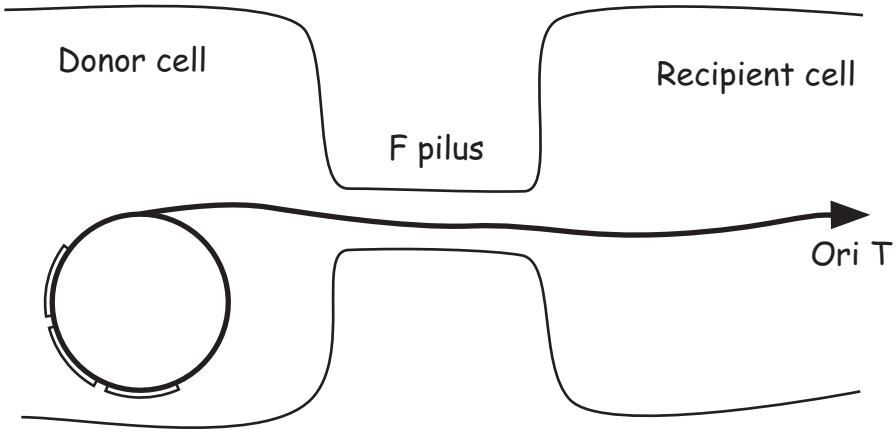
Gene Complementation in Bacteria

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: $\mathbf{F^-}$ refers to a strain without any form of **F**, whereas $\mathbf{F^+}$ refers to a strain with an **F** plasmid.



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

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

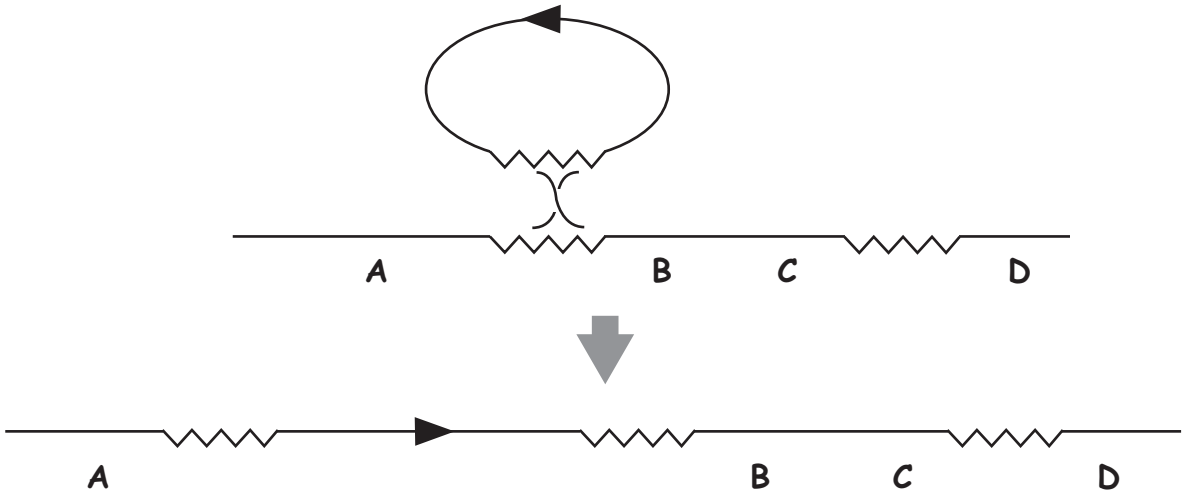
2) Does not transfer chromosomal markers

Hfr 1) Transfers some chromosomal markers efficiently

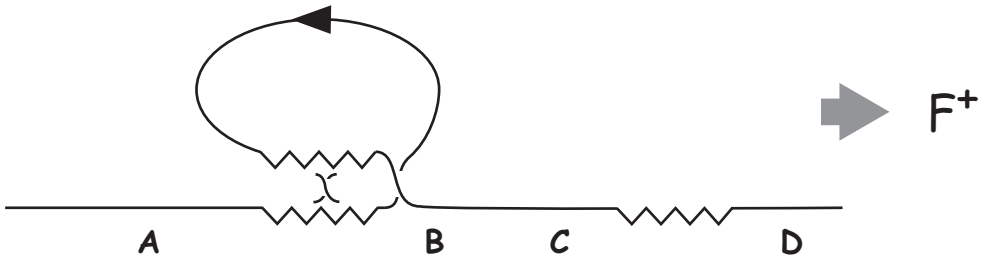
2) Other markers transferred inefficiently - Gradient of transfer

(It takes about 100 minutes to transfer the entire chromosome)

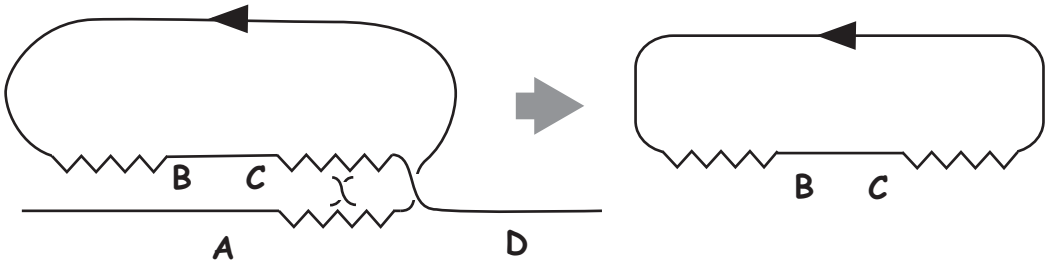
Consider an **F⁺** integrating to make an **Hfr**:



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 **Hfr**s 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.