Lecture 15

## Gene Cloning

F is one of many bacterial plasmids, most of which are also transmissible from one cell to another.

R factors - This type of plasmid was discovered in Japan in early 1950's . They came from hospital patients that were infected with bacteria that were resistant to several different antibiotics. This was surprising since antibiotics work by very different mechanisms.

For example, resistance to ampicillin, kanamycin, tetracycline, and sulfonamide could be conferred at once on transfer of a given **R** factor. In fact, most of the antibiotic resistance genes are actually in transposons that are carried on the **R** factor.
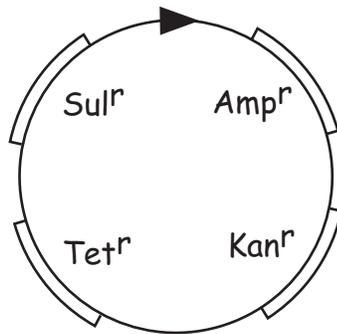
Modern cloning vectors are stripped down versions of **R** factors. They usually carry one or two drug resistance genes and an origin of replication.
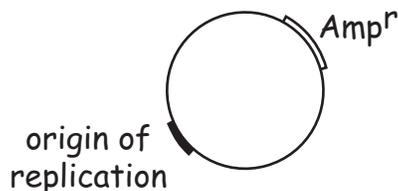

Cloning involves the use of enzymes in vitro to make plasmids carrying pieces of the chromosome. One of the important tools is a set of enzymes that can cleave DNA at specific sites. These enzymes are known as **Restriction Enzymes**. They were discovered in the following way:

	E. coli **C**	*E. coli* **K**
λ (grown on **C**)	10^8/ml	10^3/ml
λ (grown on **K**)	10^8/ml	10^8/ml

This phenomenon known as "host restriction" behaves like a genetic change that reverses at a high frequency. The explanation is that *E. coli* **K** makes enzyme that cleaves λ DNA. The **K** strain doesn't destroy its own chromosome because it also makes an enzyme that modifies the cleavage site.

The phage that grow on **K** have by rare chance escaped cleavage long enough to be modified.

The genes for restriction enzymes usually come in pairs with the gene for the restriction enzyme (**R**) residing next to the gene fr the enzyme that modifies the same sequence (**M**).
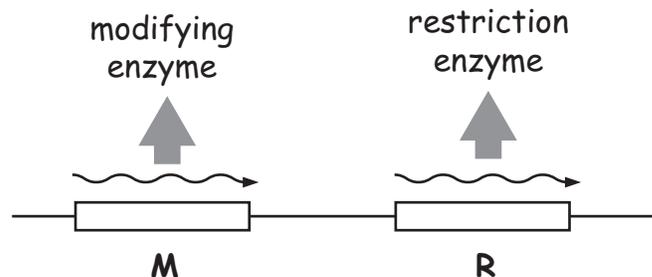
Mutants the have a mutated version of the restriction enzyme but a wild type version of the modifying enzyme (**R⁻ M⁺**) are useful because they do notshow host restriction but phage grown on these strains are resistant to host restriction. It is a useful exercise to think about why a strain with a mutated modifying enzyme but a wild type restriction enzyme (**R⁺ M⁻**) would be inviable.

A large number of these enzymes have been isolated from different bacterial species. Most of the enzymes recognize palindromic DNA sequences of 4 or 6 base pairs.

Restriction enzymes can be used to cut chromosomal DNA into fragments. These fragments can be ligated into plasmid DNA that has been cut at a single site. This procedure takes advantage of the fact that the DNA ends that remain after cleavage with a restriction enzyme will base pair with other ends cut with the same enzyme. The collection of a large number of random chromosomal fragments carried in plasmids is known as a **Library**

Generation of a library yields a a very large collection of plasmids each with a different chromosomal insert.
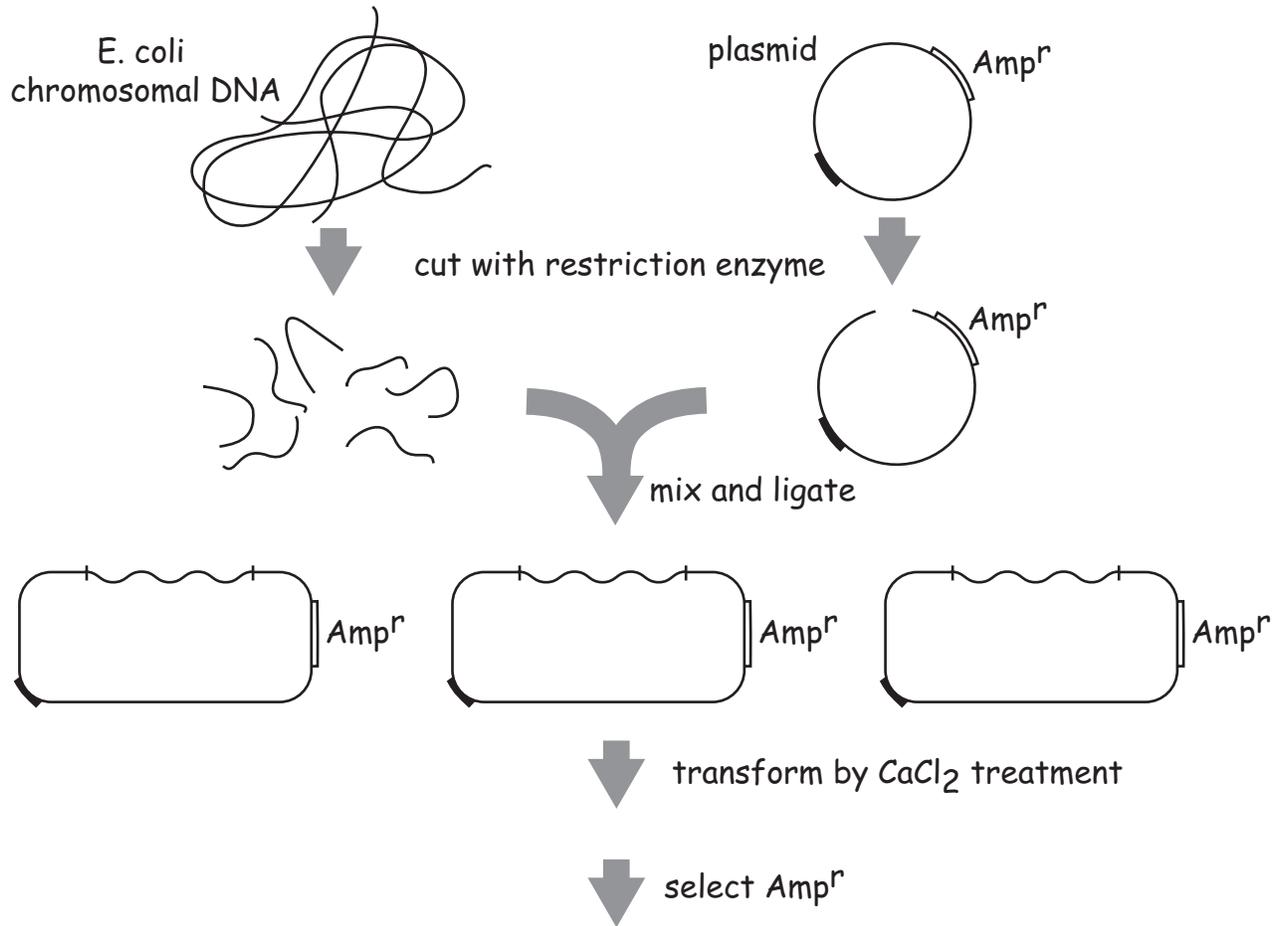
Cloning by Complementation

Say we wanted to clone the **Lac** operon. First a library would be made from DNA from a **Lac⁺** *E. coli* strain. This library would then be used to transform a **Lac⁻** strain. Transformants would first be selected by Amp^r. The resistant colonies would then be screened for the ability to grow on lactose (**Lac⁺**). These clones should contain plasmids carrying a functional **Lac** operon.

How many clones would we need to screen? Each plasmid carries about 5×10^3 bp of chromosomal DNA. The chromosome is 5×10^6 base pairs so the entire genome will be covered if several thousand clones are screened.

All sorts of genes from *E. coli* have been cloned by looking for DNA fragments that can restore function to a mutant. It is also possible to find genes from other bacteria. The following is a dramatic example of a cloning experiment to find an important protein for a pathogenic bacterium.

Yersinia is the bacillus that causes bubonic plague, a disease that killed 100 million people in the 6th century A.D.. One reason that *Yersinia* is such a deadly pathogen is that it escapes the immune system by multiplying *within* cells. The problem was to find the *Yersinia* genes that enable the bacterial cells to invade human cells. To do this an assay was needed.

A test for bacterial invasion consists of a layer of mammalian tissue culture cells. The bacteria are allowed to settle onto the cells for awhile, then the bacteria that have not entered the cells are killed with the antibiotic gentamicin, which can not cross the membrane of tissue culture cells. The bacteria that have entered cells escape gentamicin and can be recovered from the inside of the cells after the cells are lysed with detergent.

E. coli normally can not invade cells. The gene for invasion was found by transforming *E. coli* with a library of *Yersinia* DNA and then selecting for *E. coli* that had invaded cells. A single gene was found that encodes a surface protein known as **invasin**.