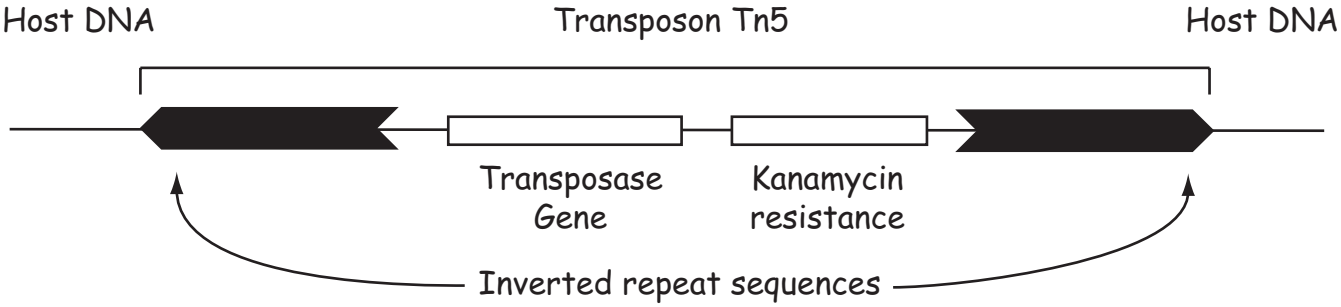
Lecture 12

Transposable elements

Transposons are usually from 10^3 to 10^4 base pairs in length, depending on the transposon type. The key property of transposons is that a copy of the entire transposon sequence can at a low frequency become inserted at a new chromosomal site. The mechanism by which transposons insert into new sites differs from one kind of transposon to another, but the details are not important to understand how transposons can be used. It is worth contrasting the recombination events that occur during transposition to the homologous recombination events that we have considered in meiosis and in phage crosses. In homologous recombination, crossovers occur between like sequences. While this type of recombination can generate new combinations of alleles the arrangement of genes is left undisturbed. In contrast, transposition involves recombination between unrelated sequences, namely the ends of the transposon and a site in the target sequence. Transposition therefore results in a new arrangement of genes along the chromosome.

The generic structure of a transposon looks like this:



Transposon Element	Function
Transposase	An enzyme that cuts the target DNA more or less at random and splices the transposon ends to the target sequences, Other steps in transposition are performed by host enzymes.
Inverted Repeats	These sequences direct transposase to act at the ends of the transposon. Note that because the sequences are inverted, the two ends have identical sequence.
Selectable Marker(s)	Transposons are thought to have evolved by providing a selective advantage to the host cell. Many transposons carry genes that confer antibiotic resistance or some other benefit to the host.

The study of transposition mechanism and the biology of transposons is an interesting subject in genetics but for our current purposes we are going to concentrate on how transposons can be used for bacterial genetic analysis. For this purpose we will focus on the transposon **Tn5** which can function in *E. coli* as well as a wide variety of other bacterial species. The selectable marker in **Tn5** is a gene that confers resistance to the antibiotic kanamycin. Thus bacteria without **Tn5** are sensitive to kanamycin (Kan^s), whereas bacteria that have **Tn5** inserted into the chromosome are resistant to kanamycin (Kan^r).

To introduce random insertions of **Tn5** into the *E. coli* chromosome we will start with **Tn5** carried on a special λ phage vector: **λ Pam int⁻::Tn5**.

Pam allows conditional phage growth. When **λ Pam** phage infect *E. coli* with an amber suppressor (Su^+) the phage multiply normally, but when **λ Pam** phage infect a nonsuppressing host (Su^-) the phage cannot replicate.

int⁻ is a mutation in the λ integrase gene. Phage with this mutation can not integrate into the host chromosome to make a stable prophage.

::Tn5 designates that the λ phage carries an inserted copy of **Tn5**.

When **λ Pam int⁻::Tn5** infects a wild type (Su^- Kan^s) *E. coli* host, the phage DNA can not replicate (**Pam**) nor can it integrate (**int⁻**) thus the only way for the *E. coli* to become Kan^r is for **Tn5** to transpose from the λ DNA to some location on the *E. coli* chromosome. This type of transposition is an inherently rare process and will occur in about one out of 10^5 phage-infected *E. coli* cells. This is how a transposon mutagenesis might be done:

1) Infect $2x10^9$ wild-type *E. coli* cells with **λ Pam int⁻::Tn5** so that each cell receives at least one phage chromosome.

2) Select for Kan^r by plating on medium that contains kanamycin. There should be a total of about $2x10^4$ Kan^r colonies. Each of these should have **Tn5** inserted into a different site on the *E. coli* chromosome.

The genes of *E. coli* are densely spaced along the chromosome and about half of the **Tn5** insertions will lie in one gene or another. There are 4,200 genes in *E. coli* so our collection of $2x10^4$ random **Tn5** insertions will likely contain at least one insertion in each gene. (Note that insertions in genes that are essential for *E. coli* growth such as the genes for RNA polymerase or ribosomal subunits will not be recovered because these insertion mutants will not form colonies on the kanamycin plates).

Let's say that we are interested in the *E. coli* genes that are involved in synthesis of histidine. To find insertion mutants that can not synthesize histidine (His^-) we could screen amongst our collection of $2x10^4$ random **Tn5** insertions to find those that are His^-. The easiest way to do this would be to plate out the collection of insertions at a density of 200 colonies per plate (100 plates total). Each of these master plates would then be replica plated (first by transfer to a sterile piece of velvet) to a plate that contains histidine and also to a plate that lacks histidine. His^- insertion mutants would be identified as colonies that can not grow on the plates that lack histidine. Note that the same collection of random Tn5 insertions can be screened multiple times to find interesting mutations with different phenotypes.

3) Identify His^- **Tn5** insertion mutants by replica plating to find colonies that specifically can not grow on plates that don't contain histidine.

Once we have a set of His^- insertion mutations (in the present example, one might expect to find 10-20 different His^- mutants), the affected gene(s) can be identified by the simple fact that they will be "tagged" by the inserted **Tn5** sequences. The easiest way to identify the site of insertion is by performing a special PCR amplification of the DNA fragment that corresponds to the novel junction between**Tn5** and the bacterial chromosomal sequences. Ordinarily PCR reactions are carried out using two DNA primers, each of which corresponding to an end of the sequence to be amplified. When we want to amplify a junction fragment we can use as one of the primers a sequence that lies near the end of **Tn5** but we won't yet know the relevant chromosomal sequence to allow the other primer to be designed. There are several tricks that can be used to circumvent this problem, which are too complicated to describe here. Suffice it to say that there are ways that the junction fragment can be amplified by PCR using only sequences defined by the **Tn5** portion of the junction fragment.

4) Use the known sequence of the end of **Tn5** to PCR amplify a fragment that spans the junction between the end of **Tn5** and the *E. coli* chromosomal site that was the target for insertion.

DNA sequencing of the amplified junction fragments will give the identity of the target sequences. Since we know the DNA sequence of the entire *E. coli* chromosome, the gene that was the target for **Tn5** insertion can be identified unambiguously.

5) The DNA sequence of the junction fragments will identify all of the genes that have been inactivated to give the His^- phenotype.

The procedure just outlined can be used to isolate and characterize a wide variety of useful mutations. A major limitation of this method is that as stated earlier, transposon mutations usually completely disrupt the target gene and therefore lead to a complete inactivation of the gene product. Often we will want to work with point mutations (such as temperature sensitive mutations or nonsense mutations). In the next lecture we will see how transposons can also be used to facilitate analysis and manipulation of point mutations.