Lecture 15

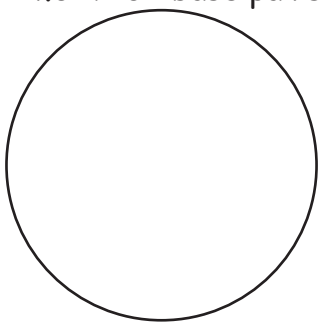
We have seen how to make mutations in bacteria using either a chemical mutagen to make base changes or by using a transposon such as Tn5 to make an insertion mutation.

The next steps of genetic analysis usually involve moving mutations from one strain to another. The most useful method for transferring DNA from one bacterial cell to another uses a phage P1 and is a method known as **Transduction**.

Transduction involves growing P1 phage on a donor strain under conditions that promote some mistaken packaging of chromosomal DNA into phage heads which will then allow this DNA to subsequently be introduced into a recipient strain.

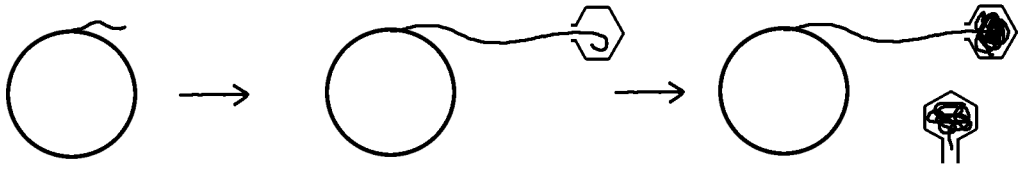
First lets see how the mistaken packaging works.

E. coli chromosome
4.6×10^6 base pairs

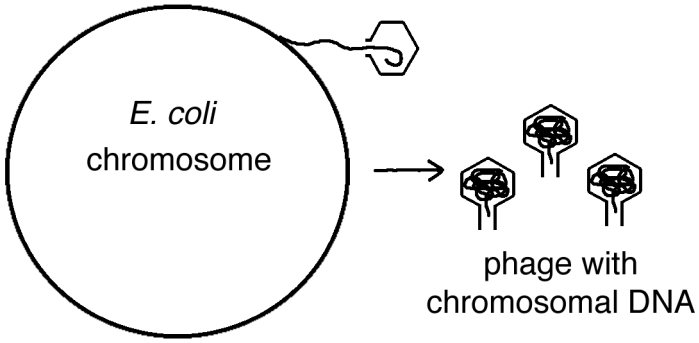


Phage P1 chromosome
10^5 base pairs

After infection of E. coli, the phage DNA is replicated by a mechanism known as a "rolling circle" and the phage is packaged into phage particles one headfull at a time:



1/300 phage mistakenly packages E. coli chromosome DNA instead of phage DNA.



Each phage particle will package about 1/50 of the E. coli chromosome. By combining probabilities we see that about 1/15,000 phage will carry a particular E. coli gene.

A basic P1 transduction experiment would allow a particular Tn5 insertion in one strain to be moved to another strain

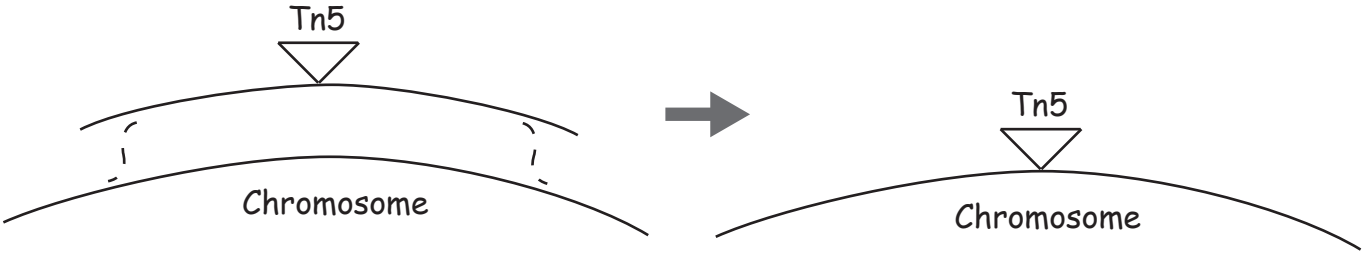
This would be done in the following three basic steps:

(1) Grow P1 phage on a strain carrying the Tn5 insertion

(2) Use the resulting phage lysate to infect a recipient strain (without a Tn5 insertion)

(3) After infection select for kan^r in the presence of citrate which removes Ca^{++} and prevents reinfection of bacterial cells with wild type phage.

About 1/15,000 phage will carry the part of the donor bacterial chromosome that includes the Tn5. When this DNA fragment enters a recipient, homologous recombination of the ends of the fragment will result in the Tn5 insertion now in the chromosome of the recipient strain. [Note that ti would be possible for the Tn5 to transpose from the incoming fragment to another site in the chromosome, but this would occur at a frequency of ~10^{-5} which is much less frequent than homologous recombination shown below which occurs at a frequency of ~10^{-1}]



Sometimes the transduction method just described will be used to move a Tn5 generated mutation into a recipient strain. However, often a Tn5 insertion, which can be used as a powerful selectable marker in a transduction experiment, will be used to facilitate transduction of a genetic marker for which there is no selection.

For example, let's say that we have isolated a new mutation in the MotA gene. MotA is a component of the bacterial flagellar motor and $MotA^-$ mutants are nonmotile, a phenotype easily detected by the inability of $MotA^-$ colonies to "swarm" outward on soft agar plates. Imagine that we want to map the MotA- mutation or to move this mutation into an E. coli strain with a new genetic background. Direct transduction of $MotA^-$ would not be possible since we have no way to select for rare (1/15,000) transductants with the nonmotile $MotA^-$ phenotype. One solution would be to use a nearby Tn5 insertion the Kan^r trait conferred by Tn5 as the selectable marker for cotransduction.

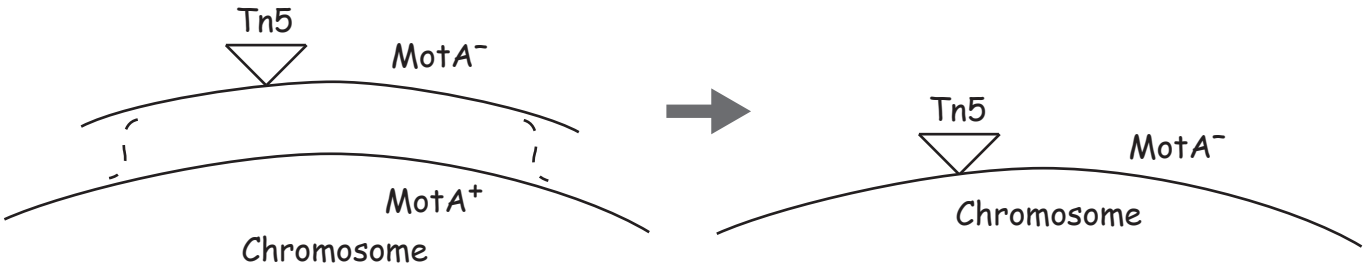
The steps to move the MotA$^-$ mutation are a simple variation on the transduction of a Tn5 insertion.

(1) Grow P1 phage on Tn5 insertion MotA$^-$ strain.

(2) Use the resulting lysate to infect wild type (MotA$^+$) recipient strain.

(3) Select for Kan^r.

(4) Screen amongst the kan^r clones for ones that Mot$^-$.



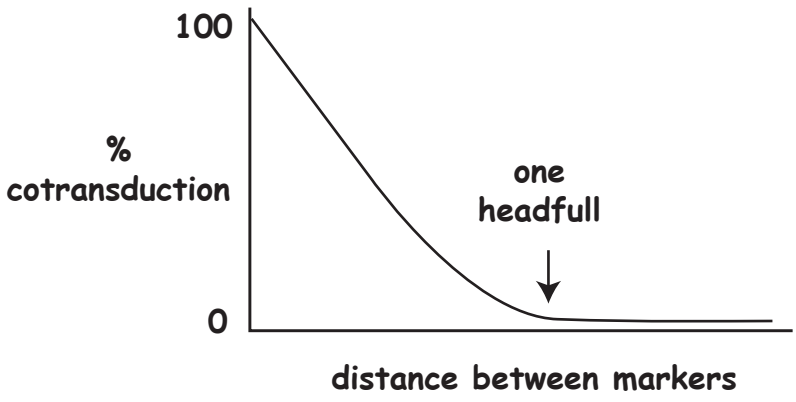
By counting the fraction of Kan^r clones that are also Mot$^-$ we can obtain a **cotransduction frequency** which can be used as a measure of genetic distance between the Tn5 insertion and MotA$^-$ mutation.

The measured frequency of cotransduction of MotA$^-$ with Tn5 gives a measure of distance according to the following rules:

- If distance between MotA$^-$ and Tn5 is greater than one headfull (10^5 bp) then there will be no cotransduction.

- If MotA$^-$ and Tn5 are very close together then there will be 100% cotransduction.

- Cotransduction frequency is an inverse measure of distance.



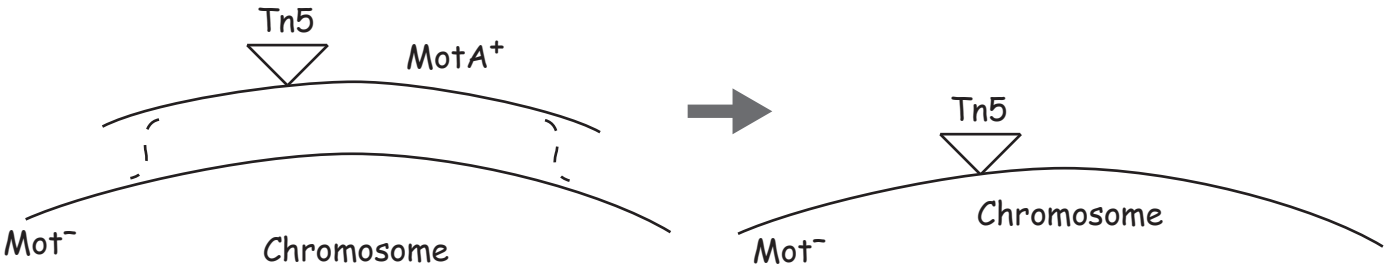
Now say we have isolated a new Mot^- mutation and we wish to know whether it ia a $MotA^-$ mutation. The criteria we will use is whether the new Mot^- mutation maps the same distance from the Tn5 insertion as the known $MotA^-$ mutation. The steps for this mapping experiment would be as follows:

(1) Grow P1 phage on Tn5 insertion $MotA^+$ strain.

(2) Use the resulting lysate to infect a Mot^- recipient strain.

(3) Select for kan^r.

(4) Screen amongst the kan^r clones for ones that Mot^+.



In this example, if the Mot^- mutation were different from $MotA^-$ and was more than 10^5 bp away from the Tn5 insertion, then all of the kan^r clones would also be Mot^- . The conclusion would be that the frequency of cotransduction of Mot^+ with Tn5 is 0 and we can conclude that Mot^- mapps to a different chromosomal location than $MotA^-$ which is is linked to the Tn5 insertion.