Lecture 16

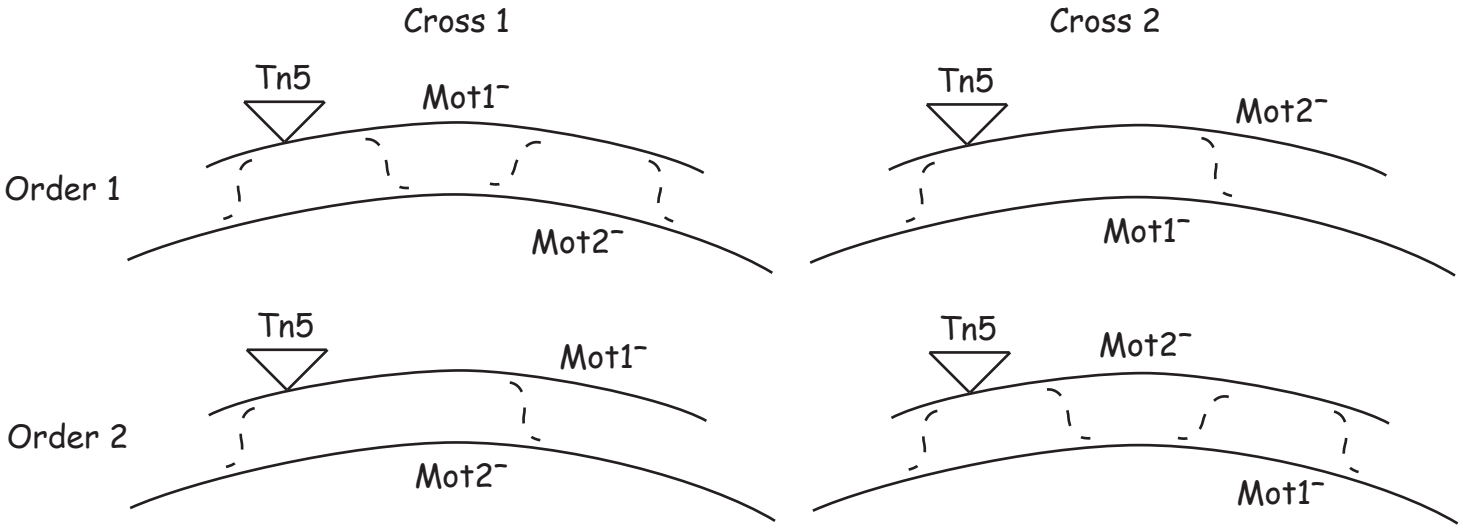
Cotransduction can also be used in the context of a 3-factor cross to determine gene order.

For example, say we found that two different Mot$^-$ mutations (called Mot1$^-$ and Mot2$^-$) were found to be linked to a given Tn5 insertion each by about 50% cotransduction each (given the the error on the measurements ther is no significant difference that can be used to obtain the order). To do a 3-factor cross to obtain the order of the Tn5 and Mot1$^-$ and Mot2$^-$ mutations, it is necessary to set up two reciprocal crosses:

Cross 1
(1) Grow P1 on Tn5 Mot1$^-$
(2) Use the resulting lysate to infect a Mot2$^-$ mutant
(3) Select for Kan^r mutation and then count proportion that are Mot$^+$

Cross 2
(1) Grow P1 on Tn5 Mot2$^-$
(2) Use the resulting lysate to infect a Mot1$^-$ mutant
(3) Select for Kan^r mutation and then count proportion that are Mot$^+$

Let's consider the crossovers that would be needed to produce Kan^r Mot$^+$ recombinants for the two possible orders.



Comparison of the frequency of Mot$^+$ for Cross 1 and Cross 2 will immediately give us the correct order of Mot1$^-$ and Mot2$^-$. For Order 1 there should be a higher frequency of Mot$^+$ for Cross 2 than for Cross 1 (since a quadruple crossover will be required for Cross 1 but only a double crossover for Cross 2), whereas for Order 2 there should be a higher frequency of Mot$^+$ for Cross 1 than for Cross 2.

Now let's consider how one might find a Tn5 insertion that is linked to a particular mutation (such as MotA$^-$) in the first place. A simple but very laborious method would be to screen random Tn5 insertions one at a time for linkage to MotA$^-$ by P1 transduction. This process would require about 50 separate P1 transductions experiments. However there is a clever way to screen that would allow the desired linked Tn5 insertion to be identified in a single transduction experiment.

1) Start with a collection of random Tn5 insertions into wild type E. coli (the isolation of such a collection was described in last lecture). Grow phage P1 on the mixture of $2x10^4$ different Tn5 insertion mutants. Note that this donor strain is MotA$^+$.

2) Use the resulting P1 phage to infect a MotA$^-$ recipient strain. Select for transduction of the Tn5 insertions by selecting for growth of the transductants on kanamycin plates. Screen for cotransduction of MotA$^+$ by testing each of the Kan^r transductants for motility on soft agar. The desired cotransductant will be Kan^r and will be motile. Given that one P1 phage headfull corresponds to about 1/50 of the E. coli chromosome, about 1 in 50 Tn5 insertions will be close enough to the MotA gene to show 50% cotransduction. Thus if we test about 10^3 Kan^r transductants for motility, we are likely to find at about 10 that have cotransduced the MotA$^+$ marker.

3) Once a Tn5 (Kan^r) MotA$^+$ transductant has been identified, P1 can be grown on this strain.

4) Use the P1 phage from step (3) to infect a MotA$^-$ recipient strain. Select for transduction of the Tn5 insertions by selecting for growth of the transductants on kanamycin plates.

Test the resulting Kan^r transductants for their motility. The transductants that have cotransduced the MotA$^+$ marker will be motile, whereas the transductants still contain the MotA$^-$ allele will be nonmotile. The fraction of the total transductants that are motile will give the distance between MotA$^-$ and the Tn5 insertion as a cotransduction frequency.

A Tn5 (Kan^r) MotA$^-$ transductant isolated in step (4) can then be used to transduce the MotA$^-$ marker into a new recipient strain by cotransduction with Tn5.