Lecture 13

Gene Manipulation in Bacteria

There is no meiosis in bacteria so special techniques have been worked out for manipulating genes in bacteria so that mapping experiments, strain construction, and complementation tests can be done.

First, we need a way of getting chromosomal DNA from one cell into another. There are several ways to do this. All of the methods have in common the use of special extra chromosomal elements for mobilizing chromosomal genes; the methods differ according to which extra chromosomal element is used.
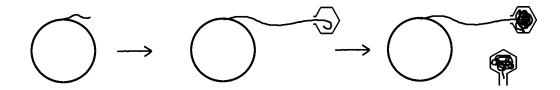
We will consider a method that uses phage and is known as **Transduction**

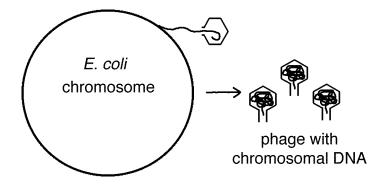*E. coli* chromosome is 4.6×10^6 base pairs

Phage **P1** chromosome is 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.

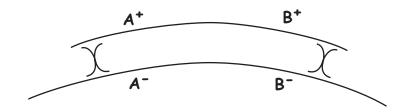E. coli chromosome

phage with chromosomal 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 transduction experiment to measure the linkage between markers **A** and **B** is done as follows:

(1) Grow P1 on **A⁺B⁺**

(2) Infect **A⁻B⁻**
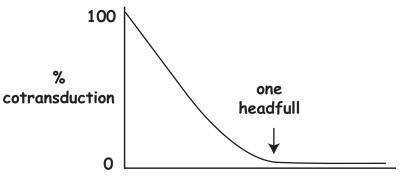
(3) Select for **A⁺** and then screen for **B⁺**

The idea is that we are looking for the rare cases where some chromosomal DNA carrying gene **A** is moved into the recipient. To find these recombinants, we select for **A⁺**. Then we screen for **B⁺** to see how often gene **B** comes along with gene **A**.

$$A^+ \qquad\qquad B^+$$

$$A^- \qquad\qquad B^-$$

The measured frequency of cotransduction of **B** with **A** gives a measure of distance according to the following rules:

• If distance between **A** and **B** is greater than one headfull (10^5 bp) then there will be no cotransduction.

• If **A** and **B** are very close together then there will be 100% cotransduction.

• Cotransduction frequency is an *inverse* measure of distance.

100

%
cotransduction

one
headfull

0

distance between markers

The experiment just described is the bacterial equivalent of a 2-factor cross and will give us relative distances between genes.

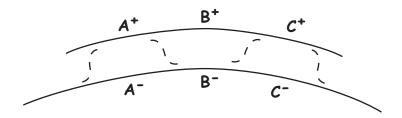We can also do a **3-factor cross** to determine gene order.

    (1) Grow P1 on **A⁺B⁺C⁺**

    (2) Infect **A⁻B⁻C⁻**

    (3) Select for **A⁺** and then screen for **B⁺** and/or **C⁺**

<u>Genotypes</u>

A⁺B⁺C⁺   2 crossovers ——— (A to C distance)

A⁺B⁺C⁻   2 crossovers ——— (A to B distance)

A⁺B⁻C⁻   2 crossovers

A⁺B⁻C⁺   4 crossovers (very rare)

(Note that there are only four possible genotypes because we select A⁺)

A limitation of transduction experiments is the need for a good selectable marker. **Tn5** insertions provide a way to extend the utility of transduction for mapping and strain construction. For example, let's say that we have isolated a new mutation in the **MotA** gene. **MotA** is a component of the bacterial flagellar moter 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. Clearly direct transduction **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 marker for which we can select to move **MotA⁻** by its cotransduction with the selectable marker.   Unfortunately, good selectable markers are not common and we are unlikely to have a good selectable marker placed within cotransduction distance of **MotA⁻** readily available. A powerful alternative approach would be to isolate a random **Tn5** insertion that is close to **MotA⁻** and to use the Kanʳ trait conferred by **Tn5** as the selectable marker for cotransduction.

The steps for finding a linked **Tn5** insertion are as follows:

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 2×10^4 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 bemotile.

Given that one P1 phage headfull corresponds to about 1/50 of the *E. coli* chromosome, about 1 in 500 **Tn5** insertions will be close enough to the **MotA** gene to show 90% cotransduction. Thus if we test about 10^3 Kan^r transductants for motility, we are likely to find at least one that has cotransduced the **MotA⁺** marker.

3) Once a **Tn5** (Kan^r) **MotA⁺** transductant has been identified, **grow P1 on Tn5 (Kan^r) MotA⁺**.

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**. Note that if we had isolated a second **MotA⁻** mutant, transduction into this strain would amount to a 3-factor cross and would provide a way to determine the order of the two different **MotA⁻** alleles.