

## Lecture 9

By way of review, let's consider the general question of how genetic mapping studies can be used to locate a gene that has been identified by an allele with an interesting phenotype. For example, the *CLOCK* mutation in mouse was identified as a semi-dominant mutation that disrupts the normal circadian rhythm. This mutation was isolated after mutagenesis of animals with ethyl-nitrosourea and then screening their offspring for abnormal activity at a time when normal mice would be sleeping.

Let's assume that we have at our disposal a large number of genetic markers spaced evenly along all of the mouse chromosomes (later in the course we will discuss DNA-based markers which can be found in an almost unlimited number in the mammalian genome) and that we have the capability to screen about 1000 mice for recombinants in a mapping experiment. The question of how precisely we can locate the *CLOCK* gene can be considered to be a question of the resolving power of a mapping experiment. Two points on a genetic map can only be resolved if a recombination event that separates them can be found. The smallest interval that can be resolved on average if 1000 progeny are screened is 1 recombinant in 1000 which corresponds to a map distance of  $\sim 0.1$  cM on either side of the *CLOCK* mutation. Thus we would be able to map the *CLOCK* mutation to an interval of 0.2 cM.

In mice, and in humans, a simple rule of thumb is that 1 cM corresponds to a physical distance of about 1 Mbp. Therefore our mapping experiment would locate the *CLOCK* gene to a region of DNA about 200 kbp in size. Such an interval may contain two to ten gene sequences so additional work using recombinant DNA methods would be needed to precisely identify the *CLOCK* gene. However, there is still no substitute for recombinational mapping studies as a way to initially narrow down the approximate location of a gene.

To review how we can categorize tetrads consider the following cross:



$\text{Trp1}^- \text{Leu2}^+, \text{Trp1}^+ \text{Leu2}^-, \text{Trp1}^+ \text{Leu2}^+, \text{Trp1}^- \text{Leu2}^-$  This is a **Tetratype**

$\text{Trp1}^- \text{Leu2}^+, \text{Trp1}^- \text{Leu2}^+, \text{Trp1}^+ \text{Leu2}^-, \text{Trp1}^+ \text{Leu2}^-$  This is a **PD**

$\text{Trp1}^+ \text{Leu2}^+, \text{Trp1}^+ \text{Leu2}^+, \text{Trp1}^- \text{Leu2}^-, \text{Trp1}^- \text{Leu2}^-$  This is an **NPD**

Now consider a cross that involves mutations that have the same phenotype.



Notice that even though we can't always unambiguously assign genotypes to each spore clone we can distinguish the three tetrad types.

<b>Tetratype</b>		<b>PD</b>		<b>NPD</b>	
<u>genotype</u>	<u>phenotype</u>	<u>genotype</u>	<u>phenotype</u>	<u>genotype</u>	<u>phenotype</u>
1 <sup>+</sup> 2 <sup>+</sup>	Leu <sup>+</sup>	1 <sup>-</sup> 2 <sup>+</sup>	Leu <sup>-</sup>	1 <sup>+</sup> 2 <sup>+</sup>	Leu <sup>+</sup>
1 <sup>-</sup> 2 <sup>-</sup>	Leu <sup>-</sup>	1 <sup>-</sup> 2 <sup>+</sup>	Leu <sup>-</sup>	1 <sup>+</sup> 2 <sup>+</sup>	Leu <sup>+</sup>
1 <sup>+</sup> 2 <sup>-</sup>	Leu <sup>-</sup>	1 <sup>+</sup> 2 <sup>-</sup>	Leu <sup>-</sup>	1 <sup>-</sup> 2 <sup>-</sup>	Leu <sup>-</sup>
1 <sup>-</sup> 2 <sup>+</sup>	Leu <sup>-</sup>	1 <sup>+</sup> 2 <sup>-</sup>	Leu <sup>-</sup>	1 <sup>-</sup> 2 <sup>-</sup>	Leu <sup>-</sup>

Here are the steps used in tetrad analysis:

**i)** Count tetrads of each type to get four numbers: PD, T, NPD, total

**ii)** If the classes are in the following ratio: **PD : T : NPD**  
 $1 : 4 : 1$

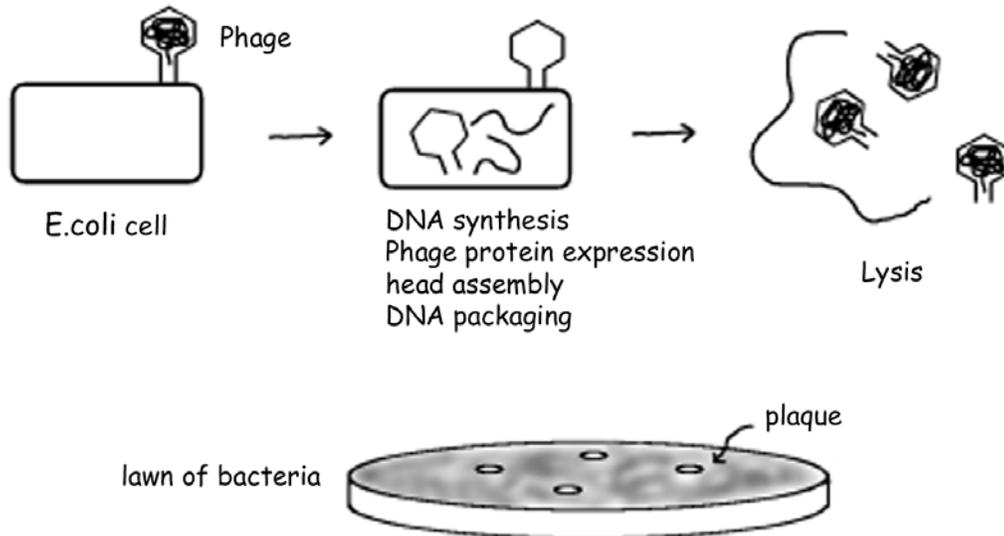
the genes are unlinked and you are finished.

**iii)** If  $\text{PD} \gg \text{NPD}$ , then the genes are linked and the distance between them is given by:

$$\text{Distance in cM} = \frac{\text{T} + 6\text{NPD}}{2 \Sigma} \times 100$$

## Gene Fine Structure

So far we have been considering the phenotypic consequences of mutations in different genes and the relative positions of genes along chromosomes. Now we are going to look at the internal structure of genes themselves and to consider the different kinds of mutations that are found in genes. For the next eleven lectures we will examine the genes of bacteria and their viruses, known as phage (some special features of eukaryotic genes will come later). First, let's see how the life cycle of phage can be exploited to carry out classical genetic manipulations.



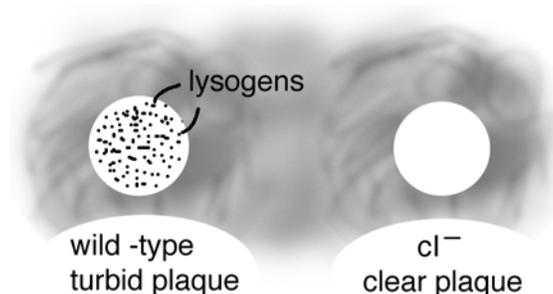
In order to do genetics with phage it is necessary to have recognizable variants of phage.

The easiest mutants to work with have obvious distinctive plaque morphologies

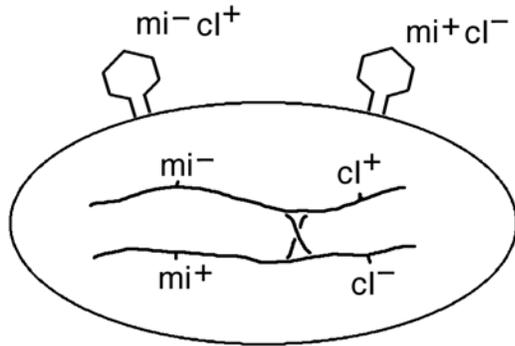
$\lambda$  phage **mi<sup>-</sup>**: make small plaques  
because fewer particles are released

$\lambda$  phage normally infects cells to make more phage. But about 1% of the time phage becomes quiescent in host cell and prevents other phage from infecting that cell. This property of phage  $\lambda$  is known as immunity. The quiescent phage is known as a lysogen.

**cI<sup>-</sup>** mutants make clear plaques because they can't form lysogens.



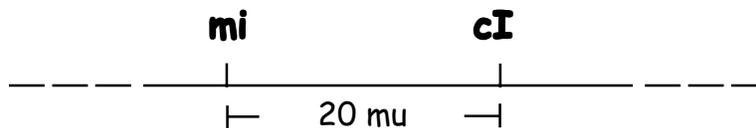
**Phage Cross:** Infect bacteria with ~10 of each phage per cell to insure that every cell has both kinds of phage replicating inside. Allow one round of growth then plate out the morphology of the plaques.



Plaque type	Frequency
lg clear (parent)	0.4
sm turbid (parent)	0.4
lg turbid (recomb.)	0.1
sm clear (recomb.)	0.1

For this cross the measured distance between **mi<sup>-</sup>** and **cl<sup>-</sup>** is:

$$\frac{\text{number of recombinants}}{\text{total}} \times 100 = \frac{20}{100} \times 100 = 20 \text{ m.u. (map units)}$$



We can also use this system to find the distance between two mutations in the same gene. For example, let's say that we have isolated two different **cI<sup>-</sup>** mutants that give clear plaques. After infection of *E. coli* with ~10 phage per cell of each mutant, the resulting phage are plated and their plaque morphology is examined. Most of the phage will still be **cI<sup>-</sup>**, but recombination between mutations will produce wild type phage with turbid plaques. If 2 out of 1,000 phage make turbid plaques then the measured map distance between the two mutations is 0.4 m.u. Note that in this example we are only counting wild type recombinants and there should be an equal number of double-mutant recombinants — both classes of recombinants must be accounted for in calculating the map distance.

