Lecture Outline 9/27

- Finish Bacterial Genetics
  - Transformation
  - Transduction via phage
  - Fine structure mapping
- Remember: EXAM 1 will be TOMORROW afternoon, 4:30 - 6:00 PM (here)
- New homework will be posted tomorrow
- First gene assignment is on the website

Review conjugation mapping

- F- strain: met- tyr- aziR strS
- Hfr strain: met+ tyr+ aziS strR
- *How would you set up the experiment to map these genes by interupted conjugation?*

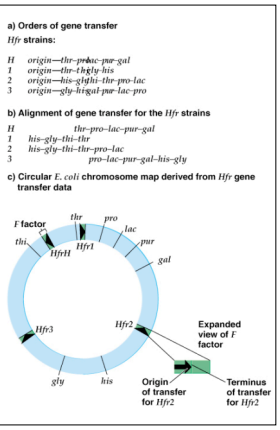
Hfr strain is the prototroph (+)

Use azide to select against Hfr donor cells

Use streptomycin to select against recipients.
What else could you use?

Variation in insertion and orientation of Hfr fragments

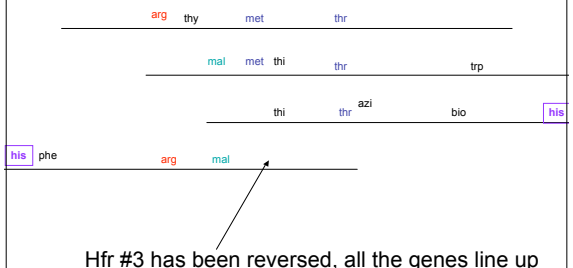
Overlap in transfer maps from different strains allow generation of a complete chromosomal map.



Example

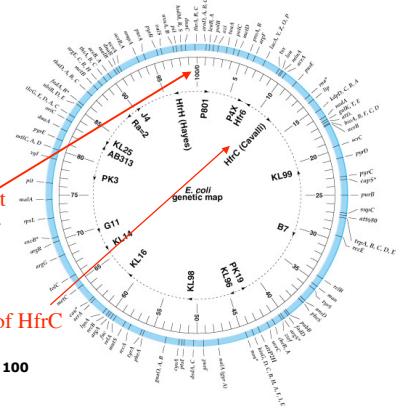
- Four different Hfr strains of *E. coli* were mated to F- recipients to determine the time of entry of various donor markers. The results are shown below.
  - Constuct a genetic map
  - What is the distance between adjacent marker pairs?

Strain	marker (time of entry)					
Hfr#1	arg (15)	thy (21)	met (32)	thr (48)		
Hfr#2	mal (10)	met (17)	thi (22)	thr (33)	trp (57)	
Hfr#3	phe (6)	his (11)	bio (33)	azi (48)	thr (49)	thi (60)
Hfr#4	his (18)	phe (23)	arg (45)	mal (55)		



Hfr #3 has been reversed, all the genes line up

Circular genetic map of *E. coli*

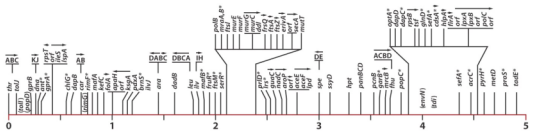


Arbitrary start point near *thr* 0 min

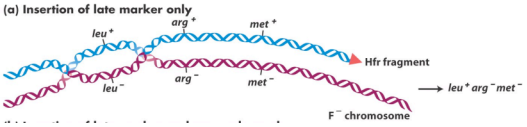
Location of HfrC

Total map units = 100 minutes

5 minute section of E. coli map

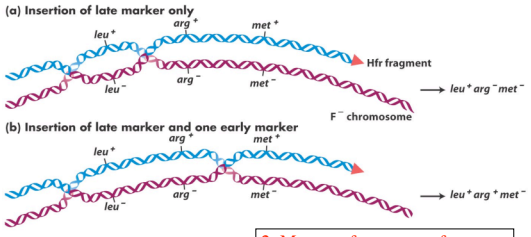


Use recombination frequency to map at finer scale



1. Select for late marker

Use recombination frequency to map at finer scale



1. Select for late marker

2. Measure frequency of arg+, met-, to get recombination between arg and met

How do you actually set up that experiment?

Q: What is the recombination distance between leu & arg and between arg & met?

Let's say you mix these strains:

Hfr leu+ arg+ met+ strS

X

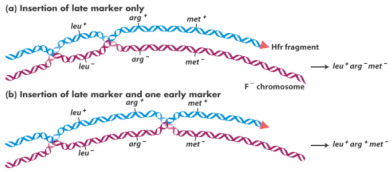
F- leu- arg- met- strR

What do you do next?

Recombination distances

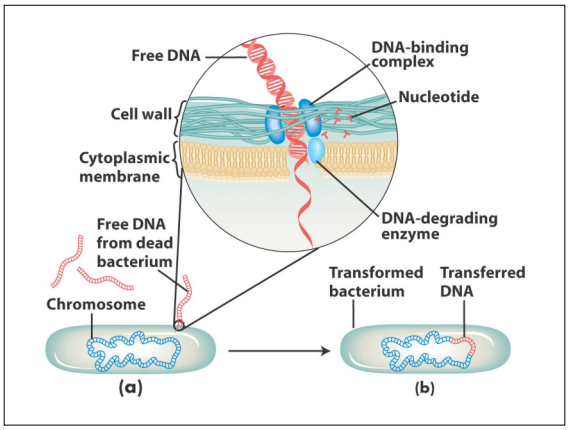
Results:

leu+ arg+ met+	85%
leu+ arg+ met-	10%
leu+ arg- met-	5% =5 cM



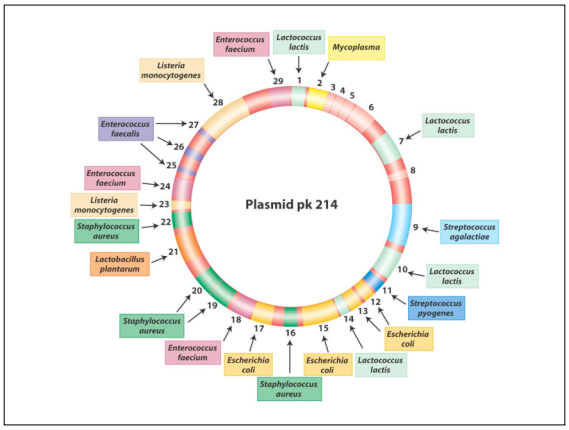
Transformation

- Remember Griffith's experiment?
- Bacteria can pick up free DNA
  - Cells must be "competent" to be transformed
  - DNA enters the cell as single-stranded DNA
  - Recombines with the homologous bacterial sequence to form a heteroduplex
    - *How does that differ from a diploid?*
  - After replication, one descendant carries the new gene



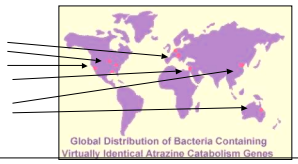
Does this happen in nature?

- In *E. coli* and *Salmonella*, roughly 17% of their genes have been acquired from other species (over 100 million years . . .)
- Such "horizontal transfer" is an important issue for the spread of antibiotic resistance

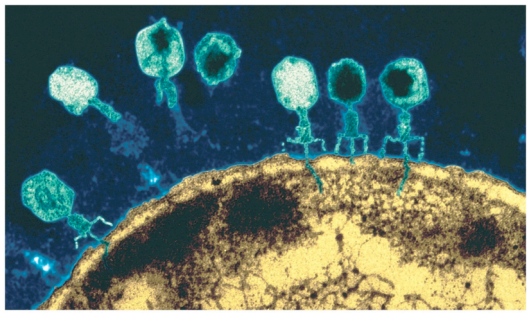


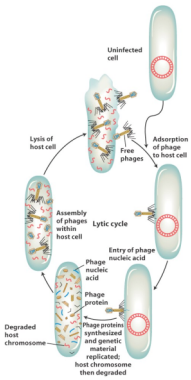
Spread of Atrizine decomposing bacteria

- A few bacterial species are capable of metabolizing the synthetic herbicide Atrizine
- All have nearly identical genes.



Transduction



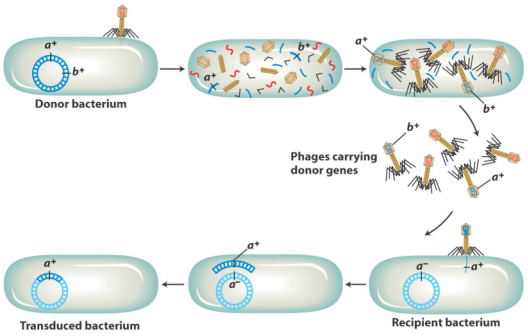


Generalized transduction

- Phage are viruses of bacteria
- Random piece of bacterial DNA incorporated into the phage
- Only small segments of chromosome can be taken up (<2 min)
- Mapping:
  - Look for co-transduction. If two genes commonly co-transduce, then they must be close together.

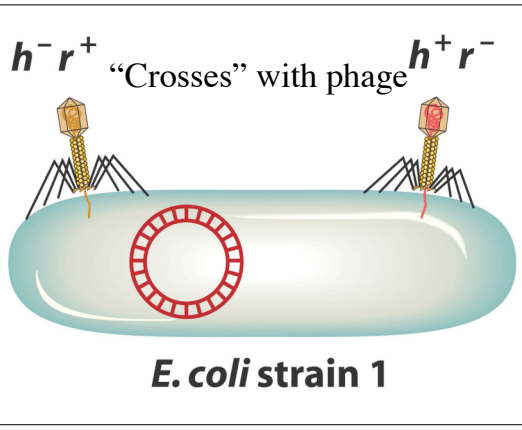
Transduction via phage

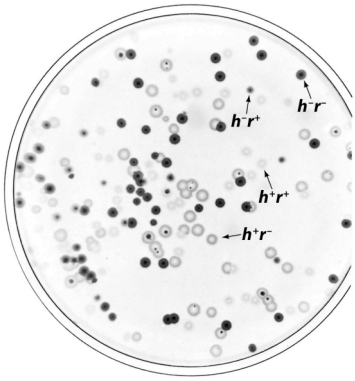
- Lambda, T1, T2, T4, etc
- Some are lytic, other lysogenic
  - (also known as "virulent" vs "temperate")
  - Temperate phage have prophage stage where they are integrated into the bacterial genome
- Transfer of genes between bacteria is still quite rare
  - *List all of the rare events that are required*



Example

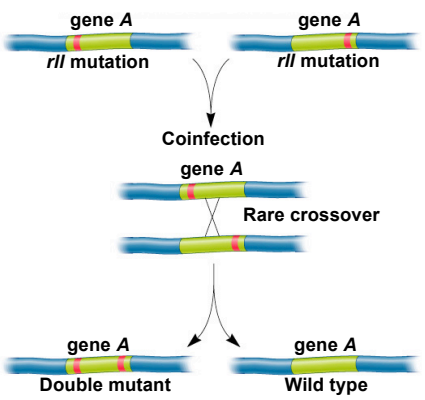
- Two E. coli strains:
  - Arg- met- Strep+ x arg+ met+ Strep-
    - Select for strep resistance and arg+
    - Then test if any of them also have met+
- Results:
  - Colonies in minimal plus met: 50
  - Colonies in minimal 21
  - 21/50 = 42% co-transduced
  - After some algebra, you can show that this corresponds to a distance of about 0.5 min
    - *If the genes were 1 minute apart, would the number of colonies growing on minimal medium be larger or smaller than 21?*

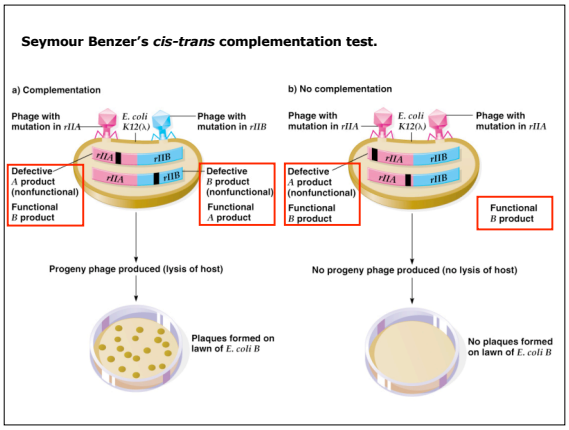


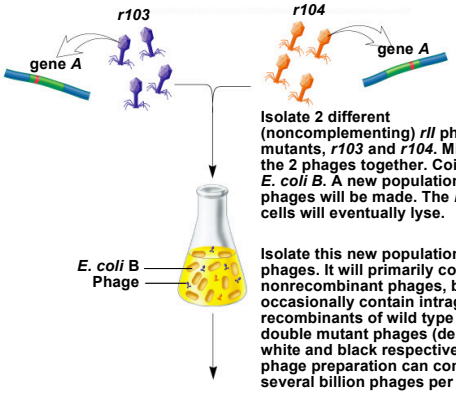


Intra-genic mapping

- Also called fine structure mapping
- Collect a huge number of independent mutants and test for complementation
- Use two non-complementing strains. If they can grow in the selective medium, the two mutants must have recombined within the gene to produce wild type.
- examine plaques for rare recombinants.
- Can find very rare recombination between neighboring mutations (down to a couple of bases).

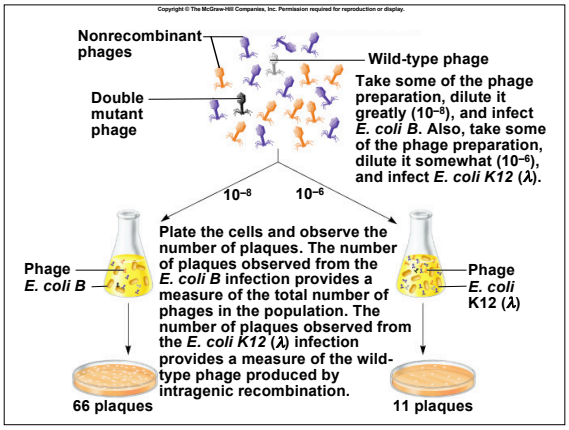


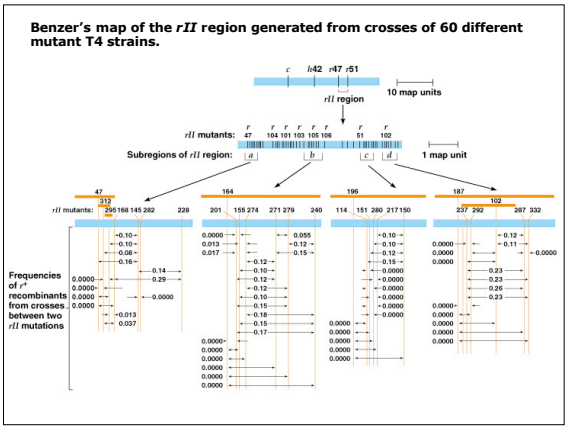




Isolate 2 different (noncomplementing) *rII* phage mutants, *r103* and *r104*. Mix the 2 phages together. Coinfect *E. coli B*. A new population of phages will be made. The *E. coli B* cells will eventually lyse.

Isolate this new population of phages. It will primarily contain nonrecombinant phages, but it will occasionally contain intragenic recombinants of wild type and double mutant phages (depicted in white and black respectively). The phage preparation can contain several billion phages per milliliter.





Benzer's deletion analysis of the rII region of T4:

No recombinants can be produced if mutant strain lacks the region containing the mutation.

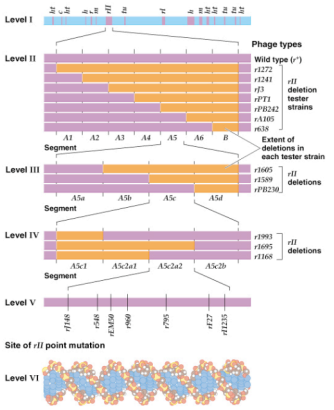


Fig. 14.22, Benzer's composite map of the *rII* region indicating >300 mutable sites on two different genes.

Small squares indicate point mutations mapping to a given site.