

Biology 322 Fall 2012

Horizontal transfer of genetic information in the bacterium Escherichia coli

⇒ *Before Thursday 11/8 read carefully through entire handout.*

- *Your Google doc submission should generally summarize what we are doing but not include specifics (ie of dilution series or plating)*
- *In your lab notebook, draw a flow chart outlining ALL dilution and plating steps*
- *Review bacterial conjugation in your genetics text.*

REQUIRED Reading Assignments:

Weapons of Microbial Drug Resistance Abound in Soil Flora

<http://fire.biol.wvu.edu/trent/trent/microbialresistance.pdf>

Superbugs on the Hoof

<http://fire.biol.wvu.edu/trent/trent/superbugs.pdf>

Triple threat Microbe Gained Powers from another Bug

<http://fire.biol.wvu.edu/trent/trent/triplethreat.pdf>

Introduction: *E. coli* strains can be divided into two groups defined on the basis of conjugational mating properties. F⁺ or male cells are able to donate chromosomal markers to recipient F⁻ or female cells, if mixed together under the appropriate conditions. F⁺ cells synthesize long, thin, protein filaments termed F pili, which are required for conjugation. (The F pili also serve as specific adsorption sites for a series of single-stranded DNA and RNA phages. These phages therefore infect only male cells.) All of the properties of the F⁺ cells are due to the presence of a small circular DNA element (plasmid) termed the F factor (or sex factor). The F factor is member of a class of elements called **episomes** which can replicate as a free circular genome in the cytoplasm or can be inserted into the main bacterial chromosome and replicate with the chromosome. Strains in which the F factor is stably integrated into the host chromosome are called Hfr strains. Not all Hfr strains are equally stable and many Hfr populations contain revertants in which the F factor is no longer integrated in the chromosome. Rare errors can occur during the excision of the F factor from the chromosome which result in the formation of **F' factors** carrying bacterial genes, for instance *lac* or *gal*. If an F' factor, for instance F' *lac*⁺ is transferred to a wild-type strain, then a partial diploid (or merodiploid) for the *lac* region is created.

Transfer of lac⁺pro⁺ from a F' to an F- strain.

Strain	Sex	Genotype
CSH23	F' ^{lac⁺proA⁺proB⁺}	ara+Δ(lacpro) spcR strS thi-
CSH50	F-	ara- Δ(lacpro) spcS strR thi-

The strR (aka strA) mutation confers resistance to streptomycin; the spcR mutation confers resistance to spectinomycin. A "Δ" indicates a deletion of the genes in parentheses. So Δ(lacpro) means lac⁻ pro⁻. thi indicates a requirement for thiamine; pro for proline. Strains with a lac

mutation cannot utilize lactose as a carbon source; strains with an ara mutation cannot utilize arabinose as a carbon source.

In the cross of strain CSH23 with strain CSH50, we wish to follow the transfer of the $F^{lac^+pro^+}$ plasmid from the CSH23 to the CSH50 genetic background. This could be done in two ways. In one, a mating mixture could be plated on glucose minimal plates with streptomycin. *What would be selected for in this case? Alternatively, we could use lactose minimal streptomycin plates. Again, what would be selected for using these plates? Will either parent survive?*


PROCEDURE:

Day 0 This will be done for you

Overnight cultures of the CSH23 and CSH50 will be set up for you in L broth (a rich medium).

Day 1 (Thursday 11/8) *SEE ALSO INSTRUCTIONS ON PG 1*

These cultures will be diluted and grown at 37° until the donor culture is $2-3 \times 10^8$ cell/ml. What is the quickest way to quickly determine #cells per ml? (This will be done for you.)

 Prepare a mating mixture by mixing 1.0 ml of each culture together in a small flask. Rotate at 30 rpms in a 37° shaking incubator for 60 minutes. At the end of the incubation:

Do serial dilutions:

Fill 6 tubes with 4.5 ml of sterile saline. Transfer 0.5 ml of the undiluted mating culture to one of the tubes. This is a 10^{-1} dilution.

Next make serial dilutions of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} & 10^{-6} . Always change pipets and mix well between dilutions.

Plate: 0.1 ml of a 10^{-3} , 10^{-4} and 10^{-5} dilution onto minimal + glucose + streptomycin + thiamine.

Plate: 0.1 ml of a 10^{-5} and 10^{-6} dilution onto a MacConkey + streptomycin plates. [A MacConkey plate is considered a rich media. It has lactose as well as other carbon sources. The phenol red dye is present to differentiate lac^+ colonies (red) from lac^- colonies (white).]

Controls:


Plate: 0.1 ml of a 10^{-1} dilution of donor (CSH23) cells on minimal + glucose + strep + thiamine plates. Repeat for the recipient (CSH50) cells.

Plate: 0.1 ml of a 10^{-5} dilution of the recipient on a MacConkey + strep plate.

Plate: 0.1 ml of a 10^{-1} dilution of donor on a MacConkey + strep plate.

Place all plates at 37° . Please don't tape your plates together. !

Day 2 (11/9 FRIDAY)

 Remove your plates from the incubator the next day. The dye in the MacConkey medium fades rapidly in the cold, so these plates need to be scored soon after incubation. Take some time on Friday to do this. You do not need to score the minimal plates on Friday. Store all plates at 4°C (refrigerator).

Day 3 (11/13 Tuesday)

1. Examine the MacConkey plates. What is the genotype of the white colonies? of the red colonies? From the total cell count on the MacConkey plates determine the total number of recipient cells per ml of the mating mixture.
2. Determine the % red colonies on a plate with 30-300 colonies. Is this a screen or selection for recombinants?
3. Count the number of colonies on the appropriate minimal plate. What is the genotype of the cells on the minimal plates? Do you expect these cells to be *lac*⁺? Are *lac*⁺ cells selected for? Determine the number of recombinants generated per ml of mating mixture.
4. Determine the percentage of cells in the CSH50 population which received the F' factor in the cross. There are two ways in which this % can be determined. Do the results of the two calculations agree?
5. Examine your control plates. What was the purpose of these controls. Be specific. Were there any colonies on your plates? How would you account for colonies on the donor plates? on the recipients plates? Which strain do you think is more likely to give rise to colonies on the control plates? Why?

→ There will be no formal lab report, but all raw data and your complete data analysis should be recorded in your lab notebook.

STUDY GUIDE FOR THIS LAB EXERCISE

1. Describe and analyze the strategy used for crossing these two strains. Which are the relevant mutations? What genotypes are selected for on each medium? Which markers are unselected? Be sure to indicate whether either parent will survive.
2. What are the results of the mating and the controls? Analyze the data as described above.
3. Be sure to address all of the questions listed in the preceding pages.
4. F' and F factors are lost fairly readily from *E. coli* cells. How would you maintain the CSH23 strain so that you were certain that only cells with the F' factor grew.
5. F' factors are useful in certain types of genetic analyses. Give an example of one.
6. There is a tremendous amount of horizontal gene transfer between bacterial cells. How is this gene transfer accomplished?
7. Describe the mode of action of streptomycin.
8. Describe at least three mechanisms by which a cell could acquire resistance to an antibiotic

DONATES AT A

<i>E. COLI</i> STRAIN	F FACTOR	HIGH FREQ.	LOW FREQ.
F⁻	none	nothing	nothing
F⁺	cytoplasmic (F factor integrated in 1/1,000 cells)	F factor	chromosomal genes (very low freq)
F[']	cytoplasmic carries a bit of the bacterial chromosome	F factor and bit of bacterial chromosome	chromosomal genes not on F' (very low freq)
Hfr → <i>an Hfr strain is a laboratory artifact</i>	integrated in all cells (but site of integration varies from Hfr strain to Hfr strain)	chromosomal genes (especially near site of integration)	F factor (very low freq)

F⁺ X F⁻ cross: Many F⁻ cells converted to F⁺; rarely: some bacterial genes transferred to F⁻ cells from rare cells with an integrated F factor

F['] X F⁻ cross: Many F⁻ cells converted to F^{'+}; rarely: some bacterial genes transferred to F⁻ cells from rare cells with an integrated F factor

Hfr X F⁻ cross: Bacterial genes transferred (from a fixed point in the chromosome in a fixed order) to F⁻ cells at a high frequency. Order of gene transfer varies from strain to strain depending on where the F factor has integrated. Few F⁻ converted to F⁺ because the F factor genes are the last to be transferred in any Hfr strain and most mating pairs don't stay together long enough for the entire chromosome to be transferred.