Part II: Transfer of genetic information in the bacterium
*Escherichia coli* with Hfr strains

You will receive separate homework and data analysis assignments related to this lab

**Introduction:** *E. coli* strains can be divided into two groups on the basis 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. Whereas every cell in an F+ population can transfer the sex factor to a female recipient, only a small fraction of the cells in an F+ population will transfer chromosomal markers. Why? *E. coli* strains exist in which the F factor is stably integrated into the host DNA in every cell. These are termed Hfr strains (high frequency recombination) since every cell now transfers chromosomal markers and the population as a whole displays a high frequency of transfer relative to an F+ population.

Once an Hfr strain has been isolated from an F+ population and purified, each cell transfers the chromosome in a linear fashion from a fixed starting point or origin, O. Hfr strains can be easily employed to provide mapping data based on the time of entry of markers. The first suggestion that the *E. coli* chromosome was circular came from such genetic studies that showed the genome to be a closed, continuous linkage group.

**Transfer of chromosomal markers from a Hfr to an F- strain.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>CSH 119</td>
<td>Hfr C</td>
<td><em>car::Tn10 Δ(gpt-lac)5</em> cysG metB</td>
</tr>
<tr>
<td>CSH 130</td>
<td>F-</td>
<td><em>ara C Δ(gpt-lac)5 gyrA rpoB</em></td>
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The gyrA mutation confers resistance to nalidixic acid and the rpoB mutation confers resistance to rifamycin. Δ(gpt-lac)5 is a deletion that removes a chunk of the *E.coli* chromosome including the proA and proB genes and the lactose utilization genes. *car::Tn10* is a complicated mutation. It results from an insertion of an *E. coli* transposon (called Tn10) in the *car A* gene. This insertion in the *car*
gene makes the cell auxotrophic for arginine and uracil. The Tn10 transposon itself carries a gene for \textit{tet}^R.

All genes not listed under genotype are assumed to be wild-type. The F factor in the Hfr C strain is integrated at about 12 minutes on the \textit{E. coli} map and transfers genes in the counterclockwise direction around the map.

**Day 0:** (This step will be done for you.) Cultures of CS119 and CSH130 will be set up and incubated overnight at 37\(^\circ\) C without aeration.

<table>
<thead>
<tr>
<th><strong>TUESDAY NOV 10</strong></th>
<th><strong>Day 1: Set up Hfr X F- cross</strong></th>
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<tbody>
<tr>
<td><strong>Day 1:</strong> (These two steps will also be done for you.) The overnight cultures will be diluted into fresh media two hours before setting up the following cross. In a large test tube, mix 0.5 ml of the donor strain with 0.5 ml of the recipient strain and let sit in a 37(^\circ) incubator for 90 minutes. Also set up a control with the donor strain alone and the recipient alone.</td>
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\begin{itemize}
  \item \textbf{Students start here:} After 90 minutes, add 5 ml of nutrient medium to each tube and incubate for an additional 1-2 hours (to allow the generation of stable recombinants). (Nutrient medium is a rich medium containing all the supplements that any auxotroph might need.)
\end{itemize}

**Plating of Mating Mixture:**
Plate 0.1ml of undiluted culture on a nutrient agar plate containing nalidixic acid and tetracycline. Also plate out 10\(^{-1}\) and 10\(^{-2}\) dilutions. Set up two plates of undiluted culture, two of the 10\(^{-1}\) dilution and one of the 10\(^{-2}\) dilution.

**Plating of Controls:**
Plate 0.1 ml of a 10\(^{-1}\) dilution of donor cells onto L + nal + tet plate Repeat for the recipient strain.
Plate all plates upside down at 37\(^\circ\) overnight.
**WEDNESDAY NOV 11: Day 2 Set up 50 colony grid**

Examine your cross and control plates for colonies

After setting up the colony grid (see below) store these plates at 4°C.

**Each student should set up one 50-colony grid. If you can’t grid your plate yourself, please have someone else set it up for you.**

Grid 50 recombinant colonies from the CSH130 X CSH119 cross onto an L plate.

- Place a fresh agar plate on the grid template.
- Be sure to place a mark on the top of your plate so you can reorient the colonies with respect to the grid on Friday. *NOTE: Your should NOT draw a grid on the bottom of the plate.*
- Touch a single colony on the cross plate lightly with the toothpick and then very gently touch/stroke the agar on the grid plate.
- **You should NOT be transferring visible amounts of bacteria.** In other words, a glob is too much.
- Use a different sterile toothpick for each colony.
- Don’t grid a given colony more than once
- Each student should grid 50 colonies
- Incubate plate upside down at 37°C overnight.

**THURS NOV 12: Day 3 Transfer colonies to selective media**

1. Count and record the number of recombinant colonies on the mating plates. Determine the number of recombinant cells per ml of mating mixture. Record observations on control plates

2. **EACH student should grid each of 50 colonies onto the 5 plates shown in the table below** [4 minimal plates (A-D) with various components added and rich media plus rifamycin (R)]

3. Also grid parental strains (plates will be available in the lab)
Supplements added | Marker selected
--- | ---
Plate A | proline, arginine, uracil
Plate B | proline, arginine, uracil, methionine
Plate C | proline, arginine, uracil, cysteine
Plate D | proline, arginine, uracil, methionine, cysteine
Plate R | Rich media with rifamycin

growth on plate?

<table>
<thead>
<tr>
<th>genotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>met+ cys+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>met+ cys G</td>
<td></td>
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</tr>
<tr>
<td>met B cys+</td>
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<tr>
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**Tuesday Nov 17th**

**Day 4 Determine genotype of each recombinants:**

- For each plate type (A-D) determine which markers are being selected for. In other words, which genotypes will or won't grow on each type of plate?
- Also score growth in the presence of rifamycin
- Determine the genotype of each recombinant clone based on its growth on the various media.
- Record your data on the Class Data Sheet
- Retest rare recombinants on a fresh set of selective media