

Interrupted Mating Experiments

Interrupted mating experiments are completed to map the order of genes on a chromosome. To complete these types of problems, it is pertinent to know how conjugation works and which cells can be donor cells. You will also need to understand the meaning of the data in an exconjugant graph as well as the fundamentals of selective plating. This worksheet integrates these concepts together through multi-step problems.

Background Review:

General Set-up and Procedure:

In a lab completing interrupted mating experiments, bacteria are allowed to undergo conjugation. A donor cell is selected that has a fertility factor (Hfr) and functional wildtype alleles for genes of interest. The selected recipient cell, noted as an F⁻ cell, has mutant non-functional alleles for the genes of interest. Genetic information can only move to the recipient cell because the recipient cell does not have a fertility factor. As conjugation occurs, the recipient cell integrates wildtype alleles for the genes into its chromosome or produces plasmids. Later, the bacterium can use these genes to survive. Any recipient cell that has received any genetic information from a donor is called an exconjugant.

At specific times, a scientist will interrupt the process of conjugation and see what genes the recipient cell has received. This is completed by plating recipient cells on selective media and noting which plates they grow on. Multiple plates are tested each one with a different combination of toxins and nutrients. Only bacteria which have a resistance for a toxin can grow on a plate with a toxin and only bacteria with a functional nutrient gene can grow on a plate where that nutrient is absent (not supplemented/provided). Bacterial growth, also known as colonies, is an indicator of bacterial genotype because of these properties.

Understanding the Data:

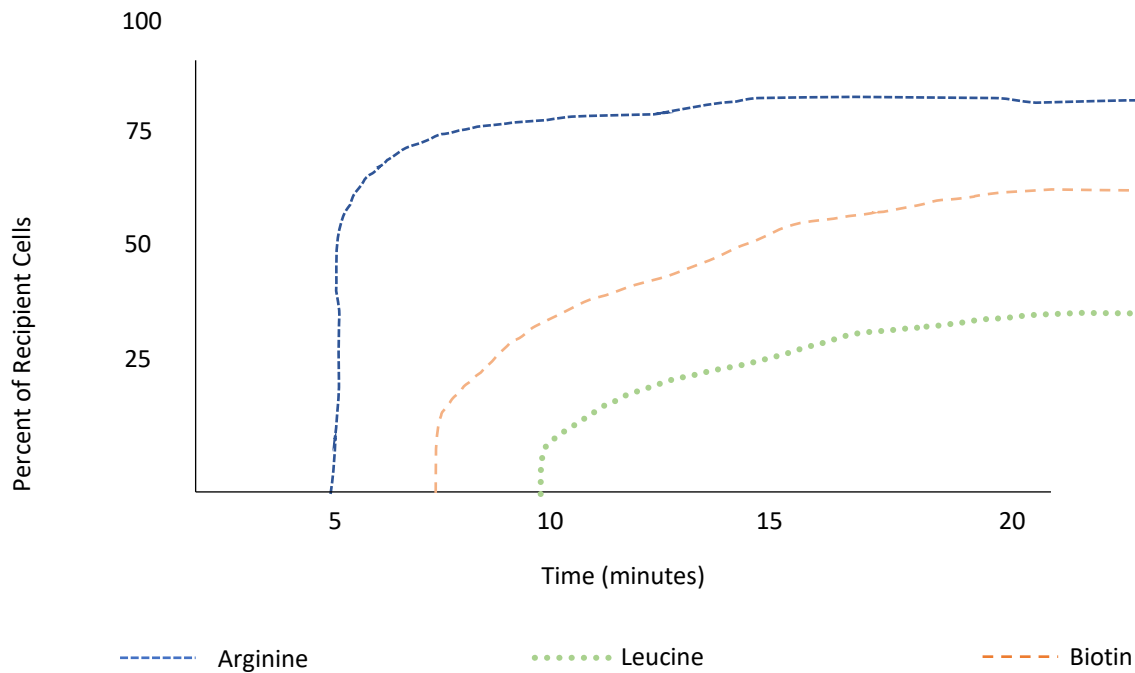
On an exconjugant graph, each line represents a gene. The x-intercept (where the line starts on the horizontal axis) is the exact moment the gene can first enter a recipient cell. Only after the gene enters the recipient cell can it be integrated into a chromosome or plasmid and then used for survival. The y-axis represents the amount of recipient cells with the gene. The further a gene is from the origin of transfer, the less likely it will enter a recipient cell before the sex pilus breaks. The further the gene is from the origin, the number of recipient cells with the gene decreases. The transfer of a fertility factor is rare, but it can occur. The larger the chromosome is, the less likely it will be donated, however.

All lines on an exconjugant graph have a similar shape. From the x-intercept, the line will have a steep increase until it nears an asymptote. Because the sex pilus can break, no lines will reach a point where the number of recipient cells is 100%. Instead, they will reach near their maximum amount of cells and then remain nearly constant. Genes that are further from the origin will have a horizontal asymptote at a much lower level than those genes that get transferred earlier.

Practice:

To fully understand how interrupted mating experiments work, you should complete a lot of practice problems. Be able to explain your reasoning for each problem and understand why the answer is correct due to the process of conjugation.

1. A *bio⁺ leu⁺ arg⁺* Hfr cell and a *bio⁻ leu⁻ arg⁻* *F* cell are used in an interrupted mating experiment. Use the following graph to answer the problems below.



- a. What is the genotype of the donor cell and what is the genotype of the recipient cell? How do you know which is the donor and which is the recipient?
- b. Which gene entered the recipient cell first?
- c. At exactly 5 minutes, approximately how many recipient cells have a functional wildtype arginine allele?

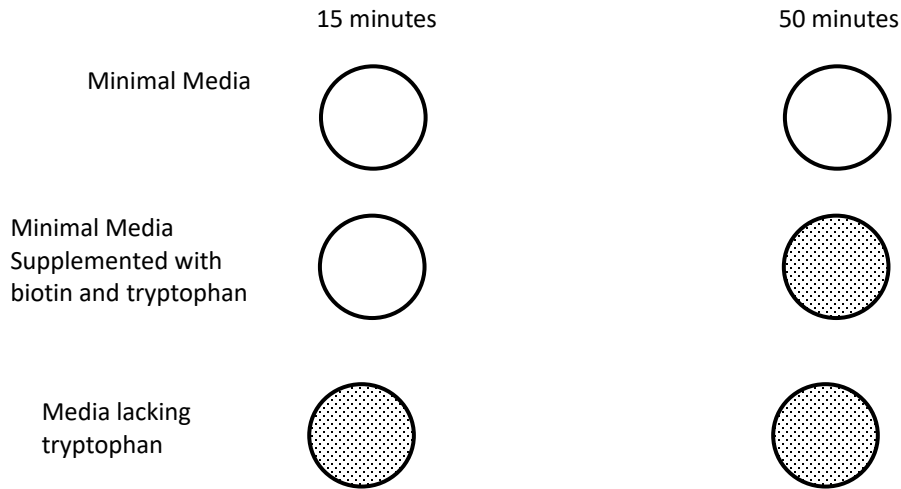
- d. At 5 minutes, would you expect to see exconjugant growth on a media lacking arginine?

- e. At 6 minutes, would you expect to see exconjugant growth on a media lacking arginine?

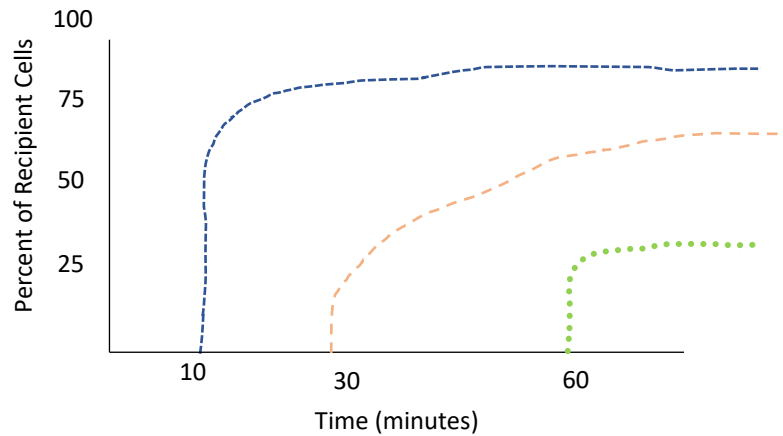
- f. This graph does not show the fertility factor gene. Draw in a sketch of a possible line for this gene on the graph above.

- g. Sketch the bacterial chromosome. Make sure to mark the origin and direction of transfer.

2. Use the following plates to answer the questions below. Dots represent colonies (growth).

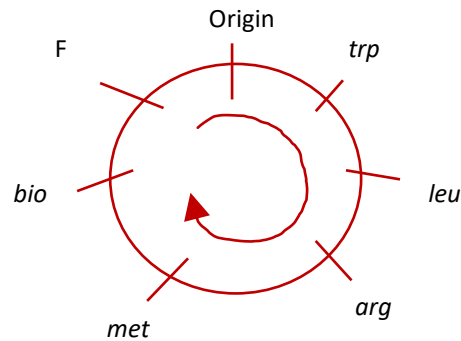


a. Identify which line represents biotin, leucine, and tryptophan genes.

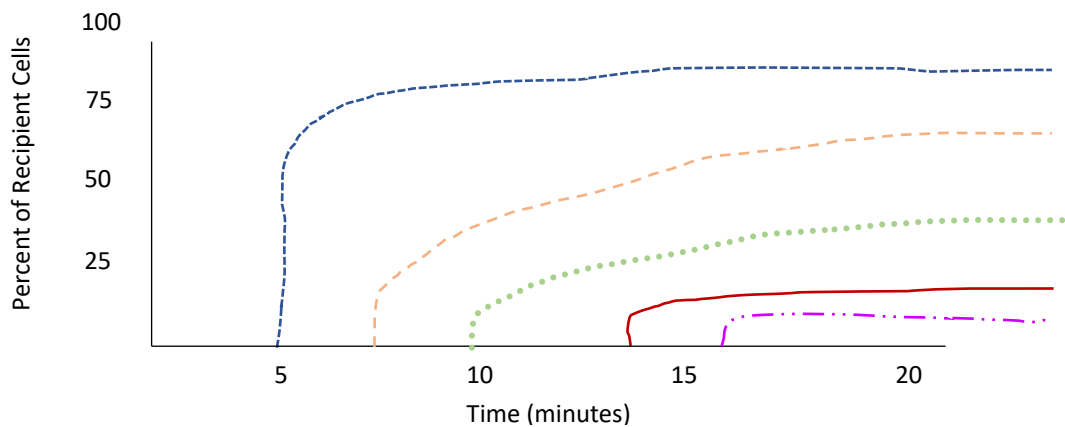


b. Sketch the bacterial chromosome. Make sure to mark the origin and direction of transfer.

3. You mate a *trp⁺ leu⁺ arg⁺ met⁺ bio⁺* Hfr cell with a *trp⁻ leu⁻ arg⁻ met⁻ bio⁻* F⁻ cell. Use the following chromosomal map to answer the problems below.



- a. Identify the lines in the following exconjugant graph.



- b. Indicate whether you would see growth on the following plates at 15 minutes:
- ☐ A plate supplemented with only biotin and arginine.
 - ☐ A minimal media with leucine and arginine.
 - ☐ Complete media.
- c. Indicate whether you would see growth on the following plates at 5 minutes:
- ☐ A plate supplemented with only biotin and arginine.
 - ☐ A minimal media with leucine, arginine, methionine, and biotin.
 - ☐ Complete media.