

# Chapter 1

## Bacterial Gene Transfer

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## Introduction

This laboratory exercise is used at Northwestern for the introductory biology sequence during the quarter that covers the topics of Genetics and Evolutionary Biology. This is the first quarter of the introductory biology sequence. The majority of the students take this course during their sophomore year and most are either biology majors or students taking the pre-medicine curriculum. The students are taught in groups of 24 and work in pairs. This laboratory is relatively inexpensive to run and spans three 2 1/2 hour long laboratory sessions. It concentrates on some important microbial techniques including the students first exposure to sterile technique. The bacterial strains can be purchased from Cold Spring Harbor Laboratory Press. Additional information found in *A Short Course in Bacterial Genetics* (Miller, 1992)

## Student Outline

### Introduction

The ability to exchange genetic material between two cells is essential for the evolution of life. There are some important differences in how a eucaryotic cell and a procaryotic cell accomplish this task. You have already seen the eucaryotic version in your fruit fly experiments. When cells undergo the process of meiosis, genetic material is exchanged between homologous chromosomes during the first meiotic division. This process, recombination, generates new gene combinations in the gametes not found in the diploid progenitor cell. Further diversity is achieved upon the union of two haploid gametes to form a zygote which receives half its genetic material from one parent and half from the other parent.

Unlike eucaryotic cells, procaryotic cells have a single circular chromosome that is not contained within a nucleus. Bacteria are haploid and “reproduce” by simple binary fission; one cell gives rise to two new cells. There are several different ways for genetic recombination to occur in bacteria: transformation, transduction, and conjugation. The process of transformation happens when a bacterial cell picks up a piece of DNA from its environment and incorporates it into its chromosome. (Also, transformation can occur if the DNA taken up is a plasmid that is able to replicate in the host bacterium.) Transduction is the process by which a bacterial phage (virus) picks up a piece of DNA from one bacterial cell and injects it into another where it is incorporated into the chromosome. The third process, conjugation, occurs when one bacteria transfers DNA to another bacteria. It is this process that will be investigated here.

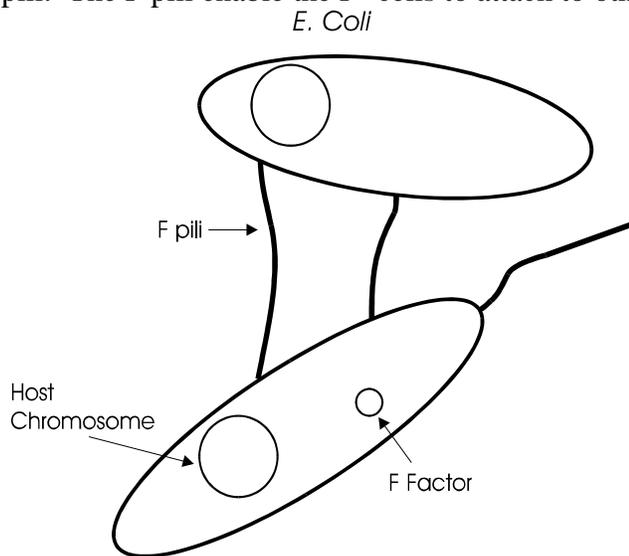
## Auxotrophs

Bacteria can be easily maintained and grown in a laboratory environment either as a suspension in a test tube filled with liquid media containing all of the nutrients needed for growth or on a solid agar support in a plastic Petri dish also containing the essential nutrients. Minimal medium contains only the essential nutrients: a carbon source (usually glucose), inorganic salts, and water. Bacteria are prototrophic organisms which means that they can produce all needed nutrients using an energy source and salts. Occasionally, mutants can be isolated that will no longer grow on minimal media. These biochemical mutants are called auxotrophs and require the addition of certain nutrients to the minimal medium in order to grow.

Auxotrophs were used by Joshua Lederberg and Edward Tatum in 1946 to show that bacteria could transfer genetic material from one cell to another. Lederberg and Tatum worked with two different auxotrophic strains of *Escherichia coli*. They mixed the two strains in a test tube and incubated them for several hours in a rich liquid media containing all of the supplements needed. Following the incubation, the cells were plated on minimal media. Since the two strains were auxotrophs for different supplements, one would expect no growth on minimal medium. Some bacteria did grow suggesting that some form of recombination had occurred between the genomes of the different strains. This recombination occurred as a result of conjugation, the transfer of DNA from one bacterial cell to another bacterial cell.

## Conjugation

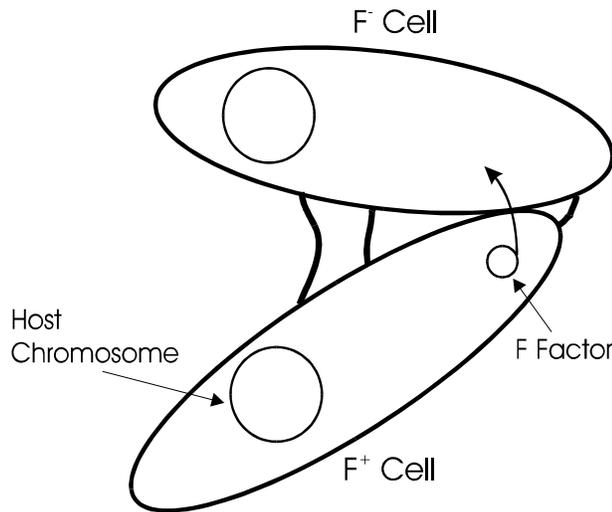
In 1952, William Hayes showed that the transfer of DNA between bacterial cells was directional with one cell acting as the donor and the other cell acting as the recipient. It was found that the donor cells contain a fertility factor ( $F^+$ ) whereas the recipient lacks the fertility factor ( $F^-$ ). F is a small circular piece of DNA called an episome or plasmid (Figure 1.1). The F factor is about 100 kbp; the *E. coli* genome is about 4,000 kbp. It can replicate autonomously within the bacterial cell or can integrate into the host chromosome. Genes found on F encode fibrous proteins that make up minute tubules called pili. The F pili enable the  $F^+$  cells to attach to other cells (Figure 1.1).



**Figure 1.1.** The F factor (F).

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Once the two cells are in contact, a pore forms that allows the transfer of DNA from the ( $F^+$ ) cell to the  $F^-$  cell (Figure 1.2). The  $F^+$  cell transfers a copy of its DNA to the  $F^-$  cell as the DNA is being synthesized. When the transfer is complete, the  $F^-$  cell contains a copy of F and is now considered an  $F^+$  cell. It is also possible for the F factor to integrate into the host chromosome and thus can transfer parts of the host chromosome. In a population of cells that have the F factor in the cytoplasm, a small fraction of the cells will have F integrated into the chromosome. This population can therefore transfer chromosomal markers at a very low efficiency due to the cells that have F integrated into the chromosome. This transfer is what Lederberg and Tatum saw in their experiment in 1946.



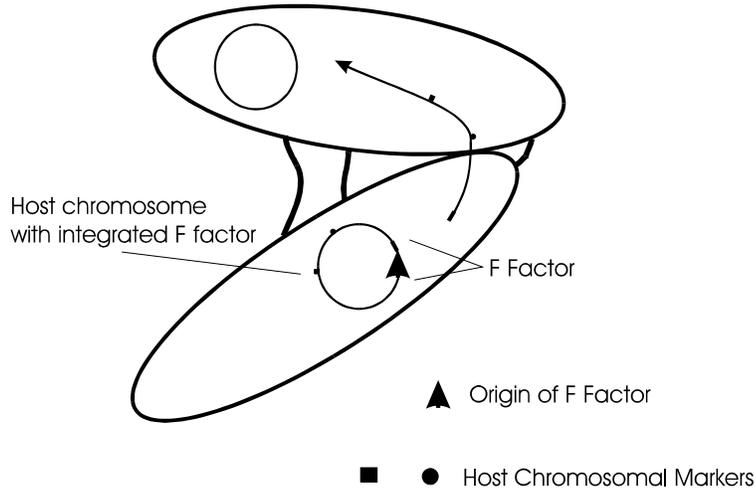
**Figure 1.2.** DNA transfer from an  $F^+$  cell to an  $F^-$  cell.

Cells containing F integrated into the chromosome can be isolated and purified so that you now have a pure population of cells. This strain of cells will be able to transfer host chromosomal markers at a high rate. Such strains are called Hfr for high frequency of recombination (Figure 1.3). Because the F factor integrates at a specific point on the circular bacterial chromosome, an Hfr strain will transfer genetic markers from the host in a fixed order from that point. This ordered transfer of markers has been used to map mutations and thus map gene locations on the bacterial chromosome.

### Chromosome Mapping and Gradient of Transfer

In 1957, Elie Wollman and Francois Jacob performed an experiment which they called the interrupted-mating procedure. They mixed an Hfr strain that was streptomycin sensitive and wild type for four different genes ( $str^S a^+ b^+ c^+ d^+$ ) with an  $F^-$  strain that was streptomycin resistant and auxotrophic for the same four alleles ( $str^R a^- b^- c^- d^-$ ). At specific times following the mixing, a fraction of the sample was removed and put in a blender for several seconds. The blending interrupted the mating between Hfr and  $F^-$  cells. The bacteria were then plated on media containing streptomycin. The Hfr strain did not grow as it is sensitive to streptomycin. Any cells that grew were transferred to media that did not contain the product encoded by genes  $a$ ,  $b$ ,  $c$ , or  $d$ . This part of the experiment tested for the presence of the alleles that were transferred from the Hfr donor. Each of the

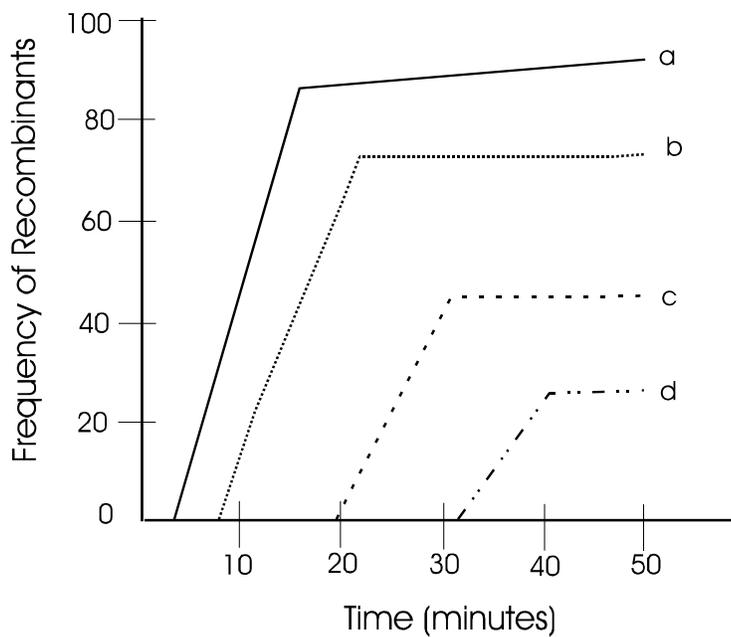
four donor alleles appeared at specific times after mating began, and a plot of frequency of recombinants for each marker



**Figure 1.3.** Hfr bacterial strains.

allele as a function of time after mating revealed that the donor alleles also appeared in a specific sequence (Figure 1.4). In addition, smaller numbers of cells were found containing the alleles that entered later. From this data, it was concluded that gene transfer begins from a specific point on the chromosome called the origin of transfer, and that the transfer occurs in a linear fashion. These results

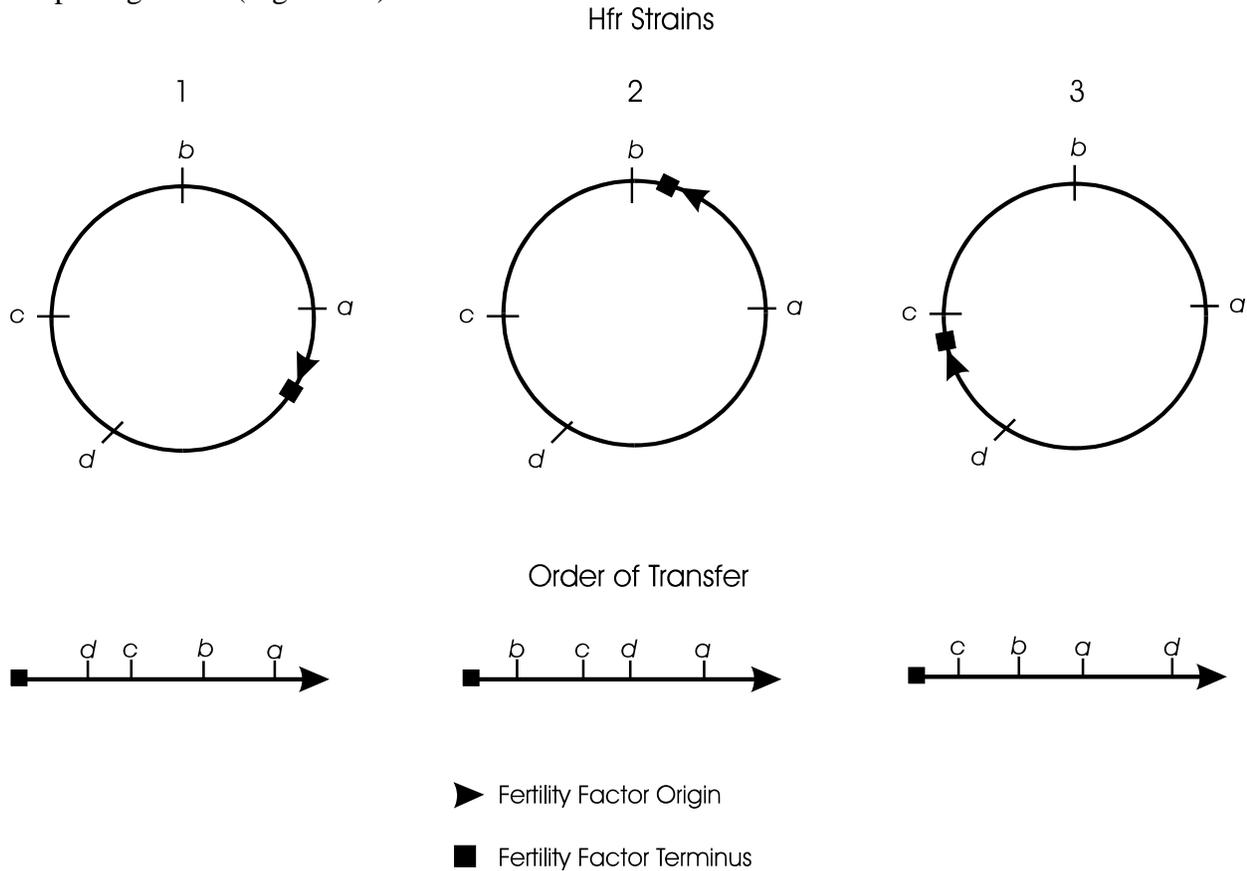
can be used to generate linkage maps of genes that are found on the bacterial chromosome. The distances are described as minutes on the chromosome as the matings were disrupted at various times (minutes) following the mating.



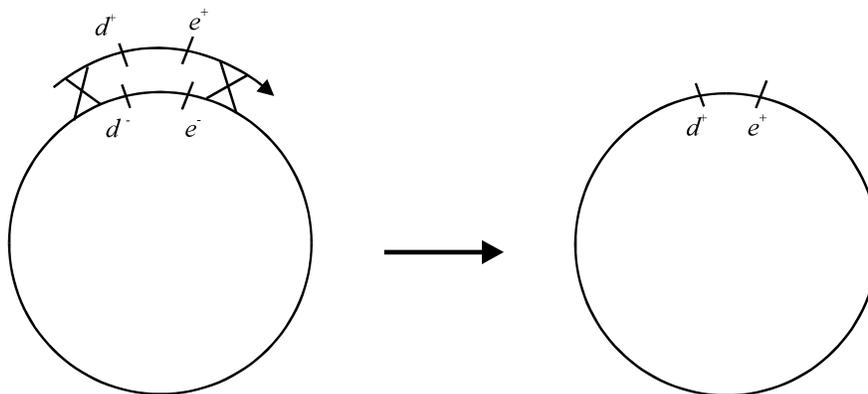
**Figure 1.4.** Frequency of recombinants for each marker allele.

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An F factor can integrate into the host chromosome at any point thus resulting in different Hfr strains that have the origin in different locations and orientations (Figure 1.5). The terminus of the fertility factor is the last part of the factor to enter a cell during gene transfer, and as a result, is transferred in only a small number of cells. After the donor genes are transferred to the recipient cell, they must become integrated into the recipients genome or they will be lost. This recombination event results from two crossovers between homologous regions of the donated fragment and the recipient genome (Figure 1.6).



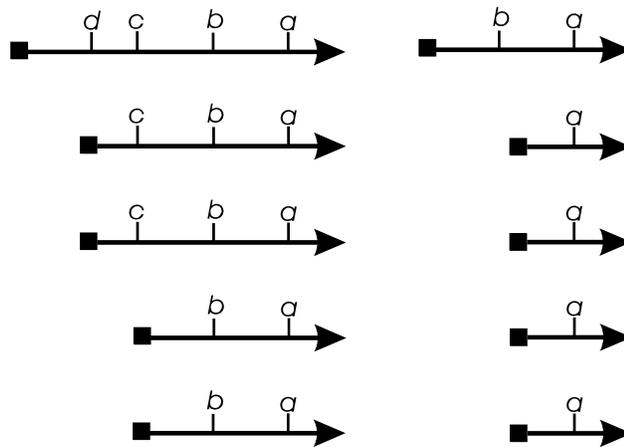
**Figure 1.5.** Origin and terminus of the fertility factor in different Hfr strains.



**Figure 1.6.** Recombination of donated fragment with recipient genome.

When an Hfr strain transfers its DNA to an F<sup>-</sup> strain there is a gradient of transmission in which earlier genes are transferred at a much higher frequency than later genes. This gradient results from the spontaneous breaking of mating pairs thus cutting short the transfer of DNA. One can conclude then that the closer a gene marker is to the origin, the greater the chance it will be transferred to the recipient during conjugation (Figure 1.7). Thus, the interrupted-mating experiment performed by Wollman and Jacob can be done using the inherent disruption of mating pairs instead of a blender.

In this exercise, you will be performing a gradient of transfer experiment to determine the location of various markers on the *E. coli* chromosome. Initially, a series of Hfr strains are used to locate the approximate position of a gene marker. After the positions of several markers have been determined, a gradient of transmission experiment can be done to locate the map position of unknown markers. Using the known position of some markers, a graph of the gradient can be made to determine the position of the unknown markers. Such a graph of percent linkage and map position in minutes is shown in Figure 1.8. In this example, an Hfr strain ( $a^+ b^+ c^+$ ) was crossed with an F<sup>-</sup> strain ( $a^- b^- c^-$ ). From previous experiments it is known that gene *a* maps to 1 minute and gene *c* maps to 20 minutes and is linked to *a* approximately 20% of the time. The location of gene *b* can be extrapolated from a graph where the map position is expressed linearly in terms of minutes from the selected marker (*a*) and the percentage of inheritance with *a*. From the graph we can determine that the map location for gene *b* is at 8 minutes, since experimentally it was found to be linked to gene *a* approximately 40% of the time.

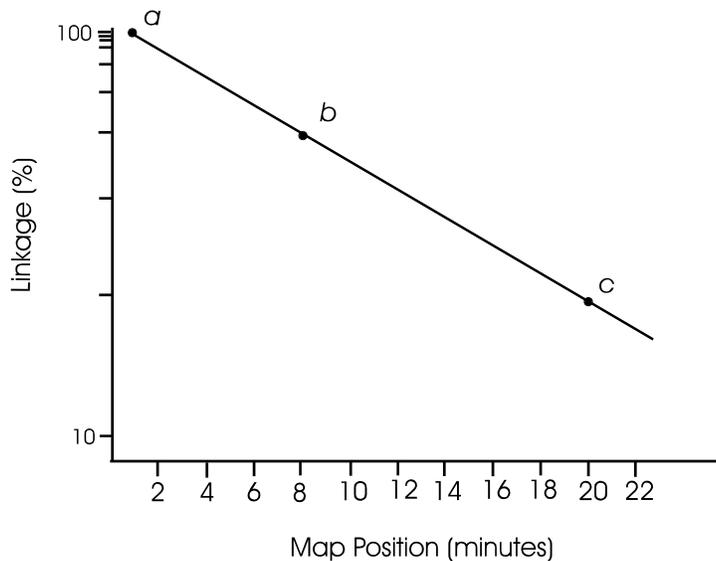


**Figure 1.7.** Distribution of fragments donated from an Hfr strain.

In the cross described above it is crucial to select for the progeny of the cross and counterselect against the parents. In this case, the Hfr donor is sensitive to the antibiotic streptomycin and the F<sup>-</sup> recipient is resistant to streptomycin. Therefore, none of the Hfr bacteria will grow on plates containing streptomycin. Following the mating, the cells are plated on minimal media with streptomycin and without the product of gene *a*. Since the F<sup>-</sup> strain is  $a^-$ , only those F<sup>-</sup> cells receiving gene *a* in the transfer will grow on this media. Subsequently, these recombinants can be tested on plates that lack the products of genes *b* and *c*. These results will give the percentage of inheritance of each allele with gene *a* thus allowing us to determine the gradient of transmission.

## Sterile Technique

Bacteria are classified into different categories based on whether they present a hazard under shipping and handling conditions. Nonpathogenic strains, such as the *E. coli* that you will be working with, present no or minimal hazards whereas *Salmonella* does, and should be treated with caution. Whenever you are working with bacteria it is a good idea to treat the bacterial strain as though it were pathogenic and thus, you will always have good sterile technique regardless of the pathogenicity of the strain. It is also important to use good sterile technique in order to avoid contaminating your sample with outside bacteria or contaminating the working area with your sample. Several rules should be followed when working with bacteria: the benchtop should be cleared of any unneeded materials; you should wash your hands thoroughly before and after the experiment; flame containers and instruments when appropriate; and dispose of all bacterial waste in the biohazard trash.



**Figure 1.8.** Gradient of transmission. Note that semilogarithmic graph paper is used.

When bacteria are plated onto a solid media at the proper dilution, a single cell will divide forming daughter cells that remain together in a clump. This clump is called a colony and contains more than  $10^7$  cells that were derived from one original cell and thus have identical genomes. Proper sterile technique is important to be able to generate single colonies. Single colonies are used to confirm the genetic makeup of the bacteria with which you are working. Single colonies also confirm that your population is not contaminated with another unwanted bacteria.

## Laboratory Procedures

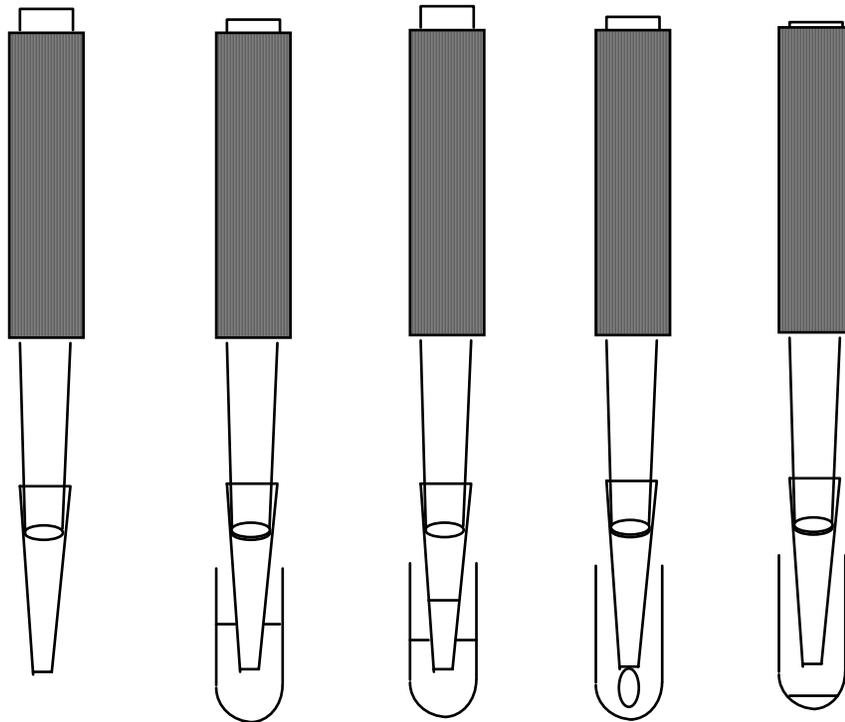
In this experiment you will be working with a laboratory partner. You will be working with two different strains of *E. coli*: CSH121 (Hfr) and CSH125 (F<sup>-</sup>). CSH121 is *Str<sup>s</sup>, Leu<sup>+</sup>, His<sup>+</sup>, Arg<sup>+</sup>, Ade<sup>+</sup>, Trp<sup>+</sup>, Tet<sup>r</sup>* and CSH125 is *Str<sup>r</sup>, Leu<sup>-</sup>, His<sup>-</sup>, Arg<sup>-</sup>, Ade<sup>-</sup>, Trp<sup>-</sup>, Tet<sup>s</sup>*. During the first week of the experiment, you will set up a mating between the two strains and plate serial dilutions onto selection plates lacking leucine and containing streptomycin. During the second week, you will be picking

isolated colonies onto several different minimal media plates: one containing tetracycline, one without adenine, one without tryptophan, one without histidine, and one without arginine. On the third week you will check for growth on each of the plates and analyze the results.

When following the laboratory procedures always read one or two steps ahead so that you are prepared for what comes next.

### **Part 1: Mating CSH121 and CSH125**

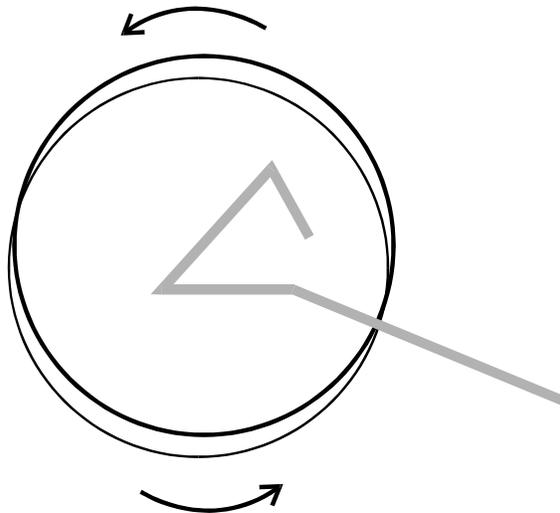
1. Actively growing cultures of both strains (in rich media) have been started for you by the preparatory staff. On the TA bench you will find several tubes containing liquid cultures of each strain. Return to your bench with these cultures and light your bunsen burner.
2. Using a marker, label three test tubes with your initials and CSH121/CSH125, CSH121, and CSH125. The CSH121 and CSH125 tubes will be your controls.
3. With one hand, remove the lid of the CSH121 container and hold it between your fingers; do not set it down. Using the other hand, pass the mouth of the container through the flame several times to warm the opening. Be careful not to overheat the container. Transfer the container to the hand that is holding the lid.
4. While you are doing step three your lab partner should be removing and flaming the mouth of the test tube that you labeled CSH121.
5. Using a pipetman and a sterile pipet tip, transfer 200  $\mu\text{l}$  of CSH121 to the test tube. Instructions for using a pipetman can be found in Step 6. Quickly warm the mouth of each container before replacing the lid. You will want to do these steps as quickly as possible so as not to contaminate your sample. Also, close the lid on the pipet tip container when you are not using it as it can easily be contaminated with bacteria from the air.
6. The TA will demonstrate how to use the pipetman. When using this kind of pipetman, you will need to first place a clean tip on the barrel of the pipetman by stabbing one from a rack. Next, depress the button on top to the first stop (Figure 1.9). You must depress the button slowly, otherwise you will not “feel” the first stop. While keeping the button depressed, place the tip into the solution. Slowly release your finger from the button and solution will be drawn up into the tip. Place the pipet with tip attached into a second tube and depress to the first notch and the solution will be forced out of the tip. Before removing the tip from the solution, depress to the second notch to expel any remaining solution from the tip.
7. Discard the pipet tip in the biohazard trash.
8. Repeat the above procedure until you have added 200  $\mu\text{l}$  of CSH121 to the tube labeled CSH121/CSH125 and 200  $\mu\text{l}$  of CSH125 to the tubes labeled CSH125 and CSH121/CSH125.
9. Place the tubes in a 37°C waterbath for one hour.
10. After one hour, using the same sterile transfer technique described above, add 2 ml of LB broth to each tube. Put the tubes into a 37°C incubator shaker for one hour.



**Figure 1.9.** Adjustable Pipetman.

11. During the incubation, label three Petri dishes containing streptomycin and no leucine (Leu<sup>-</sup>). Label the bottom side of the dish with your name, section number, date, and 1:100, 1:1,000, or 1:10,000. Also label two more plates CSH121 and CSH125 for your controls. Label around the perimeter of the plate so that it will be easier to see colonies when they grow. You will always label the bottom of a Petri dish and not the lid so that if the lid gets confused with other lids you can always identify your sample.
12. Also during this incubation, label four microcentrifuges tubes 1:10, 1:100, 1:1,000 and 1:10,000 for the mating experiment. Add 100  $\mu$ l of minimal broth to each tube. Also label and add broth to two tubes labeled CSH121 and CSH125 for your controls. You will be plating only a 1:10 dilution of the controls. Keep the microcentrifuges tubes closed until you need them.
13. Following the incubation and using the sterile transfer technique, remove 10  $\mu$ l of the CSH121/CSH125 mix and add it to the microcentrifuge tube labeled 1:10. Gently mix the microcentrifuge tube by tapping the side of the tube. Next, transfer 10  $\mu$ l of the bacterial suspension from the 1:10 tube to the 1:100 tube. Again mix the tube and transfer 10  $\mu$ l from the 1:100 tube to the 1:1,000 tube and mix. Lastly, mix the tube and transfer 10  $\mu$ l from the 1:1,000 tube to the 1:10,000 tube and mix. This process is called a serial dilution. Note that a proper 1:10 dilution would be made by adding 10  $\mu$ l into 90  $\mu$ l but we are limited by equipment.

14. You will be plating all of the bacteria from each dilution tube onto the appropriately labeled Petri dish. Plate one dilution at a time and spread the cells before proceeding to the next tube. Using a pipetman (with tip) transfer the contents of the 1:100 tube to the plate labeled 1:100. Transfer the bacteria to the center of the plate. Lift the lid of the Petri dish over the bottom of the plate and only lift the lid high enough to add the bacteria to the media.
15. You will be using a bent metal or glass rod to spread the bacteria over the surface of the media. First, dip the rod into a beaker containing ethanol. Hold the rod at a slight downward angle so that the ethanol does not drip onto your hand. Next, place the rod briefly in the flame of your bunsen burner until the ethanol ignites. Cool the rod in the air for several seconds. Lift the lid of the Petri dish and touch the glass rod to the agar surface (away from the bacteria) to cool the rod. Using the rod, spread the bacteria from one side of the plate to the other (Figure 1.10). Without setting down the rod, replace the lid and rotate the plate approximately 90°. Use the rod to again distribute the bacteria across the surface of the plate. Close the lid and rotate and spread a third time.
16. Dip the glass rod in ethanol and flame it before plating each of the 1:1,000, 1:10,000 dilutions and the controls.
17. Allow the plates to dry for several minutes. Any excess broth will be absorbed into the agar. Stack your plates on top of each other and run two pieces of tape down opposite sides of the stack. Invert the stack (bottom agar side up) and place it on a tray on the TA bench. If the lid is placed on the top side and condensation forms, it could drip on to the surface of the media and wash some cells from one colony into another colony.
18. Place the plates in a 37°C incubator for 1-2 days or until colonies appear.



**Figure 1.10.** Spreading bacteria.

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### Part 2: Gradient of Transmission

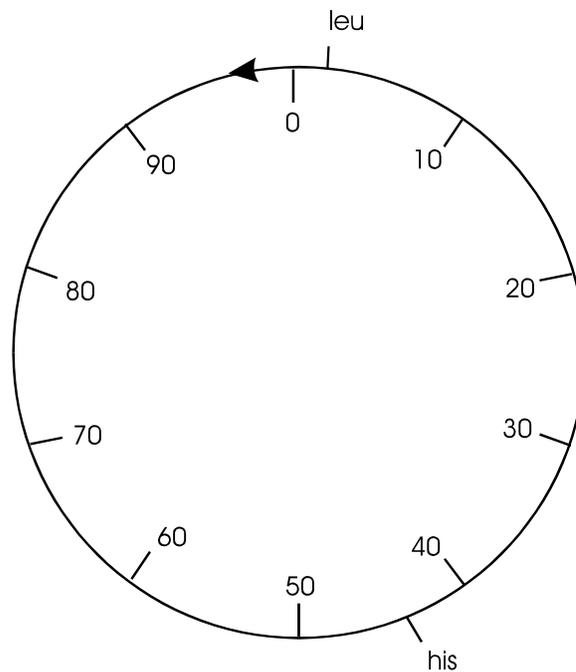
1. Retrieve your plates from the TA bench. Estimate the amount of growth on each plate (none, some, lots) and record it on the data page.
2. You will be picking a total of 100 colonies from the CSH121/125 plate(s). Identify which plate will give you that number and allow you to pick single colonies easily.
3. At the end of this exercise, you will find three pages containing grids for picking colonies. Place the seven grids on the bench in front of you and place the plates on the appropriate grids. It is useful to roll up a small piece of Scotch tape and use it to stick the plate to the grid. This will keep the plate in the proper orientation during the rest of the procedure. Using a marker, make a line on the outside edge of the bottom of the Petri dish at the location of the arrow on the grid. This will allow you to align the plates with the grids. Each grid contains a total of 50 locations. In order to pick 100 colonies you will be using two sets of plates. You should have a total of fourteen plates: four without leucine, two without histidine, two without arginine, two without adenine, two without tryptophan, and two with tetracycline.
4. At your bench, you will find a small beaker covered with foil which contains sterile tooth picks. Using the flat end of a toothpick, pick a single colony from the CSH121/CSH125 plate and pierce the agar at the number 1 location on the first grid. Remove the lid only long enough to transfer the colony. Without setting down the toothpick, pierce the same numbered location (#1) on each of the seven plates. You will always start and end with a leucine minus plate. In Part 1, you selected for colonies on a leucine minus plate and therefore, if you have picked a colony properly all 100 positions on the first plate should have growth. The last leucine minus plate is a control to make sure that you had sufficient bacteria to carry through to all seven plates. Discard the tooth pick in the biohazard trash after the seventh plate.
5. Continue picking colonies until all 100 positions have been used. Discard the tooth picks in the biohazard trash. Remember to use a new toothpick for each new colony.
6. Label the plates around the perimeter with your name, section number, and date.
7. Stack your plates on top of each other and run two pieces of tape down opposite sides of the stack. Invert the stack (bottom agar side up) and place it on a tray on the TA bench.
8. Place the samples in a 37°C incubator for 1-2 days or until colonies appear.

### Part 3: Data Analysis

1. Retrieve your plates from the TA bench.
2. Record on the data page the number of colonies that grew on each plate. Any colony that grew on the first leucine minus plate but not the last leucine minus plate should not be included in the total.
3. Return your plates to the TA bench so that they can be disposed of in the biohazard trash.

**Report**

1. Explain the purpose of the controls in this experiment.
2. It is necessary to make dilutions prior to plating in Part 1 in order to obtain single colonies. Why is it important to be able to obtain single colonies in this experiment?
3. Using the data collected, generate a graph showing percentage of inheritance with the selected marker leucine at 100%. Leu maps to 2 minutes and His to 44 minutes. Turn in the graph with your report.
4. What is the approximate location (in minutes) for Arg, Ade, Trp, and Tet?
5. Draw a map of the Hfr strain CSH121 below. The approximate location and orientation of the origin is diagrammed for you.



6. You are given another Hfr strain (CSH119) that has all of the same markers as CSH121. Design an experiment that will locate the approximate location and orientation of the origin in CSH119. Explain in detail.

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### Data

#### Part 2: Gradient of Transmission

Growth on plates:

CSH121/CSH125 1:100 \_\_\_\_\_

CSH121/CSH125 1:1,000 \_\_\_\_\_

CSH121/CSH125 1:10,000 \_\_\_\_\_

CSH121 \_\_\_\_\_

CSH125 \_\_\_\_\_

#### Part 3: Data Analysis

Number of colonies on plate #

1. Leu<sup>-</sup> \_\_\_\_\_

2. His<sup>-</sup> \_\_\_\_\_

3. Arg<sup>-</sup> \_\_\_\_\_

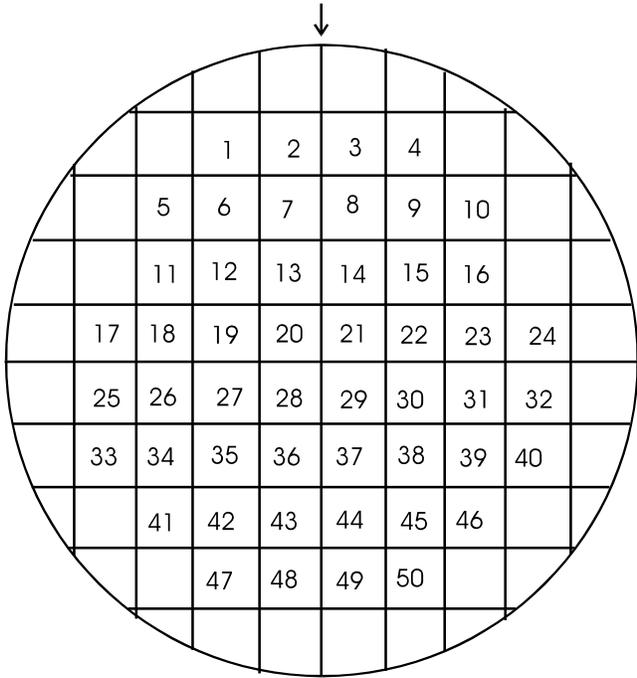
4. Ade<sup>-</sup> \_\_\_\_\_

5. Trp<sup>-</sup> \_\_\_\_\_

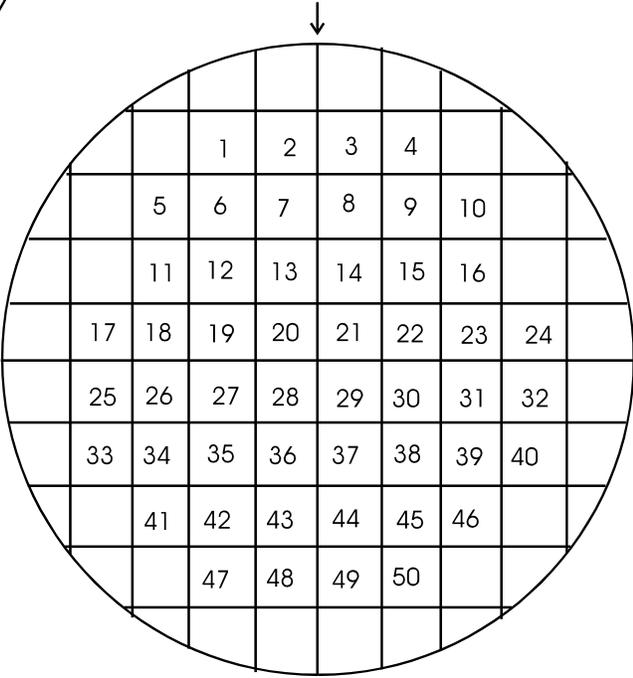
6. Tet \_\_\_\_\_

7. Leu<sup>-</sup> \_\_\_\_\_

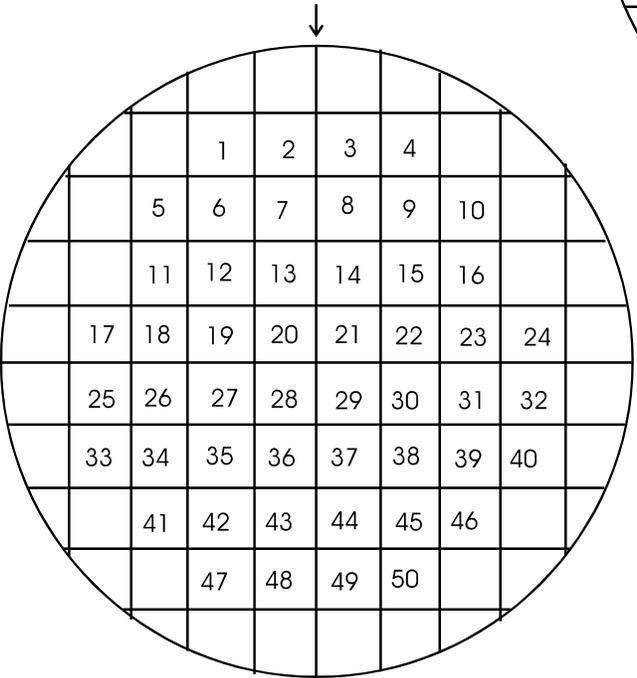
Don't forget: Do not to use any colonies that grew on plate #1 but not on plate #7.



Leu<sup>-</sup>

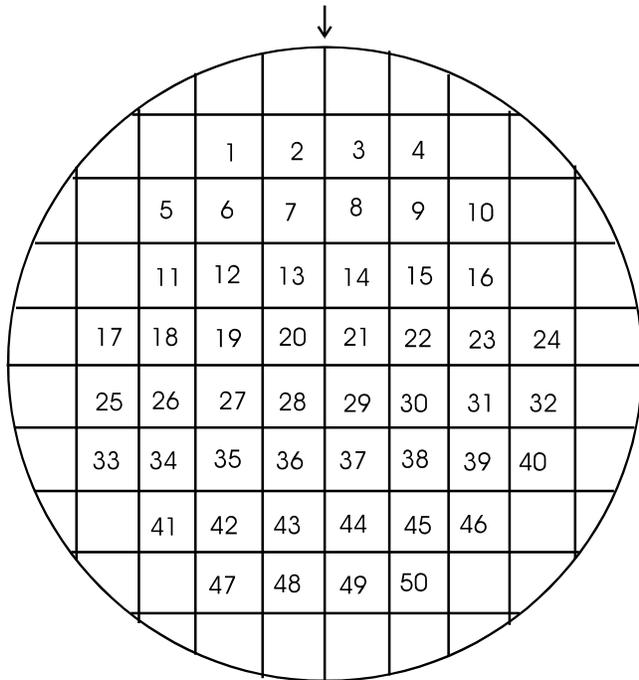


His<sup>-</sup>

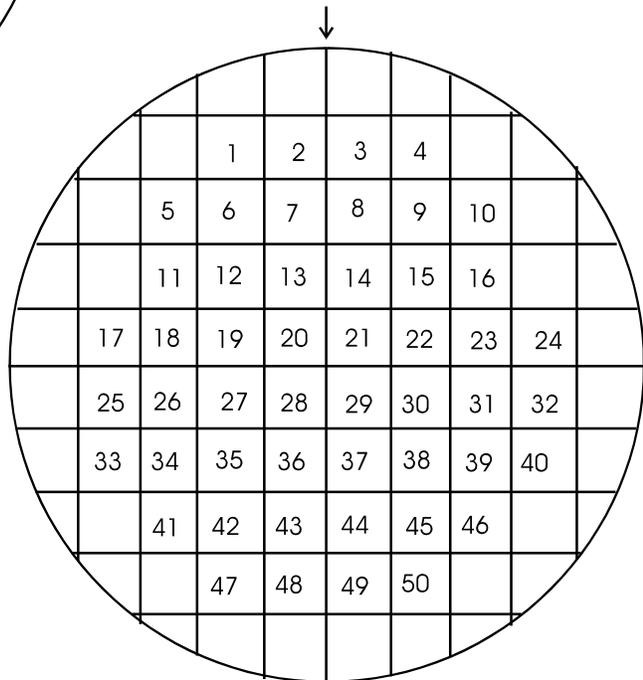


Arg<sup>-</sup>

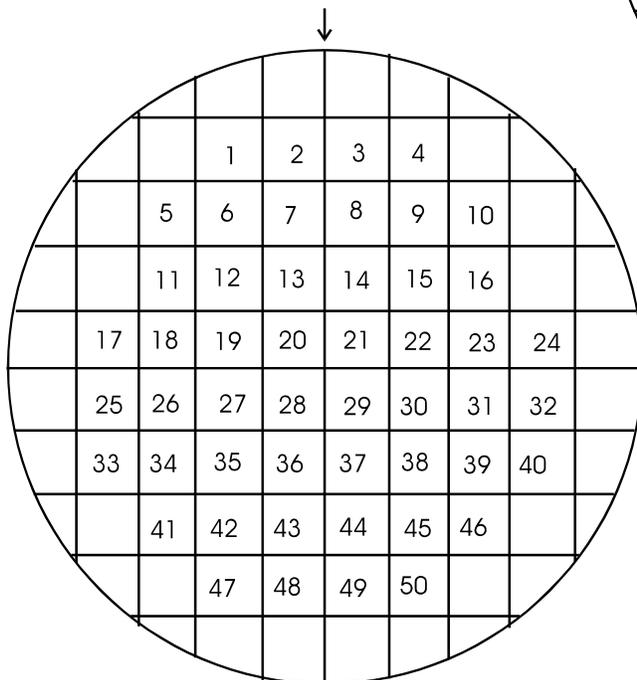
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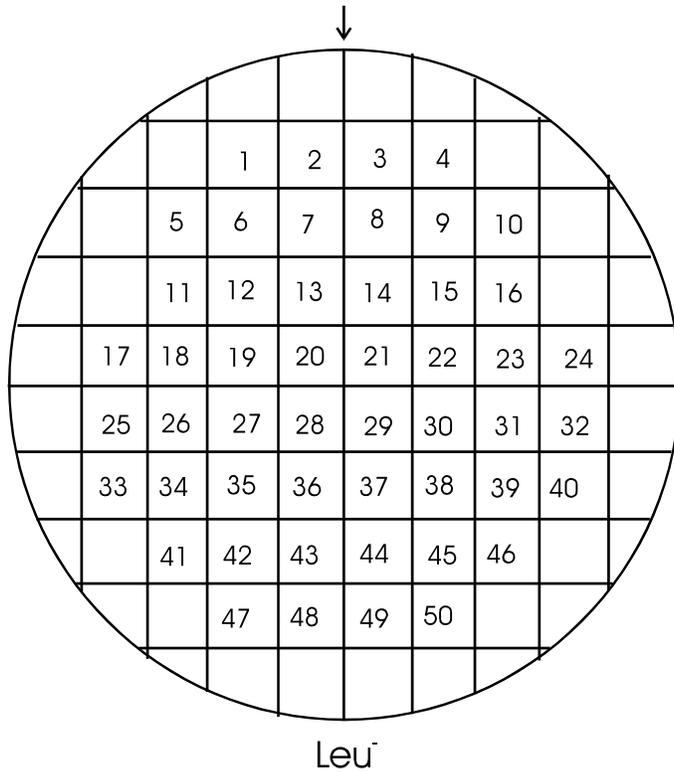
Ade<sup>-</sup>



Trp<sup>-</sup>



Tet



**Figure 1.11.** Templates for plates.

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**Appendix A**  
*Preparator's Guide*

**Materials**

**Week 1**

On lab bench:

- Piece of lab paper
- Bunsen burner
- Striker
- Plastic beaker labeled waste
- Small test tube rack
- 3 sterile capped 18 × 150 test tubes
- 2 - 13 × 100 test tubes with 5 ml each of 3 hr culture of CSH121 and CSH125
- Black marker
- 200 µl pipetman
- P-20 pipetman
- Box of sterile yellow tips
- Box of sterile blue tips
- 5 Petri dishes (C plates)
- Microcentrifuge tube rack with 6 closed sterile microcentrifuge tubes
- Metal or glass spreader
- Jar of 80% ethanol

On TA desk:

- Roll of tape
- 3 - 100 ml bottles of sterile LB broth
- 2 - 100 ml bottles of minimal broth
- 3 - 1000 µl pipetman
- 2 - 100 µl pipetman
- Box sterile yellow tips
- Box sterile blue tips

On side bench:

- Water bath set to 37°C with test tube rack for 36 - 18 × 150 test tubes
- Incubater shaker set to 37°C
- Incubater set to 37°C

**Week 2**

On lab bench:

- Small beaker with sterile toothpicks
- Plastic beaker - waste container
- Scotch tape
- 14 Petri plates: 4-C, 2-H, 2-A, 2-E, 2-G, 2-LB+Tet
- Black marker

On side bench:

Roll of tape  
 Incubator set to 37°C  
 Large biohazard bag

### Solutions

#### LB Broth (1 liter)

Bacto tryptone            10 g  
 Bacto Yeast extract      5 g  
 NaCl                        10 g

Dissolve in deionized water. Adjust pH to 7.0 with several drops of 5N NaOH  
 For LB plates, add 15 g of Bacto Difco agar per liter.

#### Plate Media

Several different types of plates are made using the minimal media formula found below.

Plate Type	Additions to minimal media								
A, Arg <sup>-</sup>	Pro		Ilv	Met	Leu	Ade	Trp	His	Strep
C, Leu <sup>-</sup>	Pro	Arg	Ilv	Met		Ade	Trp	His	Strep
E, Ade <sup>-</sup>	Pro	Arg	Ilv	Met	Leu		Trp	His	Strep
G, Trp <sup>-</sup>	Pro	Arg	Ilv	Met	Leu	Ade		His	Strep
H, His <sup>-</sup>	Pro	Arg	Ilv	Met	Leu	Ade	Trp		Strep
LB + Tet									

Note that Ilv stands for both isoleucine/valine. 4 ml of each stock is added to the media.

#### Minimal Media (1 liter)

Each of the following components should be prepared separately.

Difco agar                    15g  
 Deionized water            500 ml  
 Autoclave

#### Salts:

K<sub>2</sub>HPO<sub>4</sub>                        10.5 g  
 KH<sub>2</sub>PO<sub>4</sub>                       4.5 g  
 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>                   1.0 g  
 Sodium Citrate•2H<sub>2</sub>O      0.5 g  
 Deionized water            460 ml  
 Autoclave

1M MgSO<sub>4</sub>•7H<sub>2</sub>O            1 ml  
 Filter sterilized

1% B1 (Thiamine  
 hydrochloride)            0.5 ml  
 Filter sterilized

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20% Glucose            10 ml  
Filter sterilized

The following components are added only to certain plates described in the table on the previous page.

Streptomycin            1 ml  
100 mg/ml stock  
Filter sterilized

Amino Acids            4 ml  
10 mg/ml stock  
Filter sterilized

Tetracycline            1 ml  
15 mg/ml stock

After autoclaving the agar and salts, mix together. Then add the other components individually to the cooling agar swirling after each addition.

### **Bacteria**

Begin by growing stock cultures of CSH121 and CSH125 on LB plates for 48 hours. Grow a 250 ml overnight culture of each strain in LB broth. Three hours before lab dilute CSH121 1:25 (4 ml overnight, 96 ml LB broth) in a sterile 250 ml flask. Incubate in a 37°C water bath for 3 hours with no shaking. Also three hours before lab dilute CSH125 1:5 (20 ml overnight, 80 ml LB broth) in a sterile 250 ml flask. Incubate in a 37°C incubator for 3 hours with shaking.

At the start of each laboratory section, aliquot 5 ml of each strain into separate sterile 13 × 100 test tubes.

The bacterial strains used here are part of a set of 44 strains (Jeffrey Miller set of *E. coli* strains) that can be purchased from Cold Spring Harbor Laboratory Press (1-800-843-4388) for \$350 plus \$30 FedEx shipping charges. Allow at least 8 weeks after ordering for the strains to arrive. Similar strains can also be obtained from the *E. coli* Stock Center at Yale University (<http://cgsc.biology.yale.edu/>).

### **Appendix B** ***Results***

The markers are linked to Leu by the following percentages:  
Arg 0%, His 6%, Ade 44%, Trp 30%, and Tet 68%. Using the known locations of Leu (2 min) and His (44 min) you can graph the data and find the remaining locations: Arg (too far), Ade (14 min), Trp (19.5 min), and Tet (7.5 min). The actual locations are : Arg (69 min), Ade (12 min), Trp (28 min), and Tet (5 min). Figure 1.1 shows the results from Part 1. Figure 1.2 shows the results from Part 2.



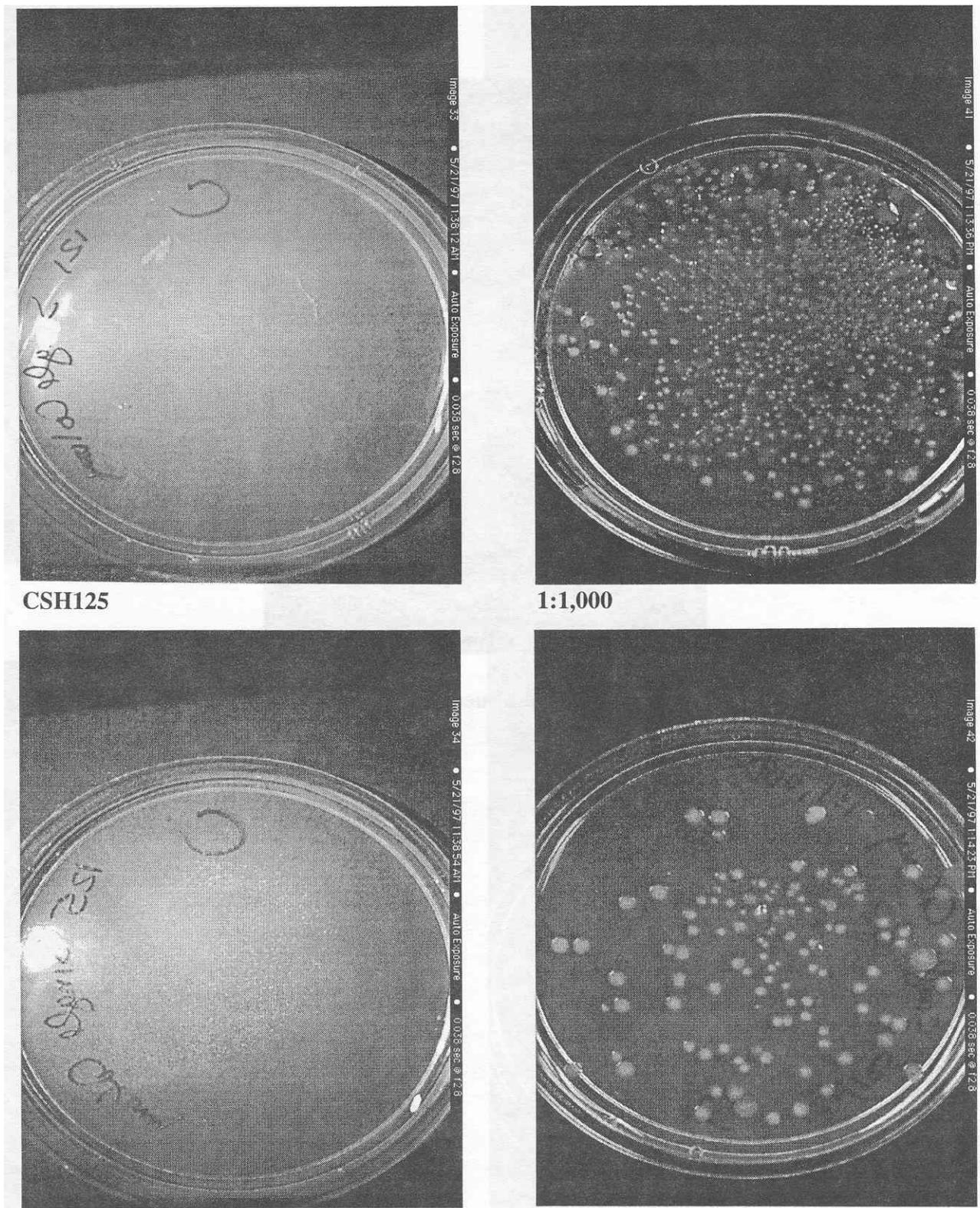


Figure 1.12. Results of Part 1.

1:10,000

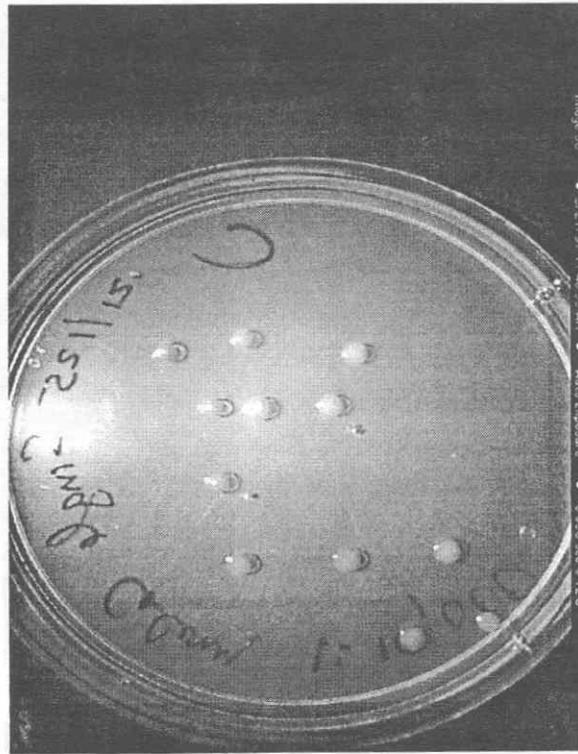


Figure 1.12, continued. Results of Part 1.

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Leu<sup>-</sup>

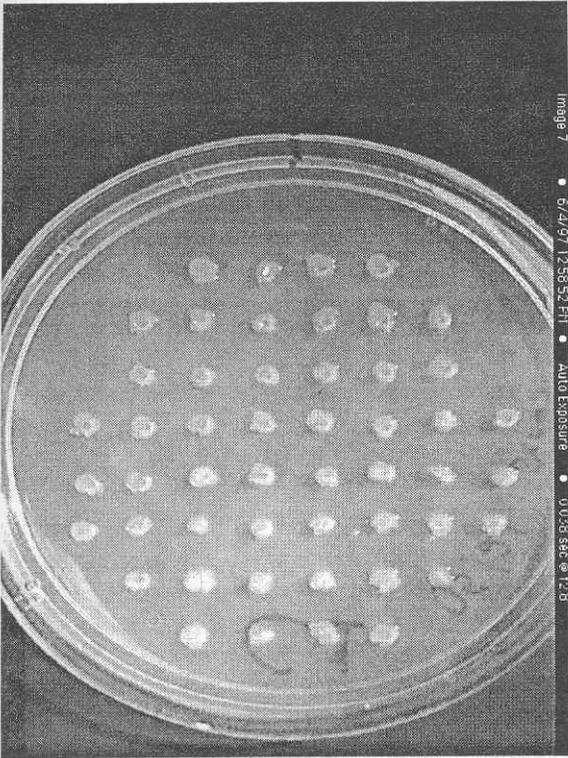


Image 7 • 6/4/97 12:58:52 PM • Auto Exposure • 0.038 sec @ f2.8

Arg<sup>-</sup>

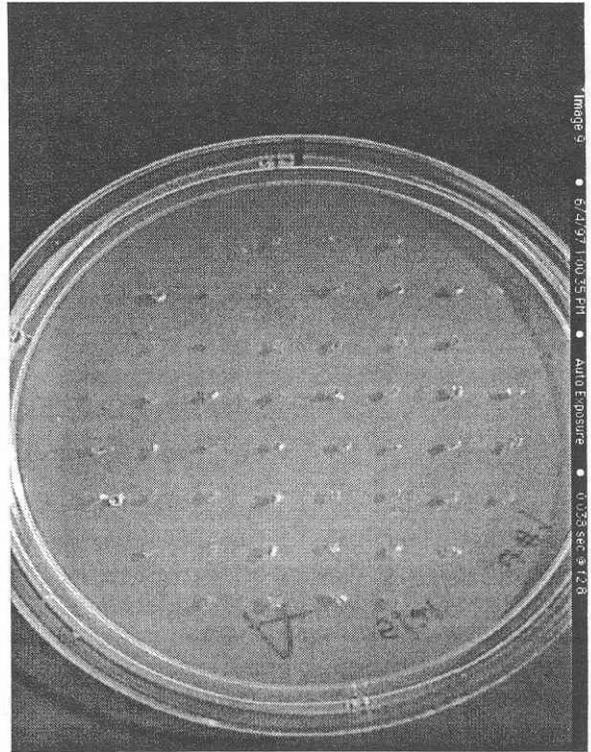


Image 9 • 6/4/97 1:00:25 PM • Auto Exposure • 0.038 sec @ f2.8

His<sup>-</sup>

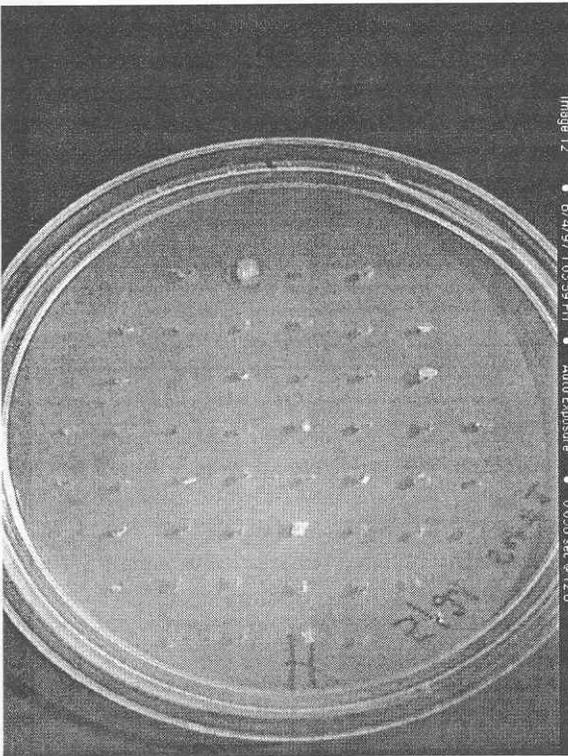


Image 12 • 6/4/97 1:03:59 PM • Auto Exposure • 0.038 sec @ f2.8

Ade<sup>-</sup>

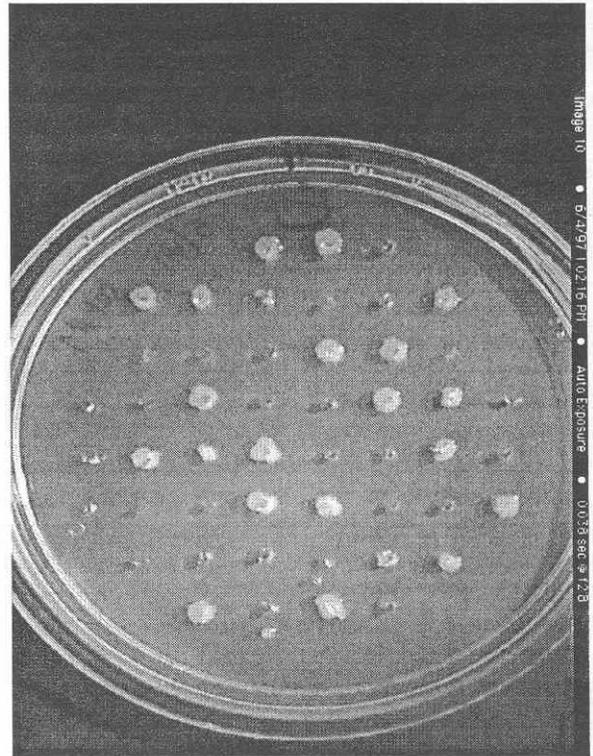
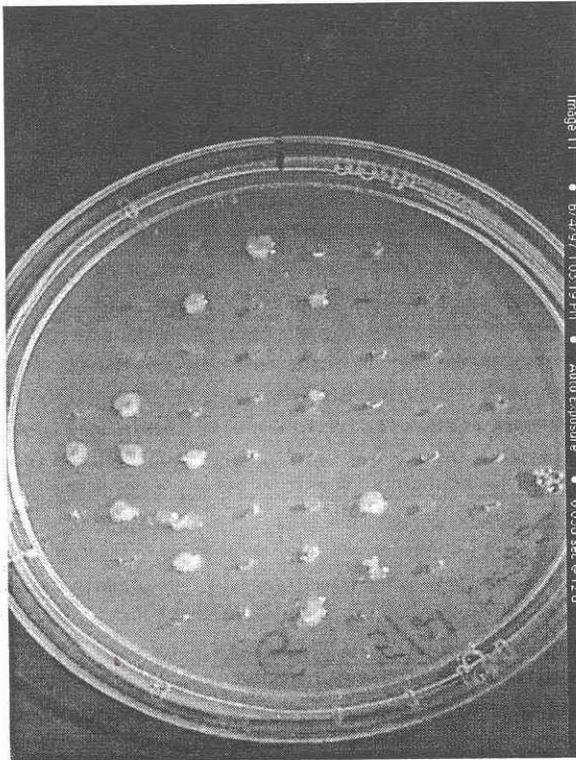


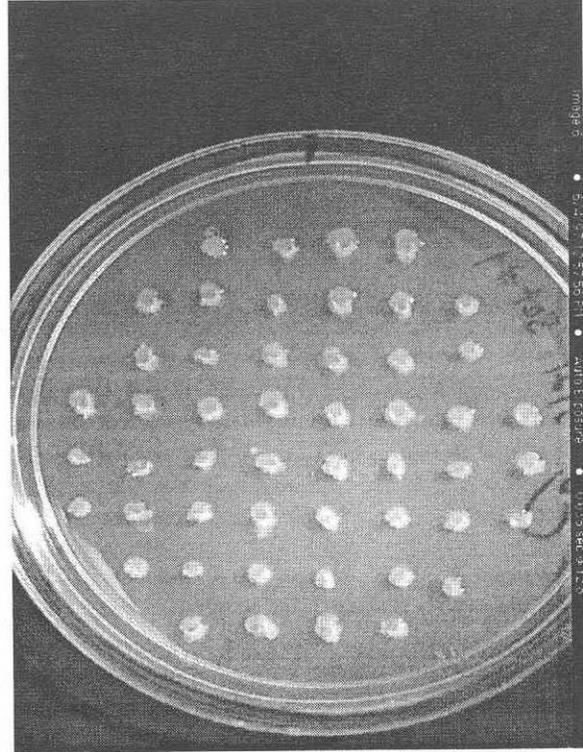
Image 10 • 6/4/97 1:02:16 PM • Auto Exposure • 0.038 sec @ f2.8

Figure 1.13. Results of Part 2.

Trp<sup>-</sup>



Leu<sup>-</sup>



Tet

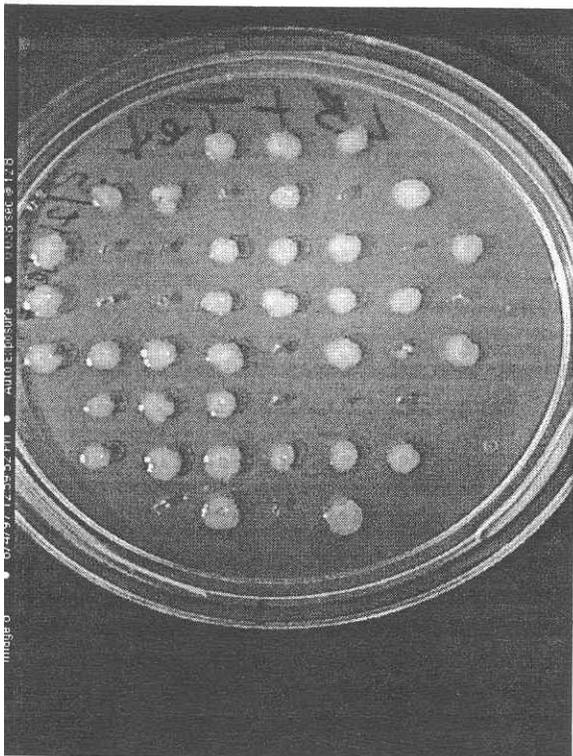


Figure 1.13, continued. Results of Part 2.