Isolation of Nutritional Mutants of *E. coli*

For the safe implementation of this lab, consult *Working with DNA & Bacteria in Precollege Science Classrooms* (1993). Toby Mogollon Horn. NABT: Reston, VA.

SYNOPSIS FOR CORE EXPERIMENT

Students will select individual colonies of *Escherichia coli* (*E. coli*) bacteria and design an investigation that demonstrates genetic variation to determine if any individual colonies are nutritional mutants.

APPROPRIATE BIOLOGY LEVEL

Advanced

TEACHER PARTNERS

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 160. For a specific variation, check the At-A-Glance Map.

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (30 minutes)
- Introduce and set up the lab.

Day 2 (45 minutes)
- Conduct the lab.

Day 3 (15 minutes)
- Observation of results and discussion.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Explain that bacterial species show genetic variations.
- Design an experiment to differentiate between mutant and wild-type *E. coli* bacteria.
- Explain the relationship between an individual bacterium and a colony.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 1 bottle antibacterial soap
- 1 BIO 101, Inc. DNA Library Media Kit, Catalog # 5800-100 (see Teaching Tips, p. 154)

OR

the components of the kit:

Rich Luria Plates
- 10.0 g tryptone
- 5.0 g yeast
- 0.5 g NaCl
- 15.0 g agar

Minimal Plates

Part A
- 10.0 g glucose
- 0.12 g $MgSO_4$
- 0.011 g $CaCl_2$
- 1.0 mg thiamine

Part B
- 15.0 g granular agar

Part C
- 7.0 g K_2HPO_4
- 3.0 g KH_2PO_4
- 0.5 g NaCl
- 1.0 g NH_4Cl

Part D
- 0.135 g $FeCl_3 \cdot 6\ H_2O$
- 0.010 g $MnCl_2 \cdot 4\ H_2O$
- 0.5 mL 1M HCl
- 1 20-mL sterile vial
- 1 0.22-μm sterile millipore filter
- 1 10-cc sterile disposable syringe

PREPARATION TIME REQUIRED

30 minutes

- Order and locate materials.

Media

2 hours

- Prepare and autoclave media and pour plates with BIO 101 media kit. Prepare bleach solutions.

OR

5 hours

- Prepare media with recipes found in Directions for Setting Up the Lab.

30 minutes

- Pour and label plates.

Library Plates

30 minutes

- Streak plates with original library culture.

(continued from p. 149)

- 3.0 L warm distilled H_2O
- 2 2-L Erlenmeyer flasks
- 80 to 120 10-cm sterile petri dishes
- 1 autoclave or pressure cooker
- 1 autoclave marking pen
- 1 100-mL Erlenmeyer flask
- 1 150-mL Erlenmeyer flask
- 1 50-mL Erlenmeyer flask
- 1 1-L sterile Erlenmeyer flask
- 1 sterile pipette
- 1 refrigerator
- 80 to 120 sterile toothpicks or 1 nichrome wire loop *(optional)*
- 1 bunsen burner *(optional)*
- 350 mL household bleach
- 2 L distilled water
- 1 1.5-L container with lid
- 12 250-mL spray bottles

You will need the following for each group of two students in a class of 24:

- 1 bottle antibacterial soap
- 1 pair disposable gloves *(optional)*
- 1 spray bottle with 10% household bleach
- 100 mL 20% household bleach
- 1 250-mL beaker
- 2 photocopied grids
- 1 rich medium plate
- 1 minimal medium plate
- 1 library plate
- 1 permanent marker
- 60 sterile toothpicks
- 1 covered, sterile toothpick container
- 1 incubator *(optional)*

SAFETY PROCEDURES


Clean lab tables with 10% household bleach solution before and after use.


Wash hands thoroughly with warm water and antibacterial soap before and after lab.


Place all materials that have touched bacteria into a biohazard bag and autoclave. If autoclave is not available, sterilize with 20% household bleach solution for one hour before disposal. Check with local universities, community colleges, or veterinary offices for assistance with disposal of materials.


Check with Student Health to determine if there are any students in the class who have immune deficiency as a result of disease or chemotherapy. These persons should not be in the room where there is experimentation with live bacteria.


Exercise caution with clothing, hair, and other flammable materials while working near open flames.


Handle glass with care. Place broken glass in a designated container, not with the general trash.


Always wear goggles and aprons.


Tie back long hair and secure bulky clothing.


Wear nonallergenic, disposable gloves when plating bacteria.

DIRECTIONS FOR SETTING UP THE LAB

Prepare BIO 101 Kit as per enclosed instructions.

OR

If kit is not purchased, prepare rich and minimal media as follows:

Five days ahead

1. Prepare rich and minimal media plates.
2. Cool to room temperature. Leave at room temperature for 2 days. Then, refrigerate until needed.

Rich Plates

For 1 L of medium:

1. Combine the following in a 2-L Erlenmeyer flask:
   - 10.0 g tryptone
   - 5.0 g yeast extract
   - 0.5 g NaCl
   - 15.0 g agar
2. Add warm, distilled water to the components of Step 1. Bring to 1 L volume.
3. Autoclave for 15 minutes at 15 pounds per square inch (psi).
4. Pour into sterile, disposable or glass petri dishes and allow to solidify. Makes approximately 40 plates.

Minimal Plates

1. For 1 L of medium prepare the following parts and autoclave separately to avoid precipitation.

Part A. M9 Mix with Thiamine

a. Combine the following in a 100-mL Erlenmeyer flask:
   - 10.0 g glucose
   - 0.12 g $MgSO_4$
   - 0.011 g $CaCl_2$
   - 1.0 mg thiamine

b. Add components of Step "a" to 45 mL distilled water.
c. Dissolve with heat and stirring.
d. Bring to a total volume of 50 mL with distilled water.
e. Autoclave 15 minutes at 15 psi.

Part B. Agar-B

Combine the following in a 2-L Erlenmeyer flask:
a. Add 15 g granular agar to 850 mL warm, distilled water.
b. Autoclave 15 minutes at 15 psi.

Part C. 10x M9 Salts

a. Combine the following in a 150-mL Erlenmeyer flask:
   - 7.0 g K_2HPO_4
   - 3.0 g KH_2PO_4
   - 0.5 g NaCl
   - 1.0 g NH_4Cl

b. Add the components of Step "a" to 95 mL distilled water.
c. Dissolve with stirring.
d. Bring to a total volume of 100 mL with distilled water.
e. Autoclave 15 minutes at 15 psi.

Part D. Trace Minerals

a. Combine the following in a 50-mL Erlenmeyer flask:
   - 0.135 g $FeCl_3 \cdot 6\ H_2O$
   - 0.010 g $MnCl_2 \cdot 4\ H_2O$

b. Add components of Step "a" to 49.5 mL distilled water.
c. Add 0.5 mL 1M HCl.
d. Swirl to mix.
e. Filter sterilize the solution in a sterile 20-mL vial with a sterile 0.22-mm millipore filter and 10-cc sterile disposable syringe. See Figure 1 for sterilization setup.
f. Do not autoclave.

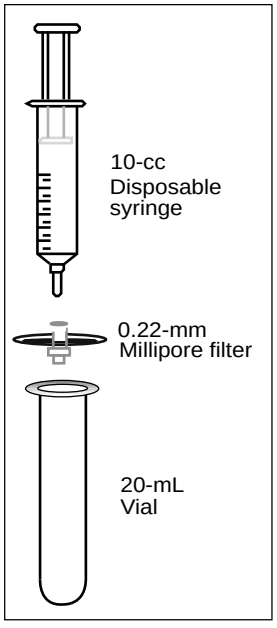

Figure 1. Sterilization setup.

TEACHER'S NOTES

TEACHER'S NOTES

2. When the Agar-B solution has cooled to approximately 55˚C, the agar will be molten and still hot to the touch. Add the M9 Mix with Thiamine and the 10x M9 Salts Solution to a sterile 1-L Erlenmeyer flask.
3. Add 0.1 mL sterilized Trace Minerals solution with sterile pipette to the Erlenmeyer flask.
4. Gently swirl to combine.
5. Pour minimal medium into sterile petri dishes and allow to solidify. Makes approximately 40 plates.

Two days ahead

1. Remove plates from refrigerator and allow to come to room temperature.
2. Streak out a plate for every two students from the library culture to allow single colonies of bacteria to grow. See Figure 2. Plates also can be streaked, grown several days ahead, and then refrigerated. See Teaching Tips. Streaking is best done with a flame-sterilized nichrome wire loop, but sterile toothpicks may be used. There should be plenty of single, isolated colonies on the streaked plates for students to select easily.

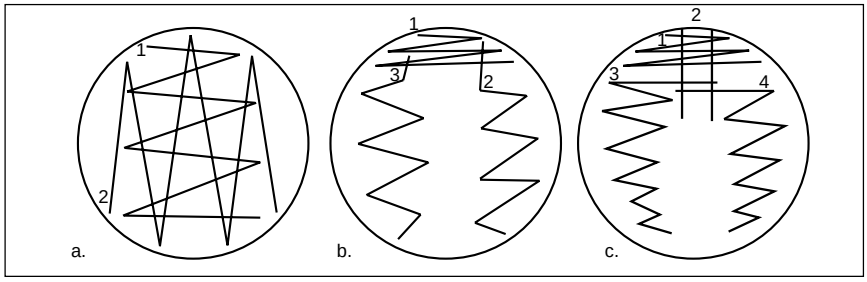

Figure 2. Suggested patterns for streaking plates. Dip the sterile loop or toothpick into the library. Then, streak the horizontal line 1 as shown. Resterilize the loop or use a fresh toothpick *without* dipping into the library for each additional streak.

3. If you find that colonies are too dense on the plate despite using the suggested streaking patterns, dilute the library 1:10 in sterile liquid medium, i.e. plate medium without agar, before streaking.

One day ahead

1. Prepare grids. See Figure 3 for grid.

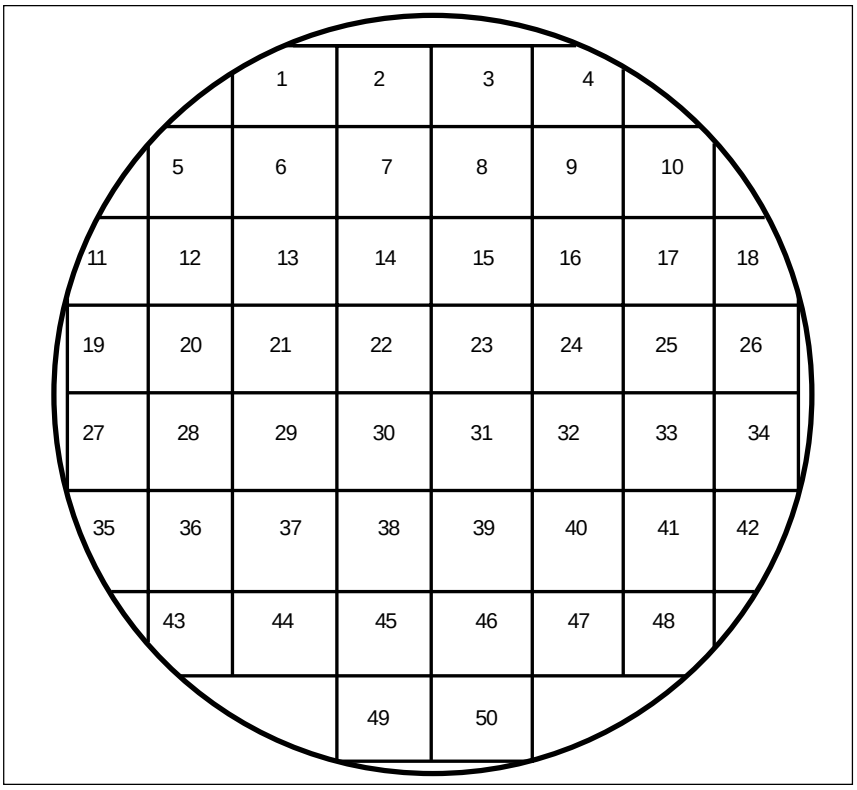

Figure 3. Grid for petri dishes.

2. Prepare 20% bleach solution. Add 200 mL household bleach to 800 mL distilled water. Store in a 1.5-L container with lid, and cap tightly.
3. Prepare 10% bleach solution. Add 150 mL household bleach to 1350 mL distilled water. Dispense 100 mL into each of 12 250-mL spray bottles.

TEACHER BACKGROUND

Content Information

Escherichia coli (*E. coli*), a bacterium that occurs naturally in the human intestine, is used frequently for bacteriology and genetic engineering experiments. The strain used in this experiment is not pathogenic and cannot cause any disease. However, students still need to use sterile technique to keep their plates free of contaminants. They should be taught the correct procedures for safely working with bacteria in the laboratory.

The *E. coli* used in this lab was made from a common laboratory strain identified as N1624. The bacteria were mutated with transposons, introduced by infecting the cells with a special strain of lambda bacteriophage. This process creates a *library* of millions of bacterial cells, each with a different mutation. Transposons or transposable elements are typically small [500 to 10,000 base pairs (bp)] pieces of DNA that can excise themselves from one position in a prokaryote's genome and insert themselves into a different place in the same genome or into a different organism's genome (Gardner et al., 1991). See Figure 4. There is often little or no specificity for the sites where the transposon integrates into the target chromosome. Typically, transposon insertion into a gene prevents that gene's expression. See Figure 4. Every bacterial cell in a transposon-mutated library contains one transposon inserted somewhere in its genome.

TEACHER'S NOTES

TEACHING TIPS

- The library of nutritional mutants is available at the cost of shipping, approximately $10, from:

  Dr. Ken Kreuzer
  Microbiology Department
  Box 3020
  Duke University Medical Center
  Durham, NC 27710
  919.684.6466
  Email:
  Kenneth.Kreuzer@Duke.edu

- Minimal medium and rich medium, Luria Broth, can be purchased from science supply houses. BIO 101, Inc., 1070 Joshua Way, Vista, CA 92083; phone 800.424.6101; fax 619.598.0116 sells the medium necessary for this lab in a kit identified as DNA Library Catalog #5800-100. Scratch recipes and directions for media are listed in Directions for Setting Up the Lab (pages 150 to 152).
- Autoclave Parts A, B, and C of the Minimal Media in separate flasks to avoid precipitation.
- Media must be autoclaved before pouring into sterile petri plates. For volumes one liter or less per container, 15 minutes at 15 pounds per square inch (psi) of pressure at 121˚C is sufficient. A pressure cooker at 15 psi for 15 minutes can serve as an alternative for the autoclave. Be sure to fill the container only half full and cover the top loosely with a cotton plug or foil. Hospitals, veterinarians, doctors, or universities may have autoclaves and are often willing to sterilize materials.
- Plastic sterile petri dishes are disposed of easily in 20% household bleach solution or by autoclaving. Glass petri dishes must be sterilized before and after use. You also may use prepoured plates, but they are expensive.
- Teach students sterile technique, also known as aseptic technique, prior to this lab.
- Have students observe *E. coli* with either prepared slides or slides they prepare themselves.
- Explain that the bottoms of petri dishes should be labeled as they contain the material of interest, not the petri dish tops that can be interchanged.

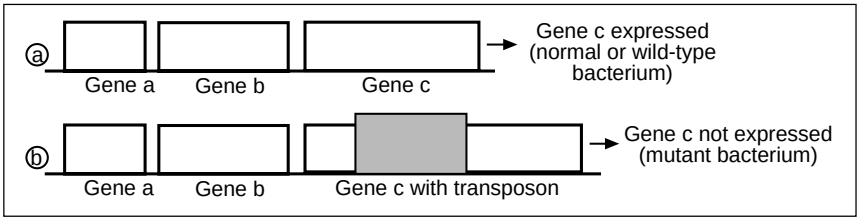

Figure 4. The effects of transposon insertion on gene expression.

Every protein is made up of combinations of 20 amino acids. Wild-type *E. coli* cells produce all 20 common amino acids. This allows the cells to produce all the proteins necessary for growth. Enzymes are proteins that catalyze the conversion of one chemical into another. Each of the 20 amino acids is synthesized by a series of enzymes. The enzymes necessary for producing one amino acid are generally different from those needed to produce a different amino acid. These enzymes for amino acid biosynthesis are each encoded by different genes in the bacterial chromosome. For example, the enzymes for synthesizing the amino acid histidine are encoded by the *his* genes. If the bacterial cell contains a mutation, such as an inserted transposon in one of the genes necessary for synthesizing histidine, this cell and its descendants will be unable to grow unless histidine is supplied. This kind of mutant is known as a nutritional mutant. A mutant requiring histidine for growth would be known as a histidine nutritional mutant.

The library of *E. coli* cells contains a collection of millions of different transposon insertion mutants with the transposon inserted randomly into the nonessential genes of the *E. coli* chromosome. If a transposon is inserted into an essential gene, the mutant cannot grow and therefore cannot be isolated. Every colony that grows on the original streak plate will be a different mutant with one transposon somewhere in its chromosome. However, because most bacterial genes are not necessary for amino acid biosynthesis, most of the mutants are not nutritional mutants. In this library, roughly one out of every 200 colonies has been found to be a nutritional mutant. See Figure 5. Usually, each nutritional mutant fails to produce only a single amino acid. On the other hand, the chemical structures of isoleucine and valine are similar. The pathways for synthesizing these amino acids share four of the same enzymes. A mutant defective in one of these enzymes will require both isoleucine and valine to grow.

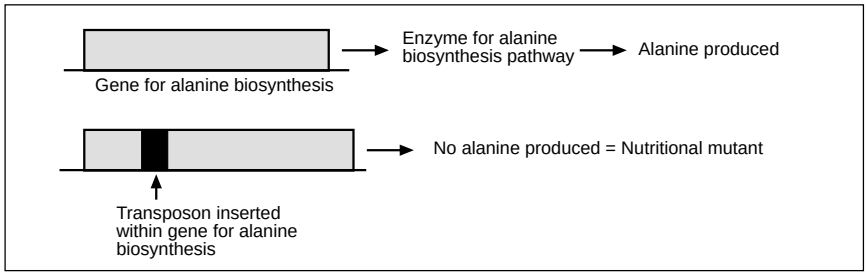

Figure 5. Transposon blocking production of amino acid.

Bacteria reproduce by fission. See Figure 6. A single cell forms a colony of genetically identical cells. *E. coli* cells divide every 20 minutes if they have sufficient nutrients and ideal conditions. With simple calculations, students can determine how many cells a single *E. coli* can produce in 24 hours. *E. coli* grow most rapidly at 37˚C. They will grow at room temperature, but it may take two days to obtain visible, isolated colonies.

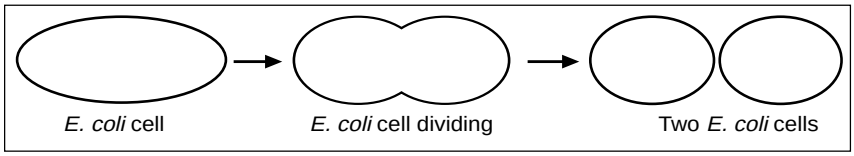

Figure 6. *E. coli* bacterium reproducing by fission.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
• Individuals within a bacterial species can be genetically variable.	• All bacteria of one species are genetically identical.
• Bacteria, like all organisms, have certain nutritional requirements.	• Bacteria can grow on anything.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Describe how the library was prepared, as described in the Teacher Background. Each team will receive a streaked plate that contains a sample from the library. Point out individual colonies on the plates and stress the fact that each grew from a single bacterial cell. They can expect about one in 200 colonies to be a nutritional mutant identifiable by its differential growth on rich and minimal media.

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

Explain that nutritionally mutant bacteria will not grow on minimal medium, but will grow on rich medium.

Sample Hypothesis

If bacteria do not grow on minimal medium, they are nutritionally mutant.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 159.

Question

Will bacteria that are not nutritional mutants grow on minimal medium and enriched medium?

Hypothesis

Bacteria that are not nutritional mutants will grow on the minimal medium but not as well as on the enriched medium.

Rationale

The minimal medium has a smaller quantity of the nutrients, such as amino acids, that allow these bacteria to grow rapidly.

Procedure

1. Wash hands with antibacterial soap and then put on disposable gloves.
2. Wipe down the lab bench with 10% household bleach.
3. Add 100 mL 20% bleach solution to a 250-mL beaker. Sterilize used toothpicks.

TEACHING TIPS

- The size of the autoclave will determine how much medium can be sterilized. One run usually takes 50 to 60 minutes.
- Demonstrate how to use the grid with an overhead projector and show what a single colony looks like.
- Agar may be colored with food coloring if you think the minimal and rich media plates may be confused by your students, or simply mark the plate edges with different colors of magic markers to indicate the type of medium they contain.
- For every two students, the teacher should streak out a plate from the library culture before the lab to allow single colonies of bacteria to grow. Good-sized, single colonies will grow in 24 to 48 hours at room temperature or overnight if incubated at 37˚C. If you grow the colonies at 37˚C, put the plates in an incubator at the end of the day and remove them in the morning to prevent colonies from growing too large. The bacterial colonies may be kept in the refrigerator for several days. Having single colonies is essential for good results. Each pair of students needs at least 50 single colonies. Therefore, it is important to perfect your streaking technique ahead of time to insure you have enough single colonies.
- Put amino acids in small salt/pepper shakers.
- Sterilize toothpicks. Place at least 60 toothpicks into a petri dish for each pair of students. Autoclave 20 minutes at 15 psi.
- Incubate plates upside down.

TEACHER'S NOTES

4. Obtain two photocopied grids. Place one grid behind the rich medium plate and the other grid behind the minimal medium plate.
5. Mark the top of each grid and the side wall of the top and bottom of each plate with a permanent marker to show alignment of the grid with the petri dish. In addition, write your initials and the date on the bottom of each petri dish. See Figure 7.

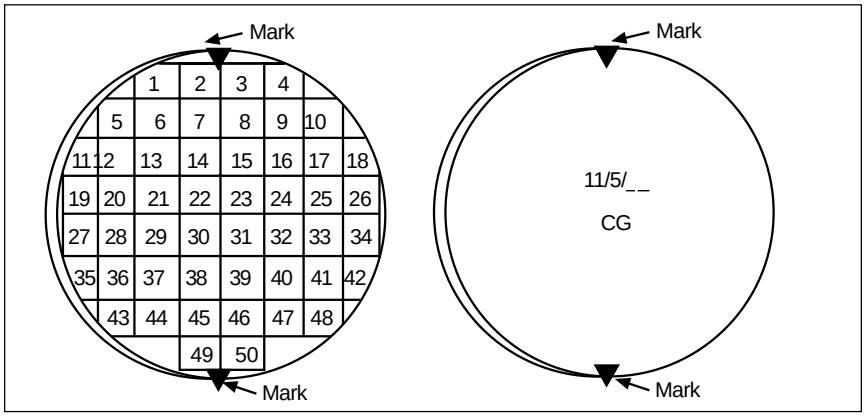

Figure 7. Labeling of petri dish.

6. Transfer 50 colonies from the library culture to one rich and one minimal medium plate in this way:
   a. *Lightly* touch a colony on the library culture with a sterile toothpick. You should not be able to see any residue on the toothpick. Be assured that you have plenty of bacteria as long as you touch the colony lightly. If you pick up too many cells, you may not have a good selection on minimal medium. Therefore, mutant colonies will be able to grow somewhat, and will be mistaken for wild-type.
   b. Touch the same toothpick to a space on the grid on a minimal medium plate and then to the corresponding space in the grid of the rich medium plate. See Figure 8.

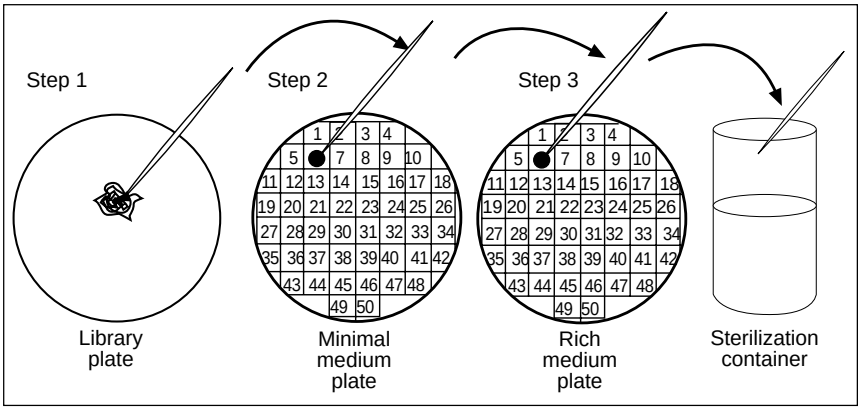

Figure 8. Transferring cultures from the library to the minimal and rich media plates.

   c. Dispose of the toothpick in the beaker with 20% household bleach solution.
   d. Repeat Steps 6a and 6b for each of the 50 colonies using a *new* toothpick and a *different* space on the grid for each colony.
7. Invert each petri dish and incubate overnight at 37˚C, or in the room 2 days at 25˚C.

8. When bacterial growth is evident, place the petri dishes over your grids. Align the marks and observe which colonies grew on rich medium versus minimal medium. Growth of a colony on rich medium, but no growth of that colony on minimal medium, indicates a mutant. The rich medium plate serves as a control, but the colony from the rich medium plate can be tested again on minimal medium to verify the results.

9. *(optional)* To verify your results and perhaps find more mutants, take any colonies that appear faint on the original minimal plate and re-streak them onto a second minimal plate. Touch a sterile toothpick to each colony on the first minimal plate and gently streak it 1 to 2 cm on a second minimal plate. Label each streak on the back of the plate or divide the plate into labeled sectors before streaking. You may find that some of the colonies will not grow on the second plate; they are probably nutritional mutants as well. Also, re-streak 1 or 2 positive controls, that is, colonies that grew normally on the original minimal plate. See Figure 9.

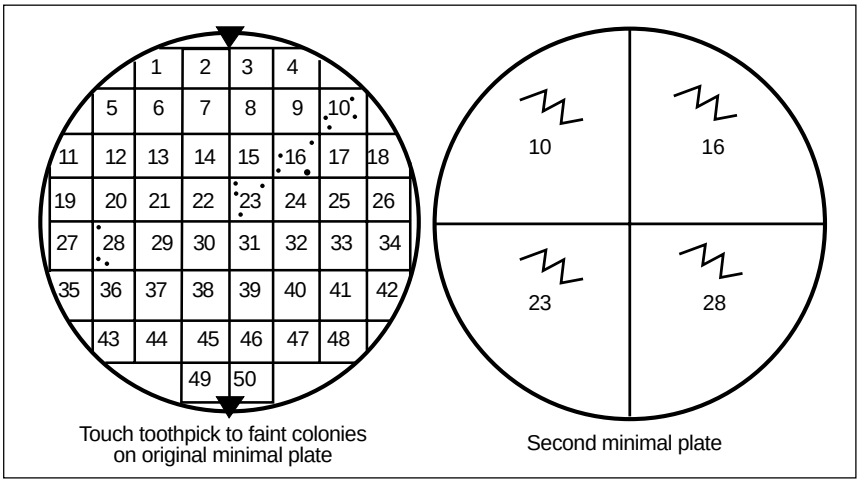

Figure 9. The spots in boxes 10, 16, 23, and 28 represent poor growth of the bacteria. It may be a single colony in each box. All other boxes will have stong growth. Streak faint colonies 10, 16, 23, and 28 found on the original plate onto a second minimal plate.

DATA ANALYSIS AND INTERPRETATION

Sample Data 1

Five colonies, A through E (see Table 1) were selected from 1100 colonies streaked from a bacterial library. The library had an expected frequency of nutritional mutants of roughly 1:200. Of these colonies, A through C grew on the rich medium, but not on the minimal medium. Colony D grew on the minimal, but not the rich medium. E grew on neither the rich nor the minimal medium.

Colony	Growth on rich medium	Growth on minimal medium
A	+	-
B	+	-
C	+	-
D	-	+
E	-	-

Table 1. Colony growth from bacterial library.

Sample Data 2

Of 450 colonies from a bacterial library, five did not grow on minimal medium. These five were plated on 20 different plates, and each contained one of the 20 different amino acids. Number 1 grew on the glycine plate, #2 still did not grow on any of the plates, #3 and #4 grew on the cysteine plate, #5 grew on the asparagine plate and on the aspartic acid plate. Number 2 mutant was replated on isoleucine/ valine, but still did not grow.

TEACHER'S NOTES

Interpretation

A through C are nutritional mutants deficient in some component provided in the rich medium that is not present in minimal medium. Colonies D and E probably were not transferred correctly since both grew on the rich medium master plate. Thus, the rate of nutritional mutation in the library was 3/1100 or 0.27%, or about half the expected rate of 0.5% (1/200).

Interpretation

Mutant #2 must require a vitamin, nuclei acid base, or other combination of amino acids.

Answers to Test Questions

1. Select individual colonies to duplicate plates. Medium type will make a difference. Incubate one plate at 42˚C, the other at 20˚C to 25˚C. Colonies that grow on the low-temperature plate, but do not grow on the high-temperature plate, are temperature-sensitive mutants.
2. Bacteria contain many genes. Only a small fraction of them encode proteins that are involved in the synthesis of amino acids. The majority of the mutants in the library have a transposon inserted in a gene that is not necessary for amino acid biosynthesis.

TEST QUESTIONS

1. You have a subset of a library containing *E. coli* mutants that fails to grow at the temperatures of 37˚C to 42˚C which are best for wild-type growth, but grow as fast as wild-type at 20˚C to 25˚C. Design a procedure for isolating these temperature-sensitive mutants.
2. Why may you have to sample hundreds of colonies before finding a nutritional mutant?

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed the data from the Core Experiment and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted the Core Experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What did you find out in the Core Experiment?
- Why do you think some individual colonies grew on the minimal medium and some did not?
- What do the results indicate about different *E. coli* bacteria? Note to Teachers: Try to draw out the idea that an individual bacterium may be genetically different.
- If you found no growth on the minimal medium, what does this suggest about the differences in composition of the two media? Note to Teachers: If students suggest the minimal medium has less material, try to determine if they mean a smaller quantity of the same nutrients or fewer kinds of nutrients. At some point, you may need to inform students that the major difference between the two media is that the rich medium contains all 20 amino acids and the minimal medium lacks all 20 amino acids.
- Given that the major difference between the two types of media is the lack of the 20 amino acids in the minimal medium and their presence in the rich medium, why do you think some individual bacteria did not grow on the minimal medium. How could you test that idea?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p.15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

There are no modifications for this experiment that could make it acceptable to be performed by visually impaired persons.

Students who are blind or visually impaired should not be in a laboratory when experiments with bacteria are being performed. Having students who are not able to participate actively in a laboratory experiment is a concern, especially where the lab is small or a passageway is narrow. The possibility of accidentally bumping the arm of someone who is in the midst of working with live bacteria or upsetting equipment is too great.

Deaf or Hard-of-Hearing

Supply written instruction for every facet of this investigation. The instructor should see personally that the student has learned the proper techniques for transfer of

bacterial colonies from plates to liquid or solid media, and that the student truly understands sterile technique.

Gifted

- Assign students to do a flow chart for the procedure as a collaborative learning experience.
- Ask students why the bacterial colonies stop growing if the bacteria can reproduce every 20 minutes.
- Have them calculate how many bacteria are in a single colony after a specific amount of time, given that bacteria can reproduce by fission every 20 minutes.
- Give students a chromosome map and have them identify the area on the chromosome where the transposon might have inserted to produce each mutant that they find.

Physically Impaired

Some colleges require students who are manually or mobility impaired to hire a person to perform their bacteriology lab experiments. Bacterial transfers are not done in a seated position.

One medical school built a frame to hold a wheelchair user in an erect position while having the experience of making a bacteriological culture and transfer.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Nutritional mutants of *E. coli* can be found by:
   A. plating just on rich medium
   B. plating just on minimal medium
   C. plating on both minimal and rich media from the same colony.
2. The nutritional requirements of an *E. coli* mutant can be identified by:
   A. plating on minimal medium containing different amino acids
   B. plating on rich medium containing different amino acids
   C. plating on both minimal and rich media from the same colony.
3. Describe an experiment that would allow you to differentiate nutritional mutants from wild-type *E. coli*.

REFERENCES AND SUGGESTED READINGS

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Lacey, J.M. & Wilmore, D.W. (1990). Is glutamine a conditionally essential amino acid? *Nutrition Reviews*, *48*(8), 297-306.

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Watson, J.D. & Crick, F.H.C. (1953). Molecular structure of nucleic acids. *Nature*, 171, 737-738.

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POSSIBLE SOURCES OF MENTORS

American Society for Microbiology

United States Department of Agriculture

Answers to Additional Test Questions

1. C
2. A
3. Bacteria from a stock culture, called a library, would have to be streaked for isolation of single colonies. These colonies then could be picked out one at a time and plated first on minimal medium, then on rich medium with the same sterile toothpick. If bacterial growth forms on the rich medium plate but not on the minimal medium plate then you have discovered a mutant that must have a nutritional need not met in the minimal medium.

Answers to Questions and Analysis on Student Page

1. They may be at greater risk because many plants do not contain essential amino acids, such as methionine.
2. If there are only small amounts of these essential amino acids in the plant tissue, their absence forces the herbivore to eat great quantities of the plant or to seek these nutrients elsewhere.
3. Compare growth of mutants on a well-characterized source of all nutrients necessary for the wild-type organism versus the same source with nutrient supplements not normally required by wild-type.
4. Depending on the particular amino acid, *perhaps* some metabolic pathway converts the other amino acid into the missing one. If you found that a particular nutritional mutant can grow in the presence of either amino acid A or amino acid B, then you might suspect that one of these amino acids can be converted into the other. However, you could not tell which way the conversion occurs without more information. Therefore, the defect in the mutant might be in the synthesis of only one of the amino acids, but the other is able to "rescue" the growth of the mutant.
5. Bacteria unable to synthesize a specific amino acid could be streaked on medium containing food. If the bacteria grow, the food probably contains the amino acid in question.
6. Cooking destroys some amino acids. You could streak bacteria with known nutritional mutations on medium supplemented with extracts of food before and after cooking to see if there were any differences in their growth.
7. A transposon could insert into the genome and disrupt the function of a gene that normally rendered the bacterium sensitive to the antibiotic. More importantly, the transposons themselves cause drug resistance because they carry drug resistance genes.

VARIATIONS OF THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATIONS 1-21

Identification of Amino Acids Not Synthesized by Nutritional Mutants of *E. coli*

SYNOPSIS

Students will use minimal plates containing different amino acids to identify which amino acid(s) are not synthesized by each mutant.

ADDITIONAL MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 20 different, sterile amino acid solutions (10 mg/mL; tyrosine at 2 mg/mL)
- 40 minimal medium plates
- 1 bent glass rod

You will need the following for each group of two students in a class of 24:

- 2 minimal medium plates
- 1 sterile amino acid solution
- 1 bent glass rod
- sterile filter paper disks *(optional)*

TEACHING TIPS

- It is best to use all 20 amino acids for this series of experiments to give students the best possible opportunity to identify mutants.
- Many amino acids can be purchased over the counter at drug stores in the vitamin section. Kits that contain all 20 amino acids may be purchased for around $70 to 90, or they may be bought separately from science supply houses. Three sources for all 20 amino acids are Sargent-Welch, Catalog #WL 4537D-60, 800.727.5229; Carolina Biological, Catalog #F6-84-3600, 800.334.5551; and Ward's, Catalog #38 W 6800, 800.962. 2660.
- Crystal Technique for Amino Acid Application
  1. Use sterile amino acids.
  2. Sterilize metal scoop by dipping in ethanol, heating in flame, dipping in amino acid, and sprinkling over the minimal medium.
  3. Allow to diffuse overnight.
- If you choose not to order sterile solutions of amino acids, you will need to make and sterilize them. Some amino acids should be filter-sterilized and others should be autoclaved, as indicated in Table 2. Preparation time will be approximately 4 hours if amino acid solutions are made and sterilized.

Autoclave	Filter
Alanine	Asparagine
Arginine	Aspartic Acid
Glutamine	Cysteine
Glutamic Acid	Tryptophan
Glycine	Tyrosine
Histidine	
Isoleucine	
Leucine	
Lycine	
Methionine	
Phenylalanine	
Proline	
Serine	
Threonine	
Valine	

Table 2. Sterilization method for amino acids.

- All 20 amino acids may be tested or a selection that is based on availability to the teacher.

(continued on page 161)

DIRECTIONS FOR SETTING UP THE EXPERIMENT

One day before

1. Spread 0.15 mL of each solution of 20 sterile amino acids onto 1 of 20 different plates of minimal medium using sterile technique. A bent glass rod should be used to spread the liquid amino acid solution. Alternately, you can apply a few crystals of amino acid powder to a minimal medium plate. Allow the crystals to diffuse into the minimal plates overnight.
2. Code each amino acid in the dish with the key found in tables, and label dishes appropriately.

HYPOTHESIS GENERATION

Question

Can the amino acid necessary for growth of a nutrient mutant be identified?

Sample Hypothesis

Most nutritional mutants require only one amino acid for growth.

Rationale

The amino acid deficiency of a nutritional mutant can be identified by growing the bacterium on plates containing each of the 20 different amino acids.

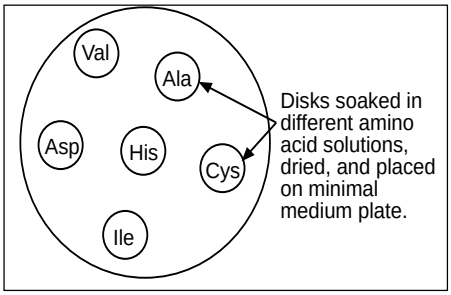

Figure 10. Application of amino acids to plates.

Sample Experimental Procedure

1. After a mutant has been identified in the Core Experiment, have students transfer it from the rich medium plate to the minimal medium plate(s) containing the amino acid(s) to be tested.
2. The mutant should be streaked onto the plate and incubated as in the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Number	Amino Acid(s)	Number	Amino Acid(s)
1	Ala	11	Leu
2	Arg	12	Lys
3	Asn	13	Met
4	Asp	14	Phe
5	Cys	15	Pro
6	Gln	16	Ser
7	Glu	17	Thr
8	Gly	18	Trp
9	His	19	Tyr
10	Ile	20	Val
		21	Ile + Val

Table 3. Key for amino acids.

TEACHING TIPS

(continued from p. 160)

- An alternative method is to apply more than one amino acid to a single plate using small filter paper disks. A sterile filter paper disk can be soaked in the sterile amino acid solution and dried inside a sterile petri dish. See Figure 10. The petri dish lid should be slightly ajar. Store in a clean, undisturbed place such as inside a drawer. Several disks then can be applied to the surface of the agar on one minimal medium plate after streaking or spreading the *E. coli* mutant across the entire plate surface.

Interpretation

Mutant A appears not to be a nutritional mutant for any of the 20 amino acids. Mutants B and C are mutants for tryptophan; mutant D for cysteine; and mutant E for aspartic acid. To determine whether mutant A is defective in the isoleucine pathway, a combination of isoleucine plus valine was tested. See plate 21 in Figure 11. Explain the results.

Answer to Test Question

The mutant does not appear to be able to make aspartic acid from asparagine, but it may be able to make asparagine from aspartic acid. Therefore, aspartic acid could be the direct metabolic precursor of asparagine.

TEACHING TIPS

- Amino acid-based food additives or supplements include monosodium glutamate (MSG) and aspartame, a dipeptide ester of phenylalanine and aspartic acid.
- MSG may be used if students have identified a glutamate-deficient mutant.
- Aspartame may be used if students have identified either a phenylalanine- or aspartic acid-deficient mutant.
- Health food or nutritional supplements also might be a good source of specific amino acids.

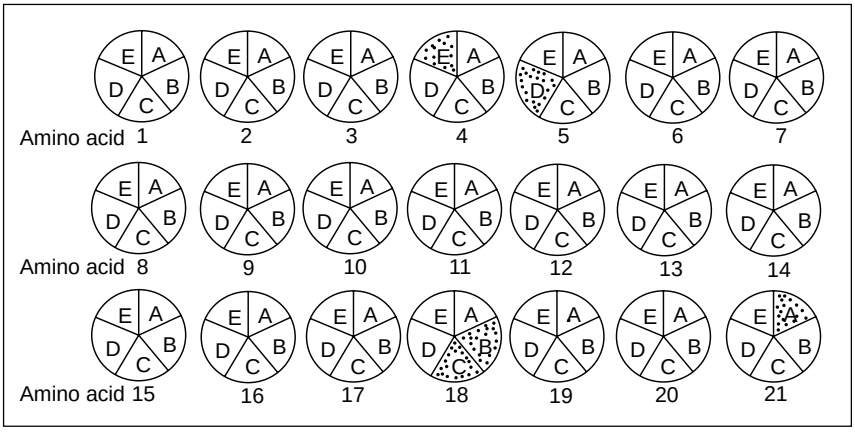

Figure 11. Each plate contains a different amino acid. See Table 3 to identify the amino acid by number. Each plate is streaked with nutritional mutants A, B, C, D, and E.

TEST QUESTION

A nutritional mutant selected from a bacterial library will grow on rich medium and on minimal medium supplemented with aspartic acid, but will not grow on minimal medium alone, or on minimal medium supplemented with a chemically similar amino acid, asparagine. What does the mutant suggest about the relationship between aspartic acid and asparagine?

VARIATION 22

The Effect of a Food Product on the Growth of a Nutritional Mutant of *E. coli*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 22.

SYNOPSIS

Students will use medium plates containing different amino acid-based food additives to identify which amino acid(s) are not synthesized by each mutant.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 4 minimal medium plates
- amino acid-base food additives or supplements.

SAFETY PROCEDURE

Do not ingest the food products.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Follow the directions for setting up Variations 1 to 21, but replace the amino acids in the growth medium with food additives known to contain the missing amino acids required by nutritional mutants.
- If autoclaving would denature the amino acid of interest in the food (see Table 2), make an extract or solution from the food and filter sterilize the extract.
- Prepare plates with a range of concentrations of the food product, as it will be difficult to determine the optimum concentration for bacterial growth beforehand.

HYPOTHESIS GENERATION

Question

Can foods or foods supplemented with amino acids provide nutritional mutants with the needed amino acid for growth?

Sample Hypothesis

If a food product contains the amino acid that a nutritional mutant is unable to make, it will have the same effect on the growth of the mutant as will the amino acid alone.

Rationale

If a food product contains amino acids needed by a nutritional mutant, bacteria will grow on minimal media supplemented with these foods.

Sample Experimental Procedure

1. Have students identify the nutritional mutant they have found using the procedures for Variations 1 to 21.
2. Identify food products that contain the missing amino acid.
3. Have students bring to class foods that they hypothesize should provide the needed amino acid for the nutritional mutant.
4. Streak bacteria on the plates prepared by the teacher to determine whether the food product that contains that amino acid will have the same effect on bacterial growth as adding the amino acid.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Mutant	Amino acid required for growth on minimal medium	Growth on minimal medium + aspartame		Growth on minimal medium + monosodium glutamate (MSG)	
		1 mg/L	10 mg/L	1 mg/L	10 mg/L
A	Glutamic acid	-	-	-	+
B	Proline	-	-	-	-
C	Aspartic acid	+	+	-	-
D	Serine	-	-	-	-
E	Phenylalanine	+	-	-	-

Table 4. Five nutritional mutants grown on minimal medium supplemented with two concentrations of MSG and aspartame.

TEST QUESTION

You are given a "mystery peptide" composed of three different amino acids and a collection of bacterial nutritional mutants each deficient in a single amino acid. Design an experiment to determine the composition of the "mystery peptide."

Interpretation

Neither MSG nor aspartame contains proline or serine, so these supplements do not promote growth of nutritional mutants B and D. Mutant A will grow on MSG that supplies its missing amino acid, glutamic acid, but only at the higher of the two concentrations. Aspartame supplies aspartic acid to mutant C, which will grow at either concentration of the additive. Aspartame also supplies phenylalanine to mutant E, but too much of the additive appears to inhibit this mutant's growth.

Answer to Test Question

Apply a sterile solution of the "mystery peptide" to a minimal medium plate. Streak each of the nutritional mutants on the plate. Identify which three mutants grow on the supplemented minimal plate.

Isolation of Nutritional Mutants of *E. Coli*

Directions for Students

INTRODUCTION

Animals, plants, and even simple organisms, such as bacteria, contain thousands of different types of proteins. With very few exceptions, all proteins are built from a set of 20 amino acid "building blocks." In order to function properly, living things must have enough of each of these 20 amino acids to make all the proteins they require.

Your body obtains amino acids whenever you eat food that contains protein. That's the only way humans can obtain certain essential amino acids that are not produced by the body, including methionine, threonine, valine, and leucine. Your body can produce the other amino acids, known as nonessential amino acids, from chemicals obtained from other non-protein sources. It also can convert some types of amino acid into others.

Different organisms vary considerably in their ability to synthesize specific amino acids. Unlike humans, the bacterium *Escherichia coli* (*E. coli*) can produce all 20 amino acids beginning with ammonia and other simple chemicals. The ammonia is changed through a series of chemical reactions into the needed amino acid. Nature efficiently changes the ammonia in a series of sequential reactions called a metabolic pathway. Through metabolic pathways, energy is captured and used efficiently to produce the needed amino acid of the bacterium. If something interrupts one of these pathways, however, the bacterium cannot grow unless the formation of the missing amino acid is provided.

In this lab, you will use a collection of mutated *E. coli* known as a library. It was created by infecting bacteria with a bacterial virus that can insert randomly one piece of its own DNA into the bacterial genome. The rest of the viral DNA has been lost, because this virus is not able to survive or grow in this bacterial strain. If the invading piece of DNA, known as a transposon, inserts itself into a bacterial gene, it typically blocks that gene's function. See Figure 1. Each bacterial cell in a transposon-mutated library contains one transposon somewhere in its genome inserted into one of its genes.

When a transposon inserts into a gene required to produce a particular amino acid of a bacterial cell, the resulting cell is known as a nutritional mutant and cannot grow and divide to form a colony unless the missing amino acid is supplied. In this library, nutritional mutants occur at the rate of approximately 1 out of every 200 colonies tested. Usually, each nutritional mutant fails to produce only one amino acid. However, in the case of isoleucine and valine, which are produced by overlapping biochemical pathways, a single mutation can block both biochemical pathways and cause a requirement for both amino acids.

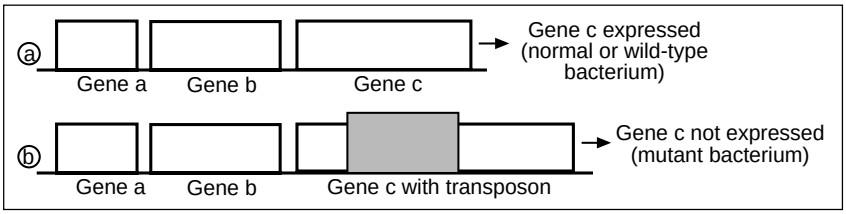

Figure 1. Diagram showing the insertion of a transposon within a gene.

OBJECTIVES

At the end of this lab, you should be able to:

- Explain that bacterial species show genetic variations.
- Design an experiment to differentiate mutant from wild-type *E. coli* bacteria.
- Explain the relationship between individual bacteria and a colony.

SAFETY NOTES

- Clean lab tables with 10% household bleach solution before and after use.
- Wash hands thoroughly with warm water and antibacterial soap before and after lab.
- Place all materials that have touched bacteria into a biohazard bag and autoclave. If an autoclave is not available, sterilize with 20% household bleach solution for one hour before disposal.
- Exercise caution with clothing, hair, and other flammable materials while working near open flames.
- Handle glass with care. Place broken glass in a designated container, not with general trash.
- Always wear goggles and aprons.
- Tie back long hair and secure bulky clothing.
- Wear nonallergenic, disposable gloves when plating bacteria.

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of potential nutritional mutants. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment which gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. Why is controlling variables important?
3. What variables must be controlled in order to investigate this question? How might this be accomplished?

PLAN OF INVESTIGATION

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow. Make a numbered list of the steps you will use to investigate the topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Are herbivores potentially at greater or lesser risk of suffering from the lack of essential amino acids? Why?
2. What are some evolutionary pressures that may have resulted in plants not containing or requiring very small amounts of certain nutrients in their tissues?
3. How can you identify a defective metabolic pathway of an organism?
4. If an organism has a defective pathway for a specific amino acid, could any other amino acid act as its substitute? Why or why not? How could you find out?
5. How might you use living organisms to determine the amino acid content of certain foods?
6. How might the amino acid content of a food be changed by the way it is prepared? How could you demonstrate this?
7. How could a transposon act to change the resistance of a bacterium to an antibiotic?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing the data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on nutritional mutants.

Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions that other students have studied include the following:

- Can the amino acid necessary for growth of a nutrient mutant be identified?
- Can food or foods supplemented with amino acids provide nutritional mutants with the needed amino acid for growth?