Biotechnology Basics™ by Ellyn Daugherty

GFP Genetic Engineering of Bacteria

(Cat. # BBED-8H)

Developed in partnership with

GFP Genetic Engineering of Bacteria

Teacher's Guide

The following laboratory activity is adapted from 2 other activities. The first, Lab 8c "Transformation of E. coli by pAmylase2014" is from Biotechnology: Laboratory Manual by Ellyn Daugherty. For more information about the program, please visit www.emcp.com/biotechnology. The second, BE-309 "Bacterial Transformation" is a hands-on teaching kit from the BioScience Excellence™ program by G-Biosciences. This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning..

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 10,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

G BIOSCIENCES®

About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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MATERIALS INCLUDED

This kit has enough materials and reagents for 8 lab groups (16 student pairs or 32 students in 4/groups).

- *E. coli* DH5α Agar Stab
- Eight culture tubes
- Ten 2ml Microcentrifuge Tubes
- 9 Sterile Packets of: 5 sterile inoculating loops, 9 small sterile and 1 large sterile transfer pipets
- 8 tubes of Competent Buffer, ice-cold (1ml)
- 9 tubes of Sterile Water (2ml)
- 8 tubes of Green Fluorescent Protein (GFP) Plasmid [5ng/µl] (15µl)
- 8 microcentrifuge tubes of sterile LB Broth (2ml)
- LB Agar, 2 bottles (200ml)
- LB Broth, 2 x 30ml
- Sterile Petri Plates (18)
- 1 vial Ampicillin
- 1 vial Sterile, distilled water (250µl)

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- Permanent lab markers, 8
- Paper towels
- Disinfectant wipes, 4 cartons
- "Trash" Beakers with 200ml of 10% bleach (8), Prepare 2 liters of 10% bleach (200 ml of bleach in 1800 ml of tap water)
- Eight Styrofoam cups with crushed or cubed ice
- Mini-centrifuges, 4
- Shaking incubator or shaking hot water bath or equivalent (37°C)
- Stationary incubator or hot water bath or equivalent (37°C or 42°C depending on the step in the procedure)
- UV lamp and UV protection goggles or UV light box with UV protective shield
- Biohazard bag

OPTIONAL MATERIALS

- Sterile flow hood or biosafety cabinet
- P20, P200 and P1000 micropipette and tips
  These can be purchased separately

Cat. #	Fisher Sci Cat #	
BT1505	501059654	Pipette, Variable, P20
BT1506	501059655	Pipette, Variable, P200
BT1503	501059652	Pipette, Variable, P1000

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SPECIAL HANDLING INSTRUCTIONS

- Store E.C. DH5α Agar Stab in a fridge or at 4°C.
- Store Ampicillin and GFP plasmid in freezer/frozen until ready to use. Thaw on ice prior to use.
- All other components can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.
- Use paper towels as lab matting

GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the Biotechnology Basics™ by Ellyn Daugherty kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDSs for chemicals in this kit is posted at www.gbiosciences.com.
- At the beginning and at the end of the lab work, all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. All used culture vials, strips, tips, or may be discarded in regular trash once they have been in 10% bleach solution for 30 minutes.

TEACHER'S PRE EXPERIMENT SET UP

- Wear protective goggles when preparing agar solution. Use heat-protective gloves when handling hot agar solutions
- Use one of the Sterile Packets of: 5 sterile inoculating loops, 9 small sterile and 1 large sterile transfer pipets for your pre-experiment set-up
- Make Agar plates a day or two before the experiment. Keep lids on the plates at all times
- Wipe down the lab tabletop with disinfectant towels before starting pouring agar plates.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

Preparation of LB Agar Plates and LB Agar + Ampicillin

NOTE: *Disinfect a lab counter prior to pouring plates or starting cultures.*

1. Label ten 100x15mm Petri Dishes "LB". 8 dishes will be shared by the students to use in the transformed colony selection lab protocol and 2 plates will be used by the teacher to set up stock cultures for the students to get bacteria colonies for their cultures.
2. Label eight 100x15mm Petri plates "LB/Amp". These will be given to students to use in the transformed colony selection lab protocol.

3. Using a small sterile transfer pipet, rehydrate the ampicillin by transferring 250µl sterile water (⅛ to ¼ volume of tube) to the vial of ampicillin. Dissolve the ampicillin by inverting the tube several times. Place on ice in the dark until ready to use.
4. There are 2 LB agar stock bottles. Label one of them with the additional label "AMP". Loosen the caps of both LB agar stock bottles (but don't open them). Heat the bottles to liquefy the agar in one of 2 ways:
   a. **Bottles may be heated at a low power in a microwave:** In a microwave (one at a time), heat bottles (with "jiggly" caps) for 4-5 minutes at 50% power. Make sure that the agar liquefies completely but does not boil. Be careful to not swirl to vigorously since the hot agar can boil over. Once liquefied, use protective gloves to take the bottles out of the microwave and place on a lab counter to cool to pouring temperature (about 55°C). Wipe the outside of the bottle with disinfectant wipes before pouring plates. ***NOTE:*** *Wear protective goggles when preparing agar. Use heat-protective gloves when handling hot agar.*
   b. **Bottles may be heated at a shallow hot water bath**. Place the agar bottles (with "jiggly" caps) in a shallow 90°C hot water bath for 40-50 minutes. Make sure that the bottles will not tip over or get contaminated. Make sure that the agar liquefies completely. Once liquefied, use protective gloves to take the bottles out of the bath and place on a lab counter to cool to pouring temperature (about 55°C). Wipe the outside of the bottle with disinfectant wipes before pouring plates.
5. Once the LB Agar bottle has cooled enough to handle the bottle, but still "hand hot" (about 50-60°C), pour a ~0.5cm (1/4") layer of agar into each of the 10 Petri dishes. This is approximately 15-20 ml. Replace the Petri Dish lids and do NOT move the plates until the agar is completely set. Leave (closed) overnight to "dry" a bit. Use 2 of the plates to set up the stock bacteria plates for students to use to make their own bacterial culture. The rest of the plates are for their transformation studies.
6. Once the LB/AMP agar bottle has cooled enough to handle, but still "hand hot" (about 50-60°C), using the small transfer pipet ***quickly*** add all the ampicillin solution in the ampicillin vial to the LB/AMP bottle.

   NOTE: *If the agar is too hot, the ampicillin antibiotic will be inactivated. The agar should be below 55°C.*
7. Swirl until the LB/ /amp agar is mixed. Do not allow to bubble or cool. Pour a ~0.5cm/ ¼" layer of LB/amp agar into the 8 labeled Petri dishes. This is approximately 15-20ml. Replace the Petri Dish lids and do NOT move the plates until the agar is completely set. Leave (closed) overnight to "dry" a bit. Cover the plates with aluminum foil to keep them dark. Ampicillin is light-sensitive.
8. Once completely set, use the plates within 24-48 hours. For long-term storage, place in a disinfected plastic bag that can be sealed shut.

Prepare Bacterial Stock Plates

NOTE: *Disinfect a lab counter prior to pouring plates or starting cultures.*

1. Set up an incubation oven or hot water bath to incubate tubes at 37°C.
2. Transfer ~0.5ml LB broth from the bottle of LB Broth to the bacterial agar stab with the large transfer pipette and incubate at 37°C for 30-60 minutes.

3. Vigorously shake or vortex for 1-2 minutes, then dip a sterile loop into the agar stab and broth and spread it across the surface of 1 LB agar Petri Plate. Streak across the top 1/3 of the agar, then turn the plate 90° and streak another 1/3 of the agar, and then repeat on the last 1/3 of the agar. Replace the lid on the plate. Repeat the process with the second LB Agar Plate. Incubate the bacterial culture plate upside down, overnight at 37°C. Disinfect the counter and wash your hands after plating bacteria cultures.

Prepare Bacterial Culture Tubes

NOTE: *Disinfect a lab counter prior to preparing.*

1. Remove the lid from the LB Broth in a sterile environment. Pour LB broth into the 8 Culture tubes and fill approximately half way.

Additional Preparation

1. Prepare cups of crushed ice for each group and set up the incubators and/or for the class to use.

2. Distribute the following items to each lab group.

    - Fine-tipped permanent marker
    - "Trash" Beaker with 200ml of 10% bleach
    - One 10-ml culture tube containing ~5ml of sterile LB broth
    - 2 Microcentrifuge Tubes/Vials, Sterile (2ml)
    - In a sterile Packet: 5 sterile inoculating loops, 9 small sterile and 1 large sterile transfer pipets
    - Cup of Crushed Ice
    - 1 tube of Competent Buffer, ice-cold (1ml)
    - 1 tube of Sterile, Deionized Water
    - 1 tubes of pGFP Plasmid
    - 1 microcentrifuge tube of sterile LB Broth (2ml)
    - 1 sterile LB Agar/amp plate
    - 1 sterile LB Agar plate

Several components will be shared by multiple groups or the whole class and should be kept on a communal, disinfected lab table

- Disinfectant wipes, 4 cartons
- Mini-centrifuges, 4
- Shaking incubator or shaking hot water bath or equivalent (37°C)
- Stationary incubator or hot water bath or equivalent (37°C or 42°C depending on the step in the procedure)
- UV lamp and UV protection goggles or UV light box with UV protective shield
- Biohazard bag

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TIME REQUIRED

- 1-2 hours for teacher preparation of agar plates and distribution of reagents
- Two 1-hour lab periods, students growing bacterial cultures, preparing competent cells and transforming cells.
- 1-hour lab period for plating cells
- 1-hour lab period for analyzing results.

NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

EXPECTED RESULTS

Reflection/Analysis

Do the results of your experiment indicate that your group was successful in transforming *E. coli* bacteria into ampicillin-resistant, GFP-producing bacteria. Give evidence to support your answer. Include a discussion of what you expected to happen in each section of each Petri plate.

Responses: Groups should expect bacterial lawns on both sides of the LB agar plate to show that both cultures survived the transformation protocol and that there are millions of cells in the culture. However, only a few dozen cells would be expected to be transformed in the pGFP culture and none in the –C culture. Therefore, it would be expected that there may be several dozen colonies growing on the GFP side of the LB/amp plate and these should glow in UV light. These are E. coli cells that have gained the plasmid and are producing new proteins that give them these new characteristics. Alternatively, the –C cells should not grow at all on the LB/amp plates since they were not given the ampicillin resistance gene and they cannot grow in the presence of ampicillin.

Were there any unexpected results on either of your Petri plates? Try to explain what may have caused these unexpected results? If there were any known technical errors, explain how they may have impacted the results of the transformation attempt and how those errors may be minimized in the future.

Responses: Cells should grow on the LB plates. If there is no growth on any sections, this indicates the cells did not survive the transformation process. If cells grow on the –C side of the LB/amp plate there may have been contamination of cells with the pGFP plasmid or the ampicillin may have been destroyed when preparing the agar.

Transforming *E. coli* cells to destroy ampicillin and make GFP demonstrates the potential of transformation science. Give some examples of how transformation technology could be used to make an important product for society.

Responses: Answers will vary but several examples of pharmaceuticals made by transforming and growing genetically-engineered cells can be found with a quick search of the Internet. Some examples include human recombinant insulin and human recombinant tissue plasmingen activator.

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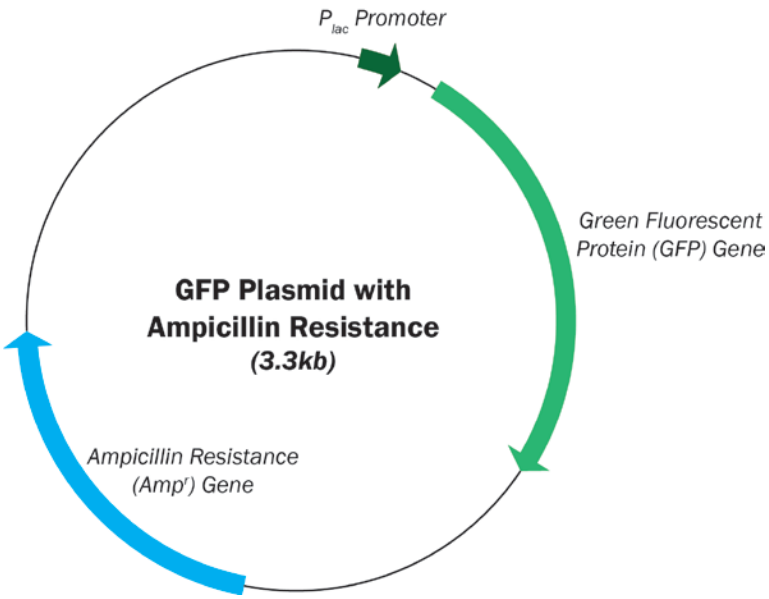
OBJECTIVES

Can *E. coli* cells be transformed into cells that have two new characteristics (ampicillin-resistance and green fluorescent protein production)?

BACKGROUND

In the 1970s, scientists learned how to transform the characteristics of bacteria cells using **plasmid** DNA. Plasmids are small extrachromosomal rings of DNA, that carry a few genes of interest and, which can be cut and combined with other pieces of DNA then repasted together to create recombinant DNA molecules.

Recombinant plasmids can carry one or more genes of interest into bacteria cells. If the newly inserted genes are expressed as proteins, the bacterial cells are said to be **transformed**. Genes of interest may be ones that make some new medical protein, industrial enzyme, or some other commercially-important protein. Genetic engineering by transformation can be used to make all sorts of protein products.



In a bacterial transformation it is important that the plasmid used, in addition to a gene to produce a protein of interest, has a selection gene that will produce a visible change in the transformed cells. The use of a selection gene ensures that the researcher will recognize when a plasmid has been taken by a cell. The most common selection gene is one that provides a specific antibiotic resistance to the bacteria. If spread on agar that contains the antibiotic, untransformed bacteria cells would not grow.

However, cells that take up plasmids carrying the antibiotic resistance gene will produce proteins that destroy that specific antibiotic in the agar. This allows transformed cells to multiple and form colonies. Since the majority of bacteria cells in a transformation procedure do not take up the plasmids, it is easy to see isolated colonies of transformed cells growing on selection media containing the selection antibiotic.

Transforming *E. coli* takes several steps. Using GFP-plasmid transformation of *E. coli* cells as an example:

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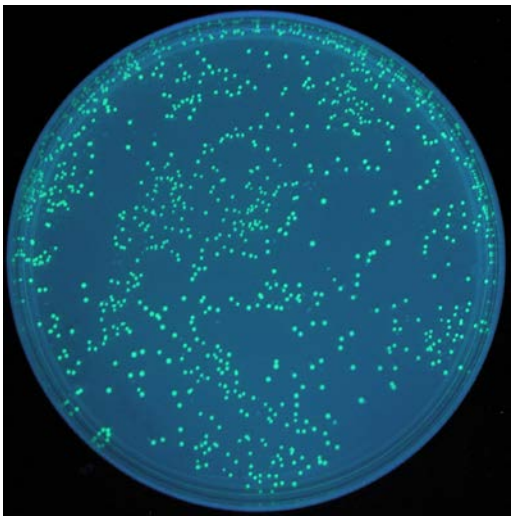
1. A recombinant plasmid is needed. GFP plasmid contains both the green fluorescent protein gene (from a jellyfish) and the ampicillin resistance gene (AmpR) from a naturally-occurring bacterial plasmid. The GFP plasmid's AmpR gene produces an enzyme that destroys ampicillin in the media on which bacteria grow. Ampicillin would normally delay the growth of *E. coli* cells, but if the cells acquire the AmpR gene, they can survive in the presence of ampicillin in agar. The GFP plasmid's GFP gene produces a protein that glows a fluorescent green when exposed to UV light. Cells that take up the GFP plasmid get both of these new characteristics.

2. *E. coli* cells must be grown in broth culture and then made "competent," or more likely to take up pieces of foreign DNA such as the plasmid to be used in the transformation. Scientists are not sure what happens when cells are made competent, but it is thought that the competency enlarges channels in the cells' membranes, making it easier for plasmids to get into the cells. Competency may be induced by treatment with divalent cations such as $CaCl_2$ or $MgCl_2$ or electrically using a process called electroporation. Electroporation is not practical for most academic labs due to the cost and special equipment required. In the following protocol, cells are made competent using $CaCl_2$.

3. The competent cells are mixed with the recombinant GFP plasmid. A heat shock, followed by a cold shock, is given to the mixture, and plasmids are drawn in and trapped inside the cells.

4. The cells are grown in recovery broth for several hours, which gives them time to repair their damaged membranes and express their new acquired genes.

5. The culture, containing a mix of transformed and non-transformed cells, is plated on selection media (LB agar containing ampicillin) and also media without the selection factor (LB agar). Only transformed cells are able to grow on the ampicillin-containing selection media while both transformed and untransformed cells (colonies) grow on the regular LB agar media.



Under the best circumstances, transformation efficiency occurs in only about one in 10,000 cells. The transformed cells are deposited on the selection media, where they grow into colonies. Maybe 10-100 or more transformed colonies may be found on a selection plate. Each cell in the colony is a clone of the original cell deposited in that location on the selection media. All the cells in the clone contain the new DNA (a new genotype), and they will express the new characteristics (new phenotypes), in this case, ampicillin resistance and green fluorescent protein production (visible using UV light).

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MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components will be shared by the whole class and should be kept on a communal table.

- Fine-tipped permanent marker
- "Trash" Beaker with 200ml of 10% bleach
- One 10-ml culture tube containing 5ml of sterile LB broth
- 2 Microcentrifuge Tubes/Vials, Sterile (2ml)
- Sterile Packet: 5 sterile inoculating loops, 9 small sterile and 1 large sterile transfer pipets
- Cup of Crushed Ice
- 1 tube of Competent Buffer, ice-cold (1ml)
- 1 tube of Sterile, Deionized Water (2ml)
- 1 tube of pGFP Plasmid (15µl)
- 1 microcentrifuge tube of sterile LB Broth (2ml)
- 1 sterile LB Agar/amp plate (20ml)
- 1 sterile LB Agar plates

Several components will be shared by multiple groups or the whole class and should be kept on a communal, disinfected lab table

- Disinfectant wipes, 4 cartons
- Mini-centrifuges, 4
- Shaking incubator or shaking hot water bath or equivalent (37°C)
- Stationary incubator or hot water bath or equivalent (37°C or 42°C depending on the step in the procedure)
- UV lamp and UV protection goggles or UV light box with UV protective shield
- Biohazard bag

PROCEDURE

- Use sterile technique throughout the procedure. Use a laminar flow hood, if available.
- Disinfect all countertops and the outside of instruments with disinfectant wipes if they have not been sterilized prior to use.
- Wash your hands with soap and water before beginning and after working with bacteria
- Keep cells on ice unless otherwise directed. *All the components of the bacterial transformation reaction and the setting up of the reaction should be done on ice. Everything remains on ice until the heat shock stage.*
- Dispose of all biohazards appropriately.

I. Preparing & Transforming Competent Cells

* Disinfect countertop and wash your hands.
* Have a cup of crushed iced at your station to place tubes on when directed.

1. Being careful to not introduce contamination, use a sterile inoculating loop to pick up a single colony of DH5α bacteria from the shared plate of DH5α bacteria colonies. Swirl the loopful of bacteria directly into the culture tube containing the 5ml of sterile LB broth. Cap the tube immediately and finger-flick the broth to distribute the

cells. Be sure the bacteria are well suspended. Label the tube with your group's initials. Deposit the used inoculating loop into the disinfectant (10% bleach solution).

2. Incubate the cultures at 37°C for 24 hours in a shaking (250 rpm) hot water bath or shaking incubator. If a shaking incubator is not available, place the cultures at 37°C and vigorously shake the culture by hand for 2 minutes ever hour (if possible).

3. Label two sterile 2-ml centrifuge tubes with the group's initials. Label one of the tubes with "pGFP" and the other "-C" (for the negative control).

4. Finger-flick the overnight culture tube (from Step 1) to resuspend the cells. Using a large sterile transfer pipet, transfer approximately 1.5ml of overnight culture to each of the 2-ml centrifuge tubes. Cap the tubes tightly. Discard the transfer pipet into the disinfectant solution.

5. Spin the 2 tubes in a minicentrifuge at 5,000 rpm for 3 minutes to pellet the bacteria cells at the bottom of the tubes. Carefully pour off and discard the supernatant from each tube into the container containing 10% bleach. **Save the cell pellets in each tube. Don't let them pour out into the bleach.** Quickly recap the tubes to decrease the chance of contamination of the cell pellets.

6. Using a small sterile transfer pipet for each, add 100µl of ice cold Competent Buffer to each pellet. Gently pipet up and down to suspend each pellet completely in the Competent Buffer. Cap the tubes tightly and place them on ice.
   The Competent Buffer contains a calcium salt that alters the outer walls of bacteria, permitting foreign plasmid DNA to enter the bacteria.

7. After 2 minutes, check to make sure that the competent cell mixture is very cloudy with bacteria. Gently finger-flick each tube to ensure that no bacteria are lying on the bottom of the tubes. Be gentle with these cells; they are fragile and can burst and die easily. Keep all the tubes on ice for 3 more minutes and keep very cold through Step 10.

8. Using a new small sterile transfer pipet, quickly open the "-C" tube and add 1 hanging drop (about 5µl) of sterile distilled water to Tube "-C" competent cells. Close the tube tightly. Mix by finger-flicking. Wrist-flick the sample to pool the reagents at the bottom of the tube. Return Tube "-C" to ice. Discard the transfer pipet into the disinfectant solution.

9. Using a new small sterile transfer pipet, quickly open the "pGFP" tube and add 1 hanging drop (about 5µl) of pGFP to the "pGFP" tubes. Pipet the plasmid **directly** into the cell suspension. Close the tube tightly. Mix by finger-flicking. Wrist-flick the sample to pool the reagents at the bottom of the tube. Return Tube "pGFP" to ice. Discard the transfer pipet into the disinfectant solution.

10. Leave both tubes of cells on ice for 30 minutes.

11. After 30 minutes, move your ice bath containing your tubes and the control tube next to the hot 42°C water bath. Make sure the tubes are very tightly closed. Hold the capped tubes at the top and as quickly as possible,

transfer both tubes from the ice to the 42°C water bath for a "heat shock" of ***exactly*** 90 sec. **Move the tubes as quickly as possible since the more distinct the heat shock, the greater the transformation efficiency.**

12. After 90 seconds, quickly return the tubes to the ice bath for 2 minutes. Move the tubes as quickly as possible from the heat to the cold. **The more distinct the cold shock, the greater the transformation efficiency.**

13. Using a small sterile transfer pipet for each, add 250µl of sterile LB broth to each tube. Close the tubes tightly. Mix each tube by finger flicking. Wrist-flick the sample to pool the reagents.

14. Make sure the labels on each tube are still visible. Incubate the tubes at 37°C for 30-45 minutes before plating. ***NOTE:*** *You may stop at this point and store the tubes in the refrigerator (4°C) overnight.*

II. Plating the Bacteria on Selection Media

NOTE: *Two LB Agar plates, without ampicillin, are shared with the entire class. Your teacher will assign you a group number and make sure you only use the assigned section. Group number: _____*

1. Disinfect the countertop by cleaning with disinfectant. Disinfect hands by thorough washing with hand soap or by using a hand disinfectant/sanitizer. Use a laminar flow hood, if available.

2. Obtain one plate of LB/amp (selection plates) and one plate of LB agar. Don't open the plates. Near the edge of the plates, on the bottom, label the plates with the group's initials. Draw a line down the center of each plate (on the bottom) and label one side "pGFP" and the other side "-C".

3. Turn the plates right side up to plate bacteria in the next steps. Only uncover the plates long enough to distribute the cell broth samples.

4. Finger-flick and then wrist flick each tube to mix the cells and pool them at the bottom. Using a new small sterile transfer pipet for each section, pipet 5-6 drops (~60µl) of the "pGFP" bacteria onto the "pGFP" section of each plate (don't touch the agar) and spread the cells around the "pGFP" section with a sterile inoculating loop. Discard the loop in the disinfectant waste container. Use a new loop for each spreading.

5. Using a new small sterile transfer pipet for each section, pipet 5-6 drops (~60µl) of the "-C" bacteria onto the "-C" section of each plate and spread the cells around the "-C" section with a sterile inoculating loop. Discard the loop in the disinfectant waste container. Use a new loop for each spreading.

6. Leave the plates, covered, on the countertop, undisturbed, flat, and right side up for 5 minutes so the suspension will be absorbed by the agar.

7. After 5 minutes, quickly invert the plates, stack them together, and place them **upside down,** in a 37°C incubator for 30-36 hours. After 36 hours the colonies should be visible. Growing overnight in the dark, at room temperature, can increase the size of the colonies. Only place plates on a surface that has been disinfected.

8. Disinfect countertop and wash your hands.

9. In your notebook, record your predictions about where colonies should grow on your plates. Consider which samples were spread on what type of agar. Think about and record the numbers of colonies you expect to see.

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10. If observations will take place after 36 hours consider storing plates, closed, upside-down at 4°C for 24 hours. For longer storage, wrap the plates in parafilm or store in an airtight container or sealed in plastic wrap. Be careful to not turn plates over until ready to observe (Part III) since they are easy to contaminate.

11. Disinfect countertop and wash your hands.

III. Observing and Recording Your Transformation Results

1. Disinfect the countertop to prepare for observations. Retrieve both Petri plates from the incubator or refrigerator. Look very closely for bacterial growth.

2. If only a few cells survived the transformation process, there may only be a few colonies on your plated sections. If many cells survived, there may be so much growth that colonies may grow together to create bacterial lawns.

3. Observe, draw, and label both plates showing **all** colonies in each section on each plate. Count the number of colonies (or do sampling and then multiply) on each plate. Record these data.

4. Make sure your drawing is as accurate as possible. Are colonies found in all sections on all plates. Why or why not?

5. Place a UV lamp behind each plate and in a dark room look for a green glow from bacterial colonies in each section of each plate. Are green-glowing colonies found in all sections on all plates? Why or why not?

Photograph the plates. When done, discard plates into the biohazard bag. Disinfect countertop and wash your hands.

REFLECTION/ANALYSIS

Describe your results. Does either suspect have a DNA fingerprint (RFLP banding pattern) that matches either of the DNA sample evidence from the crime scene? Explain. What do you conclude from the samples tested and the data collected?

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Thinking Like a Biotechnician

1. Is there any way two people could have the same DNA fingerprinting result.

_____
_____
_____
_____

2. Discuss ways that someone's DNA could be at the scene of a crime without being the victim or suspect.

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