

WHAT COLOR IS YOUR COLONY?

A HIGH SCHOOL LAB EXPERIMENT IN BACTERIAL TRANSFORMATION

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Teacher Manual

LESSON OVERVIEW

This experiment teaches students about bacterial transformation and puts emphasis on learning the science of doing the transformation. In this experiment, students do much more than look at the results to determine whether or not a transformation has occurred. The “Observations and Analysis” questions focus on what the students did that makes the experiment “good science.” Students are also asked to look at any unexpected results of the transformation as an opportunity for learning.

This lab is designed with the assumption that the students know how to set and read volumes on micropipettors and that they are familiar with the use of micropipettors. If your students need more background to help them use micropipettors in this lab you can refer to Section E of the Modern Genetics curriculum, which can be downloaded from the Washington University Science Outreach website at <http://www.so.wustl.edu>.

TIMELINE

This experiment will require approximately 45 minutes of class time for students to set up the experiment. At least 24 hours of incubation time is required. Students need about 15 minutes of post-incubation time to observe and record results. The 45-minute set-up time can be done in one day, or it can be divided into 35 minutes on one day and 15 minutes on the next day. The amount of time needed for pre-lab and post-lab discussions and time for working with “Observations and Analysis” questions depends on how the individual teacher uses these times.

MATERIALS

For each group of 4 students:

- 4 pairs of safety goggles
- 1 spray bottle of disinfectant
- 1 cup of ice
- 1 permanent marker
- 1 tube of sterile 50 mM CaCl₂ (labeled “C”)
- 1 tube of DsRed plasmid DNA (labeled “+”)
- 1 tube of sterile dH₂O (labeled “-“)
- 1 plate culture of *E. coli*
- 1 sterile toothpick (or an inoculating loop)
- 1 P1000 (100µl-1000µl) micropipette
- 1 P200 (20µl-200µl) micropipette (optional)
- 6 sterile micropipette tips (4 1000µl and 2 200µl tips if you use both micropipettes)
- 1 tube of sterile nutrient broth (labeled NB)
- 1 waste container with 10% bleach solution
- 2 sterile nutrient agar plates (labeled NA)
- 2 sterile nutrient agar + ampicillin plates (labeled NA/amp)
- 4 microcentrifuge tubes of sterile glass beads (~6 beads/tube)

Materials per class:

- 1 water bath set at 42°C
- floating racks
- incubator set at 37°C (optional)
- biohazard bag

ADVANCE PREPARATION

50 mM CaCl₂

- Mix 5 ml of 1M CaCl₂ with 95 ml of distilled or deionized H₂O. This mixture can be filter- or heat-sterilized. Store at 4°C in a sterile tube or bottle until needed.
- Make 1M CaCl₂ by dissolving 11.1 g of anhydrous CaCl₂ (f.w. 110.99) or 14.7 g of dihydrate CaCl₂ (f.w. 147.02) in 80 ml of distilled or deionized H₂O. Add water to bring the total volume up to 100 ml.
- Aliquot 550 μl of sterile 50 mM CaCl₂ in one microcentrifuge tube for each lab team. Label this tube with a "C" on the tube cap.

DsRed Plasmid DNA

- pDsRed-Express Vector, catalog # 632412 (ask for academia price)
BD Biosciences Clontech
1020 East Meadow Circle
Palo Alto, CA 94303-4230
800-662-2566, <http://www.clontech.com>
- BD Biosciences ships pDsRed at a concentration of 500 ng/μl. Dilute 1 μl of this stock solution in 99 μl of sterile TE buffer or sterile dH₂O. This makes a working concentration of 5 ng/μl (0.005 μg/μl).
- Aliquot 10 μl of 5 ng/μl pDsRed in a microcentrifuge tube for each lab team. (A total of 50 ng of plasmid DNA is used to transform the *E. coli*.) Label this tube with a "+" on the tube cap.

***E. coli* stock plate**

- *E. coli*, catalog # 4-5
Modern Biology, Inc.
111 North 500 West
West Lafayette, IN 47906
800-733-6544, <http://www.modernbio.com>
- *E. coli* should be freshly streaked for best results. This can be done 24 to 48 hours before the plates are to be used. Transformation efficiency decreases as colonies age.
- Various strains of *E. coli* have been used successfully for this transformation. Phenotypic expression of the red pigment varies among strains. The *E. coli* strain DH5 sold by Modern Biology Inc. grows and expresses most rapidly after transformation. *E. coli* strains MM294, DH5alpha, and X7029 can also be transformed.

Nutrient Broth tube

- Aliquot 750 μl of sterile nutrient broth (or LB broth) to a microcentrifuge tube for each lab team. Label this tube "NB" on the tube cap.

Luria-Bertani (LB) Broth

- To make 100 ml of LB broth, weigh out 1 g of tryptone, 0.5 g of yeast extract, and 1 g of NaCl. Stir into 100 ml of dH₂O in an autoclavable container that is at least twice as large as the volume of medium. Add 50 μ l of 4M NaOH. Autoclave medium at 15 lbs for 15 minutes. The cooled medium can be stored at room temperature.

Nutrient Agar (NA) plates

- LB agar plates can also be used.
- Four nutrient agar plates (two with ampicillin) are needed per group. A half-filled plate contains about 25 ml of medium. Produce 50 ml of nutrient agar per team and 50 ml of nutrient agar plus ampicillin per team. A plate can be filled with as little as 15 ml of medium if you want to save money.
- The final concentration of ampicillin in the NA/amp plates should be 50 μ g/ml. Add 5 ml of 10 mg/ml ampicillin stock solution to 1 liter of autoclaved nutrient agar that has cooled enough that the flask can be held. (High heat makes ampicillin ineffective.)

LB Agar

- To make 500 ml of LB agar, weigh out 5 g of tryptone, 2.5 g of yeast extract, 5 g of NaCl and 7.5 g of agar. Stir into 500 ml of dH₂O in an autoclavable container that is at least twice as large as the volume of medium. Add 250 μ l of 4M NaOH. Autoclave medium at 15 lbs for 15 minutes.

Ampicillin

- Add 0.1 g of ampicillin (sodium salt, f.w. = 371.40) to 10 ml of deionized or distilled H₂O. Stir to dissolve the sodium salt. The ampicillin solution must be filter sterilized because high heat makes the ampicillin ineffective.
- The ampicillin solution can be stored for 3 months at 4°C or for 1 year at -20°C.

Sterile Glass Beads

- 3 – 5 mm glass beads work well for spreading bacteria evenly across the media plates. These can be sterilized in the microcentrifuge tubes with the caps closed.

HINTS AND TIPS FOR STEPS IN THE STUDENT MANUAL PROCEDURE

- 4a Toothpicks taken out of a new box can be used if you do not want to sterilize toothpicks.
- 4d It is important that the *E. coli* cells be resuspended immediately after they are put in the CaCl_2 . The longer the clump of cells is in the CaCl_2 , the more difficult it is to separate them. Stress the importance of resuspending the cells **gently**. Forceful ejection of fluid from the micropipette can cause liquid to spill out of the microcentrifuge tube.
- 4f If time permits, the cells and CaCl_2 can incubate on ice up to 15 minutes before being added to the “+” and “-” tubes. This pre-incubation time has less of an effect on transformation efficiency so a pre-incubation of 3-5 minutes should give approximately the same transformation efficiency.
- 8 Heat shock is the critical step in transformation. To achieve good results, the following should be emphasized:
- Tubes should go immediately from the ice to the water bath. Therefore, do not take the tubes out of ice and carry them across the room or hold them for even a short time before putting them in the water bath.
 - The 90-second time of the heat shock should be closely followed. Variations from this time will probably negatively affect transformation efficiency.
 - Tubes should go immediately from the water bath back to the ice.
- 9e Recovery is the time after nutrient broth has been added to the cells. A recovery time of 5-30 minutes could be included but this is not necessary if the antibiotic being used for selection is ampicillin.
- 14 If you do not have an incubator the plates can incubate at room temperature. This will slow the growth of the colonies and the development of the red pigment caused by transformation.

ANSWERS TO OBSERVATIONS AND ANALYSIS QUESTIONS

Answer the following questions before you see the results of your transformation. These questions ask you to think about the experimental process.

1. What is the difference between the “+” tube and the “-” tube?
The “+” tube has plasmid in it. The “-” tube does not have plasmid in it.
2. Why is a “-” tube included in this experiment?
This tube is a control that shows how the cells are affected if no plasmid is added.
3. Why does the “-” tube contain the same volume of liquid as the “+” tube?
A good experiment keeps all “non-variables” constant. We do not want the total volume of the tubes to differ. We are not testing the effect of liquid volume.
4. What result do you expect to see on each plate?
 - a. “-” cells on NA (non-transformed *E. coli* on nutrient agar)
*We should see a lot of *E. coli* growth.*
 - b. “-” cells on NA/amp (non-transformed *E. coli* on nutrient agar + ampicillin)
*We should see no *E. coli* growth.*
 - c. “+” cells on NA (transformed *E. coli* on nutrient agar)
*We should see a lot of *E. coli* growth.*
 - d. “+” cells on NA/amp (transformed *E. coli* on nutrient agar + ampicillin)
(Think about this...Will all *E. coli* cells be transformed?)
*We should see some, but not a lot, of *E. coli* growth.*
5. Why was *E. coli* put on nutrient agar without ampicillin?
*This shows us that the *E. coli* cultures were living.*
6. Why are the two NA plates without ampicillin considered to be positive controls?
We expected growth to occur on these plates.
7. Why were non-transformed *E. coli* put on nutrient agar + ampicillin?
*We wanted to show that *E. coli* usually does not grow on plates with ampicillin.*
8. Why is the non-transformed *E. coli* on NA/amp considered to be a negative control?
*We expected no *E. coli* growth on this plate.*
9. Why were transformed *E. coli* put on nutrient agar + ampicillin?
*We wanted to show that this transformation makes it possible for *E. coli* to grow on ampicillin.*
10. Why is the transformed *E. coli* on NA/amp the experimental plate?
*This plate shows the effect of the variable, the addition of plasmid to some of the *E. coli* cells.*

Answer the following questions after you have seen the results of your transformation.

11. How many colonies are growing on each plate? Record your observations of the four plates in the matrix below. If there are too many colonies to count, record that you have a “lawn” of bacteria.

	Nutrient agar plate	Nutrient agar + ampicillin plate
Non-transformed cells (“-” tube)	<i>should have a lawn of bacteria</i>	<i>should have no bacteria</i>
Transformed cells (“+” tube)	<i>should have a lawn of bacteria</i>	<i>should have some colonies but not a lawn</i>

12. Compare the color of the colonies on the NA plates with the color of the colonies on the NA/amp plate.
- Which of the plates has *E. coli* with an unnatural color?
The NA/amp plate had red colonies on it.
 - Explain why this *E. coli* has a different color.
Transformation of the E. coli changed the color of the colonies.
13. Define transformation.
Transformation is the uptake of foreign DNA by bacteria. This DNA may then transform the bacteria by giving them some new phenotype / characteristic.
14. What two observations are evidences that transformation has occurred?
The transformed E. coli can now grow on plates containing ampicillin and the E. coli colonies are red instead of cream colored.
15. What transformed the *E. coli* in this experiment?
The plasmid that was added to the E. coli transformed the cells.

The following questions are about any unexpected observations that may have occurred. Here you need to think about what might have caused results that cannot be explained by the simple idea of transformation.

16. There should be transformed *E. coli* on one of the plates without ampicillin. Do you see evidence of transformation on this plate? If not, what result would you expect to see that you do not see?
No, there were no red colonies on the NA plate. All were the normal cream color.
17. How can this lack of evidence be explained?
Perhaps ampicillin stimulates expression of the red pigment. Perhaps crowding of the colonies in some way prevents the red pigment from being produced.

18. Do you see any colonies on the experimental plate (NA/amp) that do not appear as expected? If yes, what is “wrong” with their appearance?

There might be some white / cream colonies on the plate. Cells should not be able to grow on ampicillin plates unless they are transformed. If the cells are transformed, then the colonies should be red.

19. How can this “wrong” result be explained?

Quite often these colonies will be seen growing close to red colonies. They are probably “satellite” colonies that do not contain transformed cells. They are able to grow on the ampicillin only because transformed cells produce a product that is secreted into the medium around the red colonies. This product in some way makes the ampicillin unable to prevent growth of the satellite colonies.

This is a series of calculations that your teacher might ask you to do or that you might do on your own as an extra problem. (*The number of colonies shown below will vary.*)

20. Transformation efficiency is the number of transformed cells (equals the number of colonies that grew on NA/amp) per microgram of plasmid DNA. To determine your transformation efficiency, do the following calculations:

a. How many μl of plasmid DNA were in the “+” tube? 10 μl (A)

b. Plasmid concentration \times A = $0.005 \mu\text{g}/\mu\text{l} \times$ 10 $\mu\text{l} =$
0.05 $\mu\text{g} =$ total mass of plasmid

c. How many μl of “+” cells did you put on the NA/amp plate? 100 μl (B)

d. How many μl of cells were in the “+” tube? 510 μl (C)

e. $B/C =$ 100 $\mu\text{l} / 510 \mu\text{l} = 20\% =$ % of cells put on plate

f. Total mass of plasmid \times % of cells put on NA/amp plate =

$$\underline{0.05} \mu\text{g} \times \underline{0.20} = \underline{0.01} \mu\text{g} =$$

mass of plasmid put on the NA/amp plate

g. How many transformed colonies grew on the NA/amp plate? 50
(This answer will vary. 50 is an example colony number.)

h. # of colonies / mass of plasmid put on NA/amp plate =

$$\underline{50} / \underline{.01} \mu\text{g} = \text{transformation efficiency (colonies per } \mu\text{g)}$$

(This answer will vary. 50 is an example colony number.)

- i. What was your transformation efficiency? 5000 colonies per μg of plasmid
(This answer will vary. 5000 colonies per μg is based on 50 colonies. This is an efficiency of 5×10^3 .)

The normal transformation efficiency for colony transformations is 5×10^3 to 5×10^4 colonies per μg of plasmid.

WHAT COLOR IS YOUR COLONY?

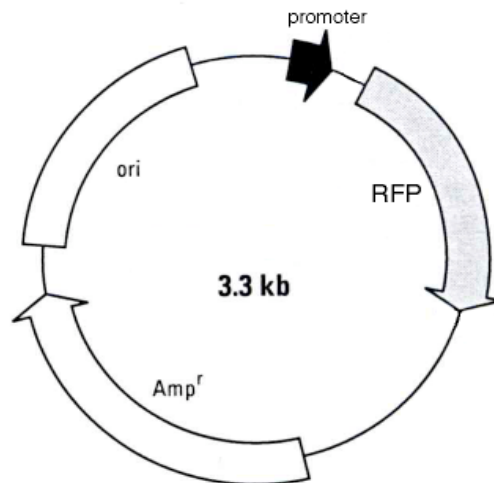
Student Manual

In 1928 Frederick Griffith demonstrated that bacteria could be genetically changed through exposure to a “transforming principle.” Sixteen years later Oswald Avery’s research team demonstrated that DNA was that “transforming principle.” This natural transformation described by Griffith and Avery is a very rare event. But in 1970, Mandel and Higa showed that *E. coli* could be “artificially” transformed if the cells were treated with a calcium chloride solution. This process made the *E. coli* competent, meaning the cells’ membranes became leaky to DNA. The form of DNA that is normally used to transform bacteria is a plasmid, a small ring of DNA.

Through the process of transformation, a gene that has been spliced into a plasmid can be inserted into a bacterium. This gene can be from another bacterium or it can be from any other organism, even a human. Because bacteria can reproduce rapidly and because each cell copies its plasmids, an inserted gene can multiply exponentially in a short period of time. This amplified gene is then available for DNA analysis or for production of a gene product. In the classroom we can use transformation to alter a visible bacterial phenotype as a quick demonstration of genetic change.

Although the principle that drives transformation is straightforward, we must be aware of the fact that variables exist during transformation that are outside of our control. We must be ready for exceptions to the expected outcome. When we see the unexpected, we need to look at the evidence and offer explanations that are consistent with the evidence.

We are going to transform a strain of *E. coli* with a plasmid that contains two genes. Look at the plasmid map and be ready to discuss what the effects of these genes might be on a transformed bacterium.



BD Biosciences Map of pDsRed-Express

Key: RFP – This is a gene for a red fluorescent protein from *Discosoma* corals.
Amp^r – This is a gene for resistance to the antibiotic ampicillin.
ori – The plasmid can replicate itself because it has an origin of replication.
promoter – The promoter is necessary for gene transcription to occur.

MATERIALS

For each group of 4 students:

- 4 pairs of safety goggles
- 1 spray bottle of disinfectant
- 1 cup of ice
- 1 permanent marker
- 1 tube of sterile 50 mM CaCl₂ (labeled “C”)
- 1 tube of DsRed plasmid DNA (labeled “+”)
- 1 tube of sterile dH₂O (labeled “-”)
- 1 plate culture of *E. coli*
- 1 sterile toothpick (or an inoculating loop)
- 1 P1000 (100µl-1000µl) micropipette
- 1 P200 (20µl-200µl) micropipette (optional)
- 6 sterile micropipette tips (4 1000µl and 2 200µl tips if you use both micropipettes)
- 1 tube of sterile nutrient broth (labeled NB)
- 1 waste container with 10% bleach solution
- 2 sterile nutrient agar plates (labeled NA)
- 2 sterile nutrient agar + ampicillin plates (labeled NA/amp)
- 4 microcentrifuge tubes of sterile glass beads (~6 beads/tube)

Materials per class:

- 1 water bath set at 42°C
- floating racks
- incubator set at 37°C (optional)
- biohazard bag

PROCEDURE

1. Find the tube labeled “C”. This tube contains sterile CaCl₂. Place the tube in the cup of ice.
2. Find the tube with a “+” on the lid. This tube contains 10 µl of plasmid DNA. Use the permanent marker to write your initials on the tube cap. Place the tube in the cup of ice.
3. Find the tube with a “-” on the lid. This tube does not contain plasmid DNA. Instead, it contains 10 µl of water. Write your initials on the tube cap. Place the tube in the cup of ice.
4. Transfer *E. coli* to the CaCl₂. To do this:
 - a. Use a sterile toothpick (or a sterile inoculating loop) to transfer 1 large colony from a streak plate of *E. coli*. Do not scrape up agar from the plate.

- b. Twirl the toothpick (loop) in the CaCl_2 against the inside of the tube to knock off the clump of bacteria. Put the toothpick in the waste container. (If you use an inoculating loop, re-flame the loop to kill bacteria left on the loop.)
- c. Set the P1000 micropipette at $500 \mu\text{l}$. Place a sterile tip on the micropipette.
- d. Resuspend the bacteria cells in the CaCl_2 by gently pipeting the fluid in and out until there are no visible clumps of cells.
- e. Write “+ cells” on the tube cap with the permanent marker.
- f. Place the tube into the cup of ice. Let the cells incubate on ice for 5 minutes.

Record the time that this incubation will be finished. _____

5. Transfer *E. coli* to the plasmid DNA in the “+” tube. To do this:
 - a. Set the P1000 micropipette at $250 \mu\text{l}$. Place a sterile tip on the micropipette.
 - b. Withdraw $250 \mu\text{l}$ of the cell suspension from the “C + cells” tube.
 - c. Expel the cell suspension into the “+” tube.
 - d. Gently mix the contents of the tube by pipeting the fluid in and out a few times.
 - e. Place the “+” tube into the cup of ice.
6. Transfer *E. coli* to the water in the “-” tube. To do this:
 - a. Put a sterile tip on the P1000 micropipette.
 - b. Withdraw another $250 \mu\text{l}$ of the cell suspension from the “C + cells” tube.
 - c. Expel the cell suspension into the “-” tube.
 - d. Gently mix the contents of the tube by pipeting the fluid in and out a few times.
 - e. Place the “-” tube into the cup of ice.
7. Let both the cells + plasmid (“+”) tube and the cells + water (“-”) tube incubate on ice for 15 minutes.

Record the time that this incubation will be finished. _____

8. At the end of 15 minutes, heat shock the two tubes at 42°C for 90 seconds. To do this:
 - a. Carry the “+” and “-” tubes in the cup of ice to the water bath.
 - b. Make sure that the water temperature is 42°C.
 - c. Remove the tubes from the ice and immediately place them in the water in a floating rack.
 - d. After 90 seconds, move the two tubes from the water bath directly back to the ice.
 - e. Leave the tubes in the ice for at least 1 minute. You can then move the tubes from the ice to a tube rack at room temperature.
9. Add nutrient broth to each tube of cells. To do this:
 - a. Set the P1000 micropipette to 250 μ l. Place a sterile tip on the micropipette.
 - b. Find the tube labeled NB. This is the nutrient broth.
 - c. Add 250 μ l of nutrient broth to each tube of cells. (If you start with the “-” tube, it is not necessary to change micropipette tips between tubes.)
 - d. Close the tube caps and gently tap the side of each tube to mix the cells into the broth.
 - e. Place the tubes back in the tube rack. You can plate the cells immediately or the tubes can be stored at 4°C overnight.
10. Prepare the nutrient agar plates for inoculation. To do this:
 - a. Write your ID and the date on the bottom of all four plates.
 - b. Write “no plasmid” on the bottom of 1 nutrient agar (NA) plate.
 - c. Write “+ plasmid” on the bottom of 1 nutrient agar (NA) plate.
 - d. Write “no plasmid” on the bottom of 1 NA + ampicillin (NA/amp) plate.
 - e. Write “+ plasmid” on the bottom of 1 NA + ampicillin (NA/amp) plate.
 - f. Pour 5-7 sterile glass beads from a microcentrifuge tube onto the medium in each plate. (These tubes can be reused. Do not throw them away.)

11. Inoculate the nutrient agar plates with *E. coli* cells. To do this:
 - a. Set a P1000 micropipette or a P200 micropipette to 100 μ l. Place a sterile tip on the micropipette.
 - b. Transfer 100 μ l of cells from the “-” tube to the “no plasmid” NA plate.
 - c. Transfer 100 μ l of cells from the “-” tube to the “no plasmid” NA/amp plate.
 - d. Place a new sterile tip on the micropipette.
 - e. Transfer 100 μ l of cells from the “+” tube to the “plasmid” NA plate.
 - f. Transfer 100 μ l of cells from the “+” tube to the “plasmid” NA/amp plate.
 - g. Hold the stack of four plates right side up. Spread the cells using a swirling motion to move the glass beads over the surface of the medium. Change the angle or the rotation of the plates to make sure that the beads cover the entire surface of the medium. Continue swirling for 2 minutes.
12. Let the plates set upright for a few minutes until the cell suspension has been absorbed into the medium.
13. Invert each plate so that the glass beads fall onto the lid of the plate. Pour the beads from the lid into a beaker. Put the lid back on the plate.
14. Stack your four plates and place them upside down into an incubator at 37°C. Incubate the plates for 12-24 hours. After a day, the plates can be kept at room temperature or in a refrigerator.
15. Clean up your lab station. To do this:
 - a. Dump your used tips into a bleach solution or a biohazard bag.
 - b. Dump your C, + and - tubes into a biohazard bag.
 - c. Make sure your ice container is empty and dump the ice into a sink.
 - d. Return all materials to the correct location.
 - e. Wipe your table top with a disinfectant solution.
 - f. Wash your hands before leaving the lab.

Name _____ Class Hour _____ Date _____

OBSERVATIONS AND ANALYSIS

Answer the following questions before you see the results of your transformation. These questions ask you to think about the experimental process.

1. What is the difference between the “+” tube and the “-” tube?

2. Why is a “-” tube included in this experiment?

3. Why does the “-” tube contain the same volume of liquid as the “+” tube?

4. What result do you expect to see on each plate?
 - a. “-” cells on NA (non-transformed *E. coli* on nutrient agar)

 - b. “-” cells on NA/amp (non-transformed *E. coli* on nutrient agar + ampicillin)

 - c. “+” cells on NA (transformed *E. coli* on nutrient agar)

 - d. “+” cells on NA/amp (transformed *E. coli* on nutrient agar + ampicillin)
(Think about this...Will all *E. coli* cells be transformed?)

5. Why was *E. coli* put on nutrient agar without ampicillin?

6. Why are the two NA plates without ampicillin considered to be positive controls?
7. Why were non-transformed *E. coli* put on nutrient agar + ampicillin?
8. Why is the non-transformed *E. coli* on NA/amp considered to be a negative control?
9. Why were transformed *E. coli* put on nutrient agar + ampicillin?
10. Why is the transformed *E. coli* on NA/amp the experimental plate?

Answer the following questions after you have seen the results of your transformation.

11. How many colonies are growing on each plate? Record your observations of the four plates in the matrix below. If there are too many colonies to count, record that you have a “lawn” of bacteria.

	Nutrient agar plate	Nutrient agar + ampicillin plate
Non-transformed cells (“-” tube)		
Transformed cells (“+” tube)		

12. Compare the color of the colonies on the NA plates with the color of the colonies on the NA/amp plate.
 - a. Which of the plates has *E. coli* with an unnatural color?
 - b. Explain why this *E. coli* has a different color.

13. Define transformation.

14. What two observations are evidences that transformation has occurred?

15. What transformed the *E. coli* in this experiment?

The following questions are about any unexpected observations that may have occurred. Here you need to think about what might have caused results that cannot be explained by the simple idea of transformation.

16. There should be transformed *E. coli* on one of the plates without ampicillin. Do you see evidence of transformation on this plate? If not, what result would you expect to see that you do not see?

17. How can this lack of evidence be explained?

18. Do you see any colonies on the experimental plate (NA/amp) that do not appear as expected? If yes, what is “wrong” with their appearance?

19. How can this “wrong” result be explained?

This is a series of calculations that your teacher might ask you to do or that you might do on your own as an extra problem.

20. Transformation efficiency is the number of transformed cells (equals the number of colonies that grew on NA/amp) per microgram of plasmid DNA. To determine your transformation efficiency, do the following calculations:

- a. How many μl of plasmid DNA were in the “+” tube? _____ μl (A)
- b. Plasmid concentration x A = $0.005 \mu\text{g}/\mu\text{l} \times$ _____ $\mu\text{l} =$
_____ $\mu\text{g} =$ total mass of plasmid
- c. How many μl of “+” cells did you put on the NA/amp plate? _____ μl (B)
- d. How many μl of cells were in the “+” tube? _____ μl (C)
- e. $B/C =$ _____ $\mu\text{l} /$ _____ $\mu\text{l} =$ _____ % of cells put on NA/amp plate
- f. total mass of plasmid x % of cells put on NA/amp plate =
_____ $\mu\text{g} \times$ _____ = _____ $\mu\text{g} =$ mass of plasmid put on NA/amp plate
- g. How many transformed colonies grew on the NA/amp plate? _____
- h. # of colonies / mass of plasmid put on NA/amp plate =
_____ / _____ $\mu\text{g} =$ transformation efficiency (colonies per μg)
- i. What was your transformation efficiency? _____ colonies per μg of plasmid

The normal transformation efficiency for colony transformations is 5×10^3 to 5×10^4 colonies per μg of plasmid.