

BACTERIAL TRANSFORMATION

Laboratory Kit Contents

Bacterial Transformation & Supplemental Activities

Part A: Bacterial Transformation Only

<u>Sent Out</u>	<u>Returned</u>	
<input type="checkbox"/>	<input type="checkbox"/>	1 ice container (cooler)
<input type="checkbox"/>	<input type="checkbox"/>	<i>E. coli</i> starter culture plate(s)
<input type="checkbox"/>	<input type="checkbox"/>	50mM CaCl ₂ solution (6 tubes)
<input type="checkbox"/>	<input type="checkbox"/>	pLUX or pVIB plasmid solution (1 microcentrifuge tube)
<input type="checkbox"/>	<input type="checkbox"/>	LB Agar (w/o ampicillin) plate (1 per class)
<input type="checkbox"/>	<input type="checkbox"/>	LB/Ampicillin agar plates (2 plates per group, 12 per class)
<input type="checkbox"/>	<input type="checkbox"/>	strips of parafilm
<input type="checkbox"/>	<input type="checkbox"/>	LB Broth (6 tubes)
<input type="checkbox"/>	<input type="checkbox"/>	sterile inoculating loops
<input type="checkbox"/>	<input type="checkbox"/>	“L” shaped bacterial spreaders
<input type="checkbox"/>	<input type="checkbox"/>	6 permanent lab markers
<input type="checkbox"/>	<input type="checkbox"/>	1.5 mL microcentrifuge tubes
<input type="checkbox"/>	<input type="checkbox"/>	6 rectangular microcentrifuge tube racks
<input type="checkbox"/>	<input type="checkbox"/>	6 100-1000 ul micropipettors
<input type="checkbox"/>	<input type="checkbox"/>	tips for 100-1000 ul micropipettors
<input type="checkbox"/>	<input type="checkbox"/>	6 2-20 ul (or similar volume) micropipettors
<input type="checkbox"/>	<input type="checkbox"/>	tips for 2-20 ul micropipettors
<input type="checkbox"/>	<input type="checkbox"/>	small autoclavable bags for tips and tubes (6 per kit)
<input type="checkbox"/>	<input type="checkbox"/>	ice bath containers (6 per kit)
<input type="checkbox"/>	<input type="checkbox"/>	water bath unit (1 per kit)
<input type="checkbox"/>	<input type="checkbox"/>	round, floating microcentrifuge tube racks
<input type="checkbox"/>	<input type="checkbox"/>	clock timer (1 per kit)
<input type="checkbox"/>	<input type="checkbox"/>	2 boxes of disposable gloves
<input type="checkbox"/>	<input type="checkbox"/>	tubes of colored water for micropipettor practice
<input type="checkbox"/>	<input type="checkbox"/>	CCA Science DVD
<input type="checkbox"/>	<input type="checkbox"/>	3-ring notebook with protocols and other supplementary materials

Pre-Lab Preparation

Student Skills/Knowledge Required for Success:

*Note: It is important that prior to performing both sessions of this lab activity, students should practice and understand the following lab techniques:

- Understanding of metric units of measurement (e.g. ml, ul, etc)
- Use of micropipettor to measure small volumes in ul using sterile tips
- Handling of microcentrifuge tubes
- Safety issues (e.g. aseptic techniques, wearing gloves, waste disposal...)

Pre-Lab Review:

1. Review the use of the metric system of measurement in the laboratory specifically the use of liquid volumes such as p.l.
2. Explain and demonstrate the correct use and handling of a micropipettor. Have students practice the uptake and transfer of different volumes of colored water.
3. Review the handling and use of microbiological equipment such as inoculating loops, petri plates, cell spreaders, water baths, ice baths, etc.
4. Review and emphasize sterile (aseptic) techniques in a microbiology lab to minimize/prevent contamination of cultures, workspace and individuals.
5. Review simple concepts of statistical analysis include sample size, average, etc.
6. Review the use of positive and negative controls in laboratory experiments and how they are used to interpret results.
7. Review the basic structure of cell membranes and membrane electrical potential and of prokaryotic/bacterial cells including plasmids.
9. Discuss methods of genetic variation in bacteria emphasizing transformation and the early experiments of Griffith using mice and different strains of pneumonia bacteria.

BACTERIAL TRANSFORMATION

Part A: Bacterial Transformation with Plasmid

Materials and Supplies Needed

- starter petri plates of *Escherichia coli* culture
- 50 mM CaCl₂ solution (1 small tube per table of two lab pairs)
- 0.005 ug/ul of pVIB or pLUX (1 small tube per class)
- LB agar plates (1 plate per class)
- LB/Ampicillin agar plates (2 small plates per lab table)
- strips of parafilm (2 strips per lab pair)
- LB broth (1 small tube per lab table)
- sterile inoculating loops (2 per lab table)
- “L” shaped cell spreaders (2 per lab table)
- permanent lab markers (1 per lab table)
- 1.5 ml microcentrifuge tubes (2 per lab table)
- microcentrifuge tube racks (1 per lab table)
- 100-1000 ul micropipettors (1 per lab table)
- box of 100-1000 ul micropipettor tips (1 box per lab table)
- 2-20 ul micropipettor (1 per class, teacher uses)
- box of 2-20 ul micropipettor tips (1 box per class)
- small autoclavable bags for used tips and tubes
- ice baths (1 per lab table)
- water bath set at 42° C (1 per class)
- floating microcentrifuge tube racks (2 per class will fit in water bath unit)
- clock timer (1 per class)
- disposable gloves (1 box per class)
- tubes of colored water for micropipettor practice

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Immediately Prior to Lab

1. Heat water bath to 42° C before lab session.
2. Right before class, fill ice bath containers at each table with crushed ice and keep both the tubes of CaCl₂ and plasmid pVIB on ice throughout the entire class session.
3. Keep starter petri plates with E. coli in the refrigerator until right before class time.

Procedures for 1st Lab Session (each lab table)

Part A: Bacterial Transformation with Plasmid

1. Use the permanent marker to label the two 1.5 ml microcentrifuge tubes as follows:
 - “+“ for the tube that will contain the plasmid
 - “-“ for the tube that will not contain the plasmid (negative control)

You will be sharing an ice bath, water bath and tube rack with other students, so you should also add your initials or some other distinguishing mark on your tubes.

2. Set the 100-1000 ul micropipettor for 250 ul and with a sterile tip, transfer 250 ul of very cold CaCl₂ to each of the above microcentrifuge tubes. Dispose of the used tip in the small autoclave bag.
3. Close the lids and place the “+“ and “-“ tubes containing the CaCl₂ in the ice bath at your table. Also return the tube with the stock solution of CaCl₂ to ice so it remains cold for the next class of students.
4. Use a sterile inoculating loop to lift off 2-3 bacteria colonies (no agar media) from the starter Petri plate of E coli and transfer to the CaCl₂ in the “+“ tube. Only open the Petri plate wide enough to reach in to remove the colonies—do not place the lid on the lab table or leave the plate exposed. Immerse the loop with the colony mass directly into the CaCl₂ solution in the tube and dislodge the mass by spinning the loop gently in the solution. Hold the tube up to the light while doing this and you should observe the CaCl₂ solution become cloudy. Make sure the colony mass does not remain on the loop or stuck to the side of the tube or in a clump on the bottom of the tube. When finished, dispose of the inoculating loop in the small autoclavable bag. Tap or invert the tube several times to make sure the solution looks homogeneous, not clumpy. Return this “+“ tube to the ice bath.

5. Use a new sterile inoculating loop and repeat step 4 above for the “-“ tube. Then place this tube in the ice bath. Now both the “+“ and “-“ tubes should be on ice.
 6. Your teacher has the tube containing the plasmid will now come around to your lab tables and with the 2-20 ul pipettor, transfer 10 ul of the plasmid solution directly into the cell suspension solution in your “+“ tube only. Close the lid on your tube and mix the contents by tapping or slowly inverting the tube several times. Try to avoid creating many air bubbles. The tube containing the stock plasmid solution should be kept on ice or in the freezer after use.
 7. Place both your “+“ and “-“ tubes on ice for 15 minutes.
 8. While your tubes are on ice for 15 minutes, obtain two small Petri plates containing LB/Ampicillin (marked LB/Amp) and label one plate “+“ and the other “-“. Label the bottoms of the plates, not the lids. Since plates from all students will be incubated together, also write your initials or name near the edge of each plate for easy identification.
 9. After 15 minutes, carry your ice bath to the hot water bath and immediately immerse both tubes in the floating microcentrifuge rack in the 42°C water bath for 90 seconds. Set the time clock or count slowly to 90.
 10. After one minute, immediately return both tubes to the ice bath for 2 minutes. For the transformation process to be successful, a distinct heat shock must occur with quick transitions from cold-to-hot-to cold.
- Note: It is okay for the tube to remain on ice longer than 2 minutes.
11. Following the 2 minute immersion on ice, use the 100-1000 ul micropipettor and a sterile tip to transfer 250 ul of LB broth to the “+“ tube. Close the lid and invert the tube several times to mix the LB broth with the CaCl₂ cell suspension. Dispose of the used tip in the small autoclavable bag.
 12. Using a new sterile tip, repeat step 10 for the “-“ tube and mix the tube contents. Discard the used tip.
 13. Place both the “+“ and “-“ tubes in the microcentrifuge tube rack and let the cells “recover” for 30 minutes at room temperature. (This time might possibly be shortened but we have had success with a 30-minute recovery time.) At this point in the procedure, if time is running short, both the “+“ and “-“ tubes can be stored on ice in the refrigerator until the next class session. Do not place the tubes in the freezer however.
 14. Following the 30-minute recovery time, use the 100-1000 ul micropipettor and a sterile tip to transfer 100 ul of the contents of the “-“ tube to the surface of the LB/Amp agar plate you labeled with a “-“. Discard the used tip. Remember to not expose the plate more than necessary to minimize contamination from other microbes.

15. Use a sterile “L”-shaped cell spreader to spread the 100 ul evenly across the entire surface of the agar plate. It works best to have one student hold the lid of the plate above the agar while the other student rotates the plate while gently holding the cell spreader on the agar surface. Close the plate immediately and let set for 1-2 minutes. Discard the cell spreader in the autoclavable bag.

16. Use the 100-1000 ul and a new sterile tip to transfer 100 ul of the contents of the “+” tube to the surface of the LB/Amp plate that you labeled “+”. Discard the used tip and remember to not expose the plate surface more than necessary. Using a new sterile “L”-shaped cell spreader, repeat step 14 to evenly spread the 100 ul across the plate surface. Close the plate and let set for 1-2 minutes.

*17. The teacher or one lab pair in the class should also set up one LB (no Amp) plate with 100 ul of the contents of one of the “-” tubes prepared in the class, following the procedure outlined above. This plate will serve as a control for the transformed bacteria. Alternatively, the starter plates of *E.coli* can function as control plates for comparison with the experimental plates.

18. After the plates have set, use parafilm strips to seal the edges of each plate closed. (Your teacher will demonstrate how to do this). Incubate your plates upside down in a warm, dark place for 1-3 days.

Procedures for 2nd Lab Session (Part A)

For each lab table

1. Examine your plates for bacterial growth by noting the presence of colonies on the surface of the plates. It is not necessary to open the plates as you can easily see the colonies through the lids of the Petri plates.
2. Record which plates show the growth of colonies and how many colonies are present. If there are numerous colonies, you may wish to use a counting grid. (introduced later in lab)
3. Take your pVIB or pLUX plates (including the control plate prepared in step 16 above) into a very dark room and observe them. What do you observe? Compare your “+” and “-” plates and also to the single control plate set up by the class or to a starter plate.

BACTERIAL TRANSFORMATION

Part B: Serial Dilution Technique**

****Materials for Part B are not included in the CCA WIRED kits**

Materials and Supplies Needed

Students work in groups of 4+ at each lab table

- Broth culture of *Escherichia coli* bacteria (1 tube per class)
- LB agar plates (3 large plates per lab table)
- strips of parafilm (3 strips per lab table)
- LB broth (1 small tube per lab table)
- sterile “L” shaped cell spreaders (3 per lab table)
- permanent lab markers (1 per table of two lab pairs)
- 1.5 ml sterile microcentrifuge tubes (7 per lab table)
- microcentrifuge tube racks (1 per lab table)
- 100-1000 ul micropipettors (1 per lab table)
- box of 100-1000 ul micropipettor tips (1 per lab table)
- counting grid for petri plate
- small autoclavable bags for used tips and tubes (1 per lab table)
- disposable gloves (1 box per class)
- tubes of colored water for micropipettor practice

Student Skills/Knowledge Required:

In addition to those mentioned for Part A.,

- Introduce the concept of a serial dilution using diagrams and a flow-chart approach.
- Review the use of fractions and exponents of base 10.

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Note:

Keep the bacterial culture tube on ice or in the refrigerator throughout the class sessions.

Part B: Serial Dilution Technique

Procedures for 1 Lab Session (each lab table)

1. Label the 7 sterile 1.5 ml microcentrifuge tubes with the following:
“1” “1/10” “1/10²” “1/10³” “1/10⁴” “1/10⁵” “1/10⁶”
2. Place the above tubes in this sequential order in the microcentrifuge tube rack.
3. With the 100-1000 ul micropipettor set for 1000 ul and a sterile tip, transfer 200 ul of the *E.coli* broth culture (your teacher will have this culture) to your microcentrifuge tube labeled “1”. This tube contains bacterial culture at full concentration. Discard the used tip in the small autoclavable bag.
4. With the 100-1000 ul micropipettor set for 900 ul and a new sterile tip, transfer 900 ul of LB broth from the stock tube of broth to each of the remaining microcentrifuge tubes (those labeled “1/10” through “1/10⁶” Discard the used tip after completing all the broth transfers.
5. With the 100-1000 ul micropipettor set for 100 ul and a new sterile tip, transfer 100 ul of the full concentration *E. coli* bacterial culture (tube labeled “1”) into the 900 ul of LB broth in the tube labeled “1/10”. Close the lid on this tube and invert gently several times to mix the contents of the tube thoroughly.
6. Transfer 100 ul of the contents of this “1/10” tube into the 900 ul of LB broth in the next tube labeled “1/10².” Close the lid and invert several times to mix tube contents.
7. Repeat this procedure for the remaining tubes in sequential order — transferring 100 ul of the previous tube into the next tube, mixing and then doing the next transfer. In this way, you are diluting each sequential tube with each transfer such that the tubes are in order of decreasing concentration from left to right. When you are finished with all the dilutions, discard the used tip.

8. Label three LB agar plates with the same notations as the last three tubes in your dilution series... “1/10⁴” “1/10⁵” and “1/10⁶.” Label the plates on the bottoms (not the lids). Since all student plates will be incubated together, also write your group’s name or initials near the edges of the plates for easy identification later.
9. Arrange the above three plates in order of decreasing concentration left to right (same as you did for the microcentrifuge tubes.)
10. With the 100-1000 ul micropipettor set for 100 ul and with a sterile tip, transfer 100 ul of the contents in the “1/10⁴” tube onto the agar surface of the LB agar plate also labeled “1/10⁴” Discard the used tip in the autoclavable bag. Remember to not expose the plate more than necessary to minimize contamination.
11. Use a sterile “L”-shaped cell spreader to spread the 100 ul evenly across the entire surface of the agar plate. It works best to have one student hold the lid of the plate while the other student rotates the plate while gently holding the cell spreader on the agar surface. Close the plate immediately and let set for 1-2 minutes. Discard the cell spreader in the autoclavable bag.
12. Use the 100-1000 ul and a new sterile tip to transfer 100 ul of the contents of the “1/10⁵” tube to the surface of the LB plate also labeled “1/10⁵.” Discard the used tip and remember to not expose the plate surface more than necessary. Using a new sterile “L”-shaped cell spreader, repeat step 11 to evenly spread the 100 ul across the plate surface. Close the plate and let set for 1-2 minutes.
13. Using a new sterile tip on the micropipettor, repeat this procedure for the remaining tube and plate labeled “1/10⁶.” Discard the used tip.
14. After the plates have set for 1-2 minutes, use parafilm strips to seal the edges of each plate closed. (Your teacher will demonstrate how to do this.) Incubate your plates upside down in a warm, dark place for 1-3 days.

Procedures for 2nd Lab Session (Part B)

For each lab table

1. Examine your plates for bacterial growth by noting the presence of colonies on the surface of the plates. It is not necessary to open the plates as you can easily see the colonies through the lids of the Petri plates.
2. Record which plates show the most and least growth of colonies and how many colonies are present. If there are numerous colonies, you may wish to use a counting grid. (introduced later in lab)