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Bacterial Transformation: Unlocking the Mysteries of Genetic Material

Teacher's Guidebook

(Cat. # BE-103)



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MATERIALS INCLUDED

This kit has enough materials and reagents for 30 students (six groups of five students).

- 1 vial Bac: E.C. DH5 α Agar Stab
- 1 vial LB Broth
- 2 bottles LB Broth
- 8 Culture tubes
- 7 Petri Dishes
- 2 bottles LB Agar (Premade)
- 1 vial Ampicillin
- 1 vial Sterile Water
- 1 vial DNA: Green Plasmid
- 1 vial TE Buffer
- 2 bottles Competent Buffer
- 7 Inoculating Loops
- 7 vials Loop Wash
- 50 Centrifuge Tubes (2ml)
- 30 Large transfer pipettes
- 20 Small transfer pipettes

SPECIAL HANDLING INSTRUCTIONS

- Store the Bac: E.C. DH5 α Agar Stab in a fridge
- Store the Ampicillin and DNA: Green Plasmid frozen until ready to use.
- All other components can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

The majority of reagents and components supplied in the *BioScience Excellence*[™] kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
L041	Loop Wash	Flammable

ADDITIONAL EQUIPMENT REQUIRED

- Waterbath or beaker and thermometer
- Shaking Incubator (Optional)
- UV lamp or UV light box
- Low speed centrifuge for 1.5-2ml tubes

TIME REQUIRED

- **Day 1:** 4 hours
- **Day 2:** 30 minutes

OBJECTIVES

- Transform bacteria cells with foreign DNA.
- Learn about plasmid DNA and bacteria.
- Insert a new gene into bacteria.
- Make bacteria produce new and foreign green protein.

BACKGROUND

Biotechnology is one of the newest and fastest growing scientific fields that has led to many new products routinely used in our day to day lives. The simplest definition of biotechnology is “applied biology”, which means the use of scientific techniques and knowledge and applying it to the development of products and new technologies. Biotechnology is more commonly referred to the use of living organisms or active molecules to make new products or control processes, such as fermentation.

Bacteria are the most frequently used living organisms in biotechnology, where they have multiple uses. Although bacteria are often associated with infection, disease and decay, there are many “helpful” bacteria. For example, bacteria are used in the fermentation of milk to produce healthy yogurts and cheeses. In the 1970s, scientist learnt how to manipulate bacteria by inserting the genes of proteins into bacteria and essentially converting the bacteria into living factories. The bacteria would produce the protein of the inserted gene and produce large amounts of the desired protein. This technique has been used for such products as human insulin for diabetic treatment, human growth hormone and several vaccines.

Early research into bacteria showed that bacteria, in addition to their chromosomal DNA, have transferable DNA known as plasmids, separate from the chromosomal DNA. The plasmid DNA is able to transfer drug resistance to other bacteria. Scientists utilized the plasmid DNA by inserting foreign DNA into the plasmid DNA and inserting this DNA into bacteria, a process known as transformation. The bacteria replicated the plasmid DNA and made the foreign protein of the inserted gene.

This kit allows students to insert a bacterial plasmid into bacteria that contains a gene from a jellyfish. This gene encodes a protein that glows fluorescent green when ultraviolet light is shone on it. This simple bacterial transformation allows students to generate green bacteria.

TEACHER'S PRE EXPERIMENT SET UP

Prepare Bacterial Stocks (Use aseptic techniques)



The bacterial stock (DH5 α) provided with this kit has been specifically selected as the most innocuous materials. The bacteria are widely used in teaching and research and are non-pathogenic to humans.

1. The day before experimentation, pour ~6ml LB broth from one of the 30ml bottles into two culture tubes. This is approximately half the volume of the supplied tubes. Save the remaining broth for the class activity.
2. Use a large transfer pipette and transfer ~1ml LB broth from the 2ml vial to the Bac: E.C. DH5 α Agar Stab. Basically, fill the tube to the top. Incubate the tube at 37°C for 30 minutes. Vigorously shake the tube after incubation.
3. With a large transfer pipette, transfer half the LB Broth from the Bac: E.C. DH5 α to each culture tube containing LB broth. Incubate the tubes at 37°C with shaking overnight.
4. The following day, pour ~2ml overnight culture into the remaining six culture tubes. Give 1 culture tube to each group of students.

Preparation of Nutrient Agar Plates (Use aseptic techniques)



Wear heat protective gloves throughout the agar melting and pouring procedure

Make Agar plates the day before the experiment.

1. Loosen the cap of the two bottles containing the agar.
2. Place the bottles in a large container, such as a beaker or saucepan and add water to the container up to the level of the agar.
3. Heat the water until it begins to boil. Simmer for 20-30 minutes and swirl the agar bottles several times until all the agar has melted.
4. Turn off the heat. Remove the bottles from the water bath and allow the agar to cool.
5. Once the agarose has cooled to the point it can be held **comfortably** in your hand, use one bottle and pour one plate with ~0.5cm/¼" layer of agar. This is approximately 15ml. This plate is the negative "Control" to be used by the teacher.

6. Next, rehydrate the ampicillin by transferring ~250µl sterile water to the vial of ampicillin with a large transfer pipette. Add water to the lowest graduation mark and dissolve the ampicillin by pipetting up and down several times.
7. Using the same pipette, transfer the contents of the vial of ampicillin to the other bottle of LB agar.



If the agar is too hot, the ampicillin antibiotic will be inactivated. The agar should be below 55 °C.

8. Pour a ~0.5cm/ ¼" layer of agar into the remaining 6 Petri dishes. This is approximately 15ml.
9. Replace the lids and do NOT move the plates until the agar is completely set.
10. Once completely set, store upside down in a refrigerator until needed. For long-term storage, wrap in a plastic bag or store in an airtight container.

Miscellaneous preparation (Use aseptic techniques)

1. Use a large transfer pipette and transfer ~0.75ml TE Buffer to the DNA: Green Plasmid vial. Fill the tube to the graduated mark between 0.5 and 1.0. Resuspend by gently flicking the tube.
2. With a small transfer pipette, dispense 6 drops to each group.
3. Use a large transfer pipette and dispense 1ml competent buffer to each group. Fill the 2ml tubes to the 1.0 mark.
4. Prepare an icebox of crushed ice and a warm waterbath to 40-45°C.

Negative "Control" (Use aseptic techniques)

1. The negative "Control" Petri dish demonstrates that the lack of an antibiotic in the agar allows all bacteria to grow.
2. Divide the Petri dish into 6 sections, by marking the bottom of the plate with a pen.
3. Select five students to streak their transformed bacteria onto a section of the plate as follows.



The same inoculating loop is being used to apply six samples to a single plate. As a result, it is critical to sterilize the inoculating loop before each application of bacteria. To do this, Loop Wash is provided, which is an alcohol based wash that kills all bacteria.

4. Dip the loop into the Loop Wash to kill any bacteria on the loop. Remove and gently shake the loop for 20 seconds to remove excess Loop Wash and allow Loop Wash to evaporate. A student then dips the loop into their bacteria and gently spreads in their section, ensuring to spread the liquid over the entire section. Repeat until all students have applied their bacteria to the agar plate.
5. In section six, have a student first sterilize the loop with the Loop Wash, as above, and then streak the untransformed bacteria, as above.

MATERIALS FOR EACH GROUP

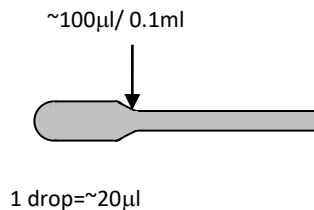
- LB Broth (shared with class)
- 1 Culture tube with 2ml Bac: E.C. DH5 α culture
- 7 Centrifuge tubes
- 3 Large transfer pipettes
- 2 Small transfer pipettes
- 1 vial Competent Buffer
- 1 vial DNA: Green Plasmid
- 1 Agar Plate with Ampicillin
- 1 Inoculating loop
- 1 vial Loop Wash

PROCEDURE

Steps 1-9 performed as a group. Step 10-19 performed individually.

1. Each student takes a tube and labels it with their name and places it on ice.
2. This experiment is carried out on ice. To prepare for ice-cold conditions, chill the remaining 2ml tubes and the Competent Buffer on ice.
3. Grow fresh bacteria: Use a large transfer pipette and add ~3ml LB broth to the culture tube containing 2ml Bac: E.C. DH5 α , by filling the tube to approximately halfway. Incubate the tube at 37°C or room temperature, with shaking for 1 hour. *Ideally, the bacteria should be grown at 37°C with continuous shaking, however if not available the bacteria will grow at room temperature and can be mixed manually every 10 minutes.*
4. Remove the bacteria from the incubator and place on ice for 5 minutes to prepare bacteria for subsequent steps.
5. Pour ~2ml bacteria to two ice-cold 2ml tubes by filling to the top of the tube. Save the remaining culture for later use as a "Control".

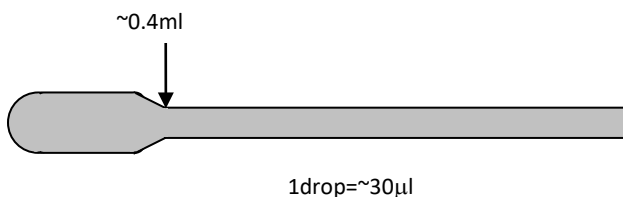
6. Centrifuge the tubes at 5,000rpm for 3 minutes to pellet the bacteria. Carefully pour off the supernatant without disturbing the bacterial pellet. Discard in the provided waste container.
7. Combine the two bacterial pellets. With a large transfer pipette, add ~0.5ml ice-cold Competent Buffer to the 0.5 mark of one of the tubes containing a bacterial pellet. Gently pipette up and down to completely suspend the pellet. Next, transfer the suspension to the second tube containing a bacterial pellet and resuspend the pellet by gentle pipetting.
8. Centrifuge the tube at 5,000rpm for 3 minutes to pellet the bacteria. Carefully pour off the supernatant without disturbing the bacterial pellet.
9. Pour the remaining ice-cold Competent Buffer on to the pellet. Gently flick and shake the tube to suspend the pellet completely. Using a small transfer pipette (see below), transfer 0.1ml bacteria to each of the five labeled tubes.



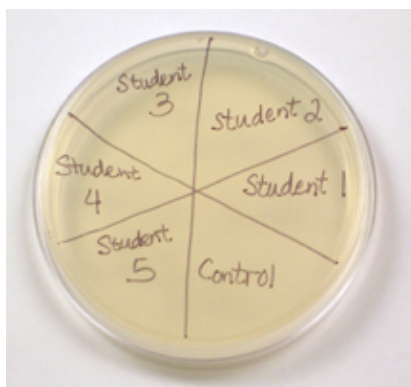
10. Now the *E. coli* bacteria cells are ready to take up the plasmid DNA, they are known as competent bacteria. Keep the competent bacteria on ice.
The Competent Buffer contains a calcium salt that alters the outer walls of bacteria, permitting foreign plasmid DNA to enter the bacteria.
11. Each student, using a small transfer pipette, adds 1 drop plasmid DNA to their own competent cells. Gently tap the tube to mix the plasmid and the competent bacteria. Place the tube on ice for 30 minutes. The plasmid has a gene for a green protein and a gene that makes bacteria resistant to the antibiotic ampicillin.
12. **Heat Shock:** *Rapid changes in the temperature of bacteria cause the bacteria to take up the foreign plasmid DNA and then subsequently seal the bacteria. After 30 minutes on ice the bacteria are transferred to warm water for a short time and then returned to the ice, this is the heat shock process.* Transfer the tube with the

competent bacteria and plasmid to a tube float in a 42°C water bath. Leave the tube in the water bath exactly 90 seconds. Do not shake the tube.

13. Quickly transfer the tube to an ice bucket and chill the cells for 2 minutes.
14. Using a shared large transfer pipette, add 0.4ml LB broth to the tube (see below) and incubate for 45 minutes at 37°C or room temperature with shaking.
Ideally, the bacteria should be grown at 37°C with continuous shaking, however if not available the bacteria will grow at room temperature and can be mixed manually every 10 minutes.



15. In the meantime, mark the bottom of the LB agar plates with ampicillin into six even sections (see below). Label sections 1-5 with the student's name and the sixth section as "Control". The ampicillin in the agar plate allows for the selection of transformed bacteria. Those bacteria that now contain the plasmid with the ampicillin resistance gene.



16. Each student in turn needs to use the inoculating loop to spread a small amount of bacteria onto their section of the agar plate. To avoid contaminating each other's bacteria, the inoculating loop needs to be sterilized in Loop Wash.
17. When it is your turn to apply your bacteria, dip the loop into the Loop Wash to kill any bacteria, remove and gentle shake the loop for 20 seconds to remove excess

Loop Wash and allow Loop Wash to evaporate. Next, dip the loop into your bacteria and gently spread in your section, ensuring to spread the liquid over the entire section. Repeat until all students have applied their bacteria to the agar plate.

18. One student from the group will wash the loop as in step 16. Next dip the loop into the original untreated bacteria from step 4 and spread in the "Control" section.
19. Invert the plates and incubate at 37°C or at room temperature overnight.
Ideally, the bacteria should be grown at 37°C with continuous shaking, however if not available the bacteria will grow at room temperature and can be mixed manually every 10 minutes.
20. View the plate with a UV lamp to visualize the green colonies the next day or a few days after. Your teacher will determine the time.
The development of the green color may take a few days. After the overnight growth of the bacteria, wrap the plate in plastic wrap and store at 4°C.

RESULTS, ANALYSIS & ASSESSMENT

Describe what you see on the plate, comparing your section with others and particularly the "Control":

Colonies are present in all the student sections, but there are none in the "Control" section.

Briefly explain the reason why there is a difference between the "Control" and your section. If there is no difference, give reasons why?

There is a difference due to the "Control" bacteria not having a plasmid; the plasmid confers antibiotic resistance to the bacteria that grew on the antibiotic plate

Observe the transformed colonies under UV light. Is the colony color different from the visible light? Why?

Under the UV lamp the colonies glow green, due to the cells expressing the green protein gene.

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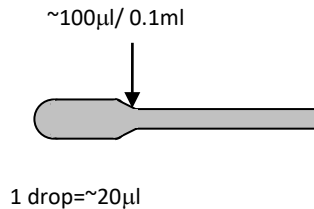
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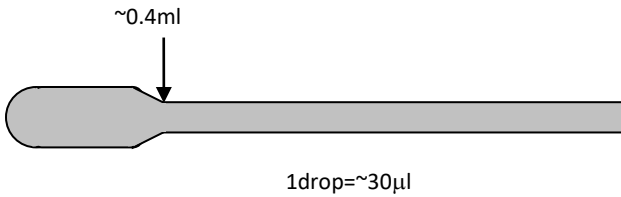
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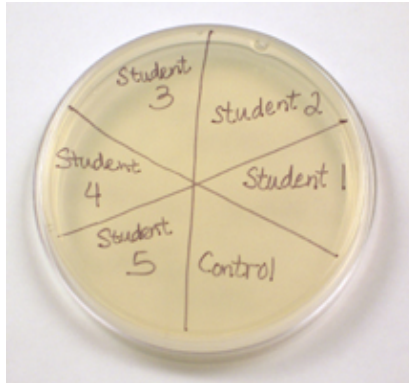
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