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Bacterial Transformation

Teacher's Guidebook

(Cat. # BE-309)



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MATERIALS INCLUDED

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 vial E.C. DH5 α Agar Stab
- 1 vial LB Broth
- 1 bottle LB Broth
- 8 Culture tubes
- 6 Petri Dishes
- 1 pack LB Agar
- 1 vial Ampicillin
- 1 vial Sterile Water
- 1 vial DNA: Plasmid 1
- 1 vial TE Buffer
- 1 tube Competent Buffer
- 6 Inoculating Loops
- 6 vials Loop Wash
- 80 Centrifuge Tubes (2ml)

SPECIAL HANDLING INSTRUCTIONS

- Store E.C. DH5 α Agar Stab in a fridge or at 4°C.
- Store Ampicillin and DNA: Plasmid 1 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 Safety Data Sheets (SDS).

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
- Low speed centrifuge for 1.5-2ml tubes
- Shaking Incubator
- Autoclave*
- UV lamp or UV light box

* 100ml premade bottles of LB agar (L011), which is melted in a boiling waterbath, can be used if an autoclave is not available. You will require 2 x LB Agar bottles.

TIME REQUIRED

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

OBJECTIVES

- Transform bacteria cells with a foreign DNA.
- Create recombinant bacteria that express a green protein.

BACKGROUND

Bacterial transformation is the process routinely used in genetic engineering to create recombinant bacteria. The bacterial transformation process involves bacteria taking up naked DNA molecules, which, if they have a compatible origin of replication, will be replicated by the bacteria.

A major consideration in bacterial transformation is to incorporate a selection factor due to the low number of bacterial cells that are transformed and the potential for the plasmid not to propagate itself in all daughter cells. The commonest selection method is to incorporate a gene that confers antibiotic resistance to the bacteria, so that untransformed bacteria will be killed off in the presence of the antibiotic.

For bacteria to be able to take up foreign DNA the most widely used technique is to treat the bacteria with calcium chloride (CaCl_2) in the early log phase of their life cycle. The precise mechanism of how calcium chloride treatment makes bacteria susceptible to transformation is unknown; however the bacterial cell membrane is permeable to chloride ions, but not calcium ions. As the charged chloride ions enter the cell they bring with them water molecules and this influx leads to the swelling of the bacteria, which is essential for DNA uptake. After treatment with calcium chloride the bacteria are now known as **competent** bacteria.

A second step in bacterial transformation is to carry out a **heat shock**. When *Escherichia coli* are subjected to 42°C heat, a survival response is triggered and a set of genes, the heat shock genes, are expressed which aid the bacteria in surviving at such temperatures. Heat shock treatment of competent bacteria is also necessary for the uptake of foreign DNA.

This kit contains all the reagents necessary to transform *Escherichia coli* with DNA that encodes ampicillin resistance and a green fluorescent protein. The presence of the green fluorescent protein allows the easy visualization of the recombinant bacteria. This kit can also be used in conjunction with the “DNA Ligation” Kit (BE-308).

TEACHER'S PRE EXPERIMENT SET UP

Prepare Bacterial Stocks

1. The day before experimentation, transfer 6ml LB broth to two culture tubes using aseptic techniques.
2. Transfer 0.8ml LB broth to the bacterial agar stab and incubate at 37°C for 30 minutes.
3. Vigorously shake or vortex for 1-2 minutes, then transfer 0.5ml LB broth from the agar stab to each culture tube containing 6ml LB broth. Incubate the tubes at 37°C with shaking overnight.
4. The following day, dispense 2ml overnight culture into the remaining six culture tubes. Give 1 culture tube to each group of students.

Preparation of LB Agar Plates



Wear heat protective gloves throughout the agar melting and pouring procedure.

Make Agar plates the day before the experiment.

1. Add the entire pack of LB Agar to an autoclavable container and add 150ml distilled water. Autoclave for 15min at 121°C.
2. Next, rehydrate the ampicillin by transferring 250µl sterile water to the vial of ampicillin. Dissolve the ampicillin by inverting the tube several times.
3. Once the LB Agar has cooled to hand hot temperature (about 45°C), add 150µl ampicillin solution to the LB agar.



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

4. Pour a ~0.5cm/ ¼" layer of agar into the 6 Petri dishes. This is approximately 15-20ml.
5. Replace the lids and do NOT move the plates until the agar is completely set.
6. 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

1. Transfer 0.2ml TE buffer to the DNA: Plasmid 1 vial. Resuspend by gently flicking the tube.



If using ligation reactions from DNA ligation kit then the Plasmid 1 is not required. Go to step 4.

2. Label six tubes with “Plasmid”. Transfer 30µl Plasmid 1 to each tube. Supply each group with a single tube.
3. Label six tubes with “Competent Buffer”. Transfer 1ml competent buffer to each tube. Supply each group with a single tube.
4. Prepare iceboxes of crushed ice for each group and a warm waterbath at 40-45°C.

MATERIALS FOR EACH GROUP

Supply each group with the following. Some reagents are shared with the whole class and can be stored on a communal table.

- 1 bottle LB Broth (shared with class)
- 1 Culture tube with 2ml bacterial culture
- 6 Centrifuge tubes (2ml)
- 1ml Competent Buffer
- 30µl Plasmid 1 (or Ligation reactions from the DNA Ligation kit BE-308)
- 1 Agar Plate with Ampicillin
- 1 Inoculating loop
- 1 vial Loop Wash

PROCEDURE



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.

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

1. Each student takes a tube and labels it with their name. A fifth tube is labeled “negative control”. Place all tubes on ice, along with two additional unlabeled 2ml tubes.
2. To prepare for ice-cold conditions, place the remaining 2ml tubes and the Competent Buffer on ice.
3. Grow bacteria in fresh media so that they are in early log phase. Add 3ml LB broth to the culture tube containing 2ml E.C. DH5 α bacteria. Incubate the tube at 37°C 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. Transfer 2ml bacteria to two ice cold 2ml tubes and 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 remove the supernatant without disturbing the bacterial pellet and discard in the provided waste container.
7. Combine the two bacterial pellets. Add 0.5ml ice cold Competent Buffer to one of the tubes containing the 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 remove the supernatant without disturbing the bacterial pellet.
9. Add 0.5ml ice cold Competent Buffer to the pellet. Gently pipette up and down to suspend the pellet completely. Transfer 0.1ml bacteria to each of the five labeled tubes. Now that the 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.

10. Each student adds 5 μ l plasmid DNA **or** 5 μ l of their ligation reactions to their own competent cells. Gently tap the tube to mix the DNA 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.

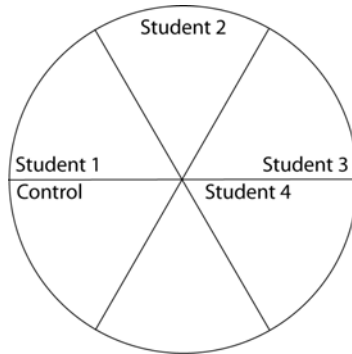
11. **Heat Shock:** *Rapid changes in the temperature of bacteria cause the bacteria to take up the foreign plasmid DNA and then subsequently seal the bacteria. After 30 minutes on ice the bacteria are transferred to warm water for a short time and then returned to the ice, this is the heat shock process.* 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.
12. Quickly transfer the tube to an ice bucket and chill the cells for 2 minutes.
13. Add 0.5ml LB broth to the tube 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.

14. In the meantime, mark the bottom of the LB agar plate with ampicillin into six even sections (see below). Label sections 1-4 with the student's name, section 5 as "Control" and the sixth section remains blank.

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.



15. 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.
16. When it is your turn to apply their bacteria, dip the loop into the Loop Wash to kill any bacteria, remove and gently 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.
17. One student from the group will wash the loop as in step 16. Next dip the loop into the original untreated bacteria from step 5 and spread in the "Control" section.
18. Invert the plates and incubate at 37°C or at room temperature overnight (16 hours).

Ideally, the bacteria should be grown at 37°C, however if not available the bacteria will grow at room temperature.

19. View the plate with a UV lamp to visualize the green colonies the next day or a few days after. This time will be determined by your teacher.

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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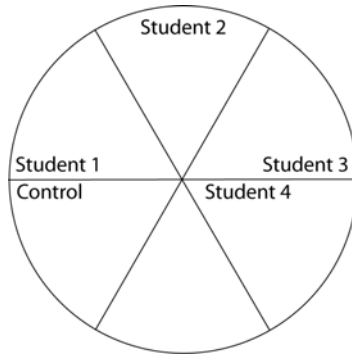
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