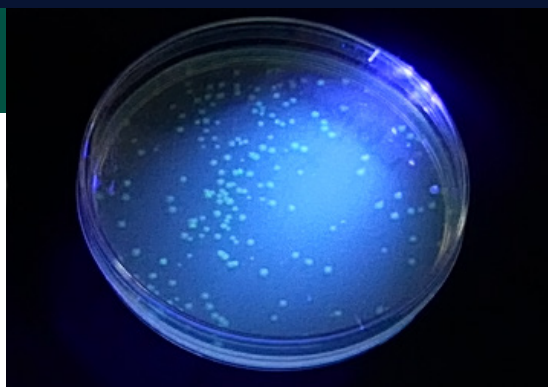
# BIO LAB:
# pGreen BACTERIAL TRANSFORMATION

TEACHER

LEVEL: Year 12 | TOPIC: Biotechnology | TIME REQUIREMENT: 90 mins



## CURRICULUM ALIGNMENT

- *The use of recombinant plasmids as vectors to transform bacterial cells.*

## BACKGROUND

DNA can mutate spontaneously or after an error is made in DNA replication. Biotechnologists have developed methods of controlled DNA mutation; such as, intentionally mutating cell DNA to alter how the cell behaves. However, it is also possible to transfer DNA from one organism into another. This method, called genetic transformation, uses an engineered molecule of DNA to transfer a gene or genes from one organism to another so that the organism is capable of producing the protein encoded by the transformed gene. One application of this process is the transformation of the human insulin gene into bacteria which enabled large scale production of the insulin protein. Genetic transformation in higher plants and animals is a costly and complicated process; however genetic transformation in Escherichia coli (E. coli) bacteria is much simpler and a great practical for students.

This practical involves transforming bacteria (E. coli) with a gene from Aequorea victoria; a bioluminescent jellyfish. If successful, the green fluorescent protein (GFP) of the Aequorea victoria will be expressed in the bacteria; causing them to glow bright green under UV light. Using bacterial plasmid-based genetic transformation, students will acquire the tools to transform E. coli bacteria to express new genetic information employing a plasmid system and applying mathematical routines to determine transformation efficiency. By playing an active role in the process of manipulation of genetic information, students will gain a greater understanding of how DNA operates; allowing them to consolidate previous learning such as cell structures of bacteria; structure and function of cell membranes, enzymes, and DNA and RNA; transcription and translation.

## MATERIALS

**Included within the Kit (8 student workstations):**

| # | # | |
|---|---|---|
| 2 | 16 | Transformation tube, 13mL, sterile |
| 10 | 80 | Transfer pipette, 1mL, graduated, sterile |
| 1 | 8 | CaCl2, 50mM, sterile, 10mL |
| 2 | 16 | Inoculation loop, sterile, disposable |
| 1 | 8 | Luria broth, sterile, 10mL |
| 4 | 32 | Inoculation spreader, sterile, disposable |
| 1 | 2 | Ampicillin powder, 30mg in vial |
| | 1 | Water, sterile, 20mL |
| | 10 | LB agar, 100mL (Melt and Pour) |
| | 40 | Petri dishes, sterile |
| | 1 | E.coli MM294, LB agar slope |
| | 1 | Plasmid, 100µL |

- # Number included within the Kit
- # Number required per workstation

**Required but not included:**

- 1 Ice bath
- 1 Fine point marker pen
- 1 Micropipette, 10µL (e.g. G40.681)
- 8 Tips for 10µL micropipette (e.g. G40.62)
- 1 Waterbath at 42°C
- 1 Stopwatch
- 1 Test tube rack
- 1 Adhesive tape (to seal plates)
- 1 Microbiological waste disposal bag (e.g. E5.37)
- 1 Thermometer
- 1 Micro centrifuge
- 1 Bunsen burner
- 1 Wire inoculation loop (e.g. E3.10)
- 1 Incubator (e.g. E7.30)
- 1 Boiling water bath (to melt LB agar)
- 1 Microtube rack to hold plasmid (e.g.G40.95, G40.97)

## SAFETY PRECAUTIONS

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE). Ensure that students understand and adhere to these practices. Know and follow all regulatory guidelines for the disposal of laboratory wastes.

## PREPARATION - BY LAB TECHNICIAN

### *Prepare LB agar plates (Can be completed up to one week before the student practical)*

1. In a large beaker, place the 10 LB agar bottles upright and pour in hot water until it nears the top of the bottle. Leave on a gentle boil until the agar is fully melted.
2. In the meantime, use a sterile transfer pipette to add 4mL of sterile water to the vial of ampicillin powder and shake well.
3. Allow the agar bottles to cool to approximately 50 – 60°C. Use a thermometer to check the temperature of the water.
4. Label the base of 24 petri dishes with **LB** . Divide 6 bottles of hot LB agar into the petri dishes with approximately 25mL per dish.
5. Using the last 4 bottles, remove 2mL of hot agar using a sterile transfer pipette. Using another sterile transfer pipette; add 2mL of ampicillin solution to each bottle. Replace the caps and gently agitate to mix the ampicillin into the molten agar.
6. Label the base of 16 petri dishes with **LB/Amp.** Divide the 4 bottles of hot **LB/Amp** agar into the petri dishes with approximately 25mL per dish.
7. Allow the agar plates to cool for 30 minutes or until they set; then store upside down in a refrigerator.
8. Place 2 **LB** plates and 2 **LB/Amp** plates on each student workstation.
9. The remaining 8 **LB** plates will be used to prepare E.coli starter plates

### *Prepare E.coli starter plates*

(Must be done no more than 12-20 hours before the student practical. We recommend preparing the starter plates on the afternoon before the student activity).

1. Line up 8 **LB** plates prepared in the previous step and label the base of each plate with **Starter plate.**
2. Aseptically transfer E. coli from the slope supplied in the kit to each plate to create 8 starter plates using a wire inoculation loop. Follow the technique required to generate single colonies.
3. Put the plates upside down in an incubator set to 37°C and incubate for 12 to 20 hours. Be careful not to leave the plates in for longer than stated, as it will negatively impact the results.

### *Prepare work stations*

1. Prepare 8 ice baths
2. Place upright one vial of CaCl2 solutions in each ice bath to chill.
3. Using a hot plate, a 500mL beaker containing 300mL warm water and a thermometer, prepare 8 water baths at 42°C. Temperature control is critical and it is important not to place the transformation tubes in the water until a stable 41°C- 42°C temperature is achieved.

### *Prepare the plasmid*

1. Thaw the plasmid microtube containing and centrifuge for 5 seconds to ensure all liquid is collected at the bottom of the tube. To keep it cold before dispensing, place it in a rack in a refrigerator or bed of ice.

## METHOD- STUDENT PRACTICAL

### *Preparing the transformation solution*

1. Label 1 transformation tubes **+ Plasmid** and the other **- Plasmid.** Keep the tubes cold by placing them upright in the ice bath. Tubes should be kept capped at all times except when in use.
2. Add 250µL of ice cold CaCl2 solution to each transformation tube, using a sterile transfer pipette. To maintain the temperature ensure the tubes are placed back in the ice bath.

### *Suspending the bacteria*

1. Transfer a single colony of E. coli from the starter plate to the ice cold CaCl2 solution in the **+ Plasmid** transformation tube using a sterile inoculation loop. To dislodge the E. coli cells from the loop, spin the loop rapidly in the solution. Observe whether the cell mass has transferred successfully.
2. Immediately pump the liquid in the tube several times to suspend the cell mass in the CaCl2 solution using a sterile 1mL transfer pipette. Do not entrain air bubbles in the liquid or allow any liquid to splash up the sides of the tube. You should see the solution begin to become milky white as cell mass is suspended. To check there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.

3. Repeating the same steps as **+ Plasmid**; transfer a single colony of E. coli from the starter plate to the ice cold CaCl2 solution in the **- Plasmid** transformation tube using a sterile inoculation loop. To dislodge the E. coli cells from the loop, spin the loop rapidly in the solution. Observe whether the cell mass has transferred successfully.
4. Immediately pump the liquid in the tube several times to suspend the cell mass in the CaCl2 solution using a sterile 1mL transfer pipette. Do not entrain air bubbles in the liquid or allow any liquid to splash up the sides of the tube. You should see the solution begin to become milky white as cell mass is suspended. To check there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.

### *Adding the plasmid*

1. The technician/ teacher will bring the plasmid to your work station. Transfer 10µL of plasmid solution to the transformation tube labelled **+ Plasmid** using a micropipette. Add the plasmid directly to the liquid in the tube without allowing it to touch the sides.
2. Immediately return the tube to the ice bath as and mix the plasmid into the bacterial suspension by rapidly spinning a sterile inoculation loop with your fingers.
3. Incubate the 2 tubes for 15 minutes on ice and without extracting them, label the 4 plates as follows:
   The first **LB** plate: **+ Plasmid**
   The second **LB** plate: **- Plasmid**
   The first **LB/Amp** plate: **+ Plasmid**
   The second **LB/Amp** plate: **- Plasmid**

### *Heat shock*

1. Extract the 2 tubes from the ice bath and transfer to the warm water bath (42°C) at the same time and hold there for 90 seconds with your hands keeping the tube caps from being fully submerged in the water. Gently agitate the tubes while they are warming up in the water Immediately move the tubes back to the ice bath when the time is up.
2. Allow the tubes to rest in the ice bath for at least 1 minute before continuing.

### *Recovery*

1. Add 250µL of Luria broth to each tube using a sterile transfer pipette. Mix the liquids at the base of each tube by gently grasping the top and tapping the base with your finger.
2. Allow tubes to recover for 10 minutes to recover in test tube rack at room temperature.

### *Plate inoculation*

1. Arrange the tubes and plates so the "- plasmid" tube is adjacent to the two **- plasmid** plates (one LB and the other LB/Amp) and the **+ plasmid** tube is adjacent to the two **+ plasmid** plates **(one LB and the other LB/Amp).**
2. Transfer 100µL of liquid from the **- plasmid** tube to the **LB - plasmid** plate using a sterile transfer pipette. Quickly spread the liquid evenly over the plate surface using sterile spreader.
3. Transfer 100µL of liquid from the **- plasmid** tube to the **LB/Amp - plasmid** plate using a sterile transfer pipette. Quickly spread the liquid evenly over the plate surface using sterile spreader.
4. Transfer 100µL of liquid from the **+ plasmid** tube to the **LB + plasmid** plate using a sterile transfer pipette. Quickly spread the liquid evenly over the plate surface using sterile spreader.
5. Transfer 100µL of liquid from the **+ plasmid** tube to the **LB/Amp + plasmid** plate using a sterile transfer pipette. Quickly spread the liquid evenly over the plate surface using sterile spreader.
6. Secure the lid of each petri dish to its base using tape. Leave plates to rest on the bench for 5 minutes and then place in a 33°C incubator for 24-36 hours. You can inspect growth after this time.

## RESULTS

Remove the plates from the incubator to check the results. Compare to what is expected.

| PLATES | EXPECTED RESULT |
|---|---|
| - PLASMID ON LB AGAR | LAWN |
| + PLASMID ON LB AGAR | LAWN |
| - PLASMID ON LB/ AMP | NO GROWTH |
| + PLASMID ON LB/ AMP AGAR | SINGLE COLONIES |