NEATH PORT TALBOT COLLEGE
COLEG CASTELL NEDD PORT TALBOT

School of Maths & Science
Science Practical

p GLO Bacterial transformation

◆ Aim

To insert the gene that codes for Green Fluorescent Protein in the Jellyfish *Aequorea victoria* into a bacterium.

◆ Introduction

Genetic transformation occurs when a cell takes up and expresses a new piece of genetic material—DNA. Genetic transformation literally means change caused by genes and it involves the insertion of a gene into an organism in order to change the organism's traits. (Remember that a gene is a piece of DNA which provides the instructions for making a particular protein.) Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest or drought resistance can be genetically transformed into plants. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the gene involved in their disease. Genes can be cut out of human, animal or plant DNA and placed inside bacteria. For example, a healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin just as they would make their own proteins.
In this practical, you will learn about the process of moving genes form one organism to the other with the aid of a plasmid.

◆ Safety

Control Measures

- The wearing of safety goggles and a laboratory coat at all times will be sufficient to take account of most hazards and significant risks.
- All waste is to be placed in the labelled container immediately after use.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.

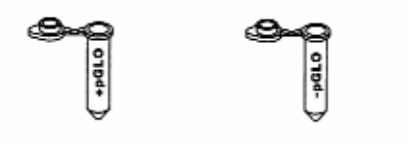
Hazards

- UV light – ultraviolet radiation can cause damage to eyes and skin. Avoid looking directly at the UV source.

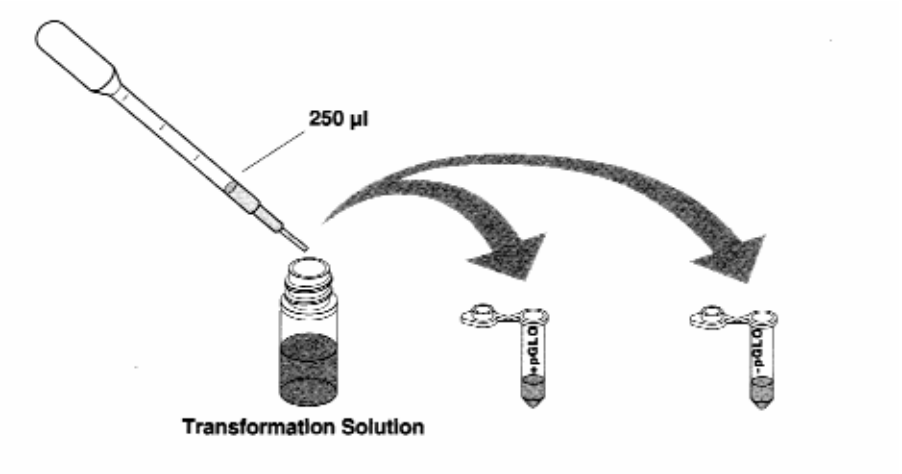
- Biohazard *E.coli* K -12 – these are not pathogenic bacteria but require standard laboratory practice, please wash your hands after handling material involving organisms containing recombinant DNA and before exiting the laboratory.

◆ Procedure

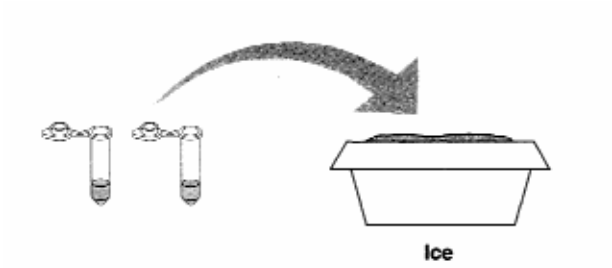
1. Label one closed micro test tube **+pGLO** and another **-pGLO.**
   Label both tubes with your name and place them in the foam tube rack.



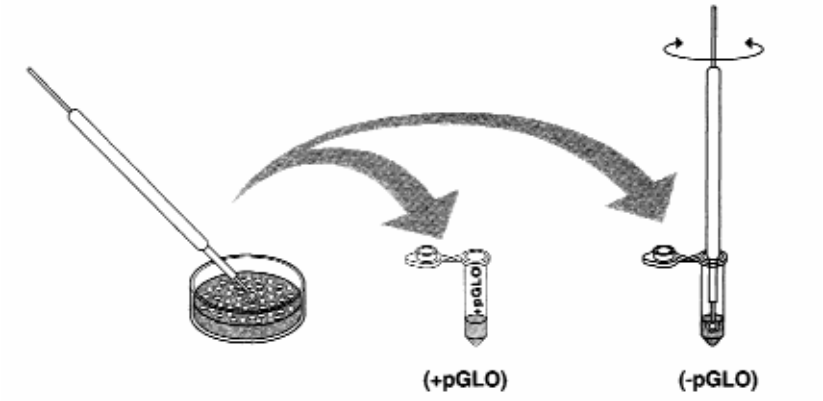
2. Open the tubes and, using a sterile transfer pipette, transfer 250 µl of transformation solution ($CaCl_2$) into each tube.



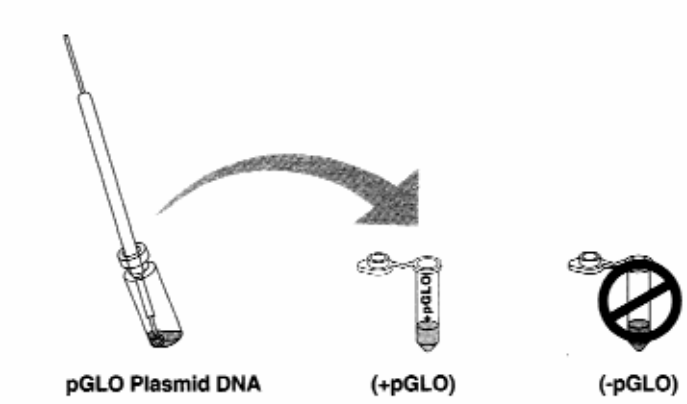
3. Place the tubes on ice.



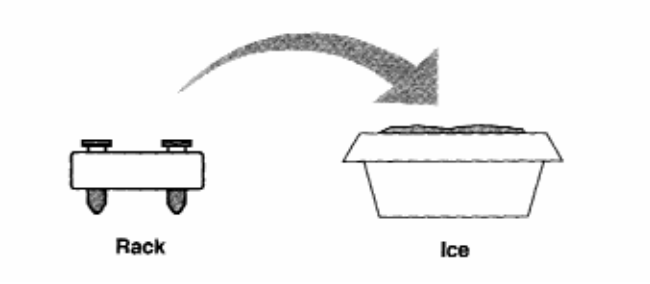
4. Use a sterile loop to pick up **a single colony of bacteria** from your starter plate. Pick up the **+pGLO** tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. **Using a new sterile** loop, repeat for the **-pGLO** tube.



5. Examine the pGLO DNA solution with the UV lamp. Note your observations. Immerse a **new sterile loop** into the pGLO plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the **+pGLO** tube. Close the tube and return it to the rack on ice. Also close the **-pGLO** tube. **Do not** add plasmid DNA to the **-pGLO** tube. Why not?



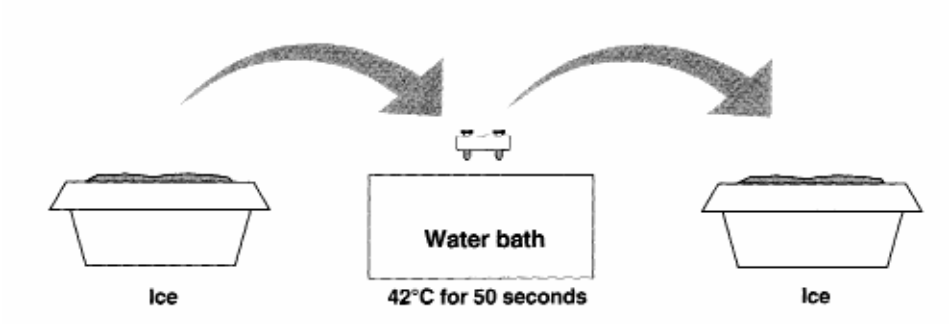
6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



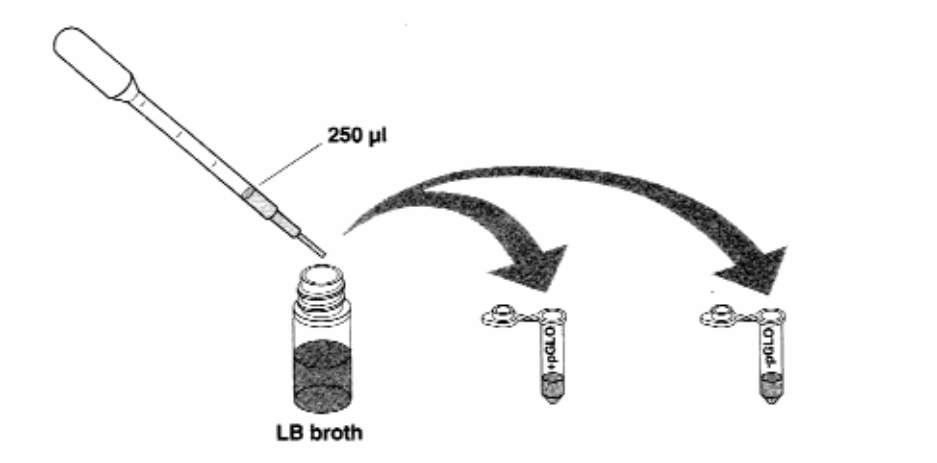
7. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom (not the lid) as follows:

- Label one **LB/amp** plate: + pGLO
- Label the **LB/amp/ara** plate: + pGLO
- Label the other **LB/amp** plate: - pGLO
- Label the **LB** plate: - pGLO

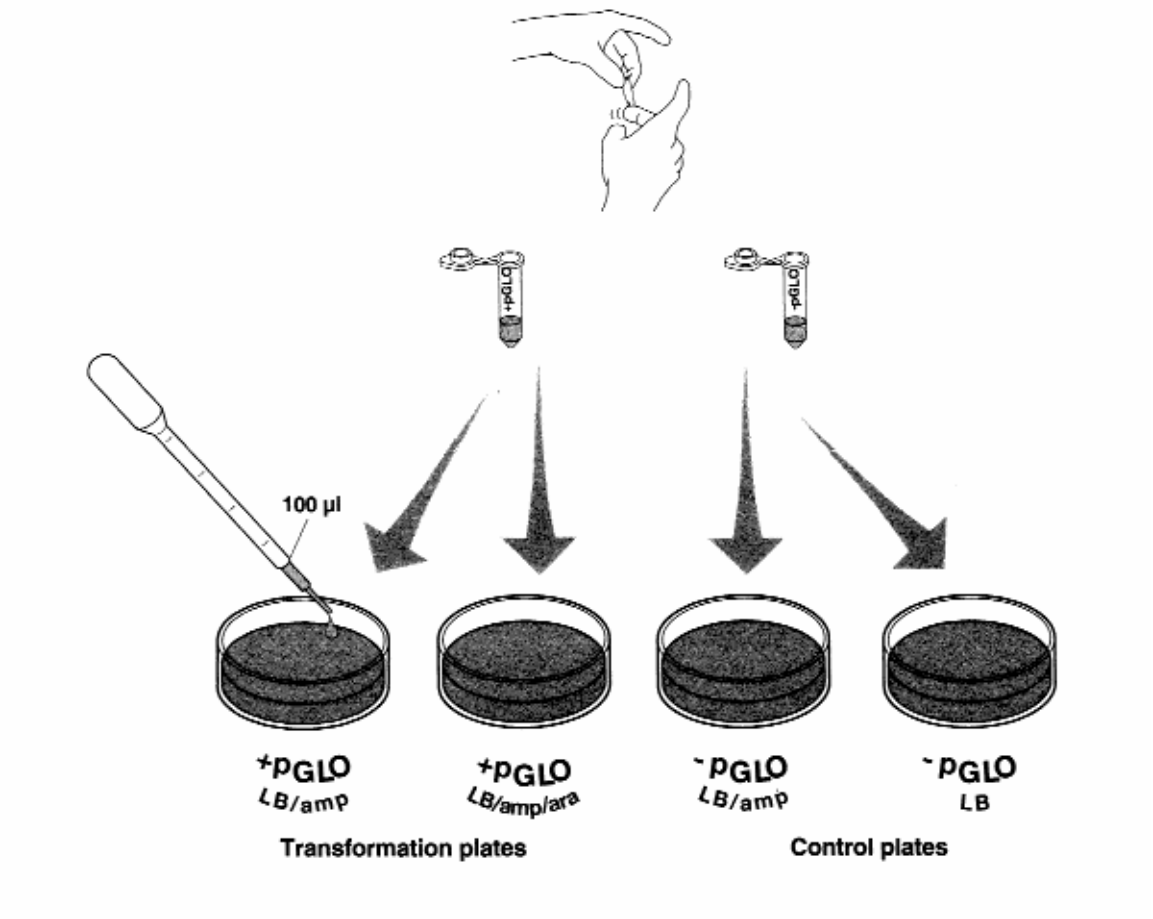
8. **Heat shock**. Using the foam rack as a holder, transfer both the **(+) pGLO** and **(-) pGLO** tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water.
   When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the transfer from the ice (0°C) to 42°C and then back to the ice must be rapid.
   Incubate tubes on ice for 2 minutes.



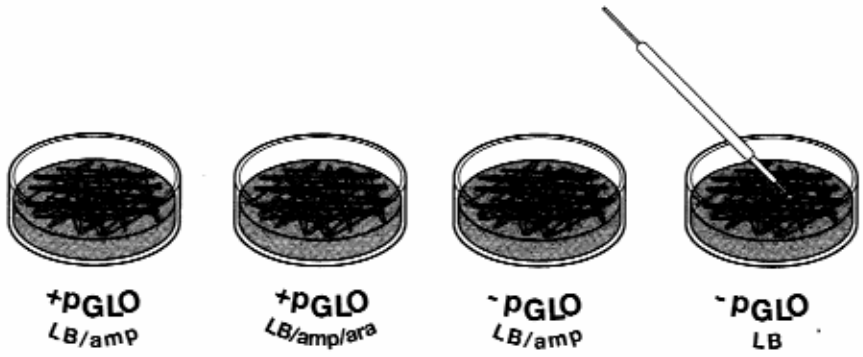
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipette, add 250 µl of LB nutrient broth to the tube and re-close it. Repeat with a new sterile pipette for the other tube. Incubate the tubes for 10 minutes at room temperature.



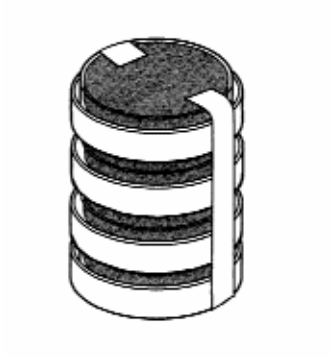
10. Tap the closed tubes with your finger to mix. Using a **new** sterile pipette for **each tube**, pipette 100 µl of the transformation and control suspensions onto the appropriate nutrient agar plates.



11. **Use a new sterile loop for each plate.** Spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of a new sterile 1oop back and forth across the plate surface. **DO NOT PRESS TOO DEEP INTO THE AGAR.**



12. Stack up your plates and tape them together. Put your name and class on the bottom of the stack and place the stack of plates **upside down** in the 37°C incubator until the next day (24 hours).



13. Examine your plates using the UV light provided, record your results on the sheet provided. **Avoid looking directly at the UV source**

Data Observation Sheet

1. Why did you label one tube “+” and the other tube “-“? What do the “+” and “-“ indicate?

2. Why did we add the bacteria to the cold calcium chloride solution?

3. Why did we put bacteria in both tubes?

4. Why did we heat shock the bacteria and place them on ice?

5. Why did we add Luria broth (LB) to the tubes? Why did we change pipettes before adding LB to the second tube?

Data Collection

Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.

1. Carefully observe and draw what you see on each of the four plates. Put your drawings in the data table in the column on the right. Record your data to allow you to compare observations of the "+ pGLO" cells with your observations for the non-transformed E. coli. Write down the following observations for each plate.

2. How much bacterial growth do you see on each plate, relatively speaking?

3. What colour are the bacteria?

4. How many bacterial colonies are on each plate (count the spots you see).