ONTARIO SCIENCE CENTRE

Teacher Guide

Way to Glow Program

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Bacterial transformation is the process by which a bacterium takes up and expresses foreign genetic material (DNA), thus acquiring a new trait(s). In this lab, you will be able to insert the GFP (Green Fluorescent Protein) gene, obtained from the bioluminescent jellyfish *Aequorea victoria*, into the bacterium *Escherichia coli* by using the pGLO plasmid as a vector (transport mechanism).

In addition to one large circular chromosome, which contains all the genes a bacterium needs for its normal existence, bacteria also naturally contain one or more tiny circular pieces of DNA called **plasmids**. Plasmid DNA contains genes for traits that may be beneficial for bacterial survival under certain environmental conditions. In nature, bacteria transfer plasmids back and forth, allowing them to share these beneficial genes. In order to do transformation, the gene to be transferred is inserted into a plasmid. For this experiment, the GFP gene has been inserted into the plasmid pGLO.

Transformation is a process that can occur in nature, although it is rare or it can be artificially induced. In order to induce transformation, the bacterial cells have to be made **competent**. This is done by placing the *E. coli* cells in a relatively high concentration of calcium ions (calcium chloride solution) and then subjecting them to rapid changes in temperature from ice cold to hot, a procedure known as "heat shock". The first step neutralizes the negative charges on the cell membrane and the plasmid DNA. The heat shock step creates a temperature gradient from the outside to the inside of the bacterial cell, causing the plasmid to get pushed into the cell. Subsequently the cells are allowed to recover in Luria Broth medium.

In order to **select** only the *E. coli* cells that have received the GFP gene, the pGLO plasmid also has a gene for resistance to the antibiotic Ampicillin. So when the *E. coli* cells are plated on agar plates that have ampicillin on them, only those *E. coli* cells that have picked up the pGLO plasmid are able to survive. Thus, the colonies growing on the agar plate with ampicillin will also be producing the protein GFP, which can be visualized by observing the *E. coli* colonies under UV light. These colonies will glow green.

The **transformation efficiency** is a quantitative number that shows the extent to which you genetically transformed *E. coli* cells in the experiment. Not all of the *E. coli* cells will be transformed in practice. In research laboratories, the transformation efficiencies range between 8×10^2 and 7×10^3 cells per microgram of plasmid DNA. Students will be able to calculate their transformation efficiency using the following formula:

$$\frac{\text{Number of transformants}}{\mu\text{g of plasmid DNA}} \times \frac{\text{Final vol. at recovery}}{\text{Volume plated}} = \text{Number of transformants per } \mu\text{g}$$

Genetic transformation has many **practical applications**. In agriculture, genes coding for traits such as frost, pest or drought resistance can be genetically transformed into plants to improve yield from crops. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes can be treated by gene therapy.

Gene expression system

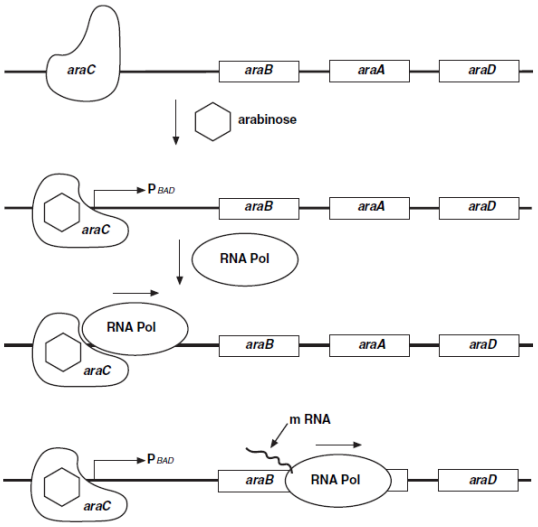
In this experiment, the goal is to express GFP in the transformed bacterial cells. In order to control the expression of the GFP gene, it has been placed under the control of a promoter, which functions as an on/off switch.

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons.

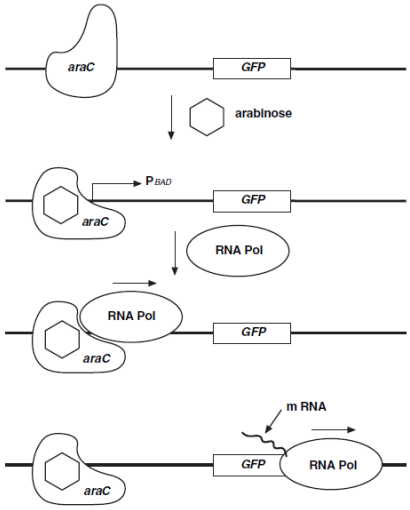
The three genes (*araB*, *araA* and *araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the **arabinose operon**. These three proteins are dependent on initiation of transcription from a single promoter, $\mathbf{P}_{\boldsymbol{BAD}}$. Transcription of these three genes requires the simultaneous presence of the DNA template (promoter and operon), RNA polymerase, a DNA binding protein called ***araC*** and arabinose. *araC* binds to the DNA at the binding site for the RNA polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with *araC* which is bound to the DNA.
The interaction causes *araC* to change its shape which in turn promotes (actually helps) the binding of RNA polymerase and the three genes *araB*, *A* and *D*, are transcribed.

The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter (P_{BAD}) and the *araC* gene are present. However, the genes which code for arabinose catabolism, *araB*, *A* and *D*, have been replaced by the single gene which codes for GFP. Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and GFP is produced.

The Arabinose Operon



Expression of Green Fluorescent Protein



WAY TO GLOW
Experimental Procedure

Setting up the transformation and control experiment

1. Label 1 microcentrifuge tube '+ DNA'. This will be the transformation tube with plasmid DNA.
2. Label a 2nd microcentrifuge tube '- DNA'. This will be the experimental control tube without plasmid DNA.
3. Using a F250, add 250 µl of $CaCl_2$ solution to each tube and place immediately on ice rack.
4. Pick colonies from the source plate of *E. coli* cells. Use the following method for each of the test tubes labeled '+DNA' and '-DNA'.
    a. Use a sterile inoculating loop to transfer several colonies (3-4 colonies, approx 2-4 mm in size) from the source plate to the microcentrifuge tube.
    b. Between your fingers, twist the inoculating loop vigorously in the cold $CaCl_2$ solution to dislodge the cells.
5. In both tubes, suspend the cells completely by tapping.
6. To the tube labeled '+ DNA', add 10 µl of pGLO twice (20 µl pGLO).
7. Incubate the 2 tubes on ice for 10 minutes.
8. Place both transformation tubes at 42°C in the dry bath incubator for 90 secs.
9. Return both tubes immediately to the ice rack and incubate for 2 minutes.
10. Using an F250 micropipette, add 250 µl of LB (Luria Bertani) Recovery Broth to each tube.
11. Incubate the cells for 20 minutes at 37°C in the dry bath incubator for their recovery period.

12. While the tubes are incubating, label 3 agar plates as follows:
    a. Label 1 ‘A’ plate: Amp/DNA-
    b. Label 2 ‘A’ plates: Amp/DNA+
    c. Put your school reference number and group number on all plates
    d. After the recovery period, remove the tubes from the water bath and place them on the lab bench. Proceed to plating the cells for incubation.

Plating the cells

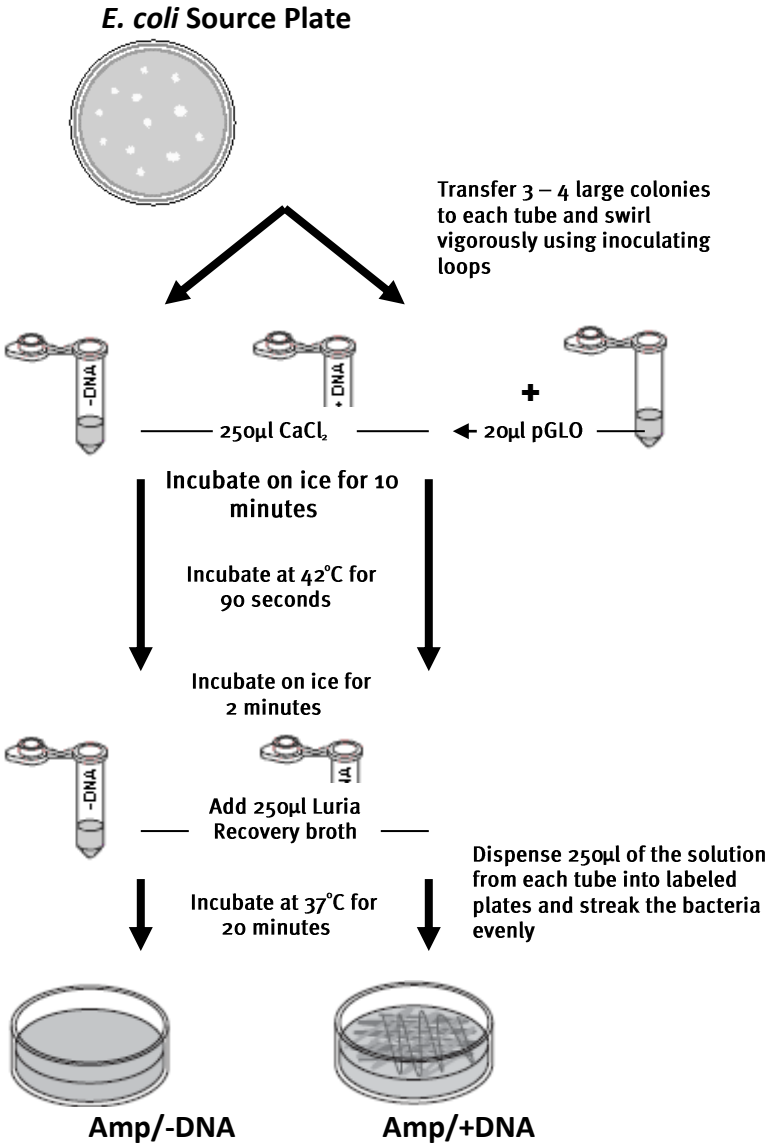
Plating cells from the tube labeled ‘-DNA’ (Control Experiment):

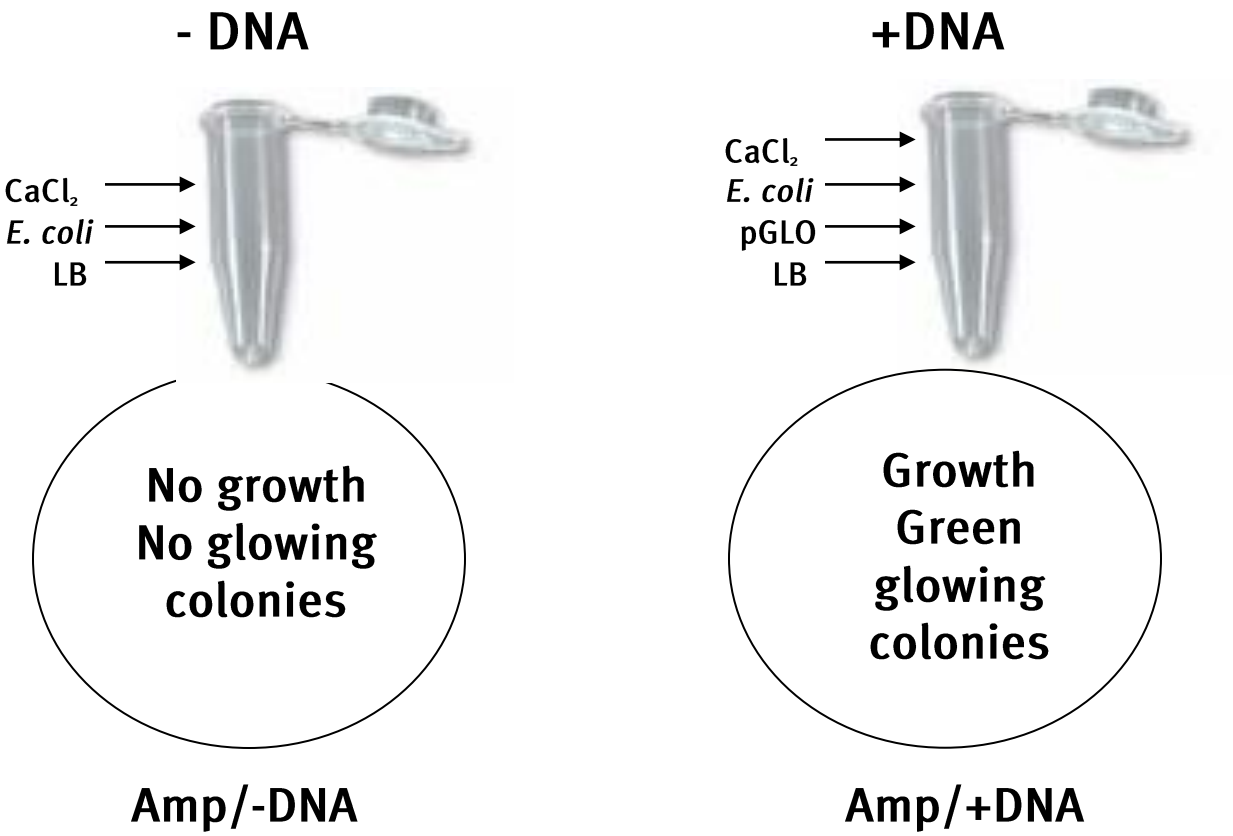
1. Use a F250 micropipette to transfer recovered cells from the tube labeled ‘-DNA‘ to the middle of the plate labeled Amp/DNA-
2. Spread the cells with a sterile inoculating loop.
3. Cover the plate and allow the liquid to be absorbed.

Plating cells from the tube labeled ‘+DNA’:

4. Use a F250 micropipette to transfer the recovered cells from the tube labeled ‘+DNA’ to the middle of each to the two plates labeled Amp/DNA+
5. Spread the cells with a sterile inoculating loop in the same manner as step 1.
6. Cover the plates and allow the liquid to be absorbed.
7. Stack your group’s set of plates on top of one another. *The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar.
8. After the cell suspension is completely absorbed by the agar, the educator will place the plates in the inverted position (agar side up) in a 37°C incubation oven for overnight incubation (24 – 48 hours).
9. Record your results after 24 – 48 hours of growth.

Experiment Overview





Calculation of transformation efficiency

$$\frac{\text{Number of transformants}}{\mu g \text{ of plasmid DNA}} \times \text{Final vol. at recovery} = \text{Number of transformants per } \mu g$$

Volume plated

Number of transformants = colony count from your plate

µg of plasmid DNA = 1.25 µg

Final volume at recovery = 520 µl

Volume plated = 250 µl

WAY TO GLOW
Post Program Activity Sheet

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1. What would happen if you illuminated:

    a) the plasmid pGLO with UV light?

    b) the transformed *E.coli* with UV light?

2. Which of the plates is the control plate? What purpose does a control plate serve?

3. Did your actual results match your expected results? If no, why not?

4. Transformation efficiency is expressed as the number of transformed colonies per microgram of plasmid DNA. In this experiment, the object is to determine the mass of pGLO that was spread on the experimental plate and was therefore responsible for the transformants observed. Answer the following questions to determine your transformation efficiency.

    a) Count the number of transformants on your AMP/+DNA plate.

    b) Determine the total mass (in µg) of pGLO used in this experiment. Remember that you used 20 µl of plasmid DNA at a concentration of 0.0625 µg/µl

    concentration x volume = total mass

    c) Calculate the total volume of the solution in the tube.

    volume of $CaCl_2$ + volume of pGLO + volume of LB = total volume

d) What volume of solution from the tube was deposited onto each plate?

e) Determine your bacterial transformation efficiency. Express answer in scientific notation.

$$\frac{\text{Number of transformants}}{\text{Mass of pGLO used}} \times \frac{\text{Total volume of solution}}{\text{Volume plated}} = \text{Number of transformants per } \mu g$$

Challenge Questions

1. What results would you expect if you plated your:

   a) –DNA tube contents on an LB agar plate without Amp?

   b) +DNA tube contents on an LB agar plate without Amp?

2. Go to www.rcsb.org/ to find the amino acid sequence of Green Fluorescent Protein (GFP). (Hint: search "Green Fluorescent Protein from Aequorea Victoria")

   a) How many amino acids is GFP is comprised of?

   b) From that sequence find out which three amino acids form part of the chromophore (*a chromophore is part of a molecule responsible for its colour).* (Hint: read this additional information on GFP: pdb101.rcsb.org/motm/42)

WAY TO GLOW
Post Program Activity Sheet with answers

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1. What would happen if you illuminated:

    a) the plasmid pGLO with UV light?

    Nothing would happen since the plasmid does not glow.

    b) the transformed *E.coli* with UV light?

    The transformed *E.coli* colonies would fluoresce due to the production of GFP from the plasmid pGLO.

2. Which of the plates is the control plate? What purpose does a control plate serve?

    The AMP/–DNA plate serves as the control plate. The control plate shows that the *E. coli* cells do not naturally have Ampicillin resistance ability and do not produce GFP.

3. Did your actual results match your expected results? If no, why not?

    Factors influencing transformation include technique errors (inaccurate micropipetting of $CaCl_2$, LB or pGLO) and, incorrect temperature and length of the incubation period.

4. Transformation efficiency is expressed as the number of transformed colonies per microgram of plasmid DNA. In this experiment, the object is to determine the mass of pGLO that was spread on the experimental plate and was therefore responsible for the transformants observed. Answer the following questions to determine your transformation efficiency.

    a) Count the number of transformants on your AMP/+DNA plate.

    Varies per student group.

    b) Determine the total mass (in µg) of pGLO used in this experiment. Remember that you used 20 µl of plasmid DNA at a concentration of 0.0625 µg/µl.

    concentration x volume = total mass

0.0625 µg/µl X 20µl = 1.25 µg

c) Calculate the total volume of the solution in the tube.

volume of $CaCl_2$ + volume of pGLO + volume of LB = total volume

250µl + 20µl + 250 µl = 520 µl

d) What volume of solution from the tube was deposited onto each plate?

250 µl

e) Determine your bacterial transformation efficiency. Express answer in scientific notation.

$$\frac{\text{Number of transformants}}{\text{Mass of pGLO used}} \times \frac{\text{Total volume of solution}}{\text{Volume plated}} = \text{Number of transformants per µg}$$

Varies per student group.

Challenge Questions

1. What results would you expect if you plated your

a) –DNA tube contents on an LB agar plate without Amp?

There will be bacterial growth since no environmental stressors are present (i.e. no Amp) but no glowing will occur since there is no plasmid.

b) +DNA tube contents on an LB agar plate without Amp?

There will be bacterial growth since no environmental stressors are present (i.e. no Amp) but no glowing will occur since there is no need to pick up the plasmid and thus successive colonies will lose the plasmid.

2. Go to www.rcsb.org/to find the amino acid sequence of Green Fluorescent Protein (GFP). (Hint: search "Green Fluorescent Protein from Aequorea Victoria")

a) How many amino acids is GFP is comprised of?

238 amino acids

b) From that sequence find out which three amino acids form part of the chromophore (*a chromophore is part of a molecule responsible for its colour).* (Hint: read this additional information on GFP: pdb101.rcsb.org/motm/42)

The chromophore forms spontaneously from three amino acids in the protein chain: a glycine, a tyrosine and a threonine (or serine).

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