

## Transformation of *E. coli* with pGAL

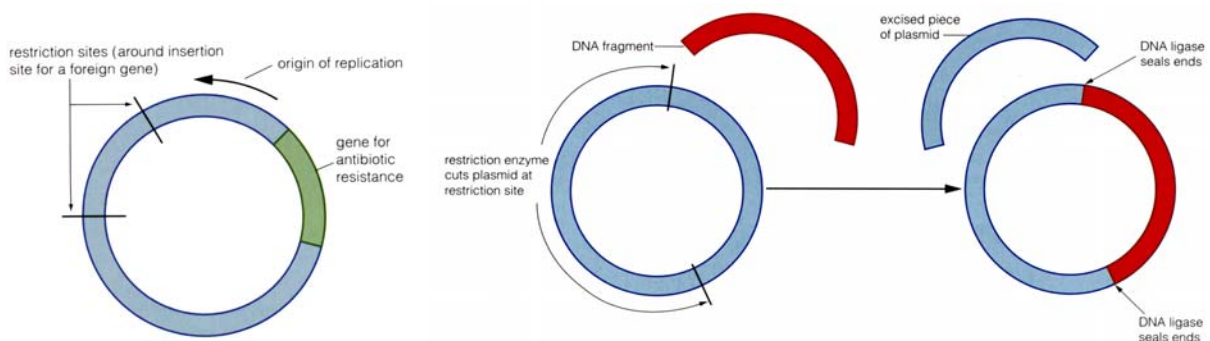
DNA can be incorporated into some bacteria by the process of transformation. The *Streptococcus pneumoniae* bacteria that Fred Griffith studied in 1928 used transformation to incorporate the genes for capsule production. Many bacteria contain plasmids, small independent DNA fragments that carry specific pieces of genetic information. In transformation, a plasmid or a piece of DNA from the environment is incorporated into the cell's genome. This is a form of genetic recombination. Transformation occurs only between closely related bacteria, and some bacteria are not known to do transformation. Those that have transformation have special proteins on their membrane that facilitate the uptake of the foreign DNA.

Transformation can also be induced by treating host bacteria cells with certain chemicals and temperature changes for a short period of time. This produces a "transient state of competence" during which the bacterial membrane is receptive to transformation. For example, competency can be induced in *E. coli* using ice-cold calcium chloride followed by a brief exposure to high temperature and then ice cold temperature again.

An advantage of using plasmids for transformation is that plasmids can incorporate into their DNA sequence pieces of DNA from different organisms. Plasmids that incorporate new DNA are called **recombinant plasmids**. Recombinant plasmids are used in biotechnology to add DNA that codes for substances such as human insulin or growth hormone into bacteria, which can then be grown commercially to provide the needed substance.

In order to obtain DNA to incorporate into a plasmid to be used for transformation, special enzymes, called **restriction enzymes**, are used. Restriction enzymes can cut DNA fragments from almost any organism. There are many different restriction enzymes, each of which recognizes one specific nucleotide sequence in any DNA molecule. Many restriction enzymes work by finding palindrome sections of DNA (regions where the order of nucleotides at one end is the reverse of the sequence at the opposite end). This way a restriction enzyme can cut tiny **sticky ends** of DNA that will match and attach to compatible sticky ends of any other DNA that has been cut with the same enzyme. DNA ligase joins the matching sticky ends of the DNA pieces from different sources that have been cut by the same restriction enzyme no matter where the DNA comes from.

A DNA fragment cut with a specific restriction enzyme can be spliced into a plasmid that has been cut by the same restriction enzyme forming a recombinant DNA plasmid. Special plasmids, which have antibiotic resistance markers, are used in this process so that a researcher will be able to tell that the desired DNA has been incorporated into the target plasmid and subsequently into the host bacterium.

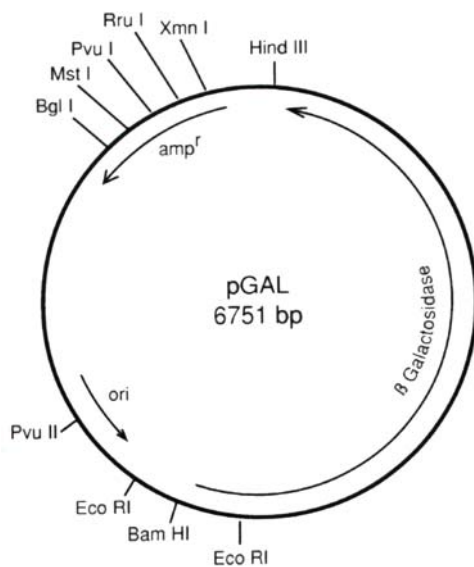


When recombinant plasmids are made, plasmids and DNA fragments are mixed in a solution of restriction enzymes and other enzymes, such as DNA ligase, needed to obtain the recombinant plasmid. But not all plasmids in the solution achieve success. When the recombinant plasmids are mixed with host bacteria, not all bacteria incorporate the plasmid and undergo transformation. Part of the procedure in any transformation experiment is identifying successful recombinant plasmids and successfully transformed bacteria. The efficiency of transformation is about 1 in 10,000. However, there are so many bacterial cells in any sample, that only a very small number of bacterial cells need be transformed to have success.

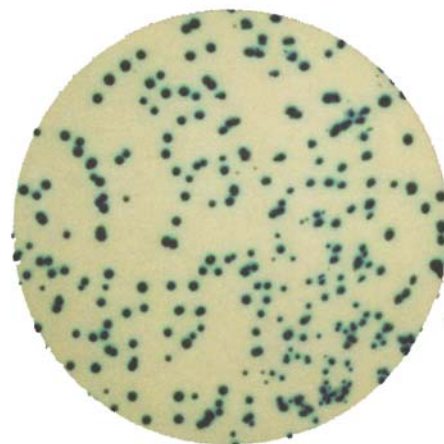
The transformation experiment you will be doing will measure transformation of special *E coli* cells that do not contain the lacZ gene that codes for  $\beta$ -galactosidase. Normally  $\beta$ -galactosidase splits an artificial galactoside (5-Bromo-4 Chloro 3 indolyl-  $\beta$ -D-galactoside) called X-GAL. Split X-GAL is pigmented. Blue bacterial colonies grow when X-GAL is split. The *E coli* in this experiment will always form white colonies since it cannot synthesize the lacZ enzyme,  $\beta$ -galactosidase.

The Edvotek proprietary pGAL plasmid contains the lac Z gene  $\beta$ -galactosidase that is always turned on (no repressor sequence). Host *E coli* cells that undergo transformation have incorporated the pGAL plasmid and will synthesize  $\beta$ -galactosidase. The  $\beta$ -galactosidase will catalyze the splitting of X-GAL and the transformed bacterial colonies will be blue.

The pGAL plasmid also contains ampicillin-resistance. pGAL plasmids have a gene that codes for  $\beta$ -lactamase, an enzyme that inactivates ampicillin. *E coli* cells transformed by the pGAL plasmid will secrete  $\beta$ -lactamase into their growth medium and any ampicillin in the area surrounding the resistant colony will be destroyed. After a longer period of incubation, small non-transformed (white) *E coli* colonies, called satellite colonies, may form in an area surrounding a blue colony because the ampicillin in the growth medium in that region has been destroyed.



The pGAL Plasmid



Transformed *E. coli* colonies

## Materials Needed

Edvo-Kit #221

LyphoCells (Special *E. coli* cells)

pGAL DNA

Control buffer lacking DNA

Ampicillin

X-Gal in solvent

Cell reconstitution medium

Solvent for competency induction

Sterile ReadyPour Media

Sterile Recovery Broth

Petri plates

10 ml pipettes

1 ml pipettes

Inoculating loops

Microtest tubes with lids

Automatic Micropipettes (5 – 50  $\mu$ l)

In the Laboratory Room

37°C Waterbath

42°C Waterbath

Incubation oven (34°C, 37°C)

Pipette pumps or bulbs

Ice

China markers

Bunsen burners

Gloves

## Procedure

Although the *E. coli* used in this experiment is non-pathogenic, it is essential to use the following safety guidelines.

- Wear the provided laboratory "coat".
- Wear gloves and goggles.
- Place all materials used in the designated containers for disinfection before permanent disposal.
- At the completion of the lab, wipe all lab surfaces with the designated disinfectant solution.
- Wash your hands thoroughly with hot water and soap.

**Setting up the Experiment** (A summary flow chart follows the detailed instructions.)

1. Put your group identification symbol on tubes labeled "pGAL DNA" and "Control Buffer" and put them on ice.
2. Use a fresh sterile 1ml pipette to transfer 0.3ml cell suspension from the tube labeled "Cells for Control" into the Control Buffer tube. Save the pipette in its wrapper.
3. Use the same pipette to transfer 0.3ml cell suspension from the tube labeled "Cells for DNA" into the pGAL DNA tube. Dispose of the pipette.

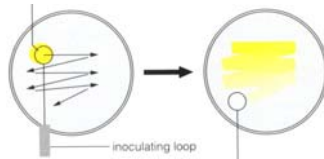
## Setting up the Transformation

4. Incubate your two tubes (pGAL DNA and Control Buffer) on ice for 10 minutes
5. Place the two tubes in the 42°C Waterbath for 90 seconds.
6. Place the two tubes back on ice for 1 minute.
7. Use a fresh sterile 1ml pipette to add 0.75ml recovery broth to the Control Buffer tube. Use the same pipette to add 0.75ml recovery broth to the pGAL DNA tube.
8. Incubate the two closed tubes (pGAL DNA and Control Buffer) in the 37°C Waterbath for 30 minutes.

## Plating the Cells

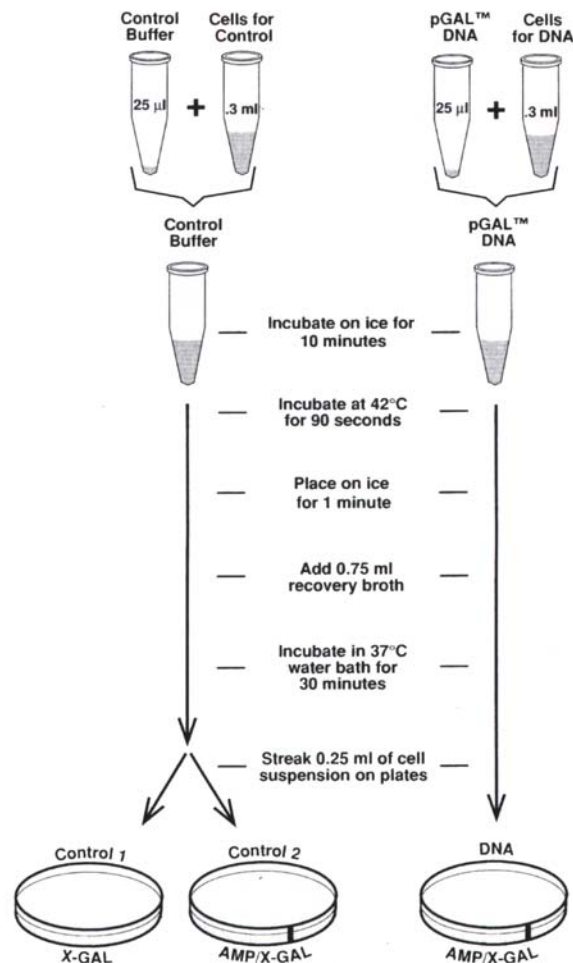
9. Label the bottom of 3 petri plates in the following way:
  - Put your group symbol on all three plates along with the date
  - Label the X-GAL plate (unstriped) "Control 1"
  - Label one AMP/X-GAL plate (striped) "Control 2"
  - Label the second AMP/X-GAL plate (striped) "DNA"
10. Use a fresh sterile 1ml pipette to add 0.25ml recovered cells from your Control Buffer tube to the petri plate labeled Control 1 (X-GAL).

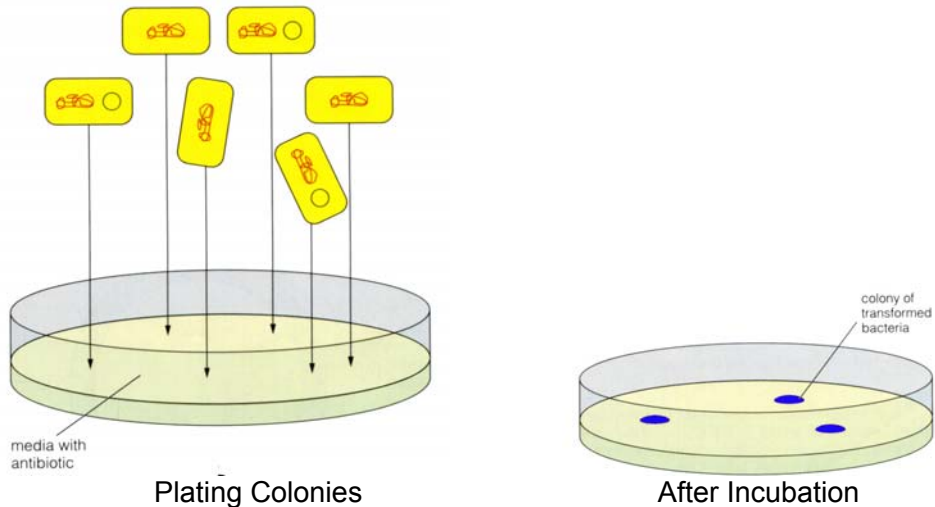
11. Use the same 1ml pipette to add 0.25ml recovered cells from your Control Buffer tube to the petri plate labeled Control 2 (AMP/X-GAL).
12. Spread the cells with an inoculating loop as shown below. Rotate your plate 90° and restreak it with the inoculating loop.



13. Cover both control plates and allow the liquid to be absorbed.
14. Use a fresh sterile 1ml pipette to add 0.25ml recovered cells from your pGAL DNA tube to the petri plate labeled DNA (AMP/X-GAL).
15. Spread the cells with an inoculating loop as shown above. Rotate your plate 90° and restreak it with the inoculating loop.
16. Cover your experimental plate and allow the liquid to be absorbed.
17. Stack your 3 plates one on top of another and tape them together. Put your group symbol on the taped set of plates. Leave the plates upright to allow the liquid to be absorbed.
18. Place your plates in the location designated by your instructor.
19. Once the plates have absorbed the liquid (in about an hour) they will be placed in an inverted position in the incubator overnight.

### Flow Chart for Setting up Transformation of *E coli* with pGAL





### Determining Efficiency of Transformation

After incubation of your plates, count the number of colonies on your DNA plate (AMP/X-GAL). Remember blue colonies are those that have been transformed.

Determine the efficiency of transformation using the following calculation:

$$\frac{\text{Number of Transformants}}{\mu\text{g of DNA}} \times \frac{\text{Final Volume at recovery (ml)}}{\text{volume plated (ml)}} = \text{Number of transformants per } \mu\text{g}$$

In this experiment, 25 ng (0.025  $\mu\text{g}$ ) of DNA was used. The recovery volume was 1 ml and the plating volume was 0.2 ml.

For example, if you have 40 colonies, your rate of transformation would be:

$$\frac{40 \text{ Transformants}}{0.025 \mu\text{g}} \times \frac{1 \text{ ml}}{0.2 \text{ ml}} = 8000 (8 \times 10^3) \text{ transformants per } \mu\text{g}$$

### Discussion Questions

1. Did you observe any satellite colorless colonies surrounding the blue transformed colonies?
2. Why did the competent control cells not grow on the plates containing ampicillin?

