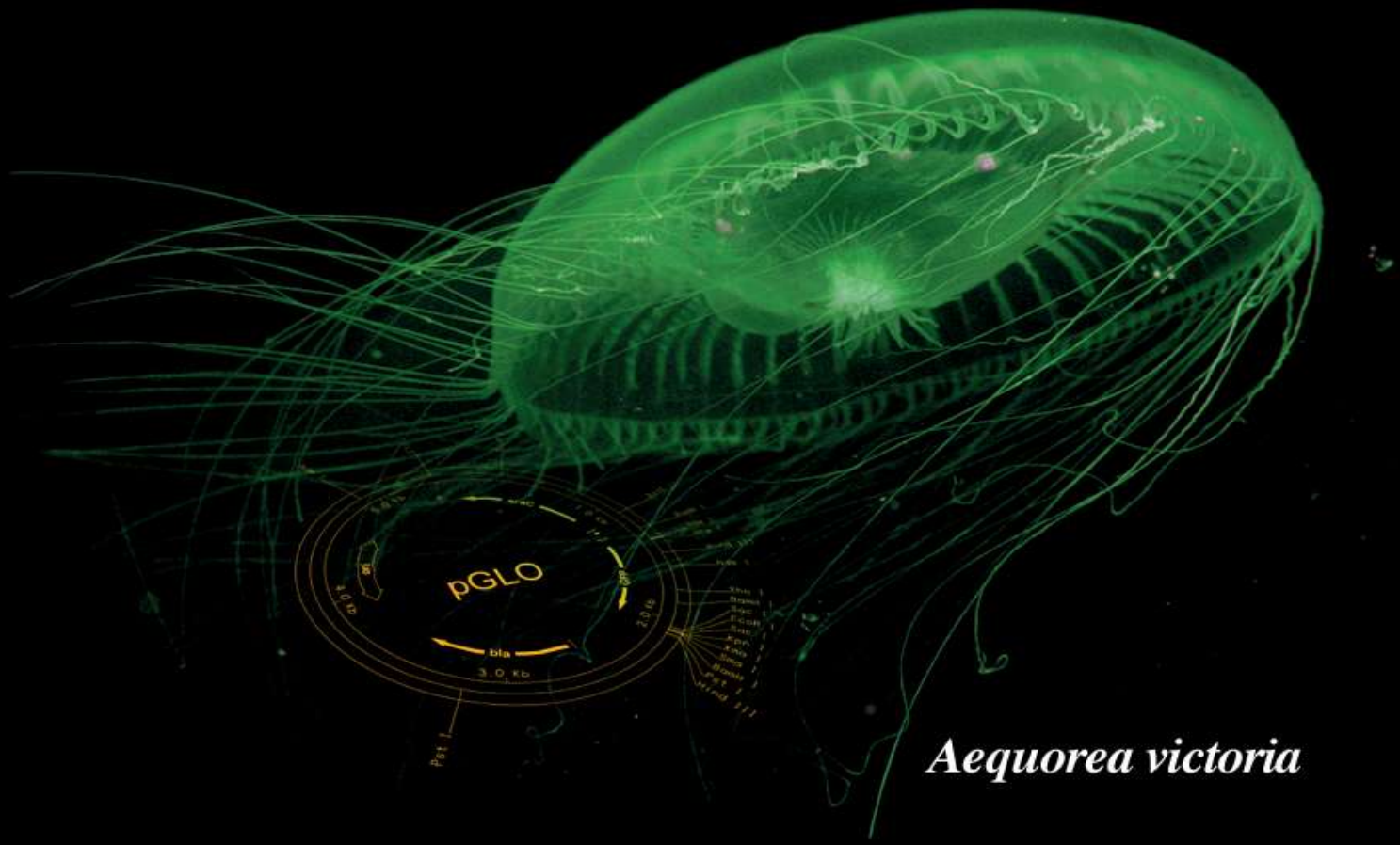
Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

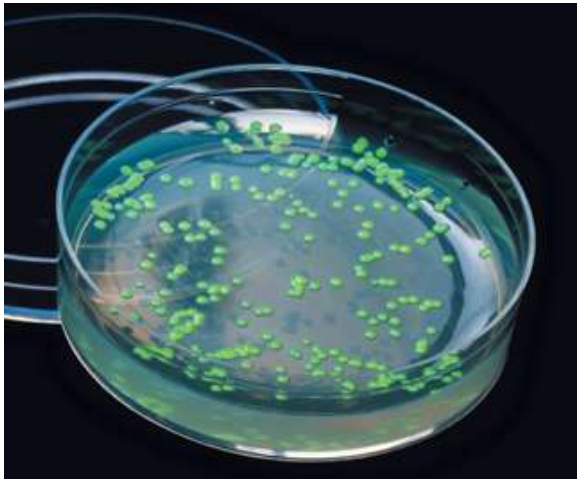
BIO-RAD

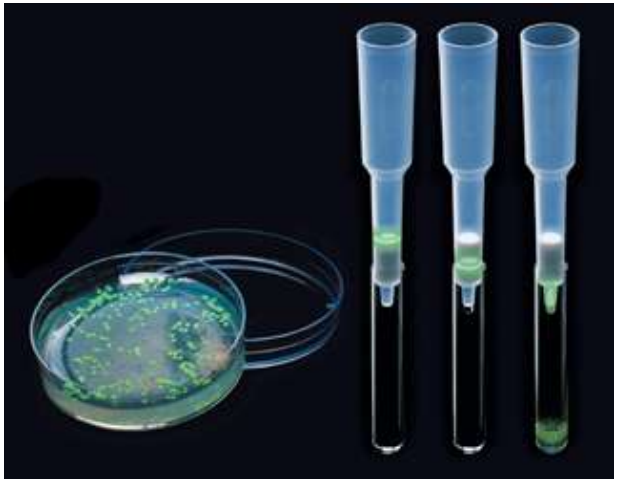
pGLO™ Transformation



Workshop Time Line

- **Introduction**
- **Transform bacteria with pGLO plasmid**



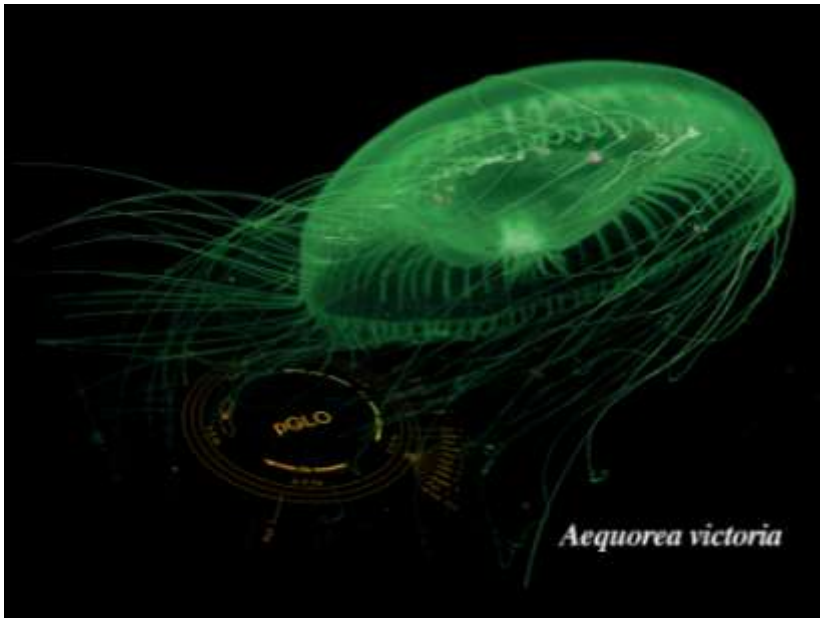


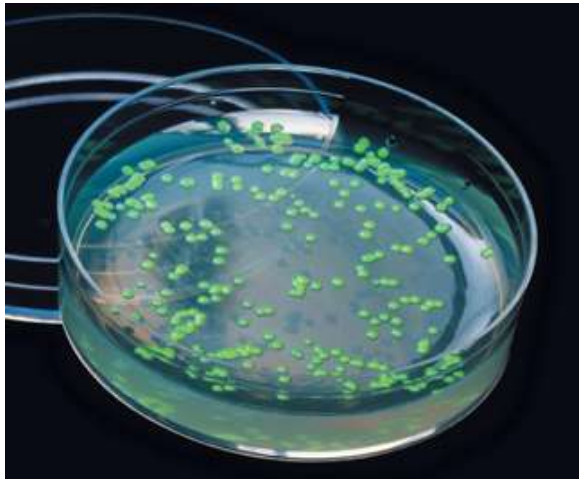
Central Framework of Molecular Biology

DNA → RNA → Protein → Trait

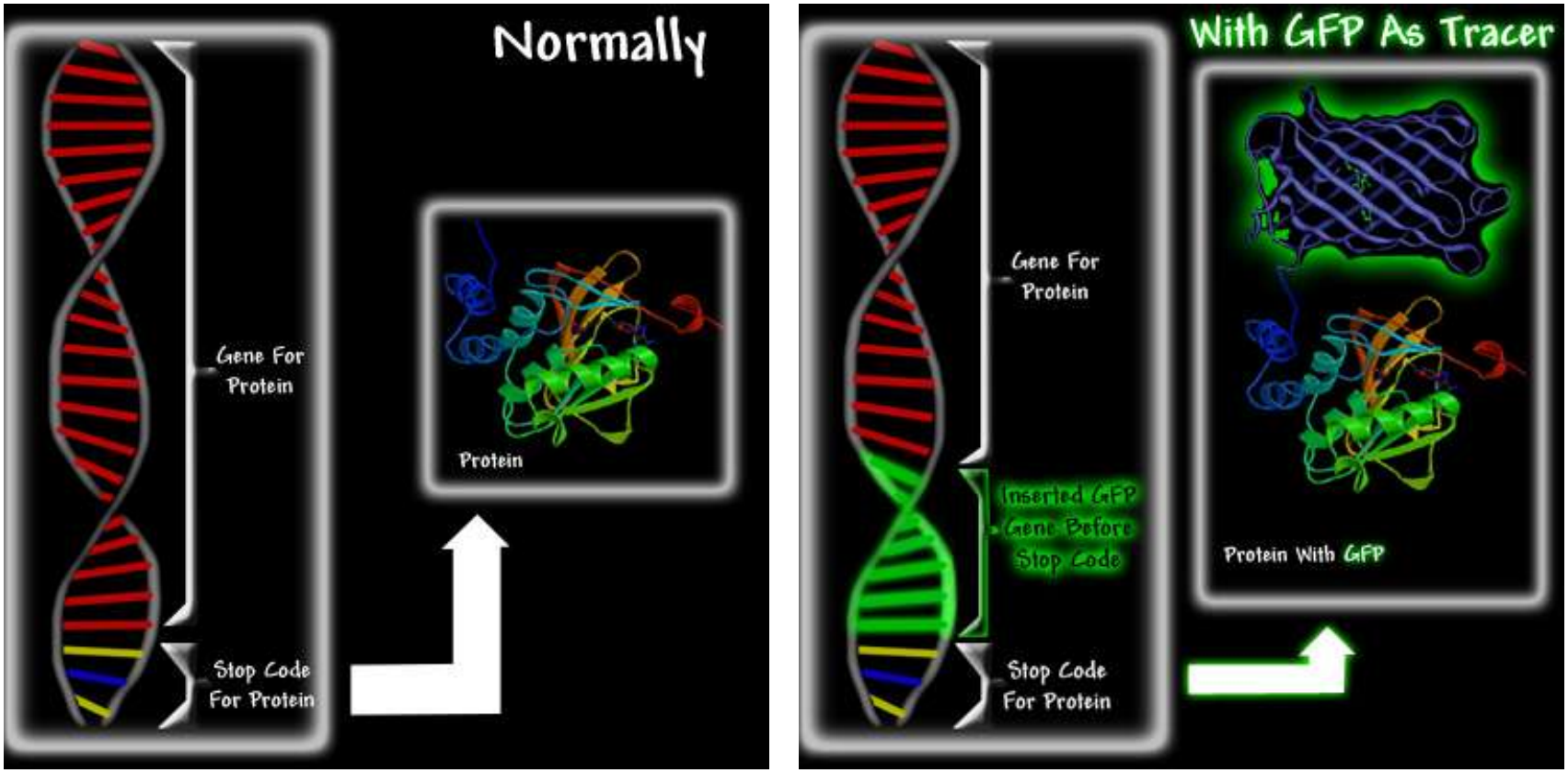
Links to Real-world

- **GFP is a visual marker**
- **Study of biological processes**
  (example: synthesis of proteins)
- **Localization and regulation of gene expression**
- **Cell movement**
- **Cell fate during development**
- **Formation of different organs**
- **Screenable marker to identify transgenic organisms**


pGLO
Aequorea victoria



Using GFP as a biological tracer

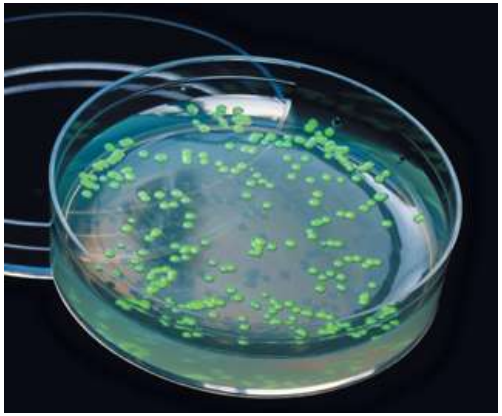


http://www.conncoll.edu/ccacad/zimmer/GFP-ww/prasher.html
With permission from Marc Zimmer

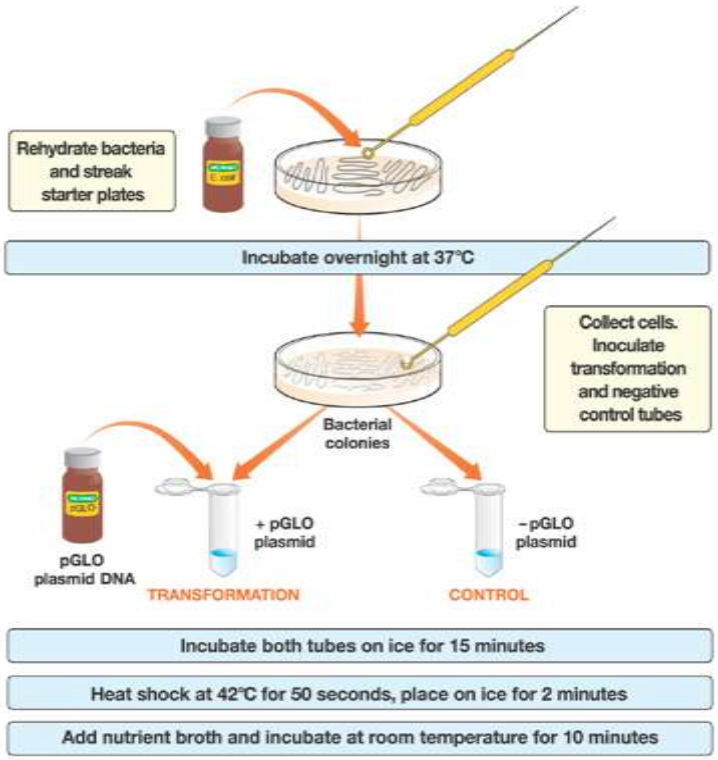
Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

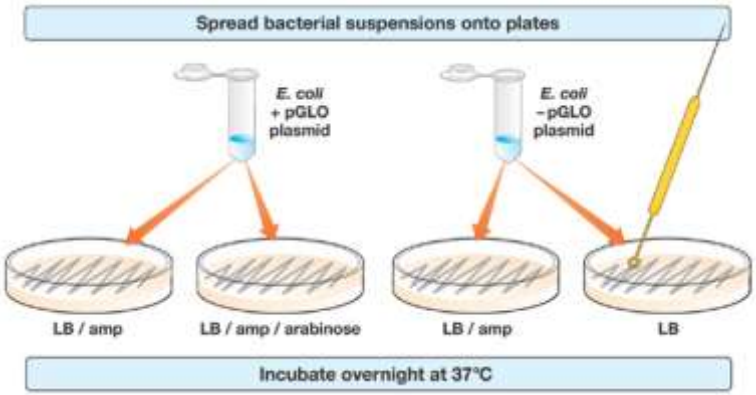
BIO-RAD

pGLO Bacterial Transformation Kit

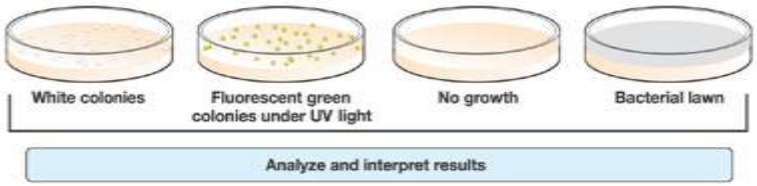


Transformation Procedure Overview





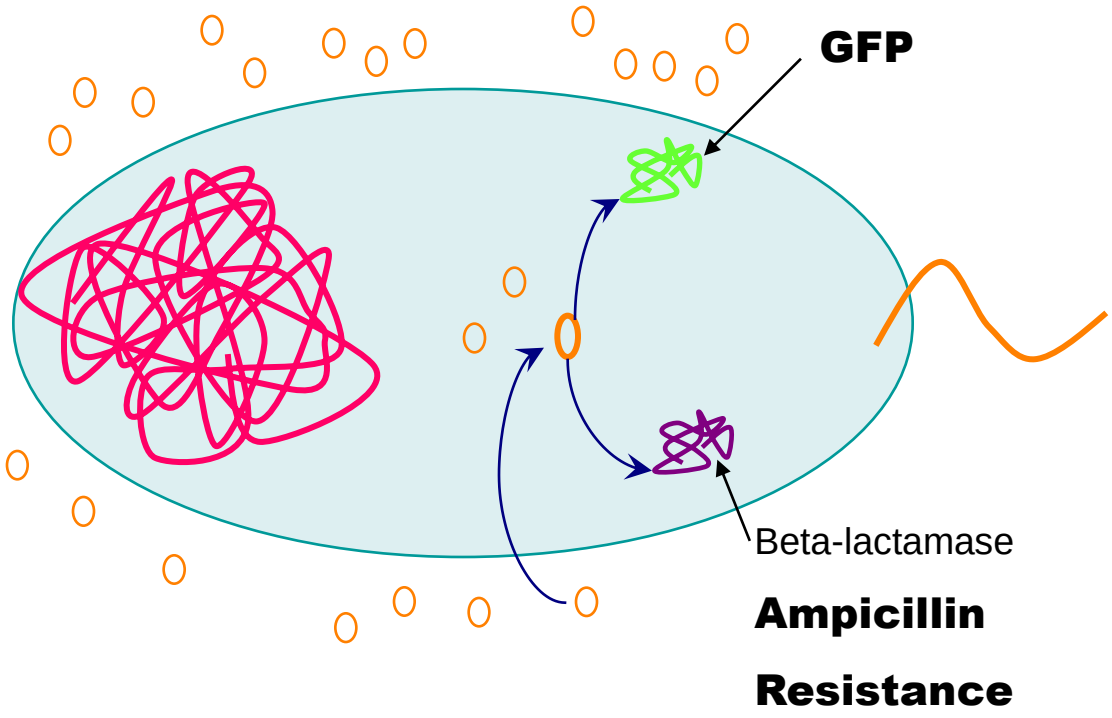
Day 1



Extension: GFP chromatography kit, pp. 22–23

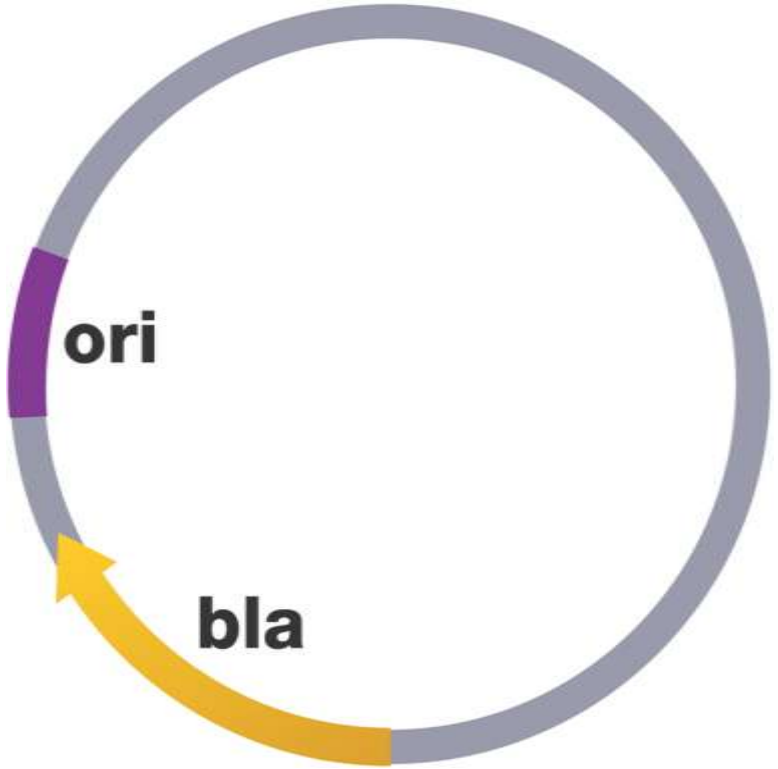
What is Transformation?

- **Uptake of foreign DNA,** often a circular plasmid

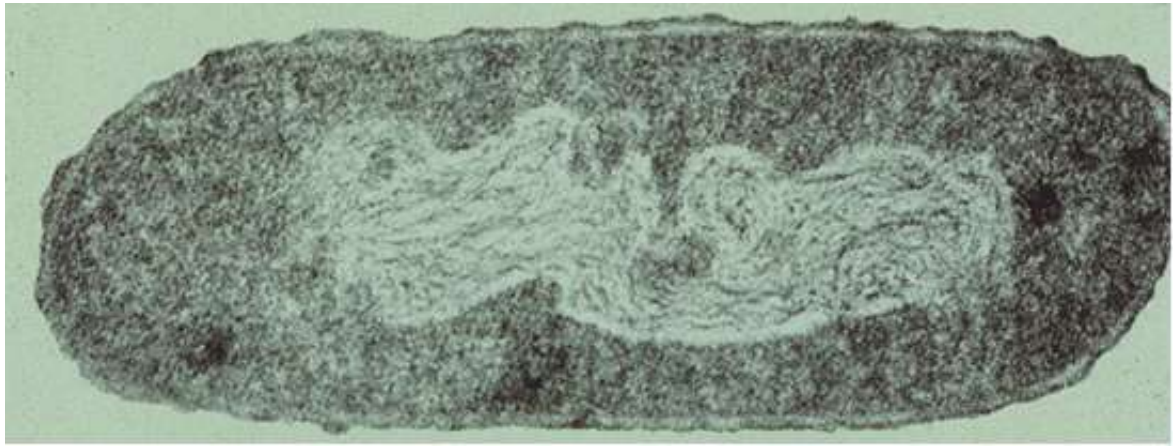


What is a plasmid?

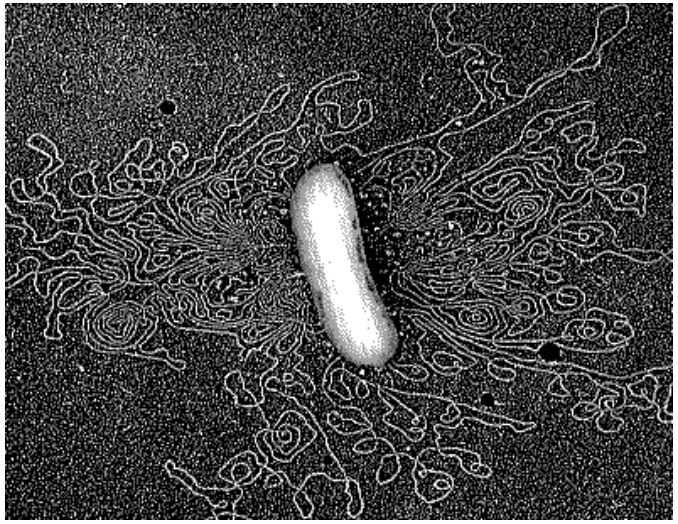
- A circular piece of autonomously replicating DNA
- Originally evolved by bacteria
- May express antibiotic resistance gene or be modified to express proteins of interest



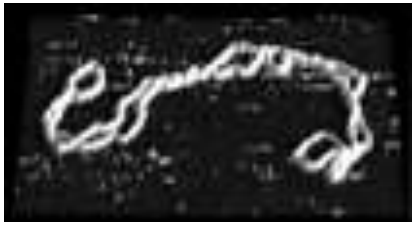
Bacterial DNA



Bacterial cell

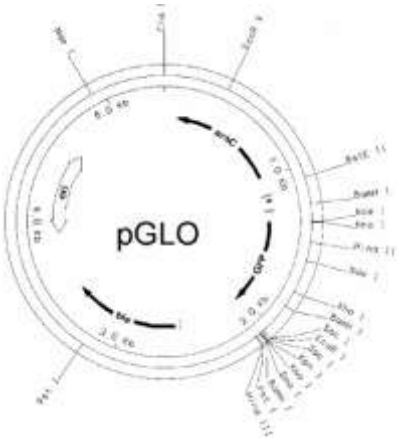


Genomic DNA



Plasmid DNA

The Many Faces of Plasmids

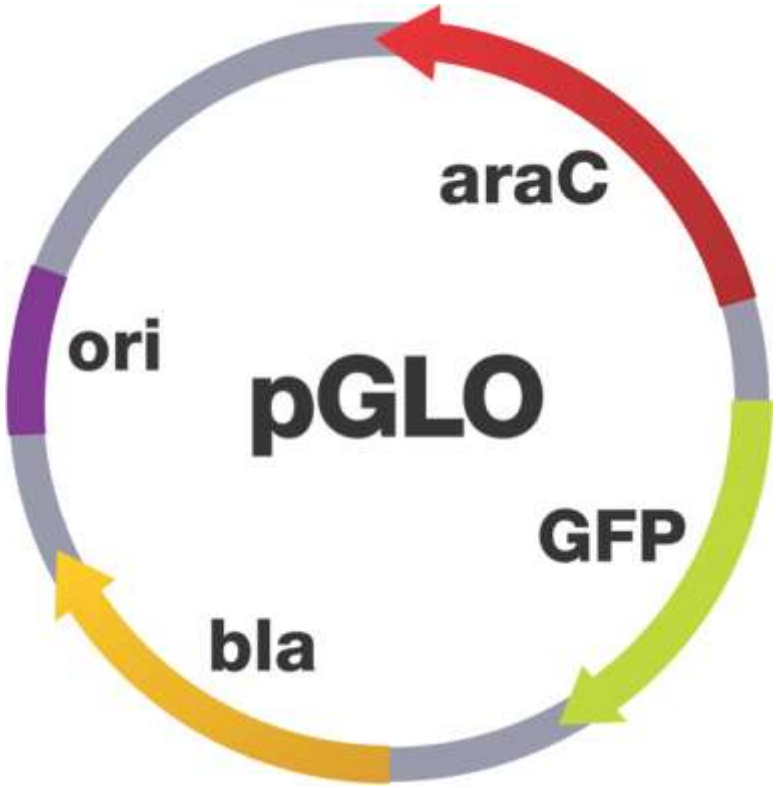


Graphic representation

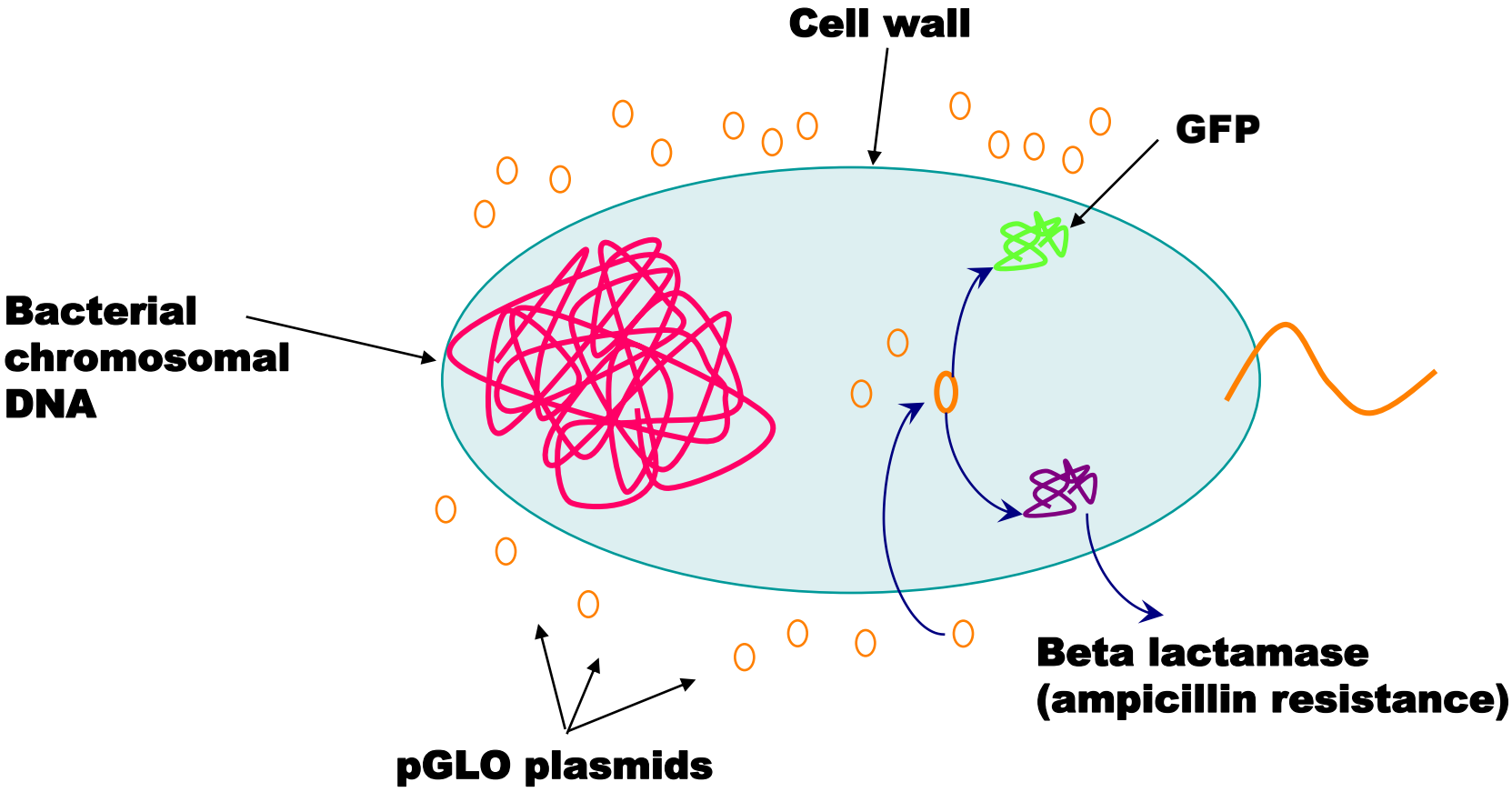
Scanning electron micrograph of supercoiled plasmid

Gene Expression

- **Beta Lactamase**
  - Ampicillin resistance
- **Green Fluorescent Protein (GFP)**
  - *Aequorea victoria* jellyfish gene
- **araC regulator protein**
  - Regulates GFP transcription

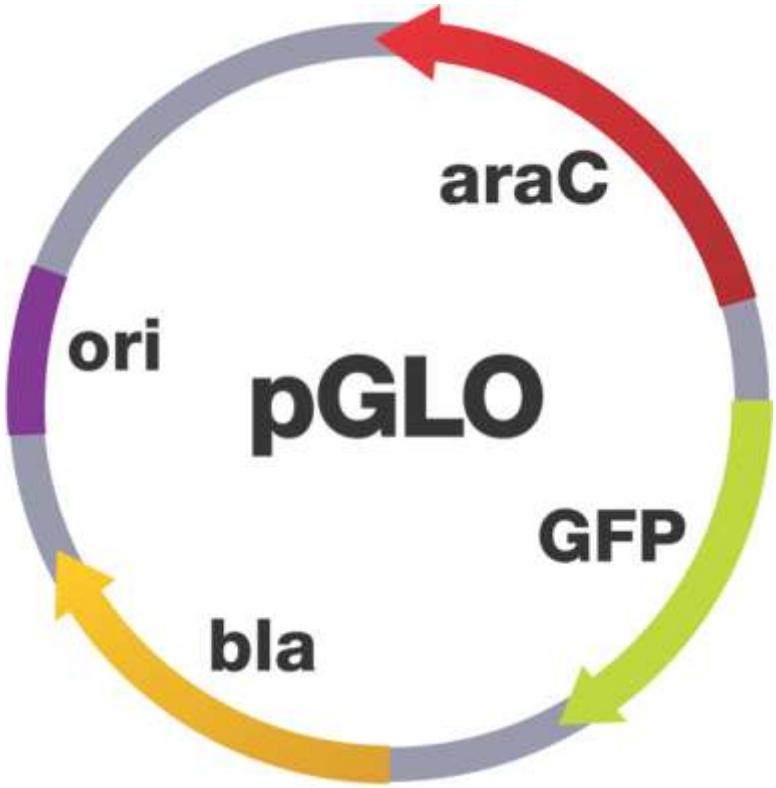


Bacterial Transformation

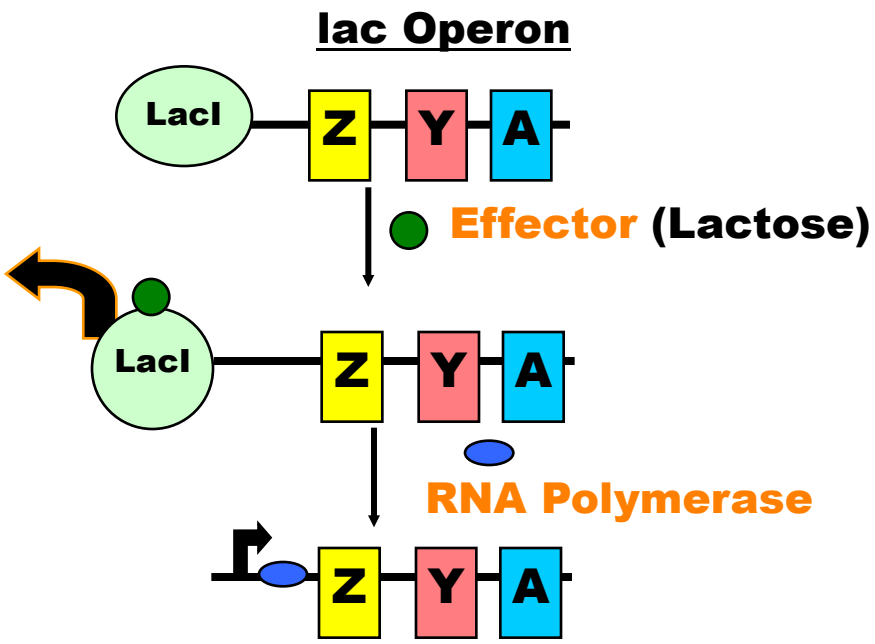


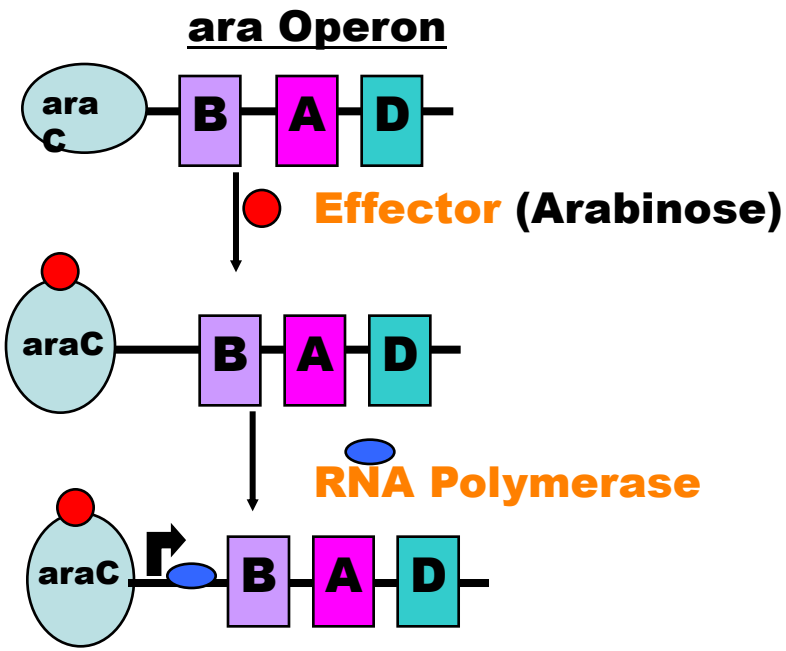
Transcriptional Regulation

- **Lactose operon**
- **Arabinose operon**
- **pGLO plasmid**

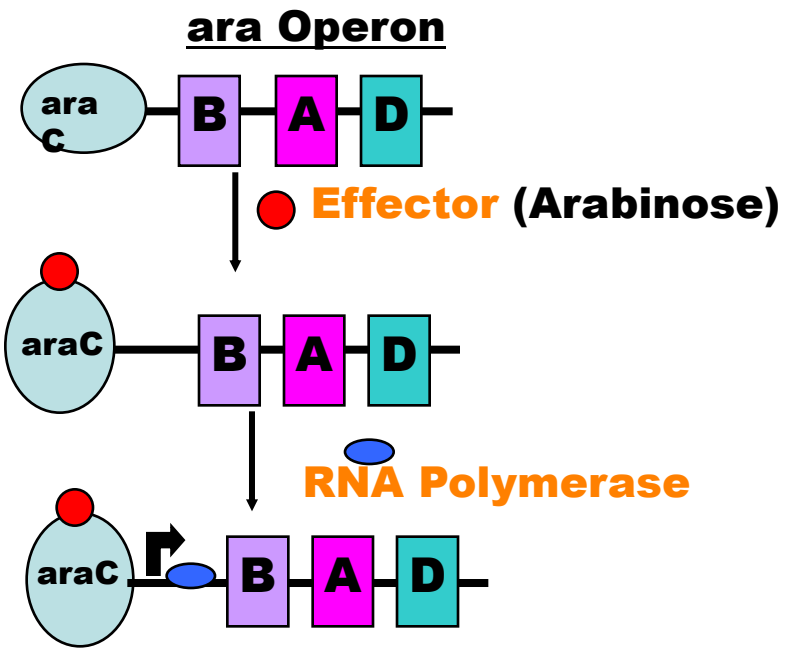


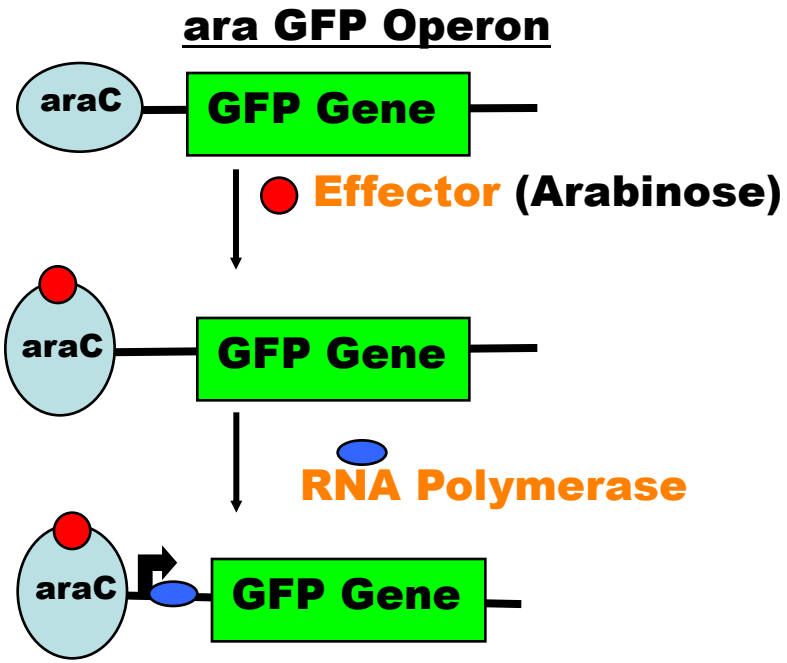
Transcriptional Regulation





Gene Regulation





Methods of Transformation

- **Electroporation**
  - Electrical shock makes cell membranes permeable to DNA
- **Calcium Chloride/Heat-Shock**
  - Chemically-competent cells uptake DNA after heat shock

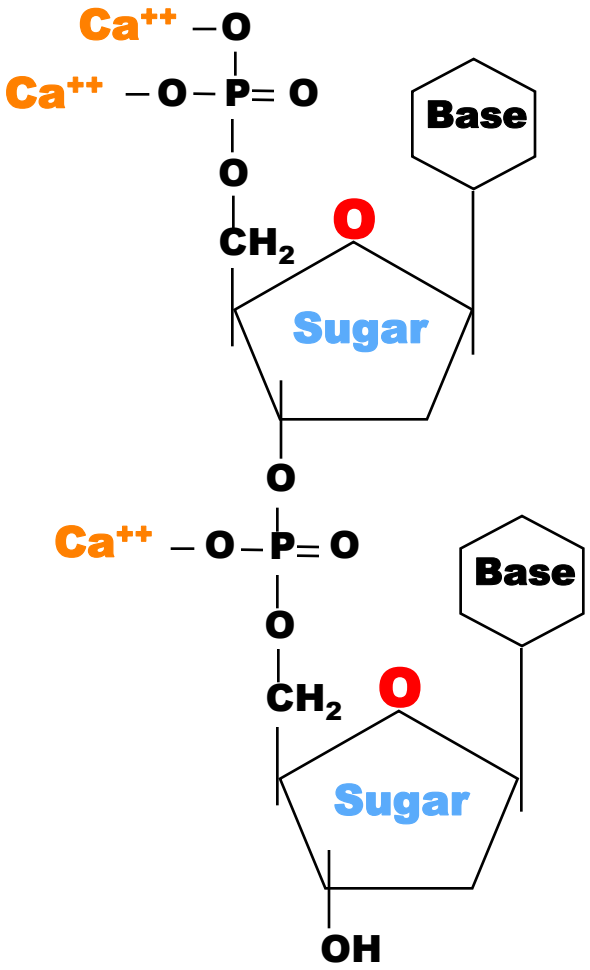
Transformation Procedure

- Suspend bacterial colonies in Transformation solution
- Add pGLO plasmid DNA
- Place tubes on ice
- Heat-shock at 42°C and place on ice
- Incubate with nutrient broth
- Streak plates

Reasons for Performing Each Transformation Step?

1. **Transformation solution = $CaCl_2$**

   Positive charge of Ca^{++} ions shields negative charge of DNA phosphates

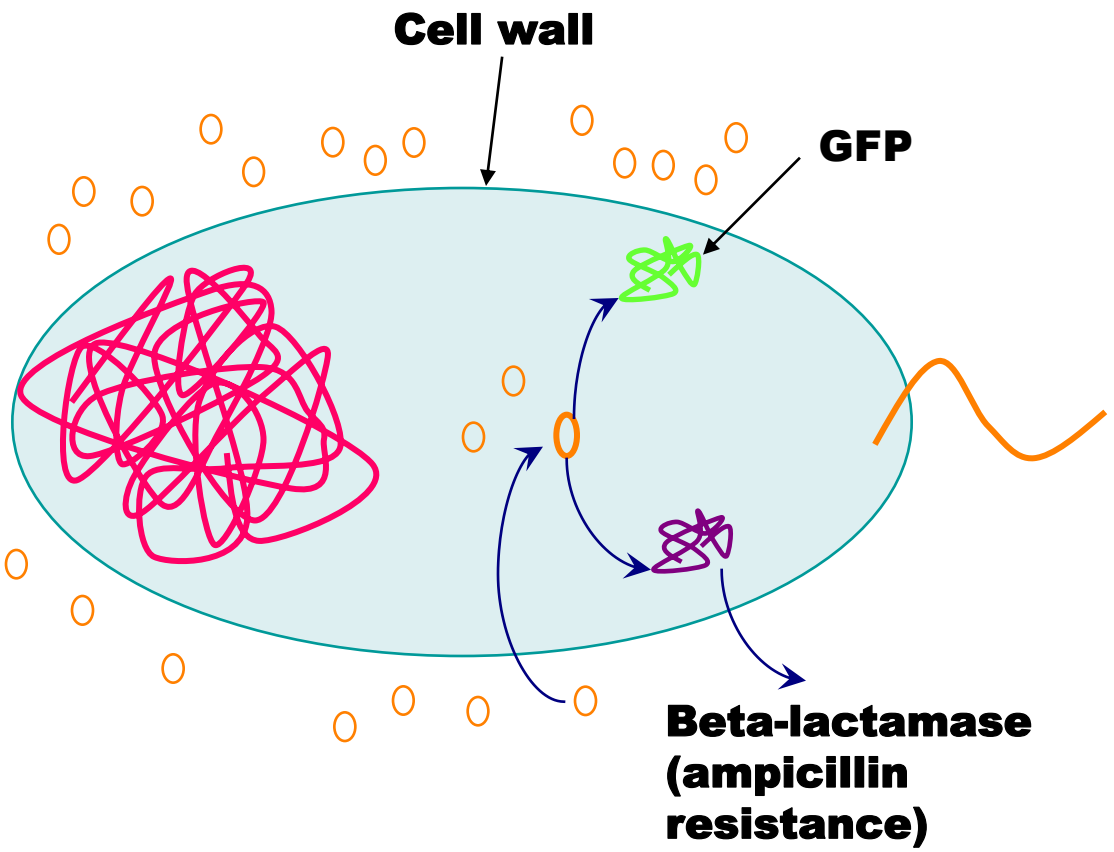


Why Perform Each Transformation Step?

2. Incubate on ice
slows fluid cell membrane

3. Heat-shock
Increases permeability of membranes

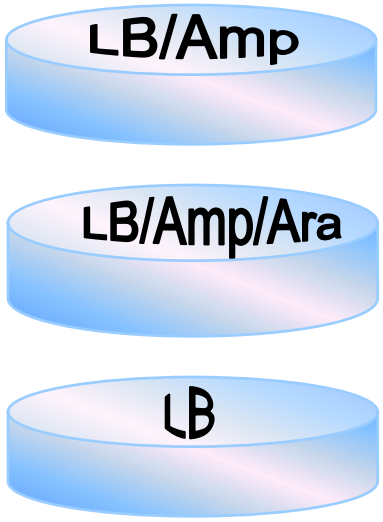
4. Nutrient broth incubation
Allows beta-lactamase expression



What is Nutrient Broth?

- **Luria-Bertani (LB) broth**
- **Medium that contains nutrients for bacterial growth and gene expression**
  - Carbohydrates
  - Amino acids
  - Nucleotides
  - Salts
  - Vitamins

Grow?
Glow?



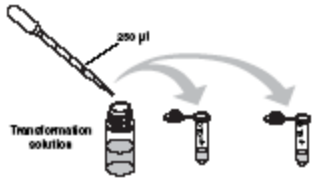
- **Follow protocol**
- **On which plates will colonies grow?**
- **Which colonies will glow?**

Laboratory Quick Guide

Transformation Kit—Quick Guide

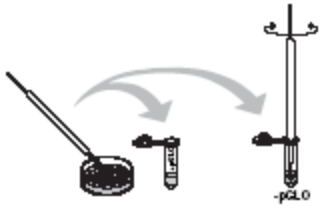
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.

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



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 plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the 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.

Volume Measurement