
Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

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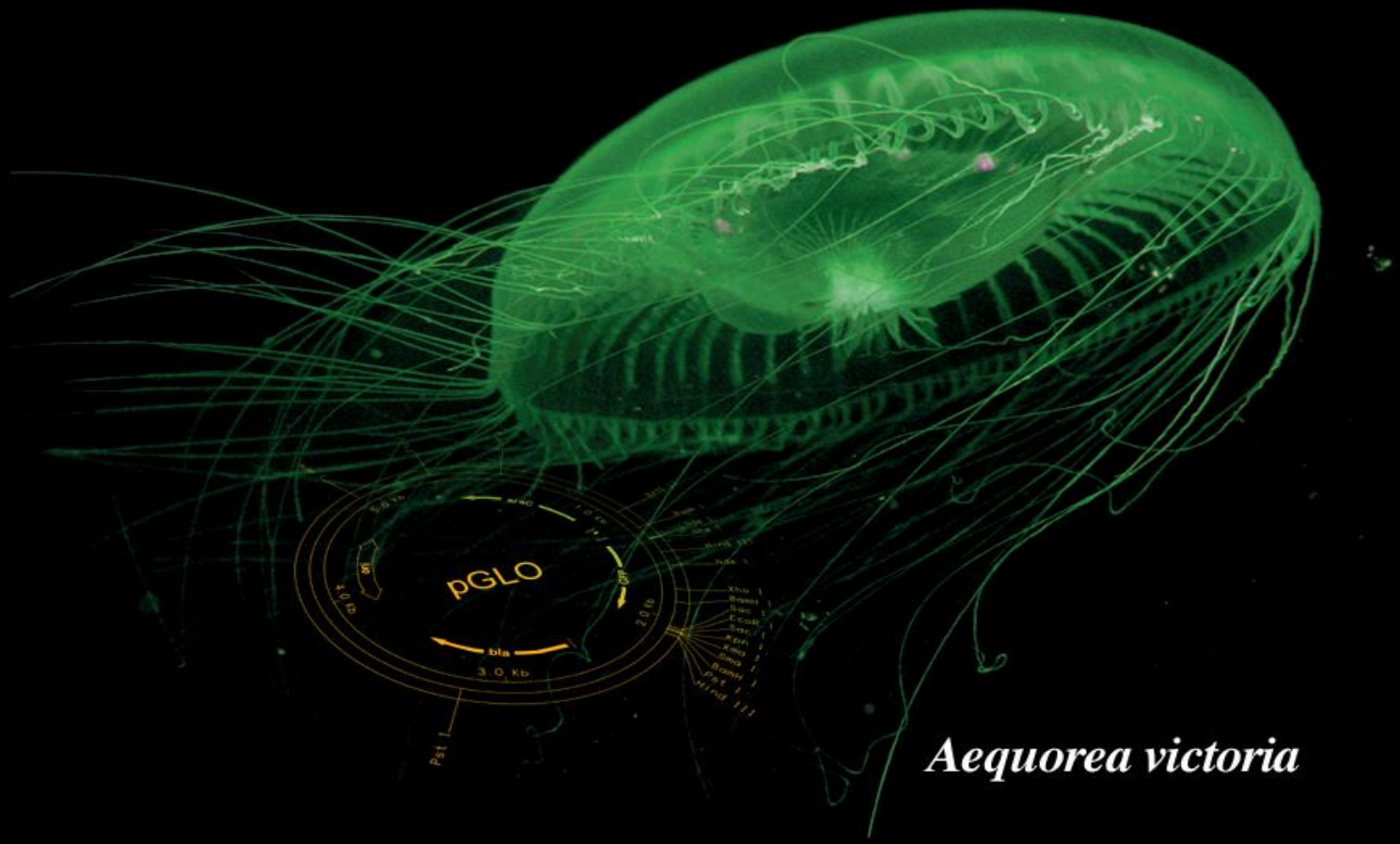
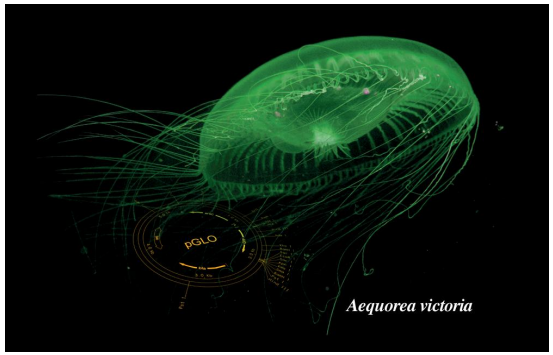
pGLO™ Transformation and Purification of Green Fluorescent Protein (GFP)



Instructors



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Why Teach

Bacterial Transformation and Protein Purification?

- Powerful teaching tool
- Laboratory extensions
- Real-world connections
- Link to careers and industry
- Standards based

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Scientific Inquiry

- Genetic engineering of organisms
- Use of experimental controls
- Interpretation of experimental results
- Calculate transformation efficiency

Chemistry of Life

- DNA structure, function, and chemistry
- Chemical properties of biological molecules
- Effects of temperature and pH on biochemical reactions

Genetics

- DNA > RNA > protein > trait
- Bacterial transformation
- The *lac* operon
- Creating genetically engineered organisms (GMOs)
- Structure and function of genes
- Gene regulation and transcription factors

Evolution

- Antibiotic selection and resistance genes
- Selection mechanisms
- Adaptation to environment
- Bacterial conjugation and gene transmission

Cell and Molecular Biology

- Prokaryotic cell structure and cell division
- Selective growth media
- Bacterial metabolism

Environmental and Health Science

- Genetically modified organisms (GMOs)
- GMOs in research, medicine, nutrition, and bioremediation
- Global challenges of GMOs
- Microbiology

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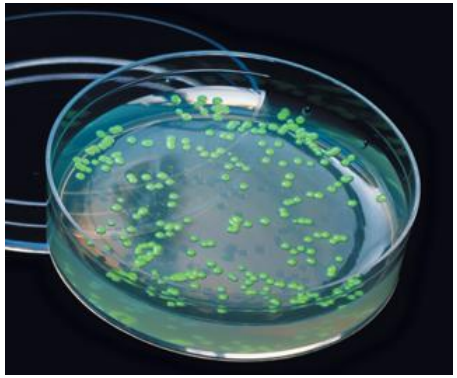
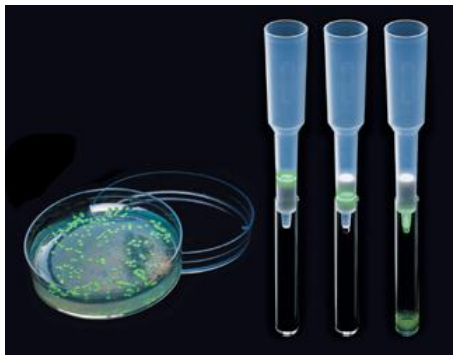
pGLO™ Bacterial Transformation Kit



Bio-Rad pGLO Kit Advantages

- **Standards-based**
- **Comprehensive curricula for inquiry-based investigations**
- **Compatible with 50 minute class periods**
- **Serves entire class of 32 students (up to 4 students per group)**
- **Cost-effective**
- **Success in student's hands**
- **Safe**
- **Striking results!**

Green Fluorescent Protein (GFP) Chromatography Kit



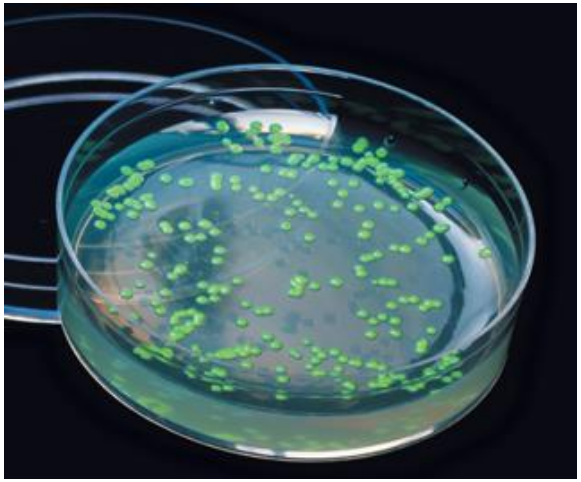
GFP Purification Kit Advantages

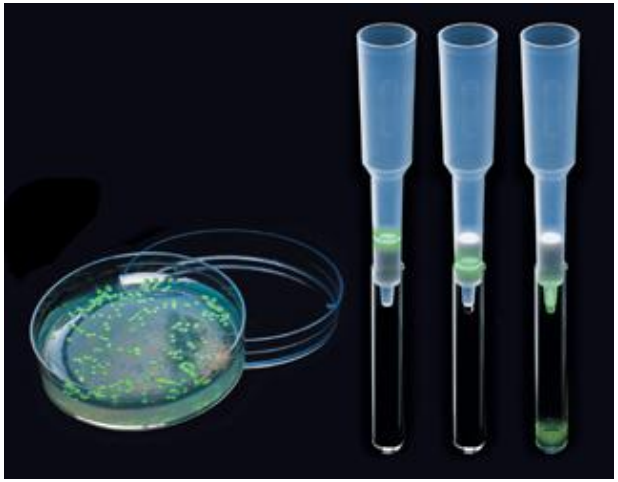
- **Cloning in action**
- **Links to biomanufacturing**
- **Biopharmaceutical development**
- **Amazing visual results**

EasyPack Column GelTec Column

Workshop Time Line

- **Introduction**
- **Transform bacteria with pGLO plasmid**
- **Purify GFP using column chromatography**



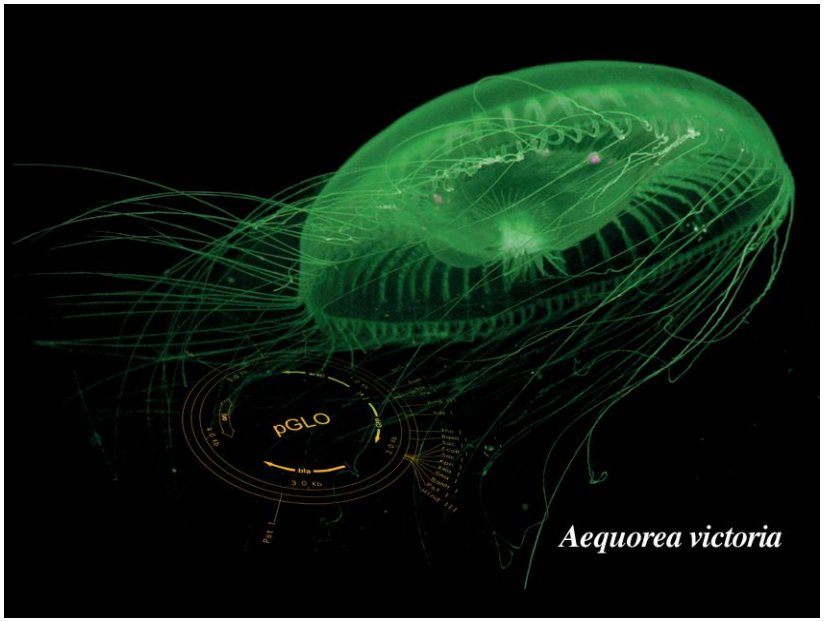


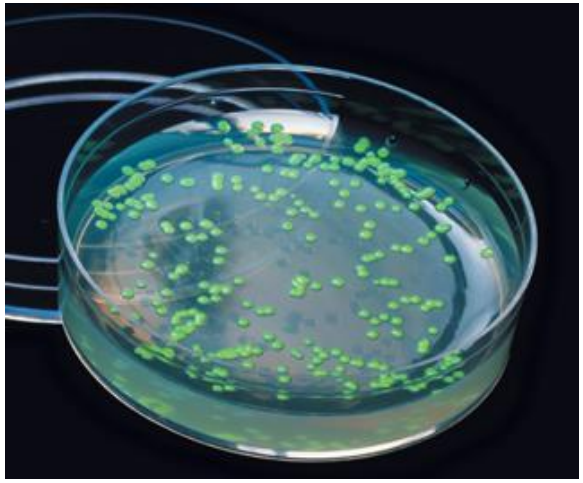
Central Framework of Molecular Biology

DNA → RNA → Protein → Trait

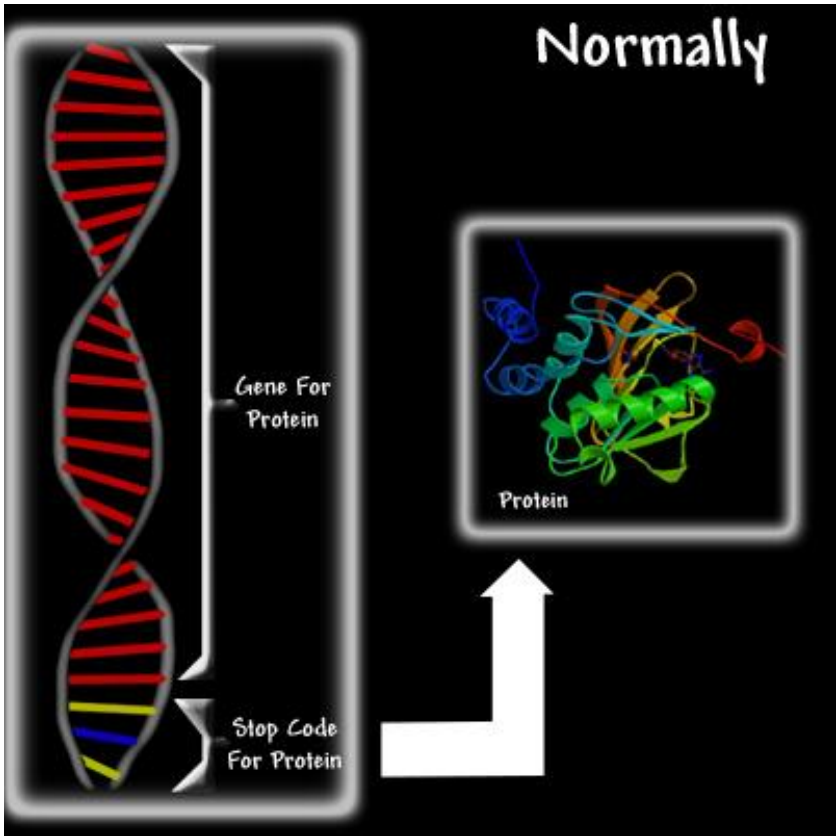
Links to Real-world

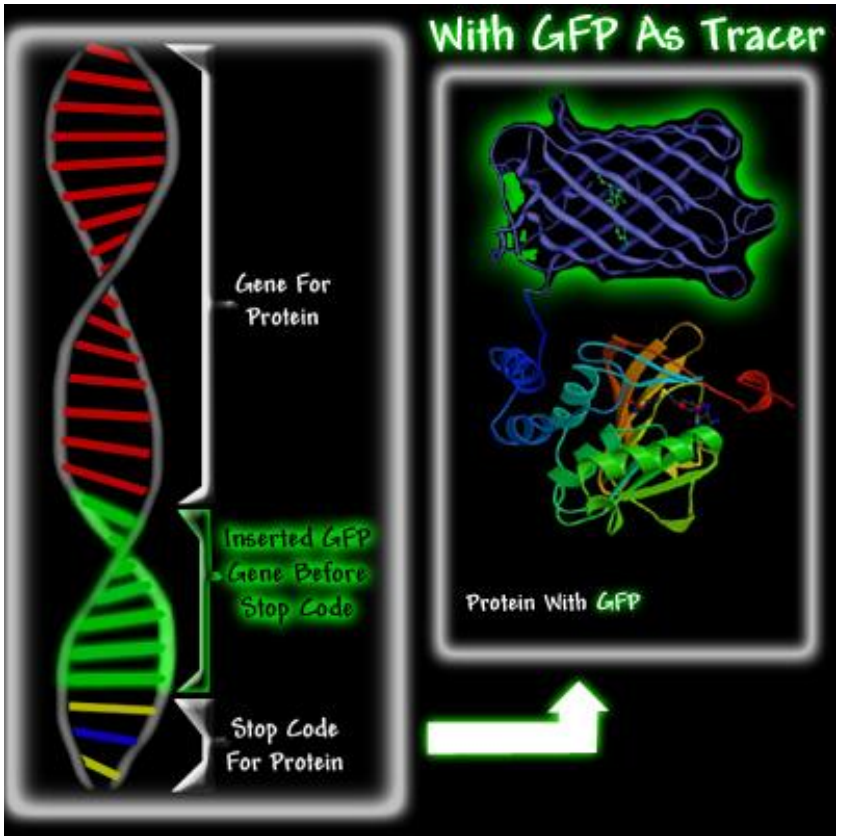
- **GFP is a visual marker**
- **Study of biological processes**
  (example: synthesis of proteins)
- **Localization and regulation of gene expression**
- **Cell movement**
- **Cell fate during development**
- **Formation of different organs**
- **Screenable marker to identify transgenic organisms**


pGLO
bla
3.0 Kb
Aequorea victoria



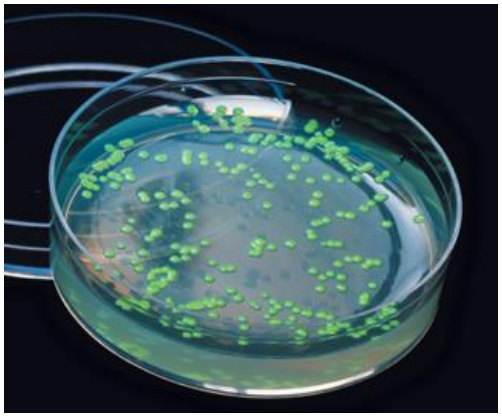
Using GFP as a biological tracer



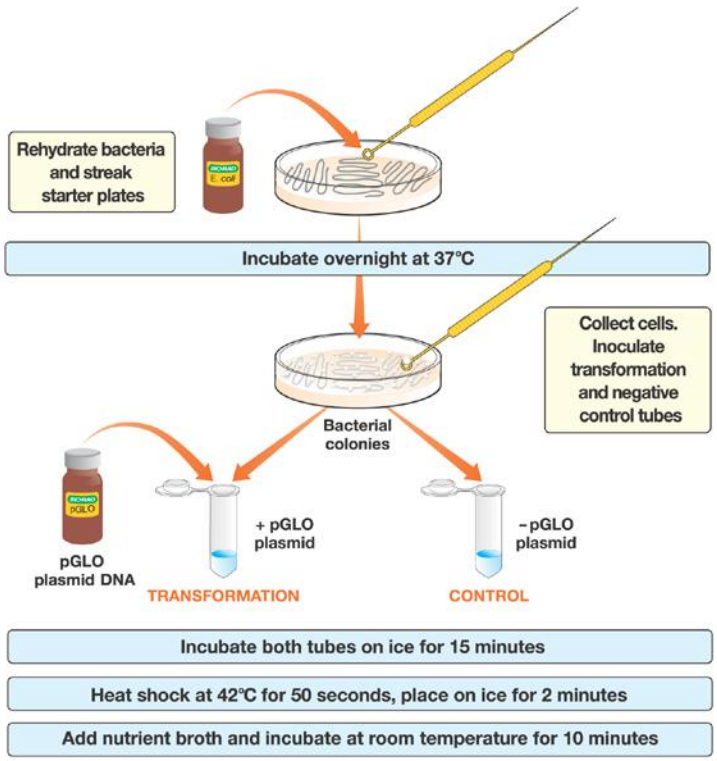


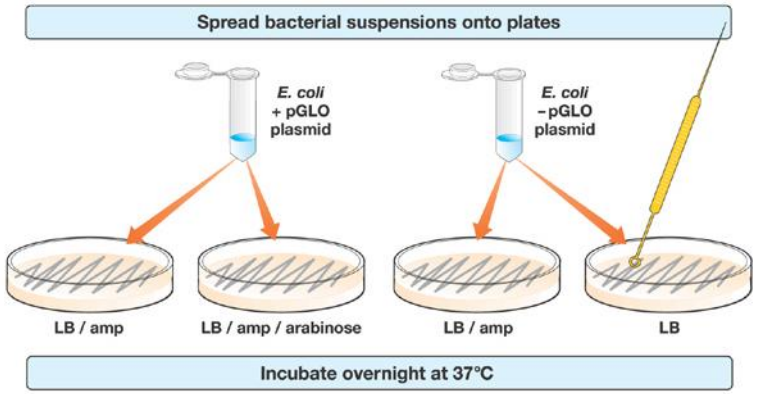
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/prasher.html
With permission from Marc Zimmer

pGLO Bacterial Transformation Kit

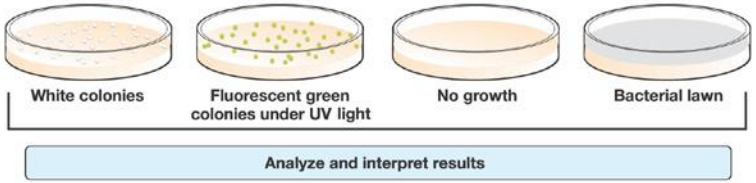


Transformation Procedure Overview





Day 1

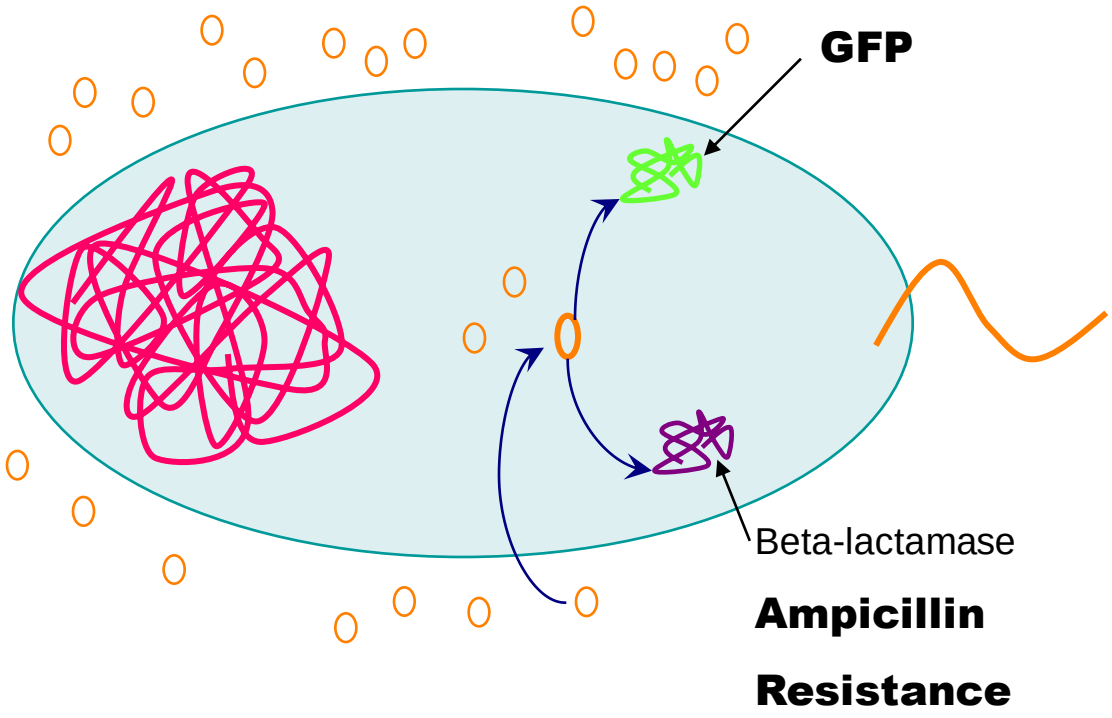


Day 2

Extension: GFP chromatography kit, pp. 22–23

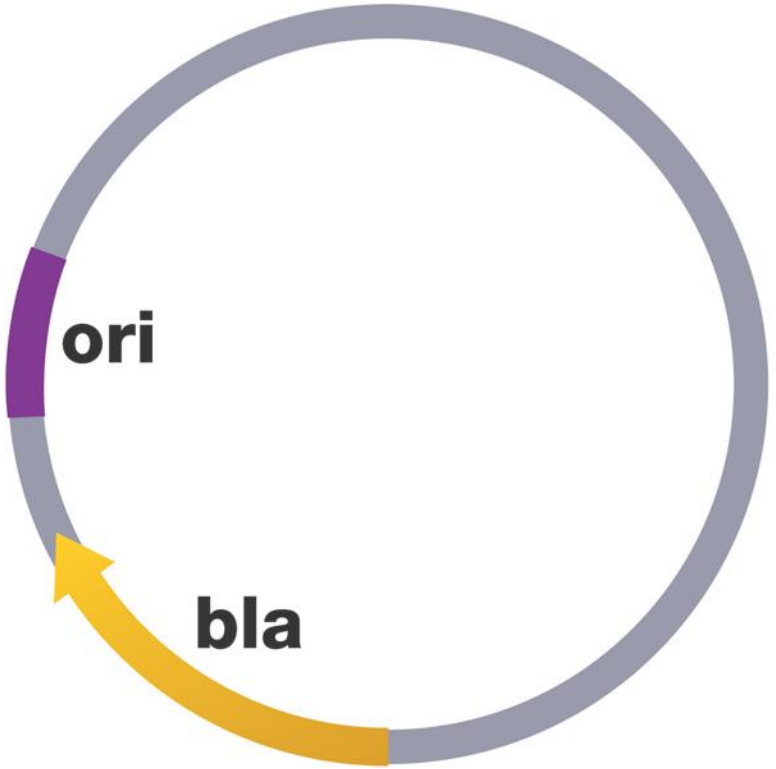
What is Transformation?

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

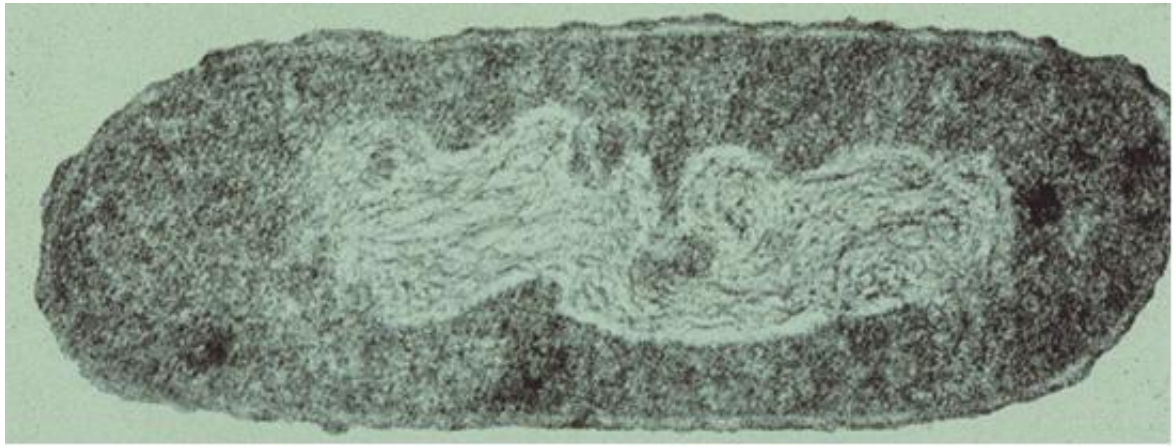


What is a plasmid?

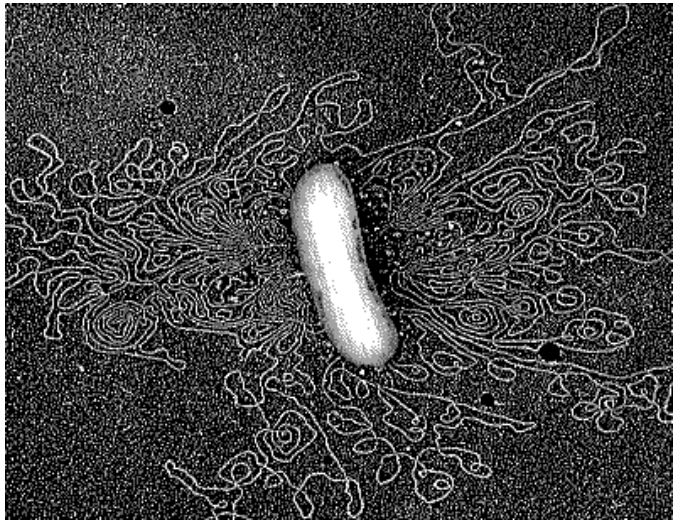
- A circular piece of autonomously replicating DNA
- Originally evolved by bacteria
- May express antibiotic resistance gene or be modified to express proteins of interest



Bacterial DNA



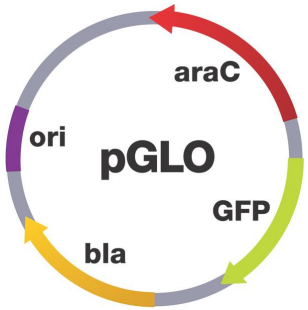
Bacterial cell

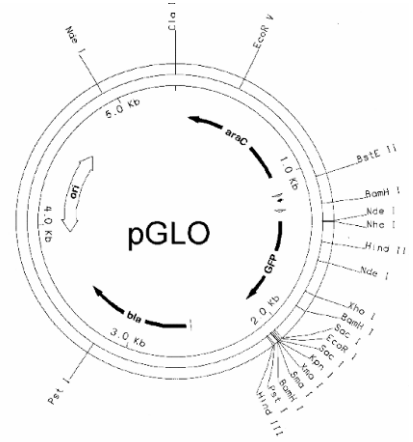


Genomic DNA

Plasmid DNA

The Many Faces of Plasmids



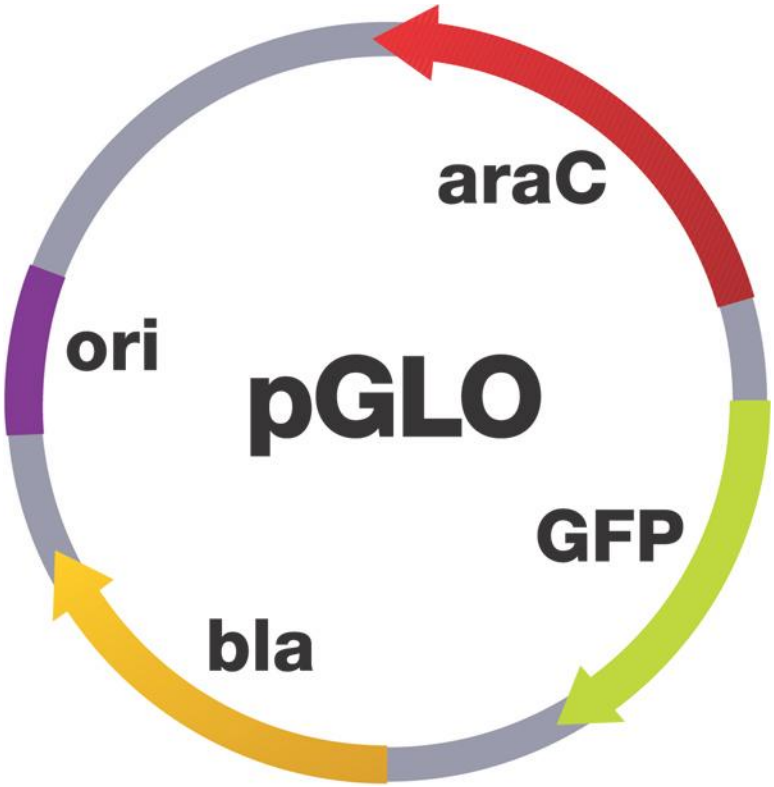


Graphic representation

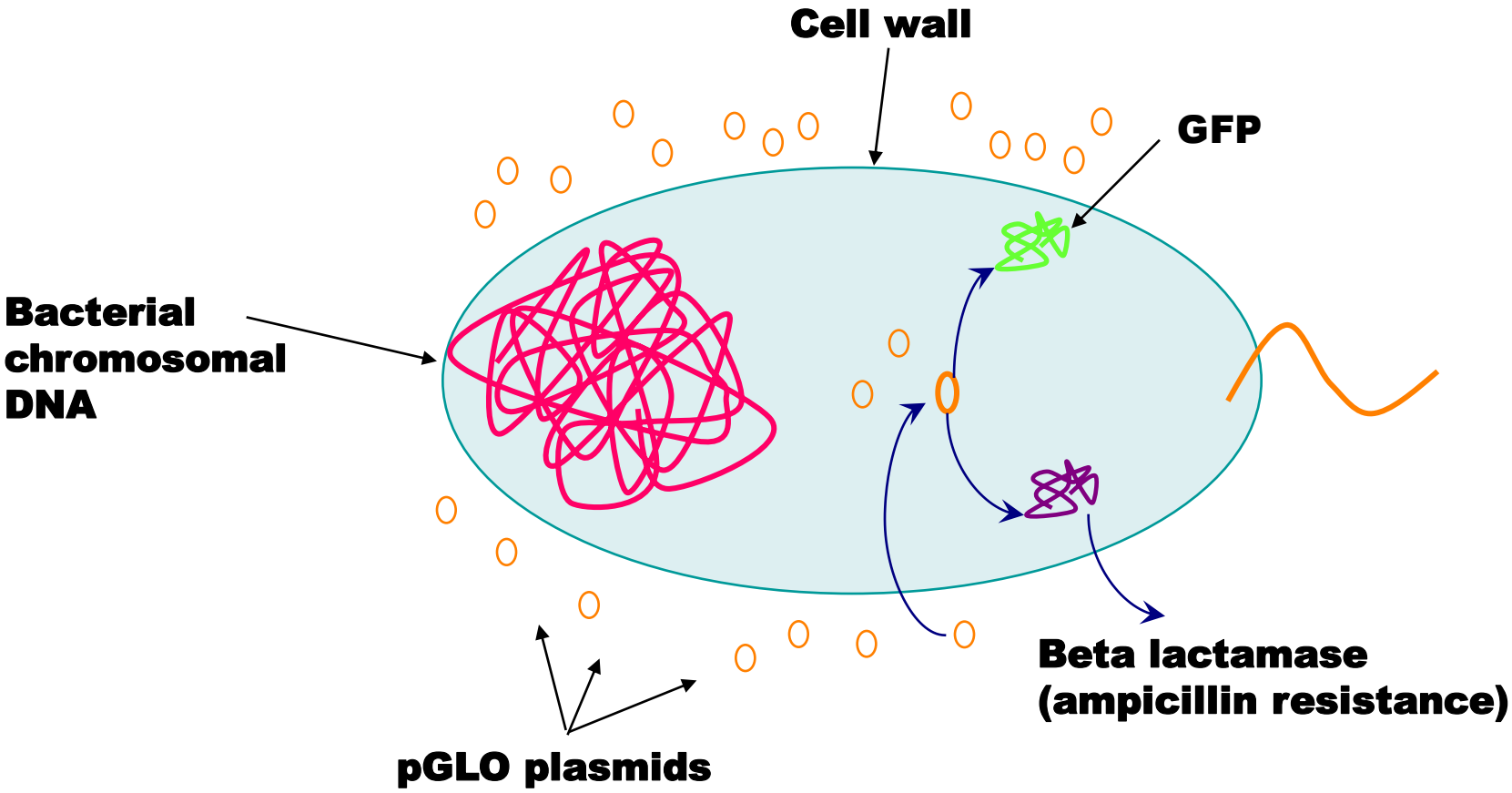
Scanning electron micrograph of supercoiled plasmid

Gene Expression

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

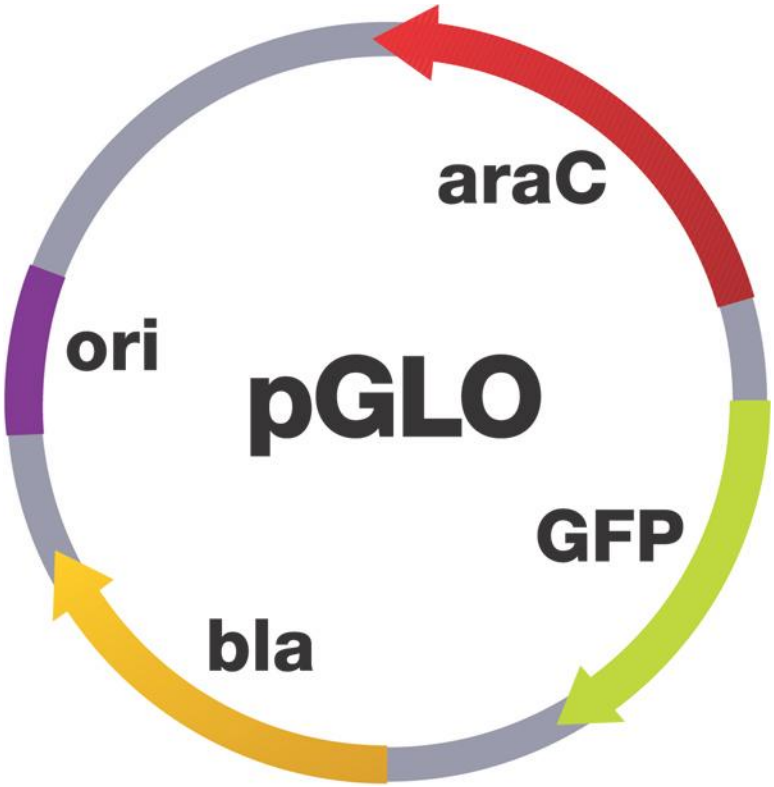


Bacterial Transformation

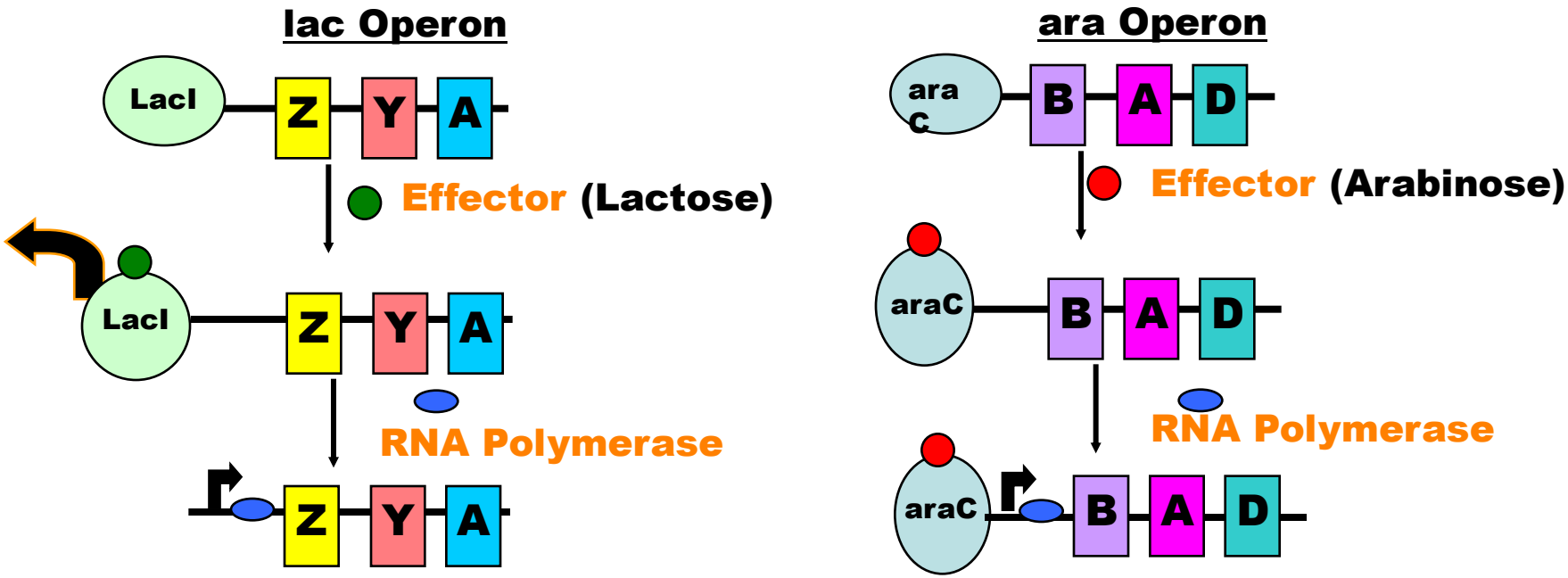


Transcriptional Regulation

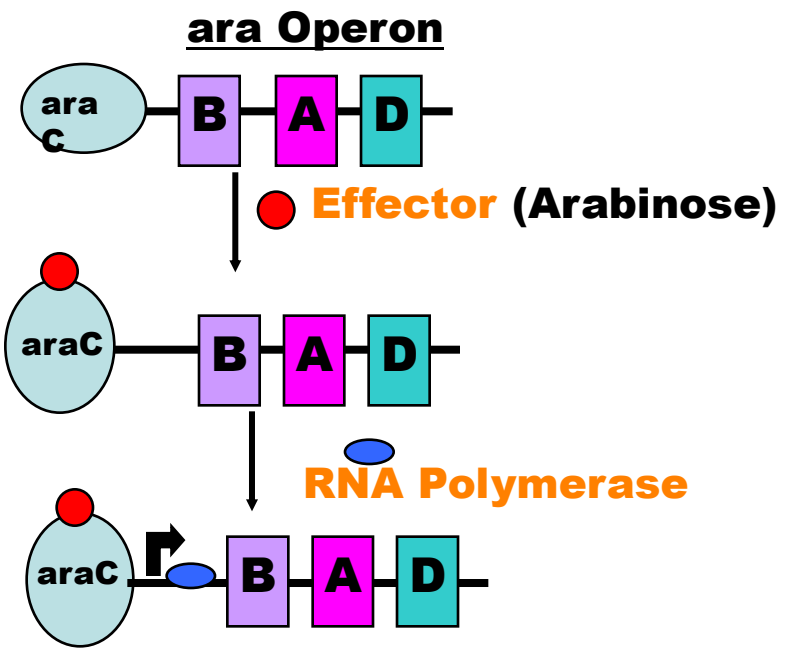
- **Lactose operon**
- **Arabinose operon**
- **pGLO plasmid**

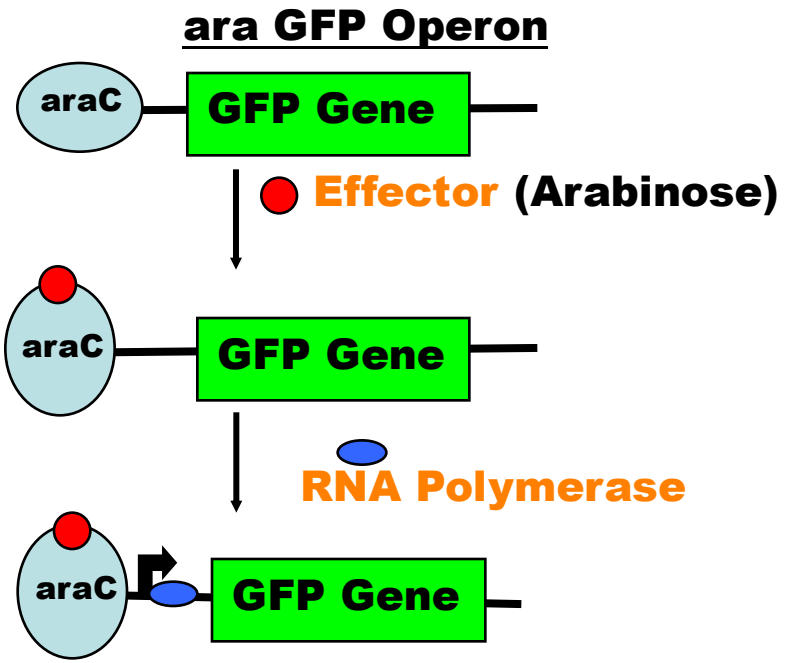


Transcriptional Regulation



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

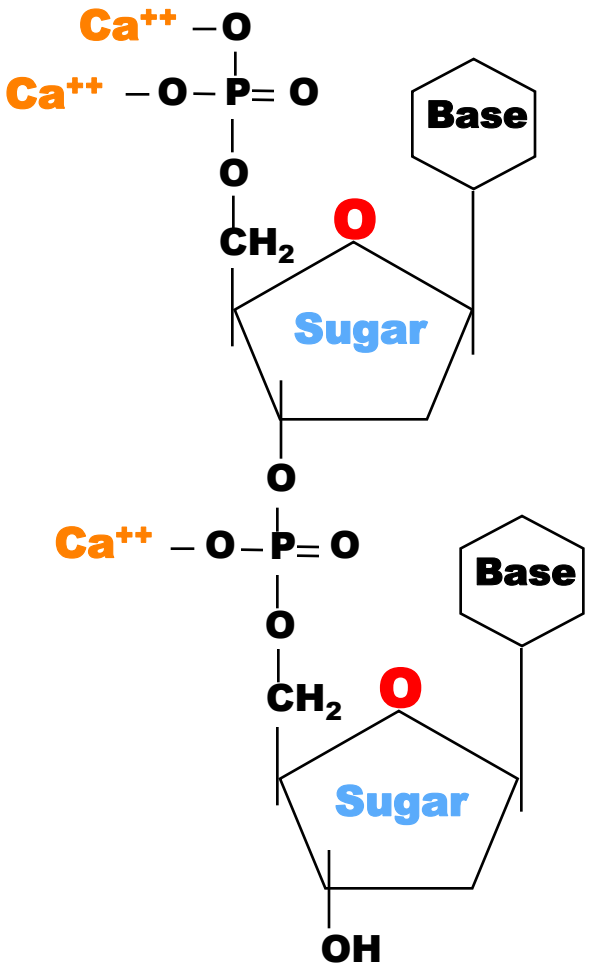
Transformation Procedure

- 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

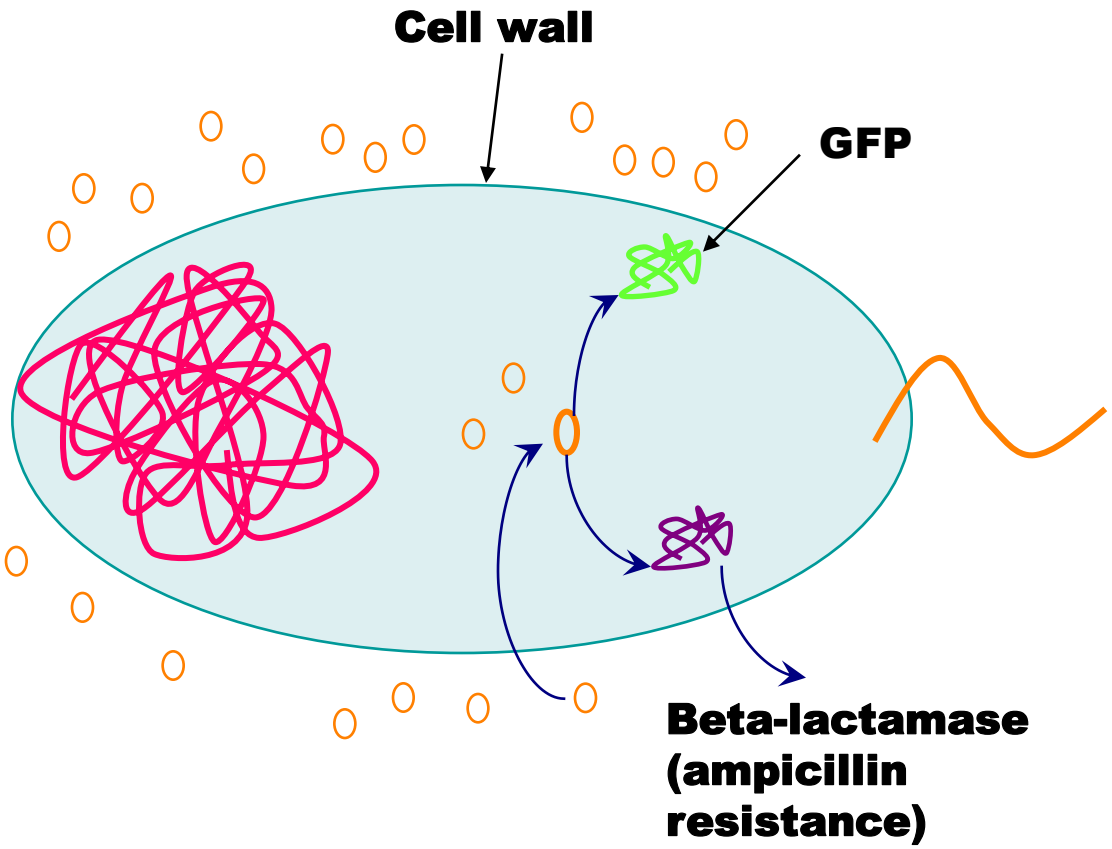


Why Perform Each Transformation Step?

2. Incubate on ice
slows fluid cell membrane

3. Heat-shock
Increases permeability of membranes

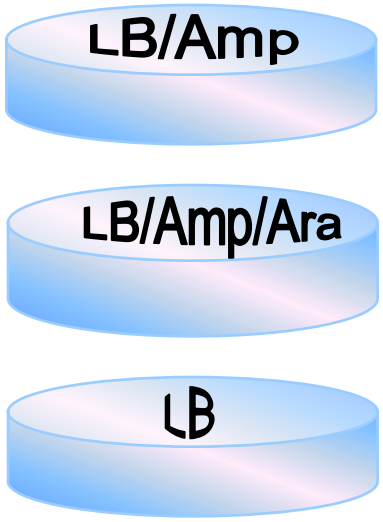
4. Nutrient broth incubation
Allows beta-lactamase expression



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?**

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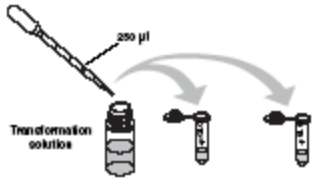
Laboratory Quick Guide

Transformation Kit—Quick Guide

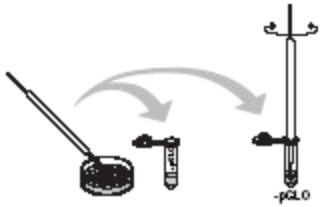
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.

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.

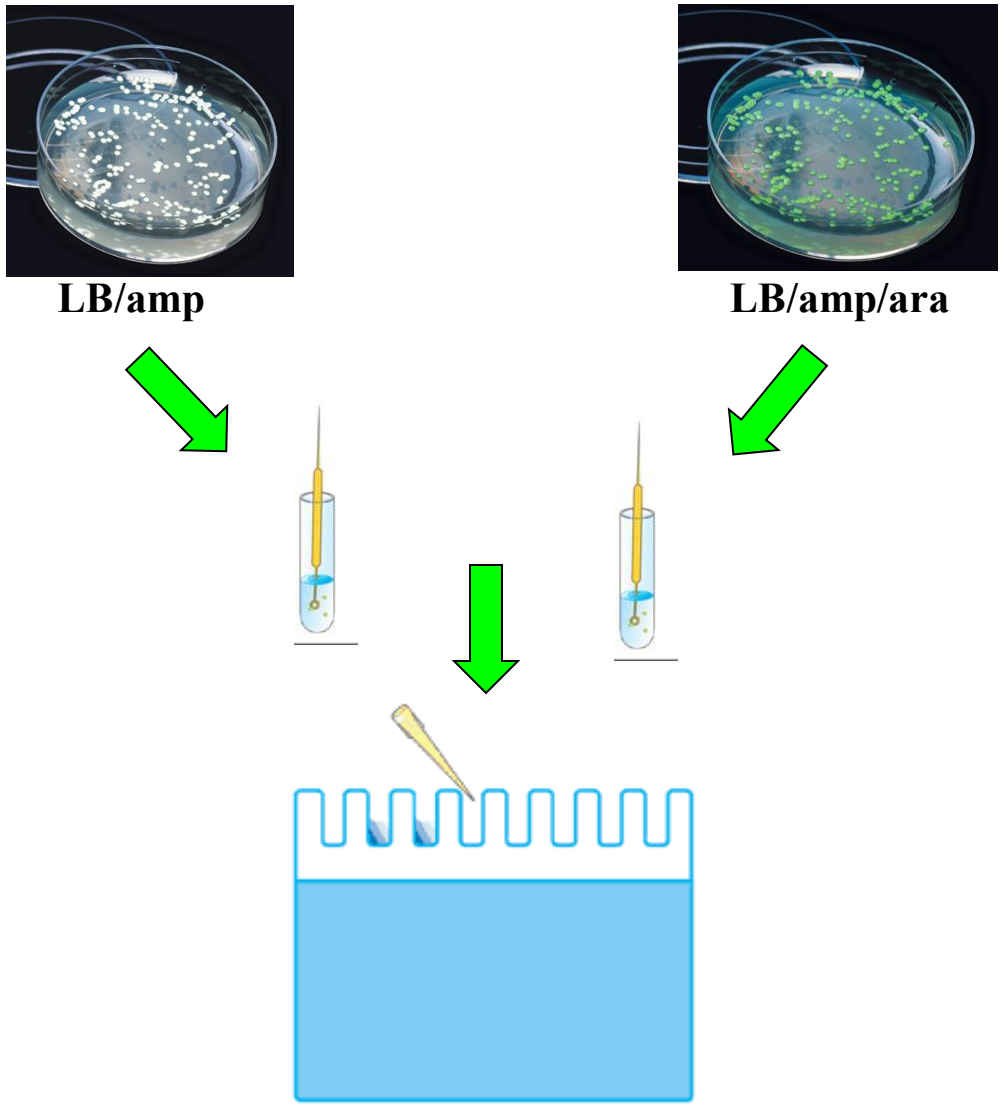


5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. 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.

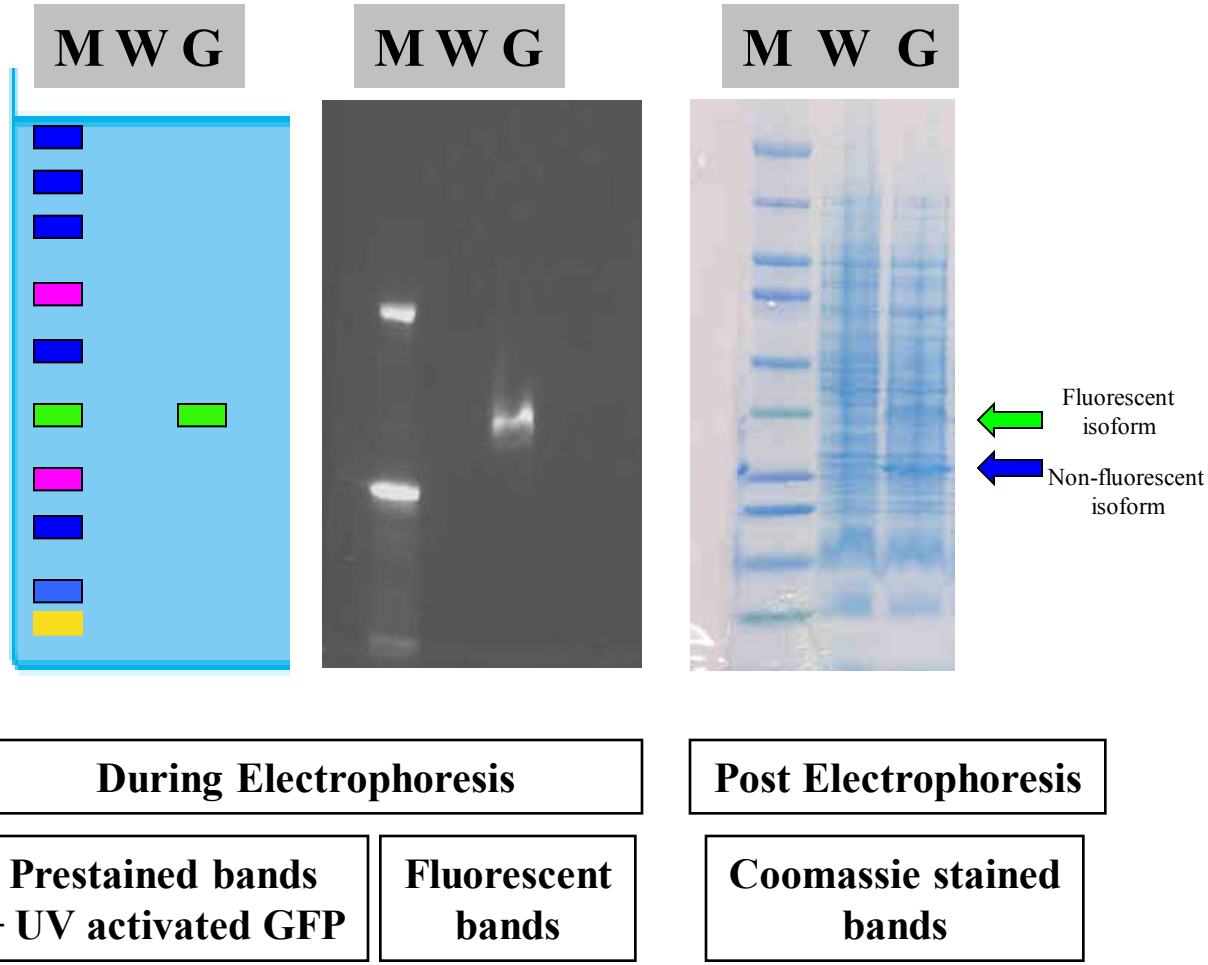
GFP Electrophoresis Extension

- **SDS PAGE sample preps are made from white and green colonies**
- **Bacterial lysates are prepared in Laemmli buffer**
- **Samples are loaded onto polyacrylamide gels**

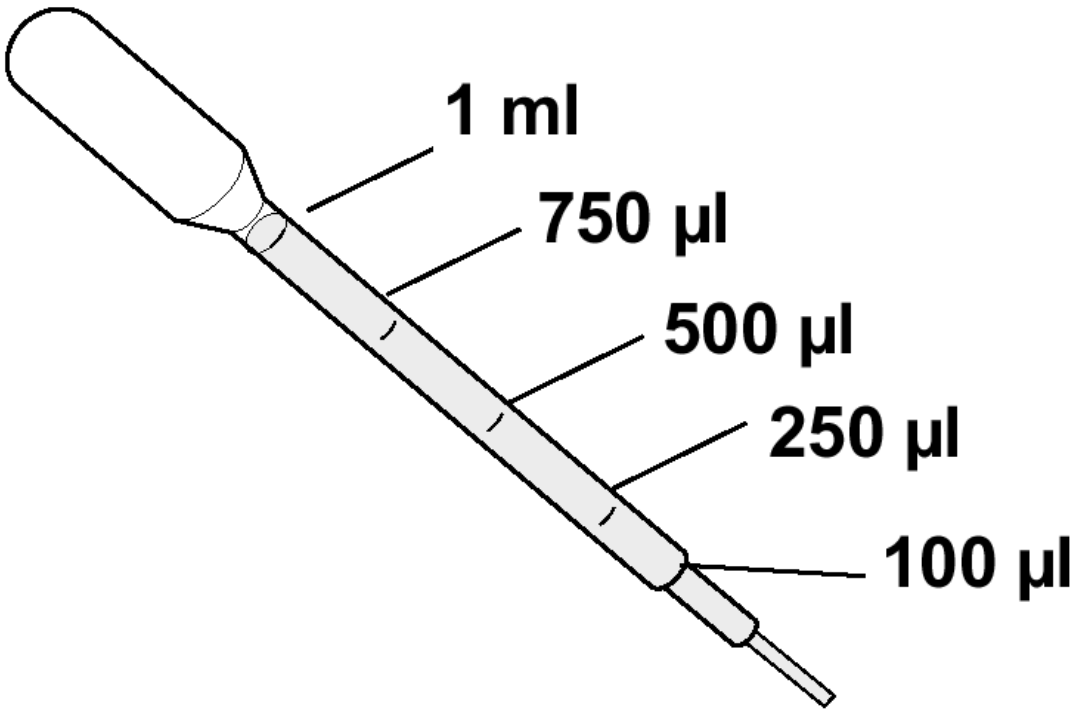


GFP Visualization- During & Post Electrophoresis

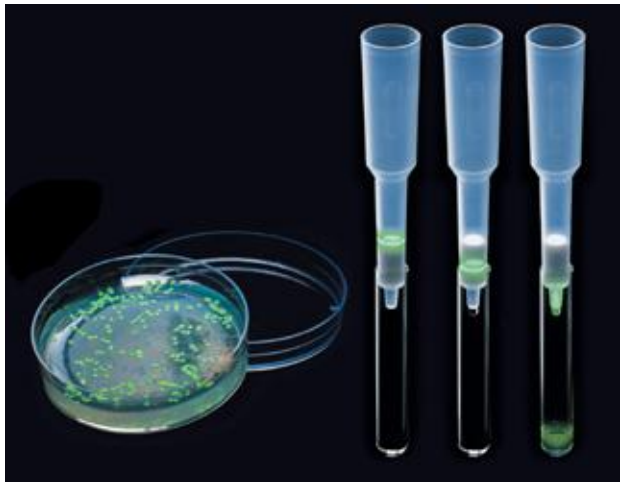
- **Samples are electrophoresed**
- **Fluorescent GFP can be visualized during electrophoresis**
- **Coomassie stained gels allow for visualization of induced GFP proteins**



Volume Measurement



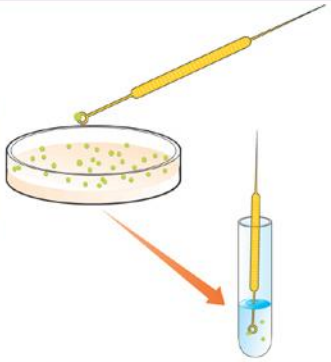
GFP Chromatography Kit



GFP Purification Procedures Overview

Start with bacterial colonies transformed with pGLO plasmid DNA

Pick a single fluorescent green colony from the agar plate using a sterile inoculation loop

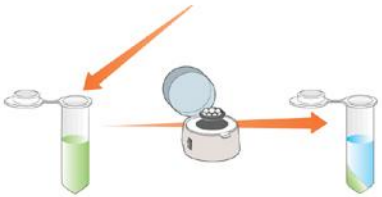


Inoculate into nutrient broth containing ampicillin and arabinose

Grow overnight at 32°C or 2 days at room temperature with shaking

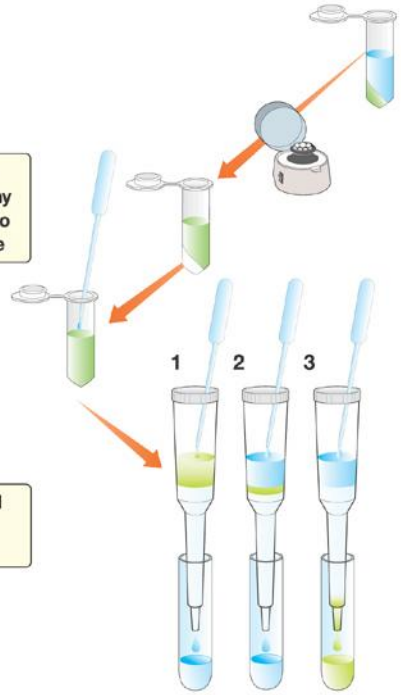
Day 1

Transfer cell culture to micro test tube, then centrifuge and pellet cells



Day 2

Add high-salt chromatography binding buffer to bacterial lysate



Resuspend cells, add lysozyme, and freeze to rupture cell membranes; then centrifuge bacterial lysate to pellet membranes and debris

1. GFP binds to chromatography matrix in high-salt buffer

2. Add medium-salt buffer to wash bacterial proteins from column

3. Add low-salt buffer to elute GFP

Load bacterial lysate onto columns

Collect three fractions

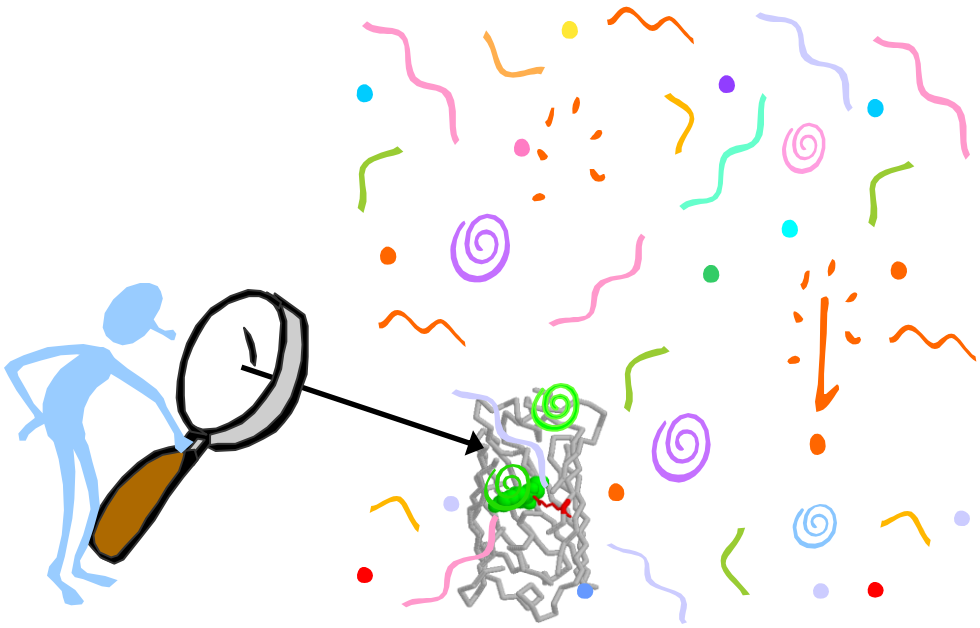
Separate GFP from bacterial proteins

Extension: Use protein gel electrophoresis to conduct quantitative and qualitative analysis of fractions

Day 3

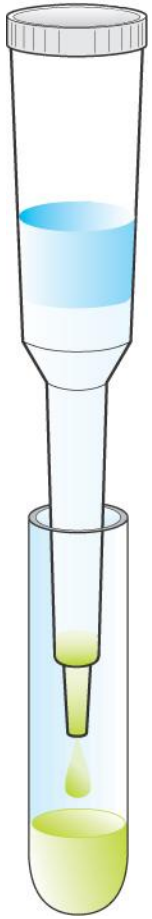
Why Use Chromatography?

- **To purify a single recombinant protein of interest from over 4,000 naturally occurring *E. coli* gene products.**



Column Chromatography

- **Chromatography used for protein purification**
  - Size exclusion
  - Ion exchange
  - Hydrophobic interaction



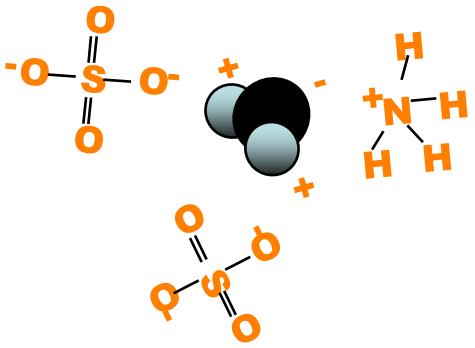
Hydrophobic Interaction Chromatography: (HIC)

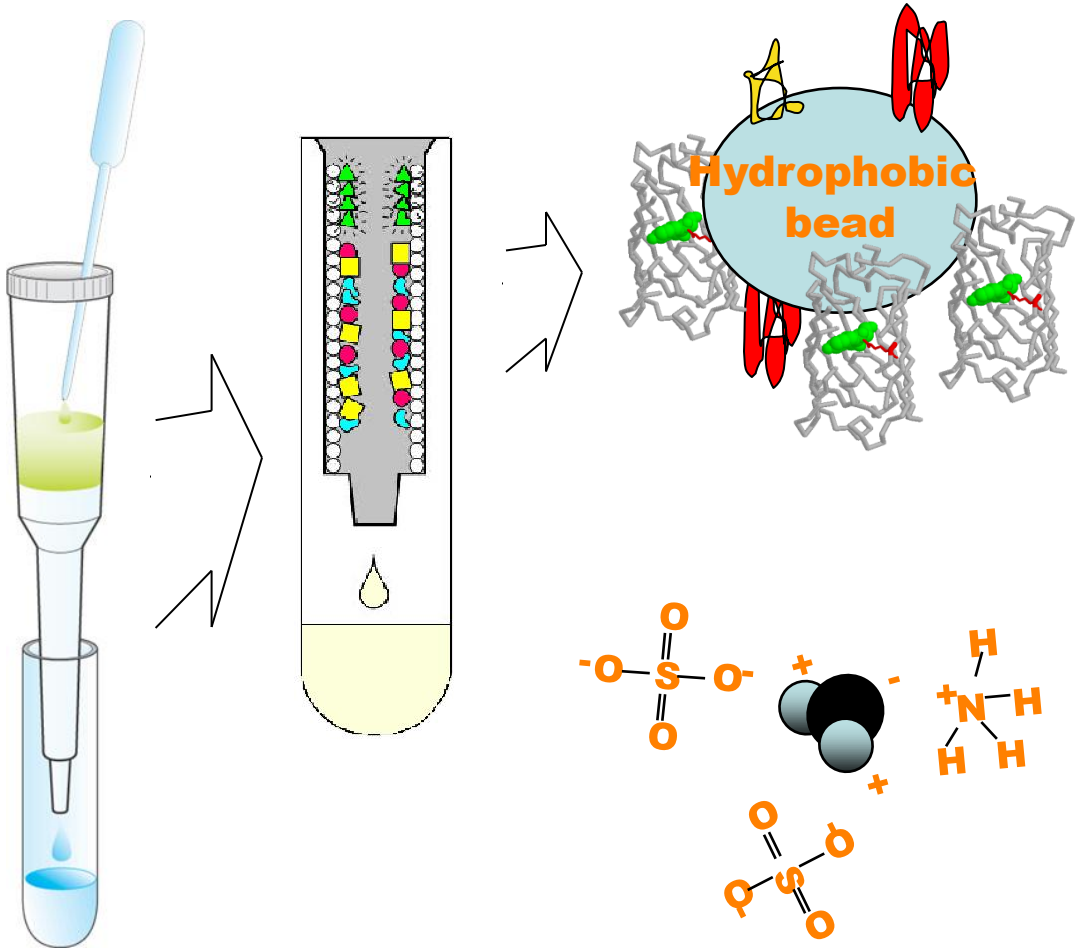
Steps 1–3

1. **Add** bacterial lysate to column matrix in **high salt buffer**
2. **Wash** less hydrophobic proteins from column in **low salt buffer**
3. **Elute** GFP from column with **no salt buffer**

Step 1: Hydrophobic Interaction Chromatography

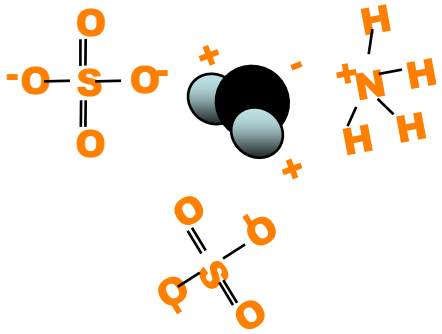
- **Add bacterial lysate to column matrix in high salt buffer**
  - Hydrophobic proteins interact with column
  - Salt ions interact with the less hydrophobic proteins and H_2O

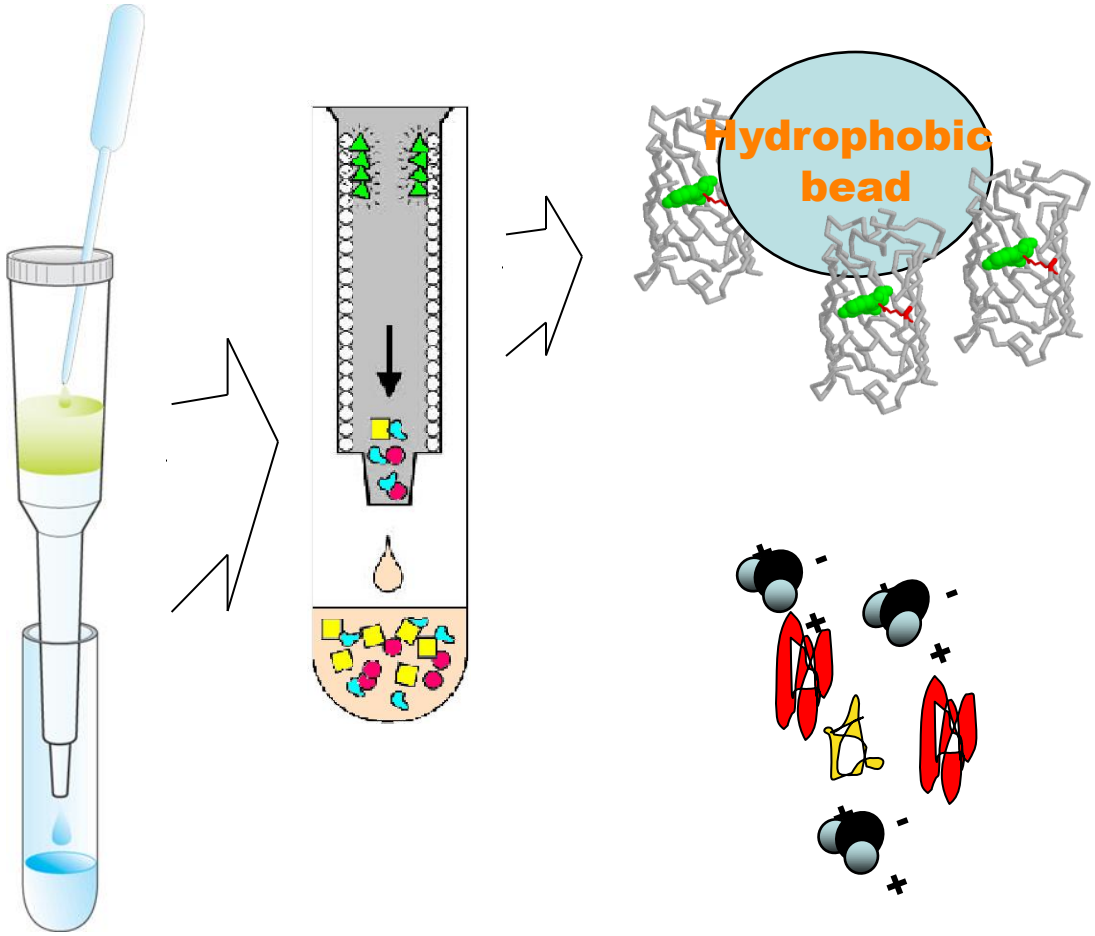




Step 2: Hydrophobic Interaction Chromatography

- **Wash less hydrophobic from column with low salt buffer**
  - Less hydrophobic E. coli proteins fall from column
  - GFP remains bound to the column



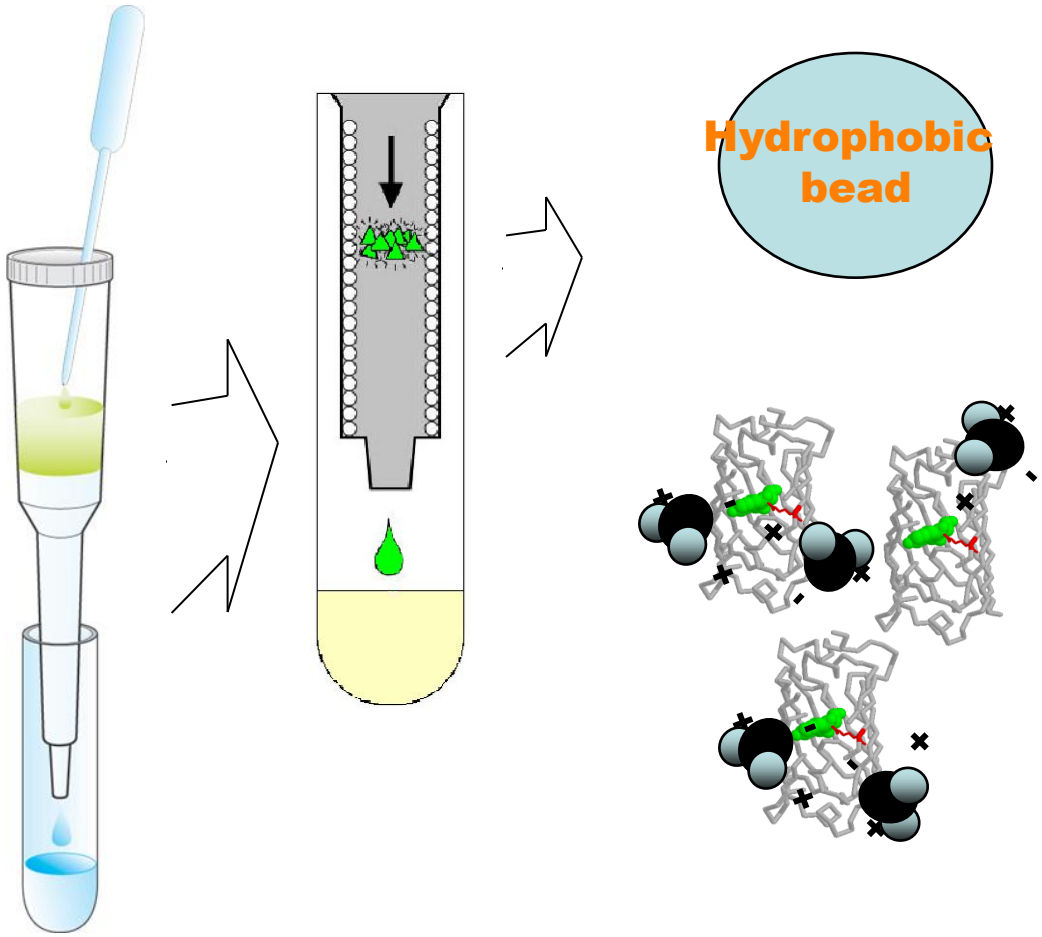


Step 3: Hydrophobic Interaction Chromatography

- **Elute GFP from column by adding a no-salt buffer**

GFP

- Released from column matrix
- Flows through the column

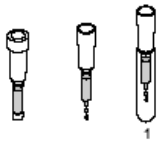


Laboratory Quick Guide

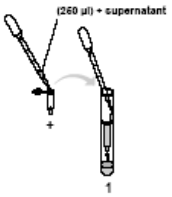
Lesson 5 Purification Phase 3

Protein Chromatography

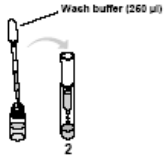
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step below.



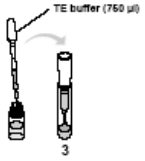
2. Using a new pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping transfer the column to collection tube 2.



3. Using the rinsed pipette, add 250 µl of wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



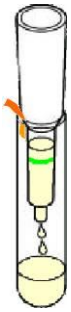
4. Using the rinsed pipette, add 750 µl of TE Buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.



5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm or Saran Wrap the tubes and place in the refrigerator until the next laboratory period.

Helpful Hints: Hydrophobic Interaction Chromatography

- **Add** a small piece of paper to collection tube where column seats to insure column flow



- **Rest** pipet tip on side of column to avoid column bed disturbance when adding solutions

- **Drain** until the meniscus is just above the matrix for best separation