Bring Biotechnology to your Classroom

- Demonstrate the central framework of molecular biology
- Transform bacteria into glowing colonies
- Select for transformed Cells by antibiotic resistance
- View operon control over pGLO protein production
- Introduction to Biomanufacturing
GFP (Green Fluorescent Protein)

- Naturally produced in Jellyfish—*Aequorea victoria*
- Discovered in 1960’s
- Source of bioluminescence when exposed to UV light

Structure of the GFP Protein

Img Src: http://wwwchem.leidenuniv.nl/metprot/armand/images/0291.jpg
Detecting Gene Activity

- PGLO gene is inserted into DNA near a gene of interest
- It acts as a reporter gene
  - linked to another gene & glowing protein appears if it is expressed
- Expressed in entire animals
Other Fluorescent Proteins
3 genes of interest:

- **GFP gene**
  - Codes for the GFP protein
- **Bla gene**
  - Codes for the enzyme β-lactamase
  - β-lactamase destroys the antibiotic ampicillin
- **araC regulator protein**
  - Controls expression of GFP
Overall Goal of Lab Experiment

- Use genetic engineering techniques to insert the GFP gene into *E. coli*
Selectable Marker: Trait that helps identify a transformed cell by conferring resistance to ampicillin

Ampicillin presence in LB Agar will kill wild type E.coli

BUT

Transformed E. coli survive in the presence of ampicillin in LB Agar
The arabinose operon in bacteria consists of the following:

*Usually, the araC protein binds to the arabinose operon operator → prevents transcription*

When arabinose is present, it binds to the araC protein → can’t bind to operator → RNA polymerase can continue
Scientists modified the arabinose operon in pGLO to express the GFP gene.

*araC protein binds to the operator* \(\rightarrow\) *prevents transcription*

When arabinose binds to araC it can no longer bind to operator \(\rightarrow\) GFP gene is transcribed and translated
Central Dogma of Molecular Biology
• Spread E. coli without plasmid (- DNA) on plain LB agar
  – Wild type E. coli can grow demonstrated
• Spread E. coli without plasmid (- DNA) on LB/amp
  – E. coli aren’t already resistant to ampicillin
Transformation Yields Product

- What does this lead to?
  - Ability to produce a protein we need but can't make
  - Cell acts as the factory for the product under the correct conditions
  - Increased cell number yields increased product
Transformation Procedure

- **Step 1** Prepare appropriate plates
- **Step 2** Suspend cells in CaCl\(_2\) solution
- **Step 3** Add pGLO plasmid to cells/put onto ice
- **Step 4** Heat Shock at 42oC /put onto ice
- **Step 5** Add nutrient broth to cells
- **Step 6** Streak cells on to appropriate plates
Transformation Time Line

- First step: Grow up colonies of E.coli
- Second step: Prepare Selective media
- Transform cells with pGLO plasmid
- Detect transformed cells
- 2-3 days required
- 1 day
- 45 minutes
- Results in 24 hours
- Supplies for up to 32 students
Cells containing pGLO plasmid are now resistant to ampicillin.

Cells containing pGLO plasmid will also glow green when arabinose.
Upstream Processing: Growing genetically transformed cells that produce a desired protein

Downstream Processing: Separation and purification of that product for human use