GFP Transformation Lab

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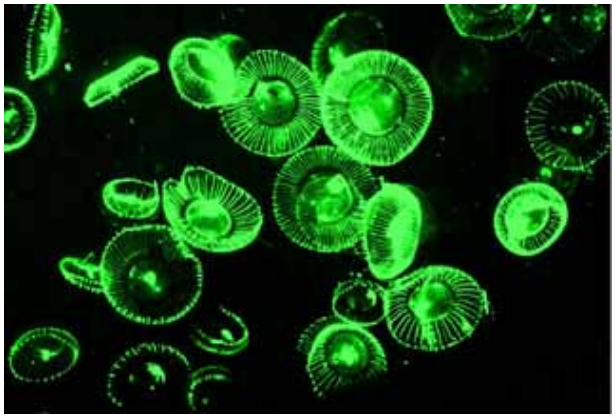

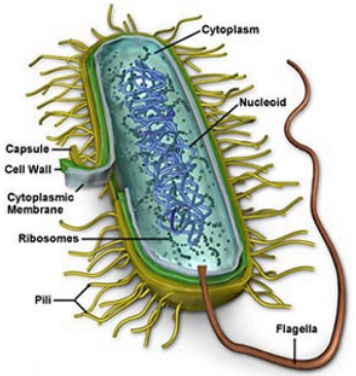

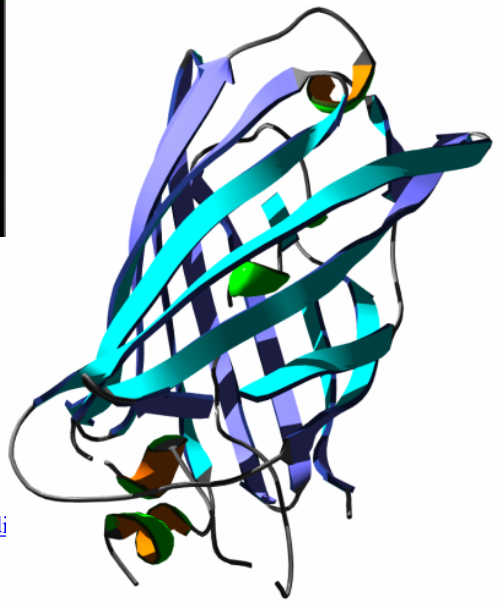

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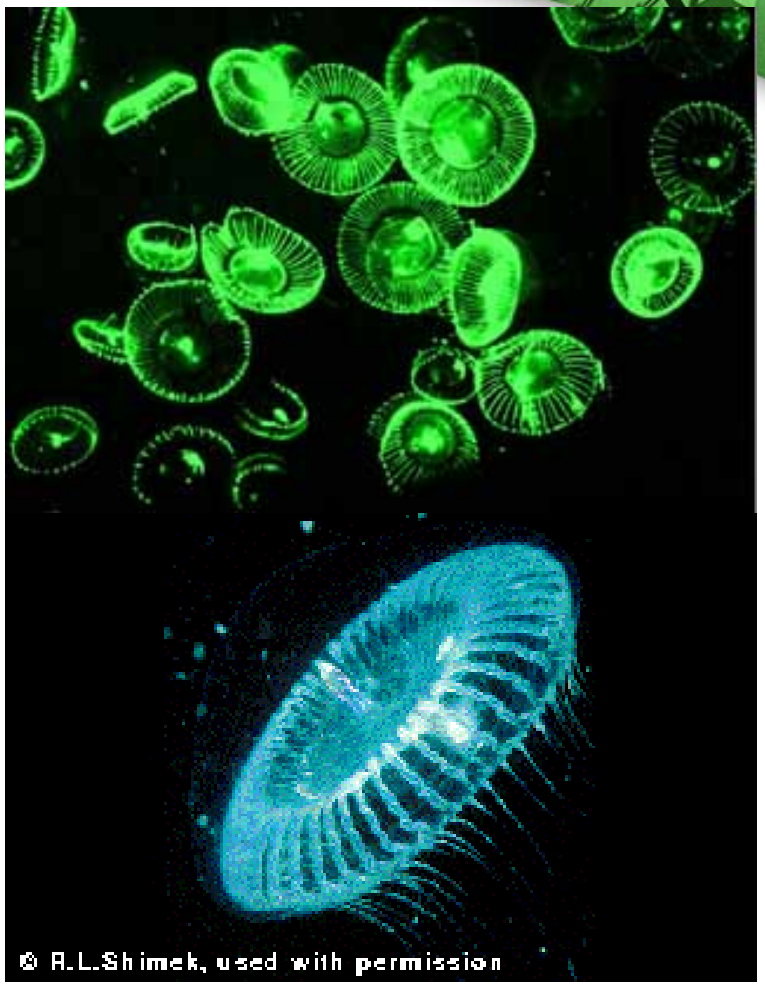
http://bioinfo.biotec.or.th/Picture/Cell%20Tutorial/image005.jpg,
http://www.plantsci.cam.ac.uk/Haseloff/SITEGRAPHICS/Jellyfish.jpeg

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

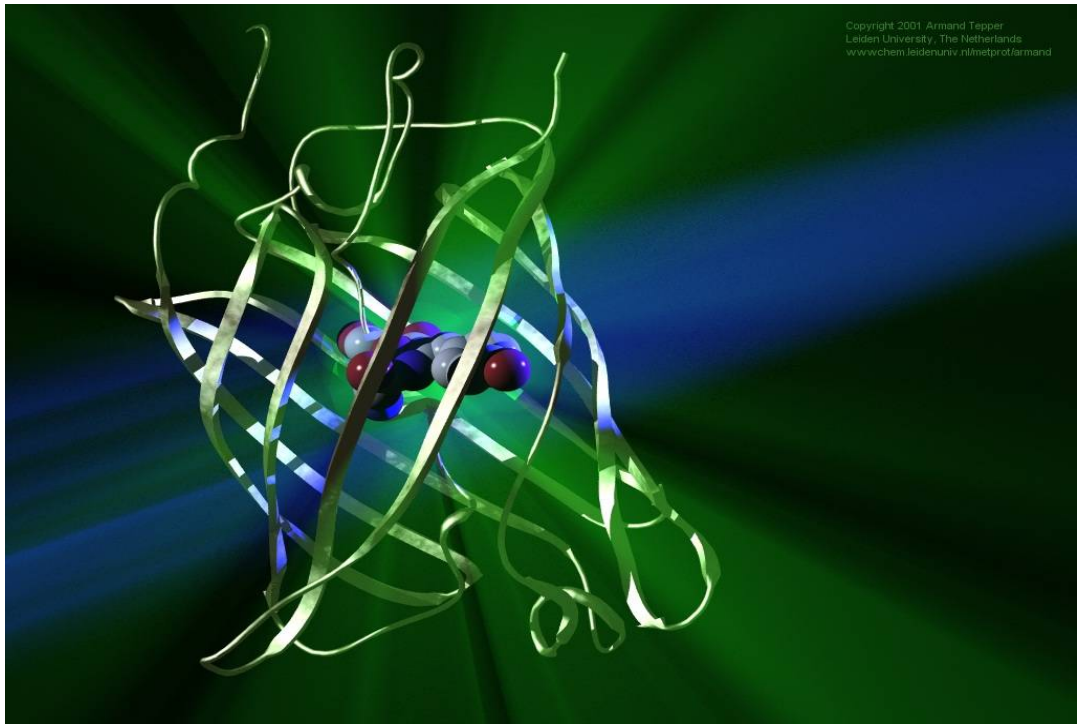
GFP (Green Fluorescent Protein)

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



Img Src: http://icbxs.ethz.ch/members/leu/jellyfish.gif , http://www.plantsci.cam.ac.uk/Haseloff/SITEGRAPHICS/Jellyfish.jpeg

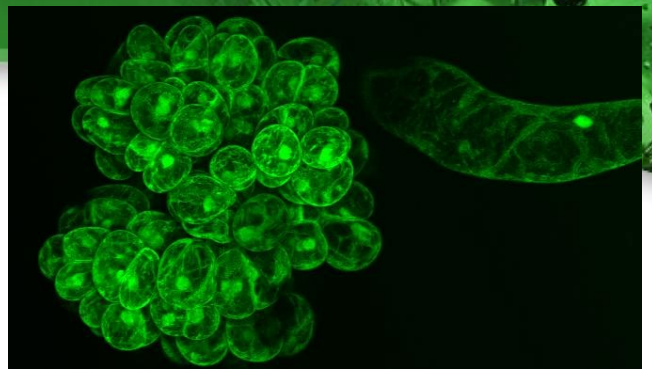
Structure of the GFP Protein



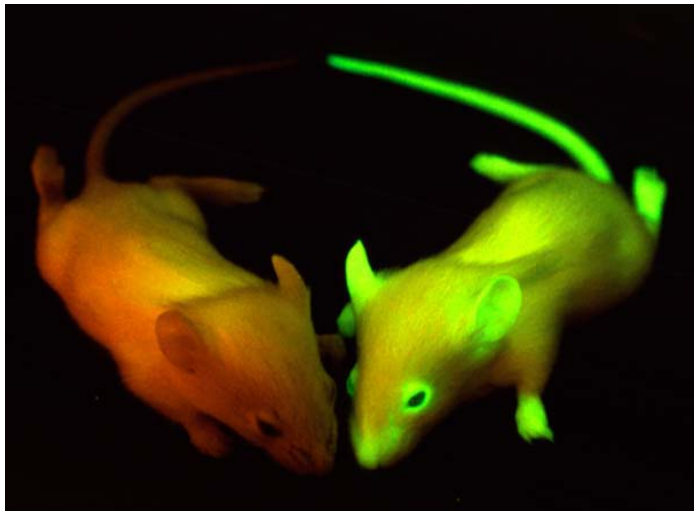
Img Src: http://wwwchem.leidenuniv.nl/metprot/armand/images/029l.jpg

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



Img Src: http://www.bio.umass.edu/microscopy/images/gfp.jpg

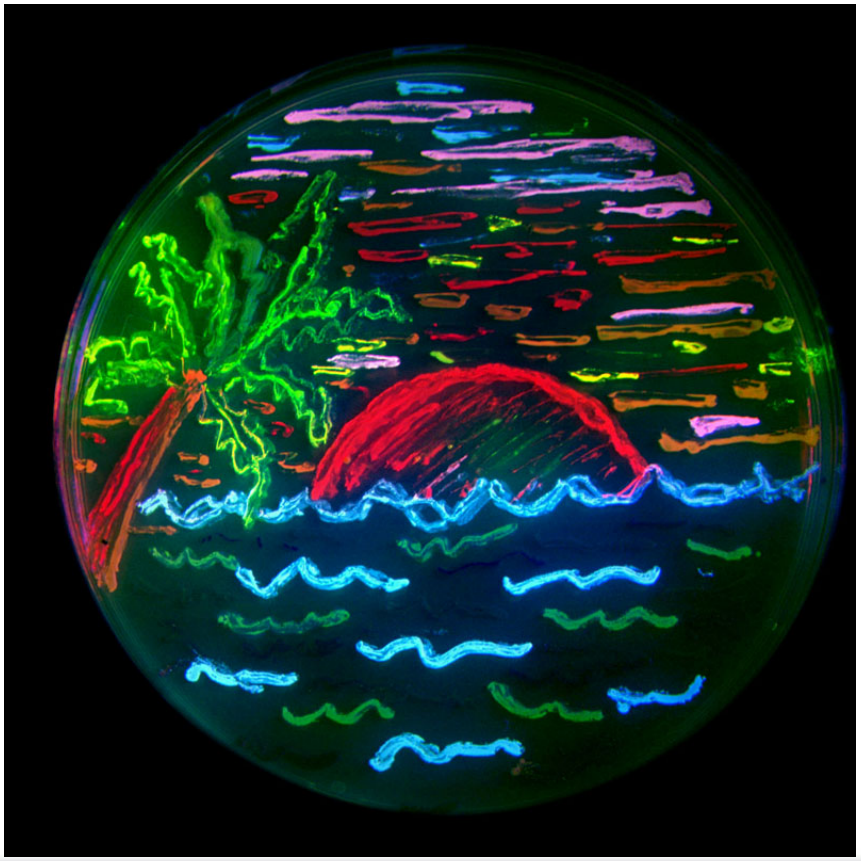


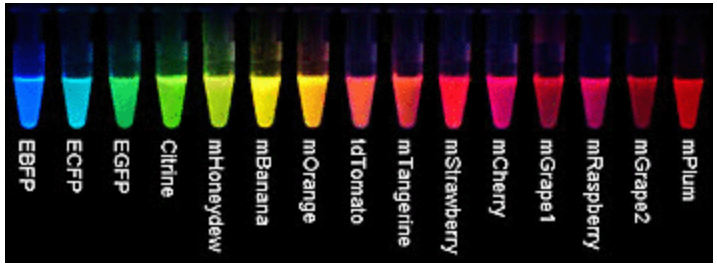
Img Src: http://www.mshri.on.ca/nagy/graphics/GFP%20mice.jpg

Img Src: http://www.antville.org/img/pop/gfp.jpg

Img Src: http://www.computerra.ru/pubimages/73944.jpg

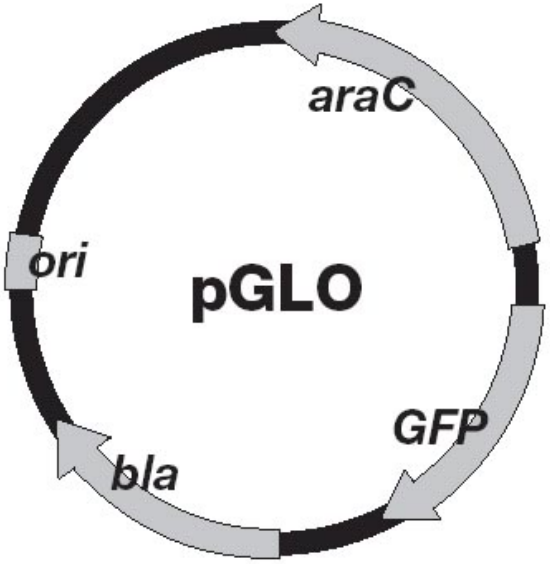
Other Fluorescent Proteins





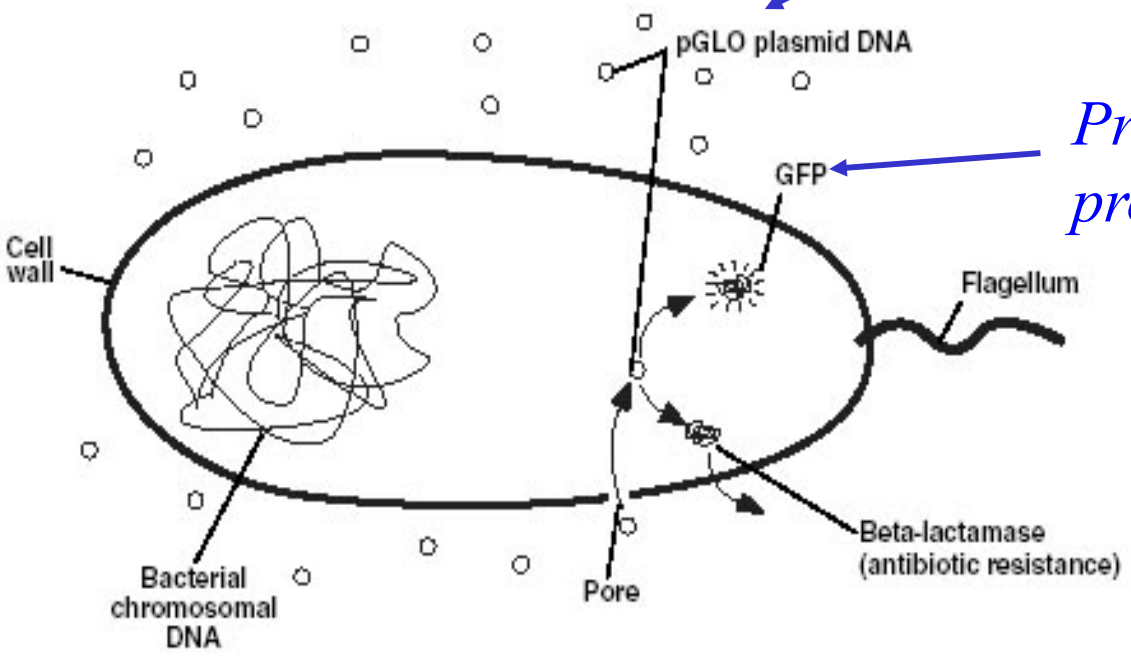
pGLO plasmid

- 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*

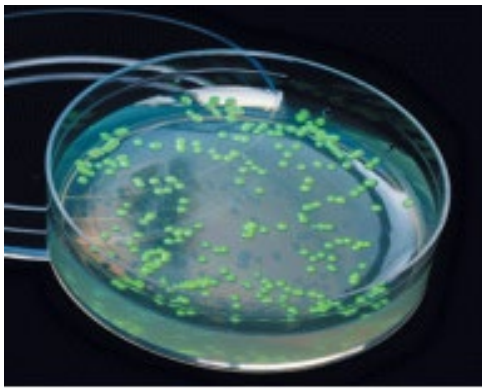


Plasmid containing gene of interest

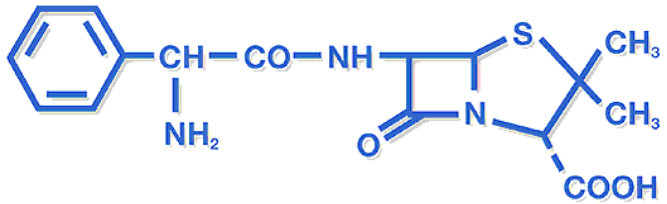
Protein to be produced

Selecting for Transformed Cells

- Selectable Marker: Trait that helps identify a transformed cell by conferring resistance to ampicillin
- Ampicillin presence in LBAgar will kill wild type E.coli
  BUT
- Transformed E. coli survive in the presence of ampicillin in LB Agar



Transformed E.coli



ampicillin

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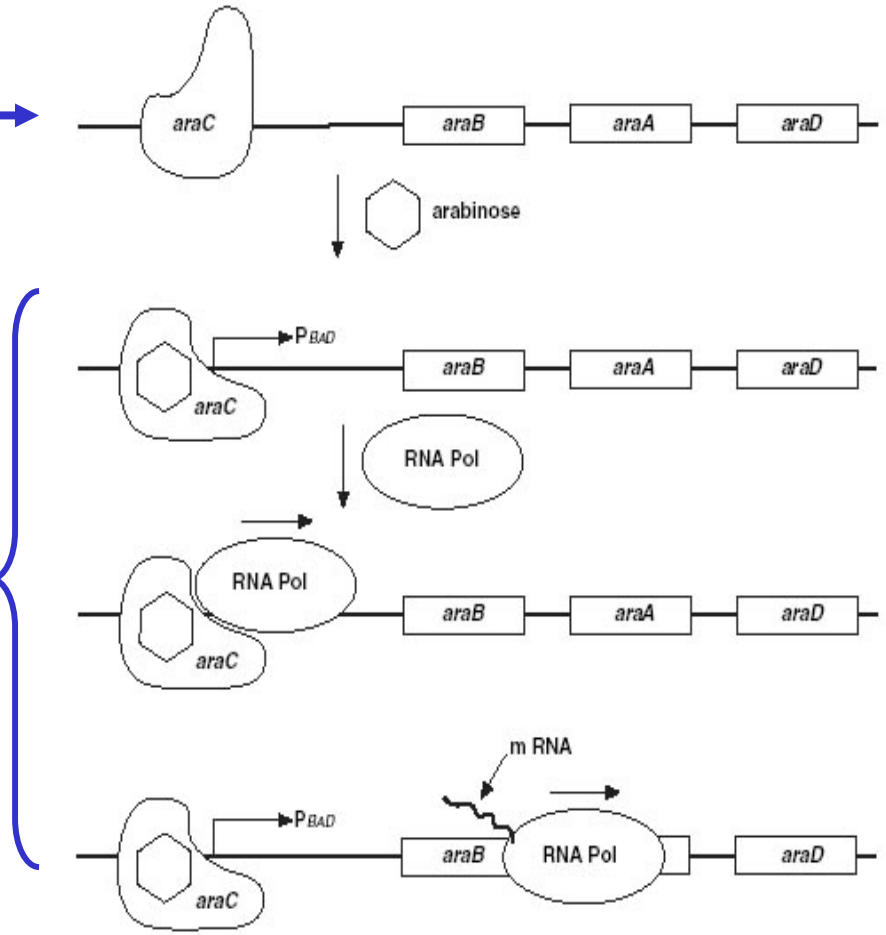
Arabinose Operon

- The arabinose operon in bacteria consists of the following:

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

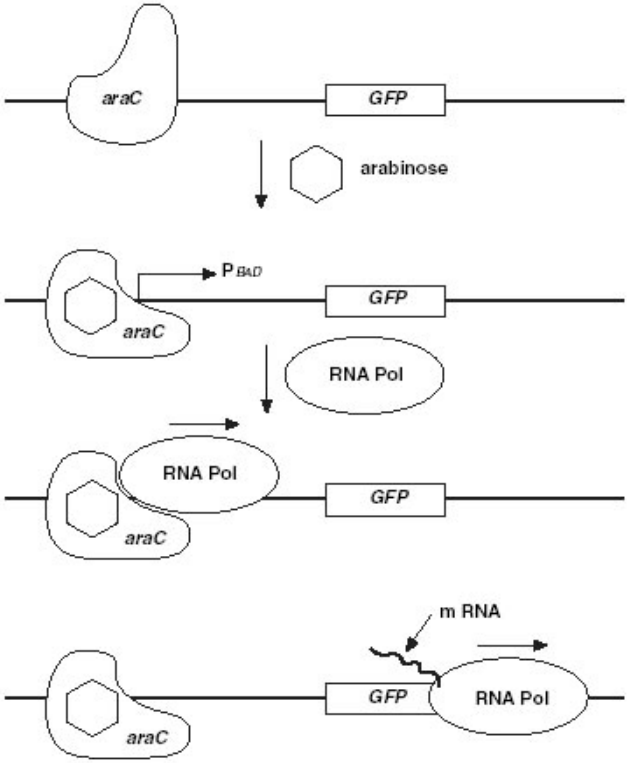
When arabinose is present, it binds to the araC protein -> can't bind to operator → RNA polymerase can continue



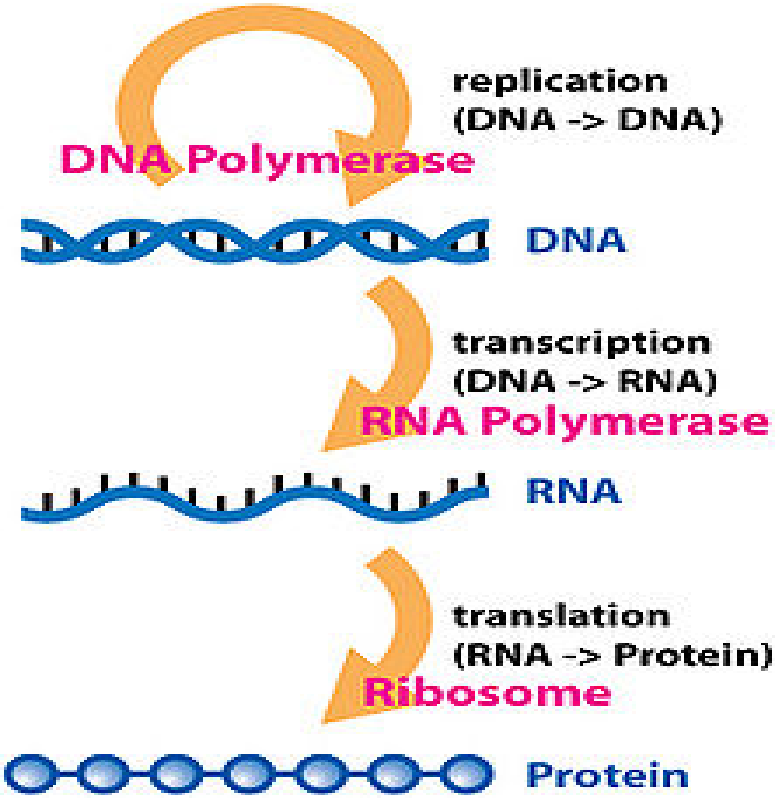
Scientists modified the arabinose operon in pGLO to express the GFP gene.

araC protein binds to the operator → prevents transcription

When arabinose binds to araC it can no longer bind to operator → GFP gene is transcribed and translated



Central Dogma of Molecular



- 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

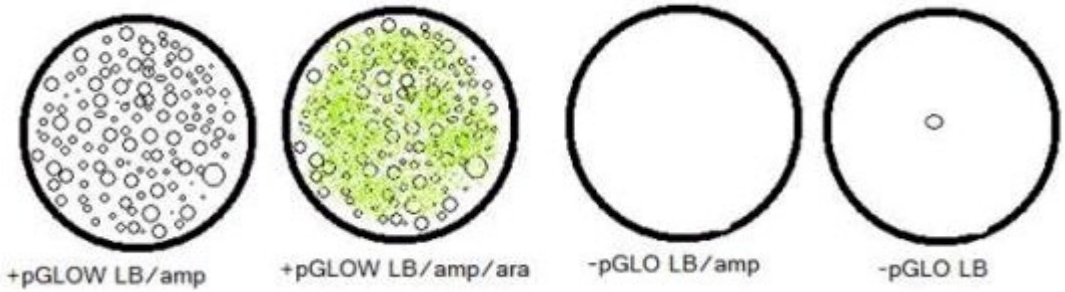
Transformation Time Line

- 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

PGLO Transformed E.coli

- Cells containing pGLO plasmid are now resistant to ampicillin
- Cells containing pGLO plasmid will also glow green when arabinose



Biomanufacturing

- Upstream Processing: Growing genetically transformed cells that produce a desired protein

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