
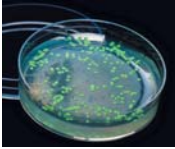




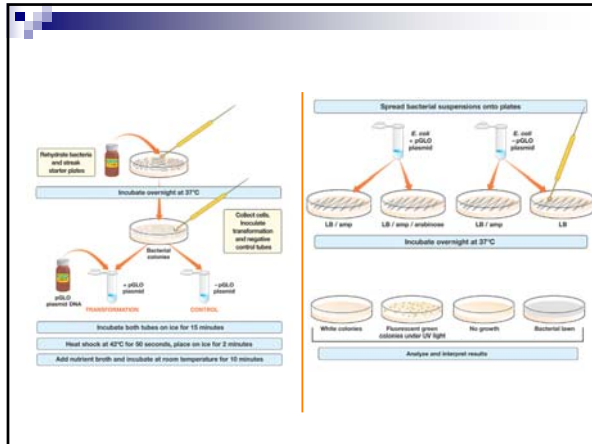
Uses of Green Fluorescent Protein



- 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



- Transformation is a natural process that Bacteria have evolved in order to obtain DNA from their environment.
- Use of the procedure enables scientists to insert genes by recombinant techniques and place the plasmid into a bacteria for expression



Timeline for Transformation

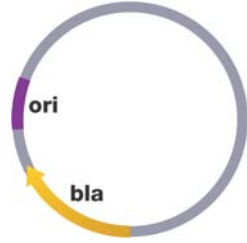
- Background
- Transform bacteria with pGLO plasmid
- Purify GFP using column chromatography

What is Transformation?

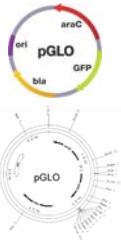
- Uptake of foreign DNA, often a circular plasmid

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



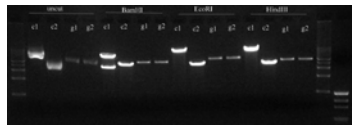
The Many Faces of Plasmids



Graphic representation



Scanning electron micrograph



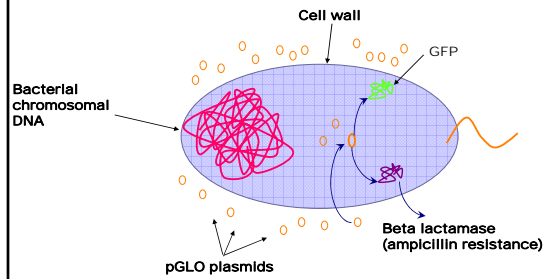
Agarose gel

Plasmid Map

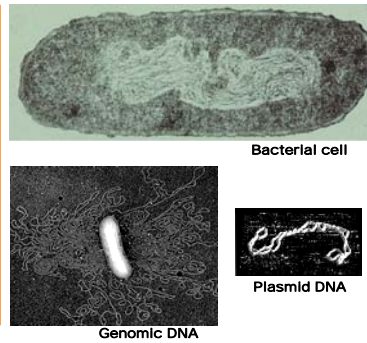
- Beta Lactamase
 - Ampicillin resistance
- Green Fluorescent Protein (GFP)
 - *Aequorea victoria* jellyfish gene
- araC regulator protein
 - Regulates GFP transcription



Bacterial Transformation



Bacterial DNA

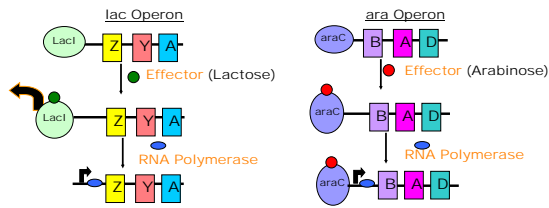


Transcriptional Regulation

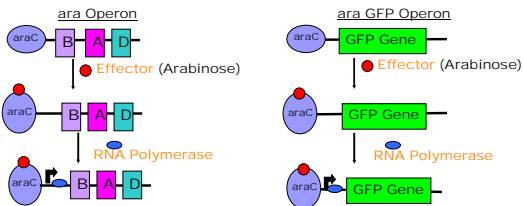
- Lactose operon
- Arabinose operon
- pGLO plasmid



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

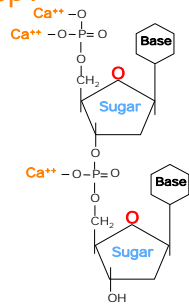
Transformation Procedure

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

Reasons for Performing Each Transformation Step?

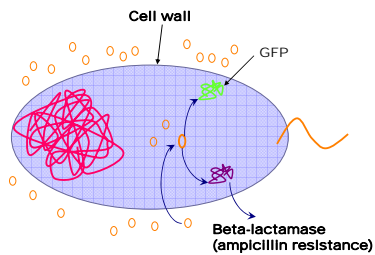
1. Transformation solution = CaCl_2

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



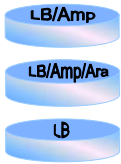
What is Nutrient Broth?



- 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**?
