Bacterial Transformation with pGlo

Overview

- **Transformation** = modification of a bacterium by the uptake and incorporation of exogenous DNA
- Determine the transformation efficiency of the competent cells.
- Amplify the pGlo expression vector.
- Express the pGlo protein.
Why is transformation important?

- A gene of interest can be inserted into a plasmid and cloned through replication.
  - Restriction enzymes can cut a plasmid and a gene can be inserted.
- A gene of interest can be isolated by inserting it into a plasmid with antibiotic resistance.
- We can determine whether competent E.coli cells have been contaminated by other plasmids.

![Transformation Diagram]

Transformation

- Calcium Chloride method
  - Good transformation efficiencies
  - Simple to complete
  - No special equipment required
- Exposure to calcium ions makes the cells **competent** (able to take up DNA)
  - The Ca\(^{2+}\) ions shield the negative charge on the DNA allowing it to pass through the cell membrane.
- The plasmid DNA and the bacteria are mixed together.
- The Plasmid DNA and cells are heat shocked.
- The cells are grown on an antibiotic containing medium to select for those cells which have been transformed.
Transformation

- **pGlo** = Plasmid which contains the gene for Green Fluorescent Protein.
- **GFP** = Green Fluorescent Protein
  - The gene for this protein comes from the bioluminescent jellyfish *Aequorea victoria*
- GFP causes the jellyfish to glow in the dark.
- The bacteria will be transformed with this gene and produce the GFP.
- The bacteria will glow in the dark when exposed to UV light.
Transformation

• The pGlo Plasmid contains the following
  – A gene for GFP
  – A gene for antibiotic resistance
  – Regulation of the GFP gene.

Gene Regulation

• Different types of cells produce different types of protein depending on their function.
  – Examples: Digestive system, immune system, skeletal system.....
• Since each cell contains the same number of chromosomes and genes your body must regulate which genes are turned on and off.
Gene Regulation

• Reasons for gene regulation
  – Developmental changes
  – Cellular specialization
  – Adaptation to the environment
  – Prevents wasteful overproduction of unneeded proteins.

Gene Regulation

• *E.coli* can digest the sugar lactose by producing several enzymes which break down lactose into galactose and glucose.

• *Lac Operon* = Regulates the expression of genes necessary for the metabolism and transport of lactose in *E.coli*

• When lactose is present *E.coli* activate a set of genes which make these enzymes.

• When lactose is not present these genes are turned off.
Gene Regulation

- **Transcriptional unit** – A set of genes which are transcribed together.
- **Promoter** – Site where RNA polymerase can bind to DNA to begin transcription
- **Operator** – Controls the access of RNA polymerase to the genes.
- **Regulatory gene** - Transcript is a Lac repressor which block RNA polymerase by binding to the operator.
- **Operon** – The combination of the operator, the promoter and all of the genes they control.
Gene Regulation

- Important locations = IPOZYA
  - I → Repressor
  - P → Promoter
  - O → Operator
  - Z → gene for LacZ (β-galactosidase)
    - Breakdown of lactose to glucose and galactose
  - Y → gene for LacY (Lactose Permease)
    - Allows lactose into the cell
  - A → gene for LacA (Thiogalactoside transacetylase)
    - Rids the cell of toxic thiogalactosidase that also gets transported in by LacY.

Gene Regulation

- **In the absence of lactose**
  - The operon is ‘off’ because a protein called a repressor binds to the operator blocking the attachment of RNA polymerase.
Gene Regulation

- **In the presence of lactose**
- The operon is ‘on’ because the repressor protein cannot bind to the operator.
  - Lactose is an **inducer**.
  - Lactose binds to the repressor protein and changes the repressor shape so it cannot bind to the operator.
  - RNA polymerase transcribes the genes which code for the enzymes which break down lactose.

[Diagram showing the Lac Operon]

Gene Regulation

- **The Lac Operon**
  - [Link to animation](http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation27.html)
LacOperon + cAMP Regulation Mechanism

- **Bacteria prefer Glucose over Lactose**
- CAP (catabolite gene activator protein) = transcriptional activator
- cAMP (cyclic AMP) = secondary messenger
  - Increases in concentration when glucose levels are low.
  - Decreases in concentration when glucose levels are high.
- CAP binds cAMP.
- CAP/cAMP complex promotes gene expression.
LacOperon + cAMP Regulation Mechanism

- Low Glucose + Lactose Available
  - Lac repressor not bound to operator
  - cAMP levels are high
  - CAP bound to cAMP
  - CAP/cAMP complex bound to promoter
  - RNA polymerase binding is enhanced
  - Lac gene expression is high

- High Glucose + Lactose Unavailable
  - Lac repressor bound to operator.
  - cAMP levels are low
  - CAP not bound to cAMP
  - RNA polymerase cannot bind to the promoter.
  - No gene expression.
LacOperon + cAMP Regulation Mechanism

- Low Glucose + Lactose Unavailable
  - Lac repressor bound to operator
  - cAMP levels high and binds to CAP.
  - CAP/cAMP bind to promoter.
  - RNA polymerase cannot bind to promoter.
  - No gene expression.

- High Glucose + Lactose Available
  - Lac repressor not bound to operator.
  - cAMP levels are low.
  - CAP not bound to cAMP
  - RNA polymerase binding is limited.
  - Lac gene expression is low.
Arabinose Operon

• **Catabolism** = transport and breakdown of nutrients.
• Arabinose
  – A sugar
  – Source of energy and carbon for bacteria.
  – Three enzymes (proteins) are needed to break down arabinose.
  – The genes are not expressed when Arabinose is absent and expressed when arabinose is present.

Arabinose Operon

• *araA, araB and araD* code for digestive enzymes which break down arabinose.
• *araP* → promoter for Arabinose Operon
• Transcription of these genes requires the three things.
  – Promoter and operon
  – RNA polymerase
  – A DNA binding protein called *araC*
  – Arabinose.
Arabinose Operon

• *araC* binds to the promoter blocking transcription by RNA polymerase.
• Arabinose interacts with *araC* and changes its shape.
  – RNA polymerase bind to the promoter.
• As a result *araA*, *araB* and *araD* are transcribed and break down arabinose until the cell runs out of arabinose.
• In the absence of arabinose, *araC* changes back to its original shape and block RNA polymerase.

GFP Modified Arabinose Operon

• The araP_{BAD} promoter is often used in expression vectors
• The pGlo plasmid has been modified to incorporate some features of the arabinose operon.
• Both the araP_{BAD} promoter and the *araC* gene are present.
• The genes which code for *araA*, *araB* and *araD* have been replaced by the gene which codes for GFP.
• In the presence of arabinose, *araC* promotes the binding of RNA polymerase to the promoter and GFP is produced.
  – Bacteria should glow under UV light
• In the absence of arabinose no GFP is produced.
  – Bacteria will not glow under UV light.
Gene Regulation

• Both the Lac and Arabinose operons are positive feedback mechanisms.
  – High Lac $\rightarrow$ high $LacZ$, $LacY$ and $LacA$ expression.
  – High Arabinose $\rightarrow$ High $araA$, $araB$ and $araD$ expression
Lab Overview

- Day 1 → Create competent cells
- Day 2 → Transformation and plating of competent cells
- Day 3 → Analysis of results

Lab Overview

- **Competent Cell** = The ability of a cell to take up extra cellular DNA
- Competent cell production protocol
  - Prepare 4 tubes each with 150ul of competent cells.
  - Wash the cells several times in a CaCl₂ solution.
  - Keep the cells and CaCl₂ solution on ice at all times!
  - Freeze the cells overnight to increase competency.
Lab Overview

• Transformation and plating.
  – Transform with pGlo plasmid by cooling, heat shocking and cooling the cells
  – Which plates are the controls? What do you expect on each plate?

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Amount of competent cells added to tube (µl)</th>
<th>Plasmid DNA concentration (ng/µl)</th>
<th>Amount of plasmid DNA added to tube (µl)</th>
<th>Plate type</th>
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<td>No DNA</td>
<td>No DNA</td>
<td>LB</td>
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<tr>
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<td>100</td>
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<td>No DNA</td>
<td>LB/amp</td>
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<tr>
<td>4</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>LB/amp/ara</td>
</tr>
</tbody>
</table>
Lab Overview

- Analysis of results
  - **Transformation efficiency** = How many bacteria were transformed per ug of DNA. (cells/ug)
  - Calculate the transformation efficiency for your experimental plates.

Transformation efficiency = \( \frac{\text{Total number of cells growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in \( \mu \text{g} \))}} \)