pGLO TRANSFORMATION LAB

One of the challenges in in studying molecular biology is that many of the events and processes are invisible. Scientists have "borrowed" the green fluorescent protein (GFP) gene naturally found in the jellyfish, *Aequorea victoria,* which causes the jelly fish to fluoresce and glow in the dark. Scientists at BIO-RAD have created a genetically engineered plasmid (pGLO) for use in biotechnology research.

In this lab you will perform a procedure known as genetic TRANSFORMATION. Genetic transformation occurs when a cell takes up and expresses a new piece of genetic material (DNA). The new genetic information often provides the organism with a new trait which is identifiable in organisms that have been transformed.

ara – arabinose sugar
amp- ampicillin (antibiotic)
amp^R- ampicillin (antibiotic) resistance gene
LB- Luria- Bertani broth (nutrient medium for growing bacteria)
GFP- Green fluorescent protein (protein glows in UV light)
pGLO-genetically engineered *E. coli* plasmid
E. coli = *Escherichia coli* bacteria

pGLO PLASMID is a genetically engineered plasmid used in biotechnology as a vector for creating genetically modified organisms. The pGLO plasmid contains several reporter genes, including green fluorescent protein (GFP) and the ampicillin resistance gene.

" REPORTER" GENES, when present, produce an observable phenotype to help identify cells that contain these genes.

pGLO plasmid contains genes for:

- *GFP* - Green fluorescent protein taken from jellyfish (*Aequorea victoria*)/fluoresces green under UV- light attached to *ara* operon/shares a bidirectional operator with *araC* gene
- *ori* - Origin of replication – allows plasmid to replicate itself
- *araC* – codes for arabin**ase** = enzyme to break down arabin**ose** sugar/part of *ara* operon
- *bla* - codes for the enzyme beta-lactamase/provides antibiotic resistance to AMPICILLIN (=amp^R)

PLASMID EXPOSURE

\+ pGLO tubes receive plasmid
\- pGLO tubes do NOT receive plasmid

MAKING CELLS **"COMPETENT"**

Bacteria that have the ability to take up the plasmid are said to be "COMPETENT".
The following steps cause changes in the bacterial cell membrane helping them to pick up the plasmid.

1. TRANSFORMATION SOLUTION containing $CaCl_2$ – Ca^{++} ions neutralize the repulsive negative charges on the phosphate backbone of the DNA and the cell membrane phospholipids allowing DNA to enter cells
2. HEAT SHOCK- moving bacteria quickly from 42 ° C (102° F) to ice makes membranes "leaky"

IDENTIFYING TRANSFORMED BACTERIA "REPORTER GENES"

If present, give cells a phenotype that is OBSERVABLE . . . so can tell which cells have picked up plasmid.
EX: ability to grow on plate with media containing antibiotic (antibiotic resistance)
ability to make GFP (glowing protein) if gene is turned on
Bacteria that have incorporated the plasmid will be able to grow in the presence of ampicillin AND GLOW in presence of arabinose

TURNING ON THE "GLOW GENE"

The GFP gene has been attached to the *ara* operon on the pGLO plasmid. Because the GFP gene and the gene for metabolizing arabinose sugar share a bidirectional promoter, the GFP gene is expressed in the presence of arabinose sugar (+pGLO/amp/ara) and will glow under UV light.