

Lab - Transformation of *E. coli* Bacteria with the pGLO plasmid

I. INTRODUCTION:

Green Fluorescent Protein is used extensively in biology research and work. It is one among several fluorescent proteins that allow DNA to be sequenced automatically by lasers. It is used quite frequently to label cells for studying how organisms develop as embryos and it is a key part of ongoing stem cell research. The protein itself does not glow but it can be made to fluoresce with a green light when exposed to ultraviolet (UV) light.

Bacteria grow well with the food source Luria Broth (LB). You can grow them either in liquid LB or on the surface of a petri dish with a mixture of LB and agar that solidifies into a thick gel. Antibiotics are sometimes also added to the agar to help control the growth of bacteria that are unwanted at the end of the experiment. Most antibiotics either kill the bacteria or keep them from reproducing. We will be using an antibiotic called ampicillin (amp).

Lab groups will prepare bacterial cells using calcium chloride to help the small plasmid circles of DNA get through the membrane of the cell. Then, you will get a plasmid into half of these cells by weakening the membrane further with heat shocks (cold, hot cold). A small percentage of the millions of bacteria in your sample should successfully take in plasmids.

Each plasmid contains a gene coding for green fluorescent protein (GFP), a gene coding for an enzyme (beta-lactamase) that breaks down the antibiotic ampicillin and a region that is like a lock or on/off switch for the green fluorescent protein gene. The on/off switch or lock is called an "inducible operon" that is usually in the "off" or locked position. The sugar arabinose (ara) is the key that opens the lock and turns on the GFP region of the plasmid.

You will put your transformation mixes (and your control sample) onto petri dishes to compare what happens with ones that have the plasmid to ones that have not received the plasmid. After the bacteria have had a chance to grow we will look at them under normal and ultraviolet light. It will take at least a day to show results.



Pre-lab Questions

1. What is a plasmid?
2. What is a gene?
3. What is luria broth (LB)?

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LM Reder



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