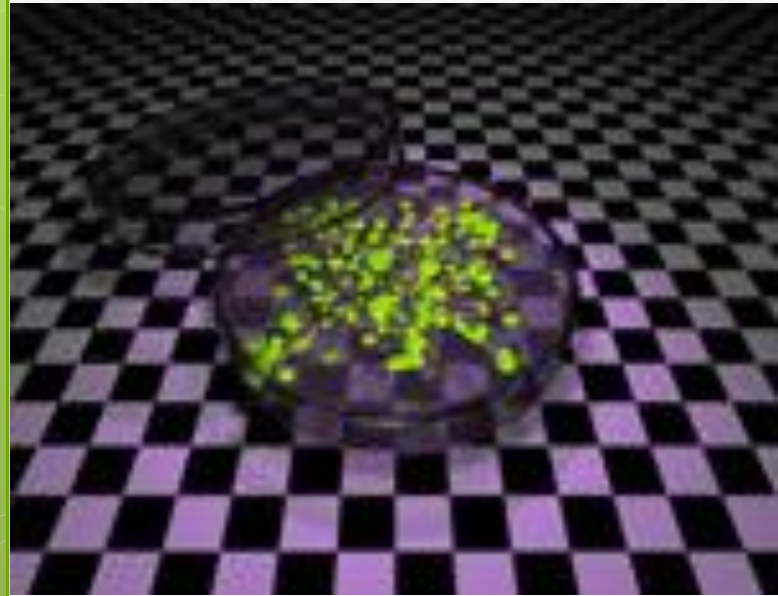


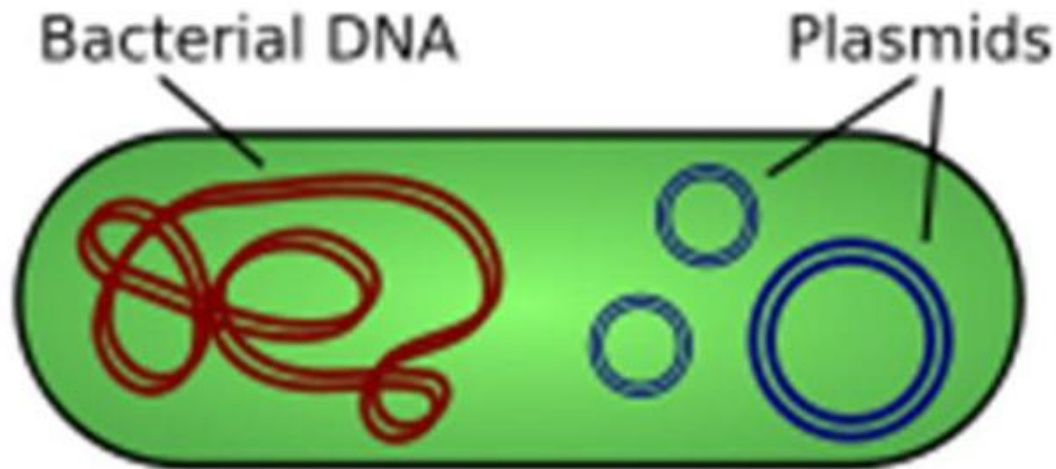
Introduction to pGLO lab

Bacteria Transformation



QQ#1: What is a plasmid?

- A plasmid is a small circular piece of DNA (about 2,000 to 10,000 base pairs) that contains important genetic information for the growth of bacteria.



How do scientists use plasmids?

- A plasmid are used as **vectors**.
 - A vector is a vehicle for transferring foreign genetic information
- Often these plasmids are altered to create *recombinant DNA* that contains and expresses new, foreign traits.
 - For example, our plasmid for this lab contains:
 - Amp^R: a gene that codes for resistance to an antibiotic (ampicillin). Bacteria with these plasmids can now live in the presence of an antibiotic.

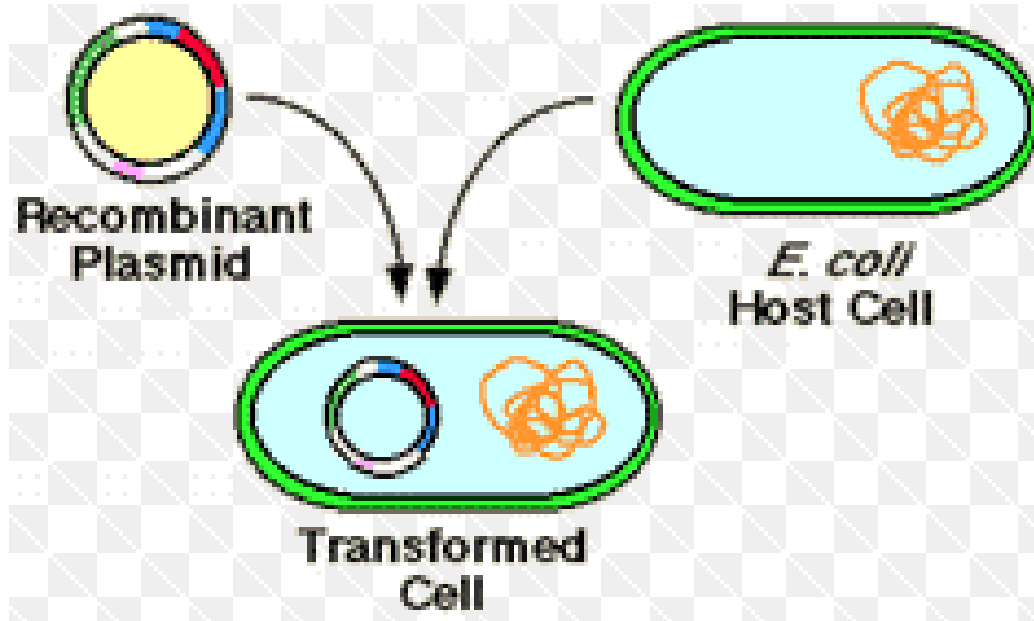
What do you do with plasmids that are now recombinant DNA?

- After following the steps to combine a bacterial plasmid with foreign DNA, scientists need to place the recombinant DNA into a living organism.
- The recombinant DNA is inserted into a bacteria.
- Then the bacteria will express the new “foreign” DNA, and the bacteria will perform new functions.

QQ#2: What is this process called?

Transformation:

The process of inserting recombinant plasmid DNA into a bacteria (or any other cell)



How do you know if Transformation occurred?

- The transformed bacteria are then spread over an agar plate that contains ampicillin.
- Because our plasmid contains the amp^{R} gene, only bacteria that have acquired the plasmid can grow on the plate.
- The ampicillin provides a **selective pressure**
- **Selective Pressure** - The same as in evolution - only the organisms that have a particular trait (in this case antibiotic resistance) will survive.
- Therefore, as long as you grow the bacteria in ampicillin, it will need the plasmid to survive

In the pGLO lab, we will be performing transformation

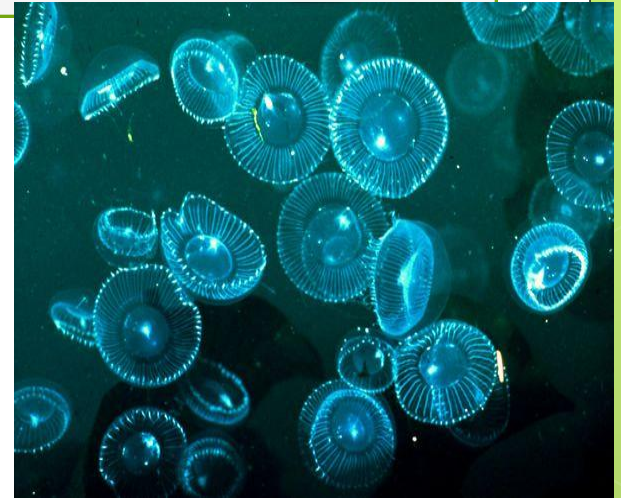
- We will take a plasmid that has been recombined into a piece of recombinant DNA that contains 3 new genes:
 - gene from a jellyfish (GFP)
 - A gene that induces GFP
 - ampicillin resistance gene.
- When transformation is complete, and we insert the plasmid into a bacteria cell, the cell will express the jellyfish gene and will fluoresce .

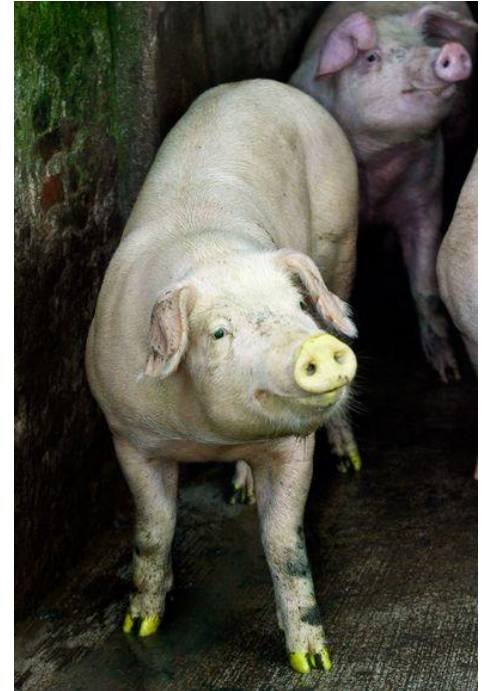
Why would we WANT to
make something glow??

http://www.youtube.com/watch?v=Qm5Bj5xNefM&list=PLNtVKBx_tJzM2jDB8-Ow67VV7otRhmSuw

What jellyfish gene will we use?

- GFP is a green fluorescent protein that normally is found in jellyfish
- Osamu Shimomura isolated the **GFP from a jellyfish**. Some of Shimomura's colleagues realized that the protein could be attached to other proteins--enabling scientists to mark proteins of their choice with a green glow.
- (GFP) has been used to decrypt previously invisible processes, like the spread of cancer or the development of nerve cells--earning Shimomura and colleagues a Nobel Prize in 2008.
- Fluorescent proteins have also been used to engineer some truly strange beasts (and the odd plant), such as the glowing puppies, monkeys, mice, fish and other animals on the following pages.





Are we going to make kittens glow? No, just bacteria.

- We are going to create transgenic bacteria by transforming them and forcing them to take up a commercially prepared plasmid that contains three genes of interest, amp^R, araC and GFP.
- Remember: Genetically modified organisms are “transgenic”



Genes of interest: amp, araC, GFP

- amp^R – this gene will give our transgenic bacteria resistance to the antibiotic ampicillin so we know if transformation occurred
- araC – this gene is induced by arabinose and creates a protein will induce (switch on) the GFP gene.
- GFP – induced with the help of araC this gene will “switch on” and cause the transformed (transgenic) bacteria to glow green
- These three genes will work together to allow gene regulation to be on, and for GFP to be expressed.

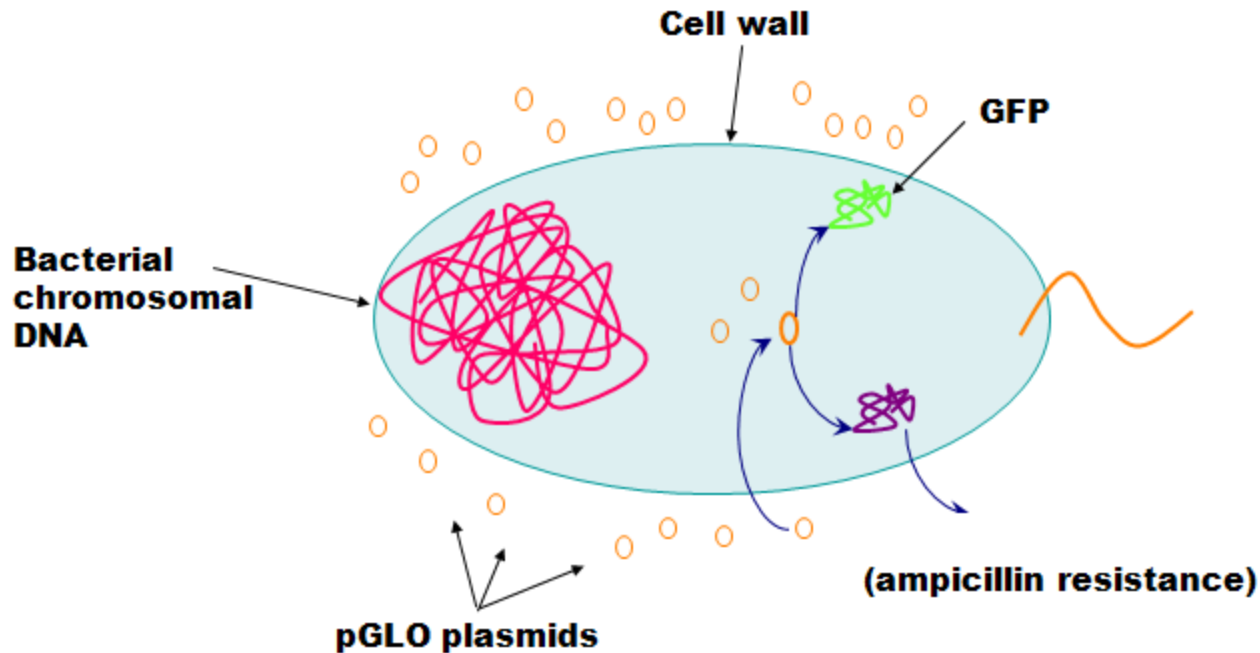
QQ#3

- Listen and draw/follow along with how your teacher explain pGLO gene regulation

- Explain in your own words the relationship between the three genes of interest.

Our Lab

- Bacterial Transformation should occur!



- We will use several different agar plates in order to see if the transformation was successful.

How will we transform the bacteria?

1. Suspend bacterial colonies in Transformation Solution to weaken the cell wall
2. Add **pGLO plasmid** DNA to +DNA tube
3. Place tubes on **ice** to make the plasmid stick to the cell wall
4. Heat shock at **42°C** to force plasmid uptake and place on **ice** to close cell wall
5. Incubate with **LB broth** to allow nutrients so newly acquired genes can be expressed.

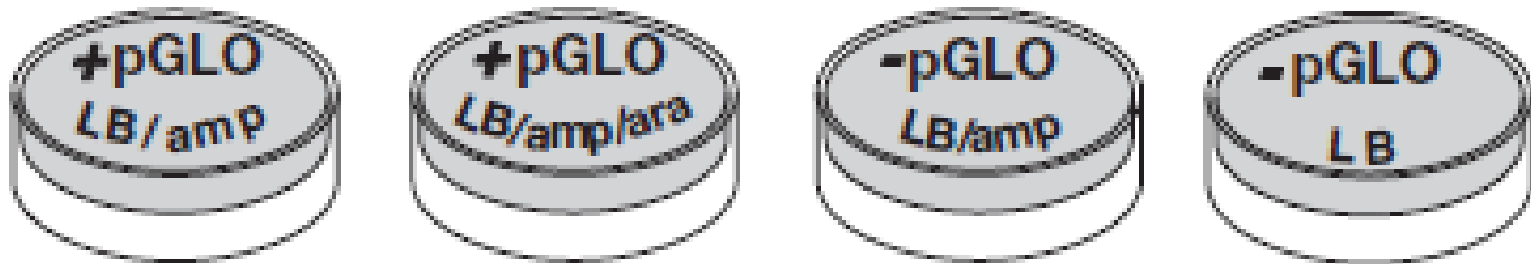
This process will make the bacteria take up the plasmid through its cell wall.

We will start with:

- **E. coli starter plate**
- This plate has the bacteria we will use growing in a luria broth (LB) agar plate.
- These bacteria are normal (have NOT been transformed)



We will use 4 different petri dish set ups to see our results



- Each plate contains different things
 - LB: luria broth-nutrients
 - Amp: the antibiotic ampicillin
 - Ara: the sugar arabinose
 - +pGLO: contains transformed bacteria
 - -pGLO: contains non-transformed bacteria

Explanation of agar plates

- **LB/-pGLO**
- This is the control plate. These –DNA bacteria are not transformed and are in normal (LB) agar.
- You should expect to see normal bacterial growth in this plate.



Explanation of agar plates

- **LB/amp/-pGLO**
- These -pGLO bacteria have not received the plasmid.
- They have not been transformed, so they do not have resistance to the ampicillin that is in the agar.
- There should be no growth on this plate- ampicillin kills bacteria



Explanation of agar plates

- **LB/amp/+pGLO**

- This plate will have *E. coli* bacteria on LB agar to which ampicillin has been added.
- The +pGLO means that the bacteria may have been transformed (if your technique is good).
- If they have been transformed, they will now have a plasmid with an ampicillin resistant site so they will not be killed by the ampicillin that has been added to the agar.



Explanation of agar plates

- **LB/amp/ara/+pGLO**
- This plate will have transformed bacteria (+pGLO) growing on agar that has both ampicillin and arabinose added to it.
- If your technique is good, you should expect to see green glowing bacteria in this plate.

