Name: _____ TOC#

Introduction to pGLO lab
Bacteria Transformation

What is a plasmid?

-

How do scientists use plasmids?

- A plasmid are used as _____
  - A vector is a _____
- Often these plasmids are altered to create _____

  _____
  - For example, our plasmid for this lab contains:
    - Amp^R: a gene that codes for _____ (ampicillin).

      Bacteria with these plasmids _____.

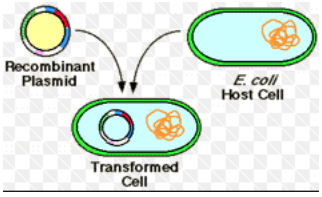
What do you do with plasmids that are now recombinant DNA?

- After following the steps to combine a bacterial plasmid with foreign DNA, _____

  _____.
- The _____ is inserted _____.
- Then the bacteria will _____.

Transformation: _____

_____



How do you know if Transformation occurred?

- The _____ bacteria are then spread over an agar plate that _____.
- Because our plasmid contains the _____, only _____

  _____on the plate.
- The ampicillin provides a _____
- **Selective Pressure** - _____
- Therefore, as long as you grow the bacteria in ampicillin, _____

In the pGLO lab, we will be performing _____

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

What jellyfish gene will we use?

- _____normally is found in _____
- Osamu Shimomura isolated the GFP from a jellyfish. Some of Shimomura's colleagues realized that the protein could be attached to other proteins--_____

  _____.
- (GFP) has been used to _____, 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 _____ by _____ them and _____

  _____ that contains three genes of interest, ____, ____ and ___
- Remember: Genetically modified organisms are "_____"

Genes of interest: amp, araC, GFP

- amp^R –
- araC –
- GFP –
- These three genes will work together to allow gene regulation to be on, and for GFP to be expressed.
- IN YOUR OWN WORDS: explain how these genes work together to show gene regulation of GFP

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.
2.
3.
4.
5.

- **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 _____ (LB) agar plate.
- These bacteria are _____ (_____)

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

- Each plate contains different things
  - LB: _____
  - Amp: _____
  - Ara: _____
  - +pGLO: _____
  - -pGLO: _____

Explanation of agar plates

- **LB/-pGLO**
  - This is the _____ plate. These –pGLO bacteria are _____ and are in _____ agar.
  - You should expect to _____ in this plate.
- **LB/amp/-pGLO**
  - These –pGLO bacteria _____.
  - They have _____, so they do _____ to the ampicillin that is in the agar.
  - There should be _____ on this plate-_____

- **LB/amp/+pGLO**
  - This plate will have *E. coli* _____ has been added.
  - The +pGLO _____ (if your technique is good).
  - If they _____, they will _____

    so they _____ that has been added to the agar.
- **LB/amp/ara/+pGLO**
  - This plate _____ (+pGLO) growing on agar that has both _____

    and _____ added to it.
  - If your technique is good, you should expect to see _____ in this plate.

Summary:

Based on your notes:

1. To the right of each "plate" below label the plate with what is added to the agar.
2. Inside each "plate" write what you expect to see that the end of the lab (i.e. growth, no growth, glowing)