Transformation of E. coli mm294 with pGFP plasmid

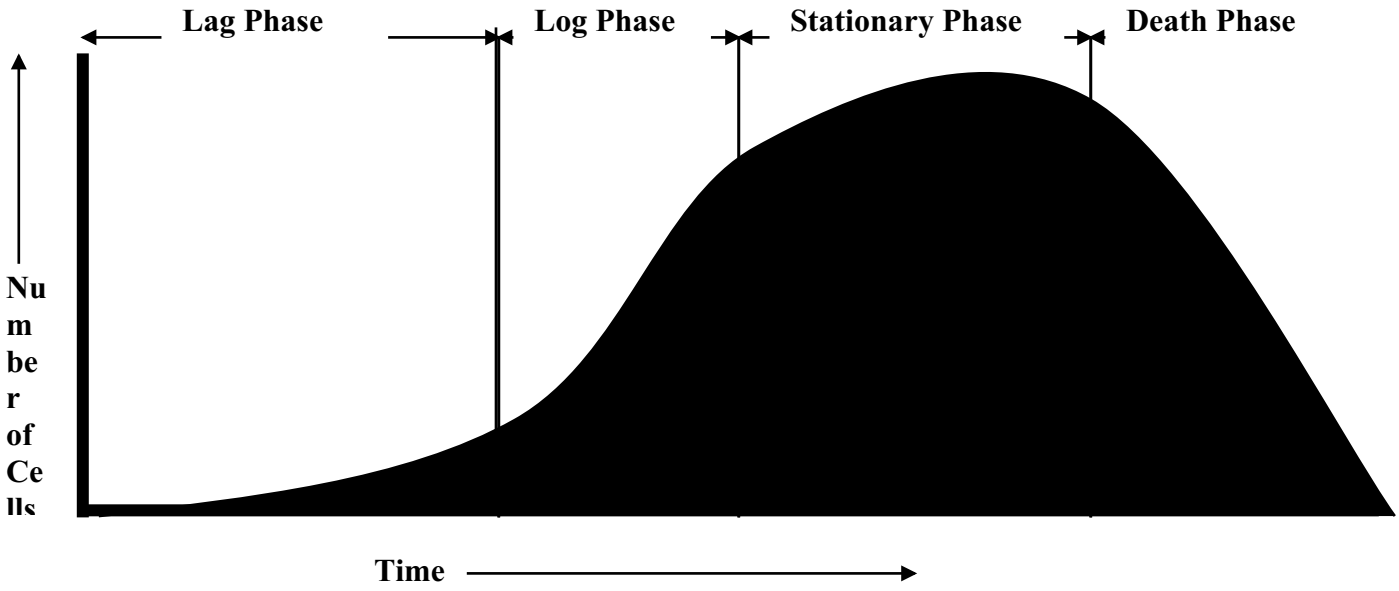
General Information:

The exact mechanism of plasmid DNA uptake by competent E. coli cells is not precisely known. However, it is believed that the DNA molecules pass through any of the several hundred channels formed at zones of adhesion, where the outer and inner cell membrane are fused to form pores in the bacterial cell wall. These zones of adhesion are only present in cells taken from cultures in the log phase of growth. As cells grow older these zones of adhesion close and transformation becomes more difficult.

Bacteria and fungi are decomposers and as such, compete with one another for food. Both bacteria and fungi produce toxins that are harmless to themselves but lethal to other decomposers. Neat adaptation....Kill your competition at the dinner table. Humans grow certain bacteria and fungi and harvest their toxins.....which humans call antibiotics.....and use those toxins/antibiotics to rid themselves of bacterial infection.

Where there are weapons there will likely be defenses and some of these decomposers (bacteria) produce enzymes that digest the toxins of their rivals. One such enzyme is beta-lactamase. Beta-lactamase digests the lactam ring found in ampicillin (an antibiotic in the penicillin family). If you have a bacterial infection and the bacterium making you ill has a plasmid in its genome that codes for beta-lactamase and your doctor prescribes penicillin antibiotic therapy, the therapy will
be unsuccessful.

The host cell we are using is E. coli strain MM294. The plasmid used in this transformation (pGFP) conveys to the transformed cell two genes: One gene is from a marine jellyfish *Aequorea victoria* that once expressed causes the host cell to produce a protein that is green and also fluorescent. The other gene codes for the enzyme beta-lactamase.



Questions to create the proper mind set

1. Describe exactly what takes place during a bacterial transformation_____

_____

2. What term describes cells that can be easily transformed? _____

3. With respect to the growth curve above, what cells can be transformed? _____

4. Why is it difficult to transform cells in the stationary and death phase? _____

_____

5. What is beta-lactamase? _____ What is its substrate? _____

6. What is ampicillin? _____ What produces ampicillin in the natural world _____

7. Why would they do that? _____

8. What is the adaptive value of beta-lactamase to any bacterial cell?

_____

Procedure:

1. Label one Falcon tube +pGFP. Cells in this tube will receive the pGFP plasmid (the independent variable)

   What do you call this tube? _____

   Label the other Falcon tube -pGFP. Cells in this tube will **not** receive the pGFP plasmid.

   What do you call this tube? _____

2. Use the 100-1000 µl micropipet to add 250 µl of $CaCl_2$ into the bottom of each Falcon tube. Remember that Falcon tubes and their caps must be flamed whenever they are open and right before they are closed.

3. Place both tubes in your ice bath.

4. Use steps (a) thru (e) below to transfer a small mass of E. coli to each tube

   a) Remove a plastic inoculating loop from the package. Open the end away from the loop
   b) Scrape up a small cell mass. **Do not remove any agar as it will inhibit transformation.**
   c) Hold the +pGFP tube up to the light and vigorously tap the loop against the wall to dislodge the cell mass. Make sure the mass is not left on the loop
   d) Place the contaminated loop in the biohazard waste beaker.
   e) Immediately suspend the MM294 cells in the $CaCl_2$ by slowly pipetting the mixture in and out until all the clumps are gone and you have a homogenous, milky white solution. Avoid making bubble or splashing. **This step is the most important variable in obtaining good transformation efficiencies!**

5. Return the +pGFP tube to the ice bath.

6. Repeat steps (a) through (e) for the -pGFP tube.

7. Return the -pGFP tube to the ice bath.

8. Incubate both the +pGFP and -pGFP tubes 15 minutes on ice. Time in _____ Time out _____

Give a molecular level explanation as to what is happening to the E. coli cells during this first incubation period.

_____

_____

9. Open the +pGFP tube and flame the tube and cap. Use the ultradigital pipet (1µl-10 µl) to add 10 µl of 0.005 µg/µl pGFP plasmid DNA solution directly into the cell suspension in the +pGFP Falcon tube. Avoid making bublles or splashing up the side of the tube. Flame the tube & cap and replace the cap.

10. Return the +pGFP tube to ice and incubate both the +pGFP and -pGFP tubes for an additional 15 minute.

Time in _____ Time out _____

At the molecular level, describe what is happening to your E. coli cells and plasmid DNA during this second incubation period._____

_____

11. Following the second 15 minute incubation period, take your tubes in your ice bath to the 42°C water bath. Hold both the +pGFP and -pGFP tubes in the same hand and quickly immerse tubes in the hot water for **90 seconds**. **You must hold on to the tubes during this heat shock period. Abruptly return both tubes to the ice bath.**

Describe the role of heat shocking in the transformation process._____

_____

When is the exact moment of transformation?_____

12. Leave your tubes on ice and Mr. H will collect them and chill them over night and we will pick up here tomorrow.

13. Flame the +pGFP tube and cap. Use the 100-1000 µl micropipet to add 250 µl of Luria Broth (LB) to the +pGFP tube. Flame the tube & cap and return the cap. Now change your tip and repeat the process for the -pGFP tube. Place both tubes in the 37°C shaking water bath for 15 minutes.

Time in _____ Time out _____

What is the purpose of the LB incubation period? (A molecular level explanation is required.)

_____

_____

What would happen if the incubation period was skipped?_____

_____

In what way will your transformed cells be genetically different from your non-transformed cells?

_____

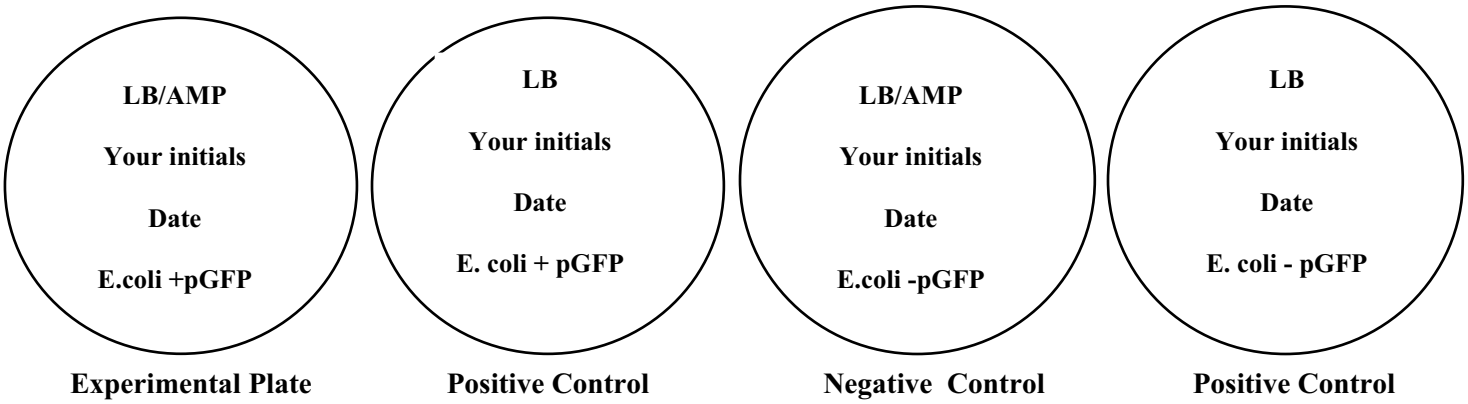
_____

In what way will your transformed cells be phenotypically different from your non-transformed cells?

_____

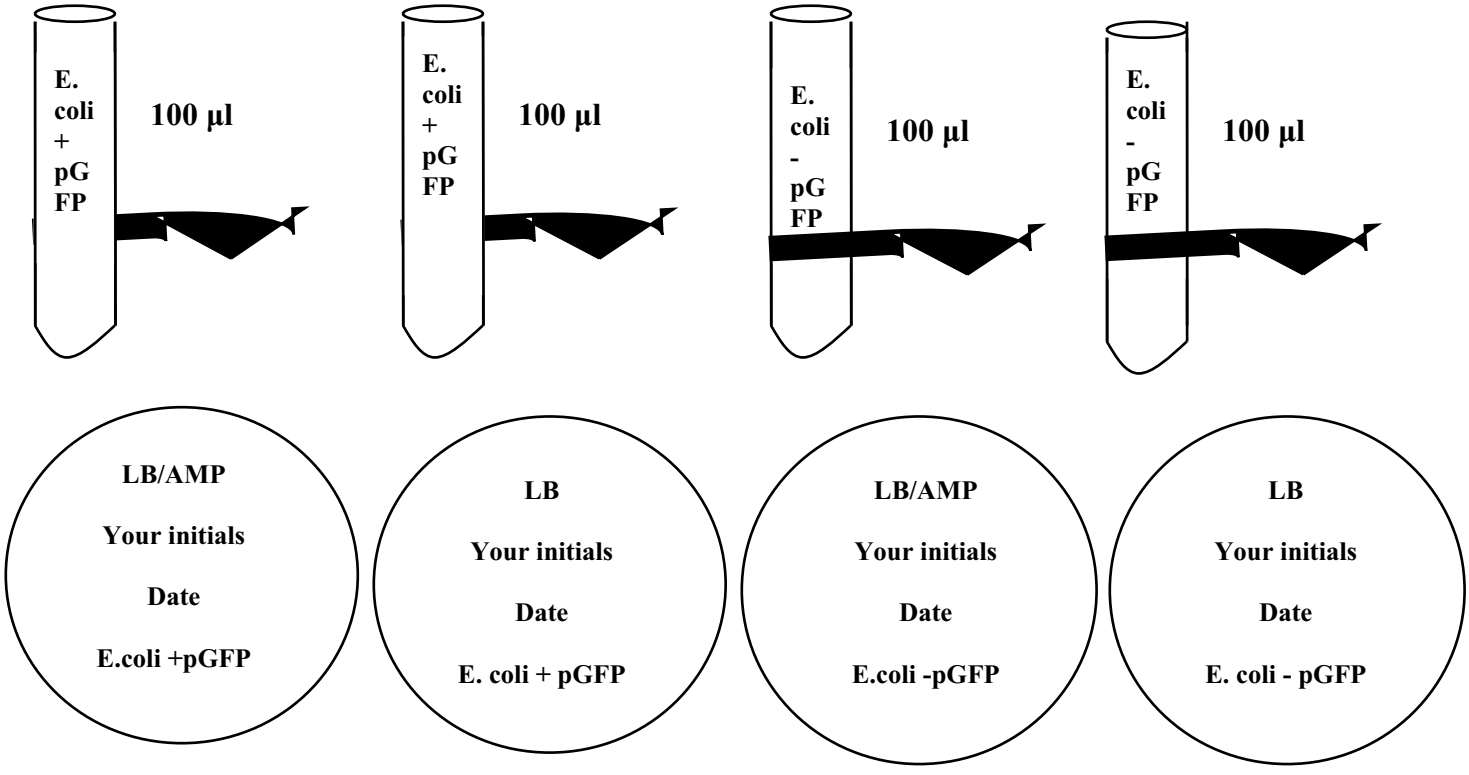
_____

14. You will be provided with 4 sterile uncolonized petri plates. 2 plates contain Luria Broth Agar and are labeled LB 2 plates contain Luria Broth Agar plus the antibiotic ampicillin and are labeled LB+Amp. Use the marker to label the plates as follows: **Place your labels close to the plate edge so as to not interfere with your future observations.**



Experimental Plate **Positive Control** **Negative Control** **Positive Control**

15 Use your the 100-1000 µl micropipet to add 100 µl of the cell suspension to the **first** of the 4 petrie plates as indicated in the illustration below:



Experimental Plate **Negative Control** **Positive Control** **Positive Control**

16) Now use a **sterile** cell spreader to spread the 100 µl of the cell suspension over the agar.

a) To sterilize the cell spreader, dip it into the beaker of ethanol for at least 30 seconds and then **briefly** pass the spreader through the Bunsen burner flame to ignite the alcohol. **Do not ignite the alcohol in the beaker!**

b) Lift the lid of the first plate only enough to allow spreading. Cool the spreader by gently rubbing it on the agar away form the cell suspension. **Do not push down on the spreader as this will tear the agar.**

c) Touch the spreader to the cell suspension and gently drag it back and forth several times across the surface of the agar. Rotate the plate one quarter turn and repeat the spreading motion. Replace the lid and return the spreader to the ethanol without flaming.

17) Repeat the above protocol to add and spread cells on the remaining three plates.

18) Let the plates sit on the desktop for 15 minutes to allow the cell suspension to be absorbed into the agar.

19) Now hypothesize the results you will see: (Below are some possibilities. Be sure to explain your reasoning.)

a) No growth.....this is a clear plate when no cells survive
b) A white lawn.....a lawn is produced when all the cells survive and completely cover the agar
c) A green lawn
d) A white lawn with green polka dots
e) A green lawn with white polka dots
f) White colonies.....colonies are produced when only a few cell survive
g) Green colonies
h) Some other novel patter that you believe to be correct

LB/AMP plate with E. coli +p GFP

_____

_____

LB/AMP plate with E. coli -p GFP

_____

_____

LB plate with E. coli +p GFP

_____

_____

LB plate with E. coli -p GFP

20) The plates will now be inverted and incubate at 37°C for 24 hours.

Results and Discussion:

1. Count the green colonies growing on the LB/Amp MM294 +pGFP plate. If you have a large number of colonies, divide your plate into four equal portions with a sharpie. Count the colonies in one quadrant and multiply to obtain a total colony count. Record your results in the space below.

Colony count_____

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2. You may notice many smaller white colonies growing around a larger green colony. These are untransformed bacterial colonies called satellite colonies.

What is a satellite colony genetically? (Circle one) **+pGFP** or **-pGFP**

How are satellite colonies able to grow in the presence of the antibiotic ampicillin?

_____

3. **Transformation efficiency** is expressed as the number of antibiotic resistant colonies produced per microgram of pGFP DNA used in the transformation.

A) Determine the total mass of pGFP plasmid DNA used to transform your MM294 cells.

Mass = concentration of pGFP plasmid DNA X Volume of pGFP plasmid DNA used

Mass = _____µg of pGFP plasmid DNA

B) Determine the fraction of the total MM294 + pGFP cell suspension spread onto the LB/Amp+MM294+pGFP plate
Fraction spread = Volume of suspension spread (µl) ÷ Total suspension volume

Fraction of the cell suspension spread = _____

C) Determine the mass of pGFP plasmid DNA in the cell suspension spread onto the LB/Amp +MM295 +pGFP plate. Remember some of the plasmid will be inside host cells and the rest in the liquid around the cells

Mass of pGFP plasmid DNA spread = Total mass of pGFP plasmid DNA X Fraction of the cell suspension spread
Mass of pGFP plasmid spread onto the plate = _____µg

D) Determine transformation efficiency

Transformation efficiency = total number of colonies ÷ mass of pGFP plasmid DNA spread

Transformation efficiency = _____ green colonies/ug of pGFP plasmid DNA