Materials List for AP Biology Lab:

- Two Luria agar plates
- Two Luria agar plates with ampicillin
- Two 15mL tubes
- One inoculating loop
- One bacterial spreader
- Several sterile micropipettes
- Calcium chloride
- Luria broth
- pAMP solution
- A Bunsen burner
- Hotplate
- Ice
- Water bath.

Procedure for AP Biology Lab:

1. There are 2 sterile 1.5 mL microfuge tubes at the lab station. Label one tube "+pGLO", the other "-pGLO".

2. Locate the tube labeled "Transformation Solution"; this contains a solution of $CaCl_2$. With a sterile pipet, aliquot 250 µL transformation solution to both the -pGLO and +pGLO tubes. Place both tubes in an ice water bath.

3. The lab station will contain a starter plate of HB101 bacteria. These bacteria contain only chromosomal DNA. Take notes on how these bacteria look in visible and UV light for comparison with the transformed bacteria later in the lab. The lab station will also include pre-packaged sterile loops (look like a very small soap bubble wand). Remove a sterile loop from the package being careful not to touch the loop on anything outside the bag. Remove the cover from the starter plate and pick a single colony of the HB101 bacteria. Immediately place the loop into the transformation solution in the tube marked "+pGLO" and spin the loop until the entire colony is dispersed into the liquid. Check to be sure there are no bacterial clumps floating in the transformation solution; cell clumps will negatively affect the efficiency of the transformation. Place the tube back in the ice water bath when the cell suspension is complete.

4. Using a new sterile loop, repeat the procedure in Step 3 for the "-pGLO" tube of transformation solution.

5. Inspect the pGLO plasmid solution with the UV lamp provided and note your observations. What do you expect to see at this step? Take the 2-20 µL pipettor, set it to 5 µL. Carefully remove 5 µL of the pGLO plasmid solution and add the DNA into the "+pGLO" cell suspension. Mix the plasmid with the bacterial cells by tapping the tube gently on the bench top. Close the tube and return it to the ice water bath.

6. Incubate both the "+pGLO" and the "-pGLO" tubes in the ice water bath for 10 min. Make sure the bottom of the tube is pushed through the foam rack and is in contact with the ice water.

7. While the tubes are incubating on ice, label the 4 agar plates at your lab station as follows:

Color code: red stripe green stripe red stripe black stripe Be sure to label the bottom of the plates (the portion containing the agar). It is very important to label each plate correctly. Remember, the color code on the side of the agar plates indicates which medium already contains the ampicillin for antibiotic selection and arabinose for GFP expression.

8. To transform the plasmid DNA into the bacteria, the cells must undergo a heat shock. This is performed by removing the foam rack from the ice water bath and placing it rapidly in a 42°C water bath. Incubate the tubes at 42°C for exactly 50 seconds. Again, it is important that the tubes are pushed down in the rack so that the bottom of the tubes have optimal contact with the 42°C water. After the heat shock, immediately place the foam rack back in the ice water and incubate for a further 2 min.

9. Remove the foam rack from the ice water bath and place the tubes in the microtube rack on the bench. Add 250 μL of LB broth to each tube, close the cap and gently tap the "+pGLO" and "-pGLO" tube to mix the contents. Incubate the tubes for 10 min at room temperature. This step of the procedure allows the cells to recover from the heat shock treatment before performing the next part of the experiment. It also lets the cells that have acquired a pGLO plasmid begin to express the β-lactamase protein (for ampicillin resistance) before the cells are placed on plates that contain ampicillin.

10. Using a new sterile pipet for each tube, pipet 100 μL of the transformation and control suspensions onto the appropriate plates.

11. Spread the suspensions evenly around the agar plate by quickly sliding the flat loop surface back and forth across the plate surface. Turning the plate in a circular motion with your fingers while swishing the loop back and forth aids in spreading the bacterial suspensions evenly. Do not press down too firmly or you will gouge the surface of the agar plate. This can complicate both the growth and analysis of the bacteria on the plates. Remember to use a new sterile loop for each plate. Let the plates sit on the bench for 2-3 minutes to allow the suspensions to soak into the plate.

12. Stack the plates and tape them together. Label the tape with the group name and class period, if necessary. Turn the plates upside down (with agar at top of plate) and place in a 37°C incubator overnight. If an incubator is not available, the plates may be grown on the bench top for 2-3 days.

Sterilizing Procedures*: (cautionary information)

- Always wear tight fitting gloves
- When pulling out the plastic loop from the bag, only touch the handle, and make sure no part of the loop touches a contaminated surface
- When pouring agar plates, minimize amount of bubbles, and make sure the solution never comes into contact with any contaminated substance or surface
- Always use pre-sterilized petri dishes
- When inoculating bacteria, minimize air contaminants and any possible contaminations that would come into contact with the process
- Always use a clean microwave to heat Agar
- Wash hands before *and* after inoculation process
- Dispose of gloves in a proper biohazard waste container

*These procedures are ALWAYS followed when working with bacteria

Purpose of conducting the AP Biology Lab:

We found that conducting a bacterial transformation under sterile conditions with the ability to observe our own success or failure would greatly contribute to our learning and experience as a team. Conducting the experiments and methods together, and seeing our success through observing the activation of GFP, was a very rewarding experience which gave each and every one lab experience in transformation and sterilization methods. The lab as a whole greatly prepared us for transformation involving AgNPs.