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* **Day 1:** streak strains from stabs onto plates
* **Day 2:** grow strains from plates as liquid overnights
* **Day 3:** b-gal assays
* **Day 4:** calculations of units and comparison of class data (could also be Day 3 if time allows)

**Annotated Laboratory Procedure**

*TEACHERS: Note that "Part 1: Culturing Bacteria" can be done by the students or by you (the teacher) depending on how much time and preparation you intend to take on/delegate.**TEACHERS: Clean-up instructions. Provide containers at each work-station for student biological waste such as pipet tips, eppendorf tubes, spreaders, innoculating loops, and plates. Be sure to follow hazardous waste procedures as set forth by your school or municipality. Generally, it is safe to soak the material in each container with a 10% bleach solution for 2 hours. Materials can then be discarded into the regular trash. You can find more information about microbiology lab safety* [*here*](http://www.carolina.com/category/teacher%2Bresources/lab%2Bsafety%2Band%2Bchemical%2Bsafety%2Binformation/general%2Bsafety%2Binformation/nine%2Bsafe%2Bpractices%2Bfor%2Bthe%2Bmicrobiology%2Blaboratory.do)

**Part 1: Culturing Bacteria**

We will be receiving our bacteria with the plasmid already inserted. This culture may come in the form of a "stab" or "slant," a test tube with a small amount of bacteria on a slanted media, in which case you will have to streak out the bacteria onto a petri dish to continue the experiment. If the bacteria have arrived on petri dishes, you can proceed to "Day 2."

**Day 1:**

1. Using a sterile toothpick or inoculating loop, gather a small amount of bacteria from the stab and transfer it to a petri dish containing Luria Broth (LB) agar plus ampicillin medium.
2. Repeat with the remaining stab samples, streaking out each onto a different petri dish.
3. Place these petri dishes media side up in a 37°C incubator overnight.

**Day 2:**
*TEACHERS: The volume of cells you'll need to grow will depend on how you are setting up your student's work. If each student or student team is to test every strain, then 2.5 ml of each culture for each of them will be more than enough. If you would like students/student teams to share the cultures that are grown when they perform "Part 2: Beta-galactosidase assay," then insure that there is at least 1 ml of bacteria for every assay to be performed.*

1. Using a sterile inoculating loop or toothpick or pipet tip, transfer a bacterial colony from the petri dish to a large sterile culture tube containing 2.5 ml LB+Amp supplemented with 25 μL IPTG. This volume is more than enough for each strain that each student or team of students must grow.
2. Repeat for each strain you will inoculate.
3. Place the culture tubes in the roller wheel in the incubator at 37°C overnight. Be sure to balance the tubes across from each other to minimize stress on the roller wheel.

**Part 2: Beta-galactosidase assay**

**Procedure using a Spec 20**

With this assay you will determine the amount of beta-galactosidase activity associated with each sample of cells. As a class you should try to perform replicate assays of each sample (so each strain gets measured two or three times) and then pool your class data to gain some confidence in the values you measure. A data table is included to help you organize your assay, but you can make one of your own if you prefer. Note that the volumes here are given for spectrophotometers that use glass test tubes (13x100 mm). *TEACHERS: In advance of lab you should prepare the four solutions necessary to run these reactions. The* ***bicarbonate buffer*** *is made by mixing 1 g of Arm & Hammer baking soda into 50 ml of distilled or bottled water. The* ***ONPG*** *is made by vortexing 10 ml of distilled or bottled water with 40 mg of ONPG. This solution may need to be warmed in your hands or a 37° incubator to fully dissolve it. The* ***dilute dish soap*** *is made by mixing one “pump” or less of liquid soap in a 50 ml conical tube with 50 ml bottled or distilled water and then inverting to mix. Finally, the* ***soda ash solution*** *is prepared by mixing 5.3 g of soda ash with 50 ml distilled or bottled water. All solutions are stable on the bench or in the fridge for at least a month.*

1. Make 3.0 ml of a 1:10 dilution (300 μL of cells in 2.7 ml of bicarbonate buffer) of each cell sample.
2. If you made the dilution in glass spectrophotometer tubes, you can proceed to the next step. If not, you will need to transfer some of this diluted cell mixture to a cuvette or glass spectrophotometer tube. The exact amount to transfer will depend on the size of the cuvette you use. Your teacher will provide further instructions.
3. Measure the Absorbance at 600 nm (OD 600) of this dilution. Record the value **X 10** in the data table. This is the density of the undiluted cells. If you do not have a spectrophotometer and are using Turbidity Standards instead, follow the instructions in the next section.
4. You can now dispose of these dilutions and tubes as instructed by your teacher.
5. Add 1.0 ml of bicarbonate buffer to 11 test tubes labeled B (blank), R (reference), and 1 though 9 (the samples). These are the reaction tubes.
6. Add 100 μl of the cells (undiluted) to each tube. Add 100 μl of LB to tube B, to serve as your blank.
7. Next you will lyse the cells by add 100 μl of dilute dish soap to each tube.
8. Vortex the tubes for 10 seconds each. You should time this step precisely since you want the replicates to be treated as identically as possible.
9. Start the reactions by adding 100 μl of ONPG to each tube at 15 second intervals, including your blank.
10. After 10 minutes, stop the reactions by adding 1 ml of soda ash solution to each tube at 15 second intervals. Ten minutes is sufficient time to provide results that are yellow enough to give a reliable reading in the spectrophotometer, best between 0.1 and 1.0. Usually this color is approximately the same as that of a yellow tip for your pipetman. Don't be surprised when the soda ash makes the reactions look more yellow. The reactions are now stable and can be set aside to read another day.
11. If you conducted the reaction in glass spectrophotometer tubes (your teacher will tell you this), you can skip to the next step. If not, you will need to transfer some of the reaction mixture from the reaction tubes to a cuvette or glass spectrophotometer tube. The exact amount to transfer will depend on the size of the cuvette you use. Your teacher will provide further instructions.
12. Read the absorbance of each sample tube at 420nm (OD 420). These values reflect the amount of yellow color in each tube. If you do not have a spectrophotometer and are comparing the color to paint chips instead, follow the instructions in the next section.
13. Calculate the beta-galactosidase activity in each sample according to the formula below.

*TEACHERS: If a microfuge is available, you can transfer some of the reaction mixture to a microfuge tube, spin the eppendorf tube for one minute, and then transfer that cleared solution to a cuvette to read the OD 420. However, the microfuge tube will not hold enough of the reaction mixture to read the absorbance using the larger glass tubes. If you must use the larger glass tubes or do not have a microfuge, you can skip this step, though allow time for the debris to settle. It is possible that the remaining cell debris will result in some negative values. These can be set to zero for calculation purposes.*

**Procedure if a Spec 20 is not available**

*TEACHERS: If a Spec 20 is not available, your students can conduct the protocol presented below. While these results will not be as precise, they do provide accurate data for analysis.*

**Estimate the OD 600**

The OD 600 can be estimated using Turbidity Standards. This method uses suspensions of a 1% BaCl2 in 1% H2SO4 at various concentrations and is modeled after the [McFarland Turbidity Scale](http://www.microbiol.org/white.papers/WP.OD.htm). These suspensions appear visually similar to suspensions of various populations of *E coli*.

1. Following your teacher's instructions, obtain small clear test tubes containing the turbidity standards. The tubes should contain enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom. Make sure each tube is properly labeled with its turbidity standard number. If you are filling the tubes from stock bottles of the standards, use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
2. Place them in a test tube rack that allows you to view them from the side. Use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
3. On a blank index card or paper use a marker to draw two thick black lines. These lines should be within the height of the standards.
4. Place the card with the lines behind the standards.
5. Make 3.0 ml of a 1:10 dilution of each cell sample, using bicarbonate buffer as the diluent.
6. To compare your bacterial cultures to the standards, you will need to place the bacterial sample in a test tube of the same size and equal volume as the standards. Be sure to label these sample tubes.
7. Place the sample tube next to the standard tubes. You should move the sample to compare it to the standard tubes with the most similar turbidity. You can make this assessment more precise by looking for a standard that most similarly obscures the black lines on the background card.
8. Use the table below to determine the comparable OD 600.
9. 1 OD 600 unit equals approximately 1 x 109 cells.



**Estimate the OD 420**

*TEACHERS: For this procedure it is not necessary to use a centrifuge.*

* The OD 420 can be estimated using Benjamin Moore paint chips. Color chips will be provided by your instructor.
1. Once the reactions have been stopped with soda ash solution, allow the debris to settle for a few minutes and then compare the solution's meniscus to the color samples provided. The approximate OD 420 value that corresponds to each color is listed in the table below.
2. Calculate the beta-galactosidase activity in each sample according to the formula below.

