

pGLO TRANSFORMATION TEACHER'S GUIDE

Class time needed: one day for introduction, one day for transformation, one day for data collection and analysis, one day for extension or wrap-up

Additional equipment needed: incubator, hot water bath, ice

PREPARATION:

Three to four days (or more) before lab: (The plates MUST dry out before you can use them)

1. Prepare LB agar
 - ◇ add 500 ml distilled water to a 1000ml flask
 - ◇ add LB agar tablets and stir (dissolve MUCH easier if crushed before adding)
 - ◇ 1 tablet for every 50 ml water
 - ◇ heat in microwave until boiling Swirl every 30 seconds or so (or do this VERY CAREFULLY on a hot plate)
 - ◇ should look clear when done
 - ◇ Allow to cool to 50°C DO NOT ADD ANYTHING TO THE AGAR WHILE HOT
2. Label plates—save the plastic sleeves for refrigerator storage.
 - ◇ label 8-16 plates LB (8 is just enough-- Plan to make a few extra plates--there is always some contamination),
 - ◇ label 2 plates LB starter, 16 plates LB/AMP, and 8 plates LB/AMP/ARA
 - ◇ **NOTE:** this is for 8 groups—there is plenty of materials in one kit to do at least double
3. Pour plates
 - ◇ when agar has cooled, pour the LB plates
 - ◇ add the vial of ampicillin, stir gently
 - ◇ pour the LB/AMP plates
 - ◇ add the vial of arabinose, stir gently
 - ◇ pour the LB/AMP/ARA plates
4. Store plates right side up (with lids on top) for two days to harden. After two days, the plates may be stored inverted for up to 3 months in the refrigerator, but be careful not to freeze.

One to Two days before the lab:

1. Streak starter plates
 - Touch sterile loop to E. coli culture in stab
 - ◇ Use sterile loop to streak starter plates, then incubate at 37°C for 24 hours or 2 days on desktop
2. Label tubes for each lab group: (can be stored in refrigerator for some time)
 - ◇ label TS and aliquot 1 ml of transformation solution
 - ◇ label LB and aliquot 1 ml of LB broth
3. Copy instructions for students—Quick Guide

Day of Lab:

1. Check starter plates for colonies
2. Set up each lab station with:
 - ◇ 1 tube of TS and 1 tube of LB
 - ◇ float rack
 - ◇ 2 empty microtubes
 - ◇ 1 waterproof pen
 - ◇ 4 disposable micropipettes**THIS WILL CHANGE TO MICROPIPETTES WITH TIPS BY THE END OF FEB 2003
 - ◇ sterile inoculating loops (I make them come and get them from me as needed)
 - ◇ tape for sealing plates after inoculation
 - ◇ 1 LB plate
 - ◇ 2 LB/AMP plates
 - ◇ 1 LB/AMP/ARA plates
 - ◇ cup with ice and water

****You will also need one incubator set at 37°C, and one warm water bath at 42°C

Clean up

All materials and equipment that has come in contact with the bacteria can be placed in a fresh bleach solution (10%) for 20 minutes for sterilization. You can have a cup with bleach at every lab station for the loops and pipettes, and a classroom container for the plates at the end of the lab. Pour excess liquid down the drain, then the plates can be treated as normal trash.

All details of the procedure and student instructions can be downloaded from the Bio Rad website at : <http://www.biorad.com> Go to Life Science Research and look for the appropriate lab. The only changes are that the ampicillin and arabinose we use is already hydrated and frozen, we use LB agar tablets (1 tablet/50 ml of water), and the E. coli comes in a stab culture, not lyophilized.