10/03/2017

Tuesday, October 3, 2017 12:26 PM

Who's in lab: Salma (11-1), Qingxi

500 mL of LB Media

- 5.11 g Tryptone
- 2.50 g Yeast Extract
- 5.03 g NaCl
- 500 mL dI water

For Qingxi:

50 mL Overnight cultures of 1539/1853

**Take out Kan, Cam, Amp, and LC1853/1539 from the –20C and put them on ice to thaw before pipetting so that they all can thaw while you are pouring LB into the tubes (see LC1539/LC1853 step 2 for info on where to find these in freezer)

Preliminary Step: Pipet 50 mL into each of the 2 large conical tubes.

1. Grab the 1 L bottle of freshly autoclaved LB media broth off of the shaker (shaker is next to the water baths) and find the 2 large conical tubes w/ green caps (in the white paper holder on top of the purple rack of large conical tubes).
    a. Conical tubes should be labeled LC 1539 and LC1853 and have today's date on them
2. Light the bunsen burner, make sure the remaining part of this protocol is completed near a bunsen flame so that the air around the benchwork is sterile
3. Remove the foil and pass the lip of the glass bottle containing the LB over the flame to sterilize it. Make sure to put the foil cap loosely on anytime the LB bottle is not being used (even if for a minute)
4. Carefully pour ~50 mL of LB into one of the conical tubes making sure not to let the LB overflow, close the green capped tube after filling it (look at the markings on the side of the tube to know how much to pour)
5. Flame the lip of the glass bottle once again to sterilize
6. Carefully pour ~50 mL of LB into the other conical tube
7. Flame the glass bottle and cover it w/ foil
8. Set the two green tubes aside

LC1539 (Kan/Cam resistance)

50 mL LB
+ 50 uL Kan (50mg/mL)
+ 75 uL Cam (33mg/mL)
+ 100 uL LC1539

1. Fill styrofoam square w/ ice, square is on benchtop near the pens, ice is in autoclave room
2. Take out Kan antibiotic, Cam antibiotic and LC1539 from –20C freezer and put on ice to thaw
   **All tubes needed are on the purple rack at front of –20 freezer;
    i. Cam is in the 10 mL tube w/ green cap, Kan is in small 1 mL centrifuge tube, LC1539 is in the small cylindrical tube w/ flat top and bottom
3. Once the antibiotics are partially thawed (after ~5-10 min on ice), pipet 50 uL of Kan into the appropriate tube of 50 mL LB (labeled "LC1539")
4. Pipet 75 uL Cam (conc 33 mg/mL) into the "LC1539" tube
5. Invert 3 times to mix
6. Pipet 500 uL LC1539 into "LC1539" tube
7. Gently invert once to mix
8. Set to incubate on the shaker in the warm room at 250 rpm
9. Return LC1539 and antibiotics to -20C

LC1853 (Amp resistance)

50 mL LB
+ 25 uL Amp (100 mg/mL)
+ 100 uL LC1853

1. Take out Amp antibiotic and LC1853 from –20C freezer and put on ice to thaw
   **All tubes needed are on the purple rack at front of –20 freezer;
   i. Amp is in the 10 mL tube w/ green cap, LC1853 is in the small cylindrical tube w/ flat top and bottom
2. Once the antibiotics are partially thawed (after ~5-10 min on ice), pipet 25 uL of Amp into the appropriate tube of 50 mL LB (labeled "LC1853")
3. Invert 3 times to mix
4. Pipet 500 uL LC1853 into "LC1583" tube
5. Gently invert once to mix
6. Set to incubate on the shaker in the warm room at 250 rpm
7. Return LC1853 and antibiotics to -20C