GROWING BACTERIA CULTURES: GENERAL

1. L Broth prep:
   - There are many different broths for growing bacteria. For most protocols performed in this laboratory we use L broth (LB) as media for growing bacteria cells in solution and/or on plates
   - We use LB tablets
   - 1 tablet / 50ml dH_2O
   - In a Erlenmeyer flask mix water and tablets
   - Cover top with a layer of tin foil
   - Autoclave flask
   - Cool broth before addition of antibiotics

2. Agar Plates for growing bacteria
   - 7.5g Agar
   - 500ml LB (or 500ml H_2O + 10 tablets LB)
   - Mix together in an Erlenmeyer
   - Tin foil top and autoclave
   - Let cool to about 50°C then add antibiotics
   - Pour agar into Petri dishes just to cover bottom of plate

3. Addition of Antibiotics
   - In this lab we mostly use Ampicillin [0.1mg/ml]
   - The stock solutions of Amp [25mg/ml]
   - Example of calculation

     300ml culture: $C_1V_1 = C_2V_2$

     (300ml)(0.1mg/ml) = (Xml)(25mg/ml)

     X= 1.2mls amp in 300ml LB

4. Overnight Cultures (for plasmid preps)

   - Add 5ml of LB broth to a 15ml conical tube.
   - Add 20ul ampicillin [stock 25mg/ml] [final 100ug/ml]
   - Pick a colony from the plate with loop and shake loop in LB amp broth or add 200µl bacterial glycerol.
   - Repeat for all plates that DNA will be obtained from.
   - Place all the tubes in the warm room and shake at 250rpm's overnight.

5. Glycerols
   - Inoculate 5ml of LBroth (w/o antibiotics) with 300µl of an overnight culture
   - Incubate with shaking at 37°C room for about 1-1.5 hour (mid-log phase)
   - Add equal amount of sterile glycerol solution (5ml) invert mix.
     - 65% glycerol
     - 0.1M $MgSO_4$
     - 0.025M Tris-Cl (pH 8)
   - Aliquot 1ml of bacteria glycerol into a cryovials
   - Store -80°C

   NB: make 10 glycerols for each new plasmid generated. This stock is used for inoculating for plasmid preps