

## Making Glycerol Stocks of New Plasmids

Once you have successfully cloned a new plasmid, it's time to make a glycerol stock of it for safe keeping!

### Materials:

2 ml screw-top cryotube

60% (v/v in water) pre-sterilized glycerol

Luria Broth or Terrific Broth

Culture Tube

Liquid N<sub>2</sub>

### Procedure:

#### *Day 1*

1. Enter the information of your plasmid into the Wang Lab Strain List google sheet, assign a number to your plasmid.
2. Transform your plasmid into DH5 $\alpha$  cells using 1-2 $\mu$ l of mini-prepped DNA plasmid that has been sequenced and confirmed to contain the correct mutation/insertion. (See transformation protocol for this.)

#### *Day 2*

3. Pick a colony from transformation plate, use this to inoculate LB or TB containing appropriate antibiotic and shake for 16-20 hours at 37°C. (Use 20 ml of media if you also need to miniprep the plasmid, and just 5 ml of media if you only need to make a glycerol stock.)

#### *Day 3*

4. Label a 2 ml screw-top cryotube with the following information: plasmid number on the top; plasmid number, plasmid name, initials and date on the side.
5. Add 0.75 ml of overnight cell culture and 0.25 ml of 60% sterile glycerol to the tube for a final concentration of 15% glycerol.
6. Freeze in liquid nitrogen, store at -80°C in the Strains box.