Glycerol Stocks

Every construct, whether created in the Stockinger lab or imported from another lab into the Stockinger lab, must be put away and maintained as a lab glycerol stock.

A. To create a glycerol stock:

(1) Pick a **SINGLE** colony of the clone off of a plate and grow an overnight in the appropriate selectable liquid medium (e.g., LB amp, SC Δleu etc.).

(2) Make a label (clone ID # and date) for the construct (use the time tape). Place this label onto a sterile screw cap microcentrifuge tube.

(3) Add 0.5ml of the o/n culture to 0.5ml of 80% sterile glycerol in the sterile screw cap microcentrifuge tube*.

(4) Screw a lid onto the tube and write the clone ID # on the lid of the tube.

(5) Vortex.

(6) Freeze the glycerol stock at –80°C.

(7) Enter any and all pertinent information (host strain, vector, cloning site(s), selection criteria, date prepared, origin/source and/or reference, and any other important information) regarding this accession into the lab stock collection book. Also include a map or sequence if possible.

(8) If this is a plasmid construct, perform a mini prep on a portion of the same culture medium that was used to prepare the glycerol construct in order to verify that it is what you think it is.

(9) Store the mini prep DNA away in the lab DNA stock box in the –20°C.

B. To streak out from a glycerol stock:

(1) Determine the location (-80°C tower #, box #, row #) of the construct.

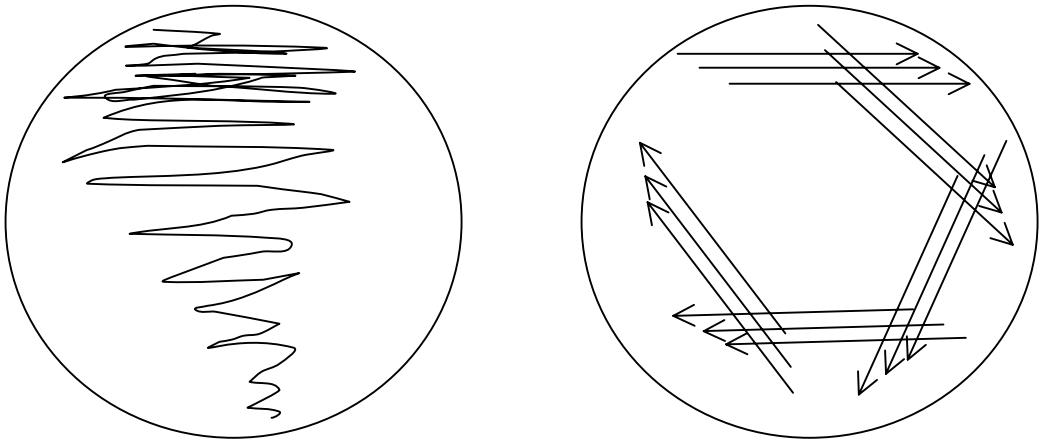
(2) Take the tube to the place that you intend to streak the clone out (e.g., your bench or the laminar flow hood, etc).

(3) Flame a metal inoculating loop until it is red hot.

(4) Scrape off a portion from the top of the frozen glycerol stock and streak it onto your plate.

(5) Return the construct to the −80°C. **DO NOT LET THE GLYCEROL STOCK THAW! FREEZE THAW CYCLES WILL KILL THE CLONE!** If you need to streak out multiple constructs take out only one or two from the freezer at a time.

(6) Flame the metal inoculating loop a second time, cool it by inserting it into the agar of the plate and finish streaking out the clone so that it will be possible to isolate single colonies. Examples of streaking patterns to obtain single isolated colonies:



Materials and Stocks needed:

1. Sterile screw cap microcentrifuge tubes.
2. Appropriate media, liquid & solid.
3. 80% Glycerol

   (1) Glycerol 80ml
   (2) dH_2O 20ml
   (3) Autoclave

Notes:

The reason for using 80% glycerol is that it is simply much easier to handle than is 100%).

*Certain antibiotics in the medium should be removed first as they are supposedly toxic over time. Tetracycline is one such antibiotic. To do this: spin the culture down and resuspend in straight LB with no antibiotic).