

Protocol for maintaining glycerol stocks

Our system for maintaining plasmids has vastly improved in the past few years but we will now store plasmids via glycerol stocks. It's easier for everyone and is a more efficient way of maintaining the integrity of our valuable constructs!

A contaminated stock is useless so please use proper aseptic technique whenever handling stocks!

Making Glycerol Stocks:

Prepare overnight cultures (1-2 ml) in LB with appropriate antibiotic. You can also just use an ml of culture from mini or maxi preps as long as they're not contaminated and are properly screened.

1. Label screw-cap 2 ml cryo-vials with as much info as possible including:
 - Initials and date
 - Vector and ORF
 - E. coli strain
 - Antibiotic resistance
2. Deposit 400 ul of 50% filter-sterilized glycerol into tubes. Final concentration of glycerol is typically 10-15%
3. Add 1 ml of overnight culture and mix.
4. Optional: Flash freeze on a mixture of dry ice with ethanol.
5. Store glycerol stock at -80 C.
6. **Fill out as much information possible on the plasmid information sheet and give to Heather for entry into the database.**

****Whenever possible, accurate electronic maps should be maintained and posted on Gobo! I use VectorNTI:**

<https://catalog.invitrogen.com/index.cfm?fuseaction=userGroup.home>

DNA strider is fine too, but does not run on PCs or newer Mac operating system