General standad protocol for preparing glycerol stocks for long term storage at -80° C

Reagents/equipment:

1. Sterile (autoclaved) 50% glycerol solution in Aqua dest. Note, glycerol is rather viscous, so pour the stock glycerol directly into a bottle and estimate the volume with your eye along the volume scale. Add Aqua dest. Prior to aliquoting the 50 % glycerol solution, add a magnetic bar and heat the solution on a magnetic stirrer. After heating, the solution can be easily pipetted and aliquoted into cryo tubes with screw caps. For example, aliquot 300 µl glycerol solution into 2 ml tubes. Autoclave.

2. Freshly grown cells:
    a. If clones, on LB medium with antibiotics (liquid or plates)
    b. If strains, on appropriate medium (liquid or plates).

3. Other items: Trays, pipettes, sterile pipette tips, freezer.

Procedure:

1) Ensure that your cells have grown up well and are in exponential growth phase prior to harvest.

2) Options for harvest:

    a. If on agar plates, scrape of biomass with a sterile inoculation loop and dissolve in a sterile liquid medium (the same as used for the agar plates or similar). Vortex and pipette aseptically into sterile cryo vials with 50 % glycerol so that the end concentration reaches between 10-15 %. For example, for 2 ml cryotubes with 300 µl 50 % glycerol solution, add 700 µl liquid sample. Vortex.
    b. If in liquid solution, vortex carefully your sample, and then simply aliquot an appropriate amount to reach an end concentration of 10-15 %. For example, for 2 ml cryotubes with 300 µl 50 % glycerol solution, add 700 µl liquid sample. Vortex
    c. Vortex and place at -80 °C (no need to freeze in liquid N2).

Comment: Prepare replicates (minimum duplicates, better triplicates or more). Store in separate boxes in different freezers.

Be sure to enter your samples appropriately into inventory lists. Samples not labeled properly run the risk of getting thrown away!

When inoculating from glycerol stocks, do not thaw up the whole tube. Keep the tube on ice and withdraw sample with a sterile inoculation loop only from the top part of the frozen solution. After this, the tube can be frozen again at -80 °C.

Make notes of how often you thaw up your glycerol stocks (e.g. make strokes on the tube or record in your protocols).

Note that not all strains tolerate freezing in glycerol at -80 °C. Consider also other long term storage options (see e.g. Tindall. Vacuum-drying and cryopreservation of prokaryotes. Methods Mol Biol. 2007;368:73-97; and Prakesh et al., Practice and Prospects of Microbial Preservation, FEMS Microbiology Letters, early view manuscript DOI: 10.1111/1574-6968.12034.