Freezer Stocks

Version 1.5

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Glycerol Stocks

1. Grow single clone / population in MSW+D-Gluc. for ~2 days
2. Prepare two replicate stocks (one working and one backup stock) using one of the following recipes (all result in final glycerol concentrations of about 20%; the choice comes down to personal preferences):
   - Add 500 µL glycerol 60% and 1000 µL liquid culture to cryotubes
   - Add 400 µL glycerol 80% using a multipipette with the 10 mL tip (one full tip is enough for 33 cryotubes) and 1000 µL liquid culture to cryotubes
3. Mix stocks well by vortexing and inverting just before freezing at −80°C
4. Add new boxes to spreadsheet on freezer door and keep track which stocks are where

Growing up strains from glycerol stocks

Sometimes strains have a hard time growing after being frozen for too long. When trying to grow up such a "difficult" strain from freezer stocks, consider the following protocol, especially for high temperature adapted strains.

1. Prepare MB agar and pour 20 mL plates
2. Streak out freezer stocks onto MB plates and incubate at 15°C or 28°C (the latter for high temperature adapted strains) for 1-2 days.
3. Pick three colonies per strain and inoculate 2 mL MSW+D-Gluc. with it. Incubate at a temperature closer to the highest one the strain is adapted to (e.g. 27°C or 28°C when working with a strain adapted to 30°C) for ~2 days.
4. Use strains as usual, for example to make copies of the stocks