Cryopreservation Protocol
Be especially mindful of sterility while preparing cryostocks

1. Transfer 1 mL of freshly saturated culture (see instructions below) of each sample to be frozen to a labeled eppendorf tube, and centrifuge at 12000 rpm for 1 minute (rich medium) or 2 minutes (minimal medium).
2. Remove supernatant by pipetting, and resuspend the cell pellet in 500 μL of fresh LB medium (via vortex mixing or repeated pipetting).
    a. *Resuspend cell pellet in LB medium BEFORE adding glycerol solution! Cell pellets are hard to resuspend in glycerol solutions.*
3. Add 500 μL of sterile 50% v/v glycerol solution to each eppendorf tube, and mix the contents by repeated pipetting.
4. Pipette contents of each eppendorf tube (~1 mL) to a sterile freezer tube. Label the tubes with strain and date.
5. Place freezer tubes in "J. Minty Backups" storage box in the -80C freezer. Be sure to record location of tubes in box.

Culturing Guidelines

Freshly saturated cultures (generally 12-24 hours old) will have best viability. Guidelines for culturing different strains:

Strain	**Medium**	**Temperature**	**Notes**
EcNR# and EcHW# strains and progeny	LB	30C	Evolved lines should be handled differently (see below)
K12, DH5α	LB	37C	
Strains harboring pLOI297, pPro24(S) series plasmids	LB + 100 μg/mL ampicillin	Depends on host strain	Antibiotics are essential for maintaining plasmids
Isobutanol tolerant lines evolved on xylose	NX50 + 0.5% w/v isobutanol	30C	EcNR1 is parent strain
Isobutanol tolerant lines evolved on glucose	NG50 + 0.5% w/v isobutanol	30C	EcNR1 is parent strain; glucose #2 line grows slowly

*notes
1. Got 3- 15 mL falcon tubes from the Lin lab
2. Got 2 – 2 mL pipettes and 1 5 mL pipette from the Lin lab
3. Went to 1230 ERB and met Kevin

4. Using sterile technique, added 2 mL to each of the 2 falcon tubes in step 1
5. Got K12 cultures from 6/29/2010 out of the incubator/ shaker
6. Prepared the K12 for cryopreservation as per protocol (above)
7. Put the K12 in the iGEM box #53 (1 vial)
8. Put the other K12 in the -20C freezer in 1239
9. Got DH5alpha out of iGEM box #4
10. Using sterile technique, stabbed cryopreserved stock and pipette up and down in LB media from tubes in step 4
11.Put DH5alpha in incubator/shaker in 1230 for 24 hours