Protocols for maintaining Salmonella culture
UBC iGEM 2021

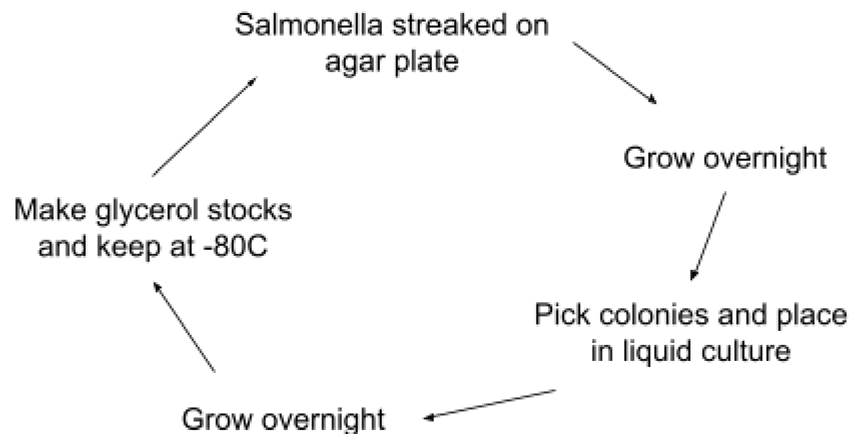
To make glycerol stocks - for the strain as it was received (SL133):

1. From the streaked out plate, pick a colony and inoculate in 2ml LB with appropriate volume of kanamycin (or streptomycin) in round bottom culture tubes. Let grow overnight. (2ml LB volume is arbitrary; just needs to be more than 500ul for step 3).
2. Make 50% glycerol mix with 20ml glycerol and 20ml water, and store in a 50ml Falcon tube for future use (the 20ml volume is arbitrary).
3. Once grown and the tube is cloudy, add 500ul glycerol mix to 500ul of grown liquid bacterial culture into a 1.7ml epi tube. This makes 25% final glycerol volume (you said we should have a range of 15%-25%) and 1ml final volume in the epi tube.
4. Store the epi tube at -80C. This makes 1ml aliquots for storage of the strain.

To make aliquots for transformation - once the strain has been made competent:

1. Follow step 1 from above.
2. Once grown and the tube is cloudy, make 50ul aliquots into PCR tubes and store at -80C.

General workflow:



Inoculation

1. Add 2mL of LB broth into 14mL round bottom culture flasks.
2. Add streptomycin kanamycin (50ng/ul) and swirl the bottle.
3. Pick a colony with a pipette tip or colony picker, wiggle the tip into the broth and place in 30 or 37C shaking incubator.

4. **Day after:** check if the tube is cloudy, if so ready to make into a glycerol stock (next protocol).

Making Salmonella glycerol stock aliquots

Glycerol stocks are long-term bacteria storage, and is the way our E. coli competent cells are stored in the -80C freezer. We can keep our Salmonella the same way, and use them for transformation the same way we use E. coli. We can also streak out glycerol stocks into a plate to pick colonies and make more glycerol stocks.

1. Add 500ul glycerol mix* and 500ul bacteria from picking into epi tubes. This makes 25% final glycerol volume.
2. Place in the -80 C freezer.

*Glycerol mix is 50% water to glycerol; mix 20mL glycerol and 20mL water in a Falcon tube and store at room temperature for future use.

Making Salmonella plates

From plates, you can pick colonies to make more glycerol stocks.

1. Streak out a glycerol stock sample with a pipette tip onto a kanamycin or spectinomycin plate and let grow overnight at 37 or 30C.
2. From here can inoculate.

Things to keep in mind :

- 2ml glycerol stock at -80C
- Electroporate using the same protocol as E. coli
- **Once already made electrocompetent:** for transformation, 50ul aliquots at -80C.
- Electroporation almost the same but - (2.5 kV for *E. coli*, and 3 kV for *Salmonella*).
- SL1344 Δ*invA* Δ*ssaR* double mutant has antibiotic resistance to both streptomycin (50 ug/ml) and kanamycin (50 ug/ml)

Protocol for Electro-Transformation of Salmonella

Preparation of Cells

1. Inoculate 1L of L-broth with 1/100 volume of a fresh overnight culture.
2. Grow cells at 37C with vigorous shaking to an $O.D._{600}$ of 0.5 to 0.8 (the best results are obtained with cells that are growing rapidly; the appropriate cell density, therefore, depends on the strain and growth conditions).
3. To harvest, chill the flask on ice for 15 to 30minutes, and centrifuge in a cold rotor at 4,000 x g_{max} for 15 minutes.
4. Resuspend pellets in a total of 1L of cold water. Centrifuge as above.
5. Resuspend in 0.5 liter of cold water. Centrifuge as above.
6. Resuspend in ~ 20 ml 10% glycerol. Centrifuge as above.

7. Resuspend to a final volume of 2 to 3 ml in 10% glycerol. The cell concentration should be at least 3 X 10^10 cells/ml.
8. This suspension may be frozen in aliquots on dry ice, and stored at -70C. The cells are good for at least 6 months under these conditions.

Electro-Transformation

1. Chill the cuvettes and the sliding cuvette holder in ice.
2. Set the Gene Pulser apparatus to the 25 uF capacitor, 2.5 kV, and the Pulse Controller Unit to 400 Ω.
3. Gently thaw the cells at room temperature and place them on ice.
4. To a cold, 1.5 ml polypropylene tube, add 40 ul of the cell suspension and 1 to 5 ul of DNA in a low ionic strength buffer such as TE. Mix well and let sit on ice for approximately 1 minute.
5. Transfer the mixture of cells and DNA to a cold, 0.2 cm electroporation cuvettes, and shake the suspension to the bottom of the cuvettes.
6. Apply one pulse at the above settings. Thus should result in a pulse of 12.5 kV/cm with a time constant of 4 to 5 msec.
7. Immediately add 1 ml of SOC medium (at room temperature) to the cuvette, and gently but quickly resuspend the cells with a pasteur pipette.
8. Transfer the cell suspension to a 17 x 100 mm polypropylene tube and incubate at 37C for 1 hour. (Shaking the tubes at 225 rpm during this incubation may improve the recovery of transformants.)
9. Plate the appropriate aliquots on a selective medium.