

Chemically Competent Bacteria

- Streak out bacteria on LB plate (I use Ecoli XL1Blue, DH5alpha work too)
- Let grow o/n at 37°C

- Inoculate 5mL LB with a single colony and grow o/n at 37°C

- Dilute 5mL in 500mL LB in a 2L flask and grow at 37°C until $OD_{660} = 0.6$ (about 2 hours)
- Chill flask in ice waterbath for 10 min
- Spin down 3500 RPM for 10 min in 3 bottles
- Gently (pipette up and down without introducing air bubbles) resuspend cells in 25mL STB per bottle
- Spin down 3500 RPM for 10 min in 1 bottle
- Gently resuspend cells in 20mL STB
- Add 1.6mL DMSO and leave in 50mL tube overnight at 4°C

- Aliquot 200mL per tube and freeze in dry ice/EtOH bath or liquid nitrogen
- Store at -80°C

STB

10 mM	Pipes
15 mM	Calcium Chloride ($CaCl_2$)
250 mM	Pottassium Chloride (KCl)

- Mix these ingredients and pH to 6.7 with Pottassium Hydroxide (KOH).
- Ingredients will dissolve while adjusting the pH

55 mM Manganese Chloride ($MnCl_2$)

- Dissolve $MnCl_2$
- Filter and store at 4°C

Test with serial dilution of DNA with a range of 1000ng to 0.1ng.