

Making Competent Cells

Day 1:

1. Grow 5 mL LB culture of cells overnight at 37°C
For XL2-Blue add 12.5 ug/mL Tetracycline

Day 2:

1. Dilute overnight culture into LB
For XL2-Blue – 1mL of culture into 500mL LB + 12/5 ug/mL Tet + 15mM MgCl₂
For DH5a – 2mL of culture into 120mL LB
2. Grow cells at 37°C until OD₆₀₀ = 0.4
3. Pellet cells at 3000 rpm for 15 min at 4°C
4. Resuspend pellet in 150 mL cold MES A
5. Incubate on ice 20 min
6. Spin down at 3000 rpm for 15 min at 4°C
7. Resuspend pellet in 30mL of cold MES A + 15% glycerol
8. Aliquot (200-300uL) and snap freeze in liquid nitrogen

MES A (500mL)

100 mL – 50 mM MES Salts, pH 6.3 with KOH (0.9762 g)

2.77 g – CaCl₂·2H₂O

5 mL – 1M MnCl₂·4H₂O (0.98955 g)

ddH₂O to 500 mL

Make fresh, filter sterilize, wrap in foil to protect from light, and store at 4°C