

Preparation of competent BL21 bacteria

1. Dilute an overnight culture of cells @ 1:100 in 50 mL of LB + chloramphenicol
2. Incubate shaking at 37°C until $OD_{600} \approx 0.5$
3. Spin out cells at 4°C
4. Resuspend pellet in 15 mL ice-cold 0.1M $MgCl_2$
5. Spin out cells at 4°C
6. Resuspend pellet in 15 mL ice-cold 0.1M $CaCl_2$
7. Incubate on ice for 45 min
8. Spin out cells at 4°C
9. Resuspend pellet in 5 mL ice-cold 0.1M $CaCl_2$, 30% glycerol
10. Aliquot into eppi tubes and snap-freeze
11. Store a -80°C