

CsCl Maxi Prep

DAY 1

1. Spin 500mL o/n cultures for 15min @ 5K in (GSA rotor).
2. Resuspend pellet in 20mL solution I. Add 2/mg/mL (40mg) lysozyme. Leave @ RT 10min.
3. Add 30mL solution II. Invert gently to mix and leave on ice 5min.
4. Add 30mL solution III, mix thoroughly and put on ice 5min, then spin 15min @ 5K, 4°C.
5. Filter supernatant through large kimwipes on a funnel into a graduated cylinder. Add 0.6 volumes isopropanol, and let sit 20min @ RT.
6. Spin 15min @ 5K, RT. Remove supernatant and air dry pellet by inverting on paper towels ~ 10min.
7. Dissolve pellet in 3mL water, bring volume up to 4mL add 4.4g CsCl (1.1g/mL) and 200µL EtBr, mix well.
8. Spin in table top @ 3K for 10min (to get rid of most RNA)
9. Transfer to Beckman opti-seal tube (362185), balance tubes, and spin o/n in NVT65 rotor @ 60K, 20°C (lower temp will ppt CsCl).

DAY 2

10. Pull bands w/ 18 gauge needle and place in second Beckman tube. Make up volume w/ CsCl balancing soln and to a second spin for 4.5hrs.
11. Pull bands again and measure volume. Extract out EtBr in 15mL conical with water-saturated butanol 3-4X or until clear. Add 4mL butanol per extraction, vortex, and allow phases to separate. (EtBr and butanol will be in upper layer)
12. Add 2 volumes water equal to volume before extraction and 2 volumes of 100% EtOH (after adding H₂O), mix by inversion and spin in table top for 10min @ 3K at RT.
13. Wash w/ 10mL ice cold 70% EtOH, spin in table top 10min @ 3K, 4°C.
14. Dissolve Pellet in ddH₂O.

Solution I	stock	500mL
50mM Dextrose		4.5g
10mM EDTA	0.5M	10mL
25mM Tris-HCl pH 8.0	1M	12.5mL

Solution II (make fresh)	stock	200mL
0.2N NaOH	10N (50X)	4mL
1% SDS	10% (10X)	20mL

Solution III (for 500mL)
147.725g KOAc
57.5mL glacial acetic acid

CsCl Balancing Solution
100g CsCl in 100mL water.