MOLECULAR WORK

Plasmid CsCl-EtBr manual

- Ultra centri

1. Add 1.01 g CsCl per plasmid DNA sol 1 g
2. Add EtBr (10 mg/ml) 100㎕ per original DNA sol 5 g
3. Centrifuge (3000 rpm, 10 min, 4°C)
4. Transfer the red solution into the ultra tube without bubbles (tube vol 12 mL in case of 90 Ti rotor)
5. Seal the tube
6. Check tube weight (0.00 acuity)
7. Centrifuge (60,000 rpm, 17-18 hr, 4°C)
8. Obtain plasmid layer (red) using a syringe

- EtBr removal (organic solvents extraction)

9. Remove EtBr using water-saturated n-butanol or isoamyl alcohol
10. Centri and remove supernatant
11. Repeat to centre to remove EtBr (you could check EtBr leftover using UV)

- CsCl removal from DNA sol

12. Add 3 volume of DW into CsCl DNA sample
13. Add 8 volume EtOH
14. Stand the sample for 15t min 4 °C (No stand sample at -20 °C to -70 °C because CsCl could precipitate)
15. Centrifuge 13000 rpm, 15 min, 4°C (You could repeat EtOH wash and precipitation)
16. Remove the supernatant
17. Add 70 % EtOH and wash (centri)
18. Dry at RT
19. Dissolve sample in DW or TE.