MOLECULAR WORK

Plasmid miniprep (Alkaline manual)

Prepare for ice and Ice-cold Sol I and III solutions

Solution I (resuspension buffer):
50 mM glucose, 10 mM EDTA (pH 8.0) and 25mM Tris-HCl (pH 8.0)
Prepare sol I from standard stocks around 100 mL and sterilize by autoclaving and store at 4°C

Solution II (lysis buffer):
200 mM NaOH, 1% SDS
Prepare sol II fresh from stocks and use at RT (room temperature)

Solution III (Neutralization buffer):
3M potassium acetate, 5M acetic acid

5M potassium acetate, 60,0 mL
Gracias acetate, 11.5 mL
H_2O, 28.5 mL
Store the solution at 4°C

Procedure

1. Transfer 1.5 ㎖ of the culture to a microfuge tube (you could store the leftover culture at 4°C)
2. Centrifuge the tube for 30sec, max at 4°C (or RT also OK)
3. Remove the supernatant
4. Add 100㎕ of ice-cold sol I and vortex the sample
5. Add 200㎕ of sol II and invert the tube and stand in ice for 3-5 min
6. Add 150 ㎕ ice-cold sol III and invert the tube and stand in ice for 5min
7. Centrifuge (5min, max, 4°C and transfer the supernatant
8. Add equal volume of phenol : chloroform and vortex
9. Centrifuge (5min, max, 4°C) and transfer the supernatant
10. Add 2 volumes of 100% ethanol and 1/10 vol 3M NaOAc (pH5.2) and invert
11. To increase yield, store the sample at 20°C 2hr-O/N or-70°C over 30min
12. Centrifuge (30min, max, 4°C) and remove the supernatant
13. Add 70% ethanol 1㎖ and invert sample
14. Centrifuge 2 min
15. Discard the supernatant
16. Dry 4-5 min
17. Add 20㎕ D.W.

* You could skip phenol step for the purpose, and you could add TE buffer for the purpose.