MINIPREP EXTRACTION WITH ABC PROTOCOL

Why to do this :

1. To extract plasmidic DNA in order to verify or to ligate vrious parts

What you need :

1. Culture media : LB
   - 10 g bactotrypton
   - 5 g yeast extract
   - 5 g NaCl
   - 0,5 mL NaOH 10N
   - Qsp 1 L
2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

→ 50 µL antibiotic / 5mL medium

3. Apparatus : centrifuge, Speed Vac

4. Material

a) An overnight culture at 37°C of the transformed strain
b) 100 µL of the A solution
c) 200 µL of the B solution
d) 150 µL of the C solution
e) 1 mL of 100% alcohol
f) 1 mL of 70% alcohol
g) 50 µL of TE containing RNAse at 20 µg.mL-1

How to do :

5. Step 1

a) Take 1.5 mL of the culture into a micro tube
b) Centrifuge for 2 minutes at 12 000 rounds per minute (rpm)
c) Remove the supernatant
d) Add 100 µL of the A solution and homogenize by pipetting
e) Let the mixture rest for 5 min at room temperature

6. Step 2

a) Add 200 µL of the B solution and shake the tube by inversion (the mixture should become viscous)

7. Step 3

b) Add without waiting 150 µL of the C solution

c) Vigorously shake by inversion, then vortex a bit
d) Let the mixture rest for about 3-5 min (a white “blob” should appear)
e) Centrifuge for 7 min at 13 000 rpm
f) Prepare an tube containing 1 mL of 100% alcohol
g) When the centrifugation is over, take up to 400 µL of the supernatant (be sure not to take anything from the precipitate) and put it in the alcohol
h) Shake by inversion
i) Rest for 2 min

8. Step 4

j) Centrifuge for 10 min at 12 000 rpm
k) Remove the 100% alcohol then add 1 mL of 70% alcohol
l) Shake, vortex a bit, then centrifuge for 3 min at 12 000 rpm
m) Remove the alcohol as much as possible
n) Evaporate the remaining alcohol in the Speed Vac for about 5 min (10 maximum)
o) Add 50 µL of the TE + RNAse and homogenize