Plasmid preparation by alkaline lysis and SDS

Materials

- 2 mL microtube
- 1.5 mL microtube
- 6 mL overnight culture
- Pre-cooled Solution 1 (50 mM Glucose, 25 mM Tris-HCl, 10 mM EDTA, pH 8)
- Solution 2 (200 mM NaOH, 1% SDS)
- Solution 3 (5 M Potassium acetate, pH 4.8)
- Pre-cooled Ethanol 100%
- Pre-cooled Ethanol 75%
- Water, nuclease free

Apparatus

- Shaker
- Incubator

Method

1. Pellet 2 mL of an overnight culture in a 2 mL microtube and discard the supernatant. Repeat this step twice.
2. Resuspend the cell pellet in 100 µL of pre-cooled resuspension buffer (Solution 1) by vortexing and incubate on ice for 5 minutes.
3. Add 200 µL of the lysis buffer (Solution 2). Mix by inversion four times and incubate at 37°C for 5 minutes.
4. Add, immediately, 150 µL of the neutralization solution (Solution 3). Mix by inversion four times and incubate on ice for 5 minutes.
5. Centrifuge at 13000 rpm for 10 minutes.
6. Transfer the supernatant to a new 1.5 mL microtube. Be careful to not transfer the white pellet.
7. Add 800 µL of pre-cooled ethanol 100% and mix by inversion two times.
8. Centrifuge at 13000 rpm for 5 minutes and discard the supernatant. Be careful to not discard the small white DNA pellet.
9. Wash the pellet in 1 mL of pre-cooled ethanol 75% and invert two times.
10. Centrifuge at 13000 rpm for 5 minutes and discard the supernatant.
11. Let the pellet air dry (tube open) to allow the ethanol to evaporate.
12. Resuspend the DNA in 50-100 µL of water.
13. Store DNA at -20°C.

Considerations: You can add RNase A (100 µg/mL) into solution 1 to reduce the RNA contamination.