Miniprep Camilla V2

Overview :

This protocol is generously provided by Camilla.
It is based on Plasmid DNA Purufication by Alkaline Lysis with SDS.
Bacteria are lysed by treatment with a solution containing sodium dodecyl sulfate (SDS) and NaOH (SDS denatures bacterial proteins, and NaOH denatures chromosomal and plasmid DNA). The mixture is neutralized with potassium acetate, causing the plasmid DNA to reanneal rapidly. Most of the chromosomal DNA and bacterial proteins precipitate (as does the SDS, which forms a complex with potassium) and are removed by centrifugation. The reannealed plasmid DNA is then concentrated by ethanol precipitation.

Procedure :

DAY 1

1. Inoculate 1 colony or few µL of *E.coli* DH5-alpha in 5mL of LB + Antibiotic
2. Shake at 180 rpm overnight at 37°C

DAY 2

1. Centrifuge 5mL culture, 1 min, 8000 rpm (eliminate last drop by centrifugation)
2. Add 100 µL sterile H20 with RNAse A 0,1mg/mL (store at 4°C)
3. Add 200 µL 0,1 M NaOH 1% SDS (Solution II) => invert carrefully 4-5 time, **2 min max** (freshly prepared)
4. Add 150 µL sol III (5M KOAc ph 5.5)
5. Put on ice for 10 min
6. Centrifuge for 15 min, 14000 rpm, 4°C
   (recover the supernatant carefully in a fresh tube - 400 µL)
7. Add 500 µL Isopropanol and mix
8. Centrifuge for 15 min, 14 000 rpm, 4°C (discard the supernatant)
9. Wash with 70% cold Ethanol (500µL)
10. Centrifuge for 5min, 14000 rpm, 4°C (discard supernatant)
11. Centrifuge for 1 min (discard last drop and dry the pellet for a few minutes)
12. Resuspend in 50 µL H2O

Solutions :

- H_2O + RNAse 0,1 mg/mL : 3 µL stock RNAse A 34 mg/mL in 1 mL H2O
- Sol II (0,1 M NaOH 1% SDS) : 1 vol 1 M NaOH + 1 vol 10 % SDS + 8 vol H2O
- Sol III (5 M KOAc ph5.5) pour 200 mL final : 120 mL 5 M KAc + 23 mL AcOOH (Acid glacial) + 57 mL H20 **=> Autoclave**