MINI PREP PROTOCOL

Buffers:

P1 = 50 mM Tris and 10 mM EDTA, pH 8.0-0.6 g Tris base and 0.37 g Na_2EDTA, add 80 mL dH2O and pH to 8.0 then QS to 100 mL with dH2O.
Note: 50 mL Final Volume = 5mL of 100 mM EDTA plus 0.3 g of Tris add dH2O to 40 mL, then pH to 8.0 with conc. HCl and QS to 50 mL.

P2 = 0.1 M NaOH and 1% SDS – 0.8g pf NaOH pellets and 5 mL 20% SDS, QS to a final volume of 100 mL.
Note: 50 mL Final volume = 1 mL of 10 N NaOH and 5 mL of 10% SDS.

P3= 1.5 M Potassium Acetate, pH 5.5 – 14.74g of Potassium Acetate and 40 mL dH2O, pH with acetic acid to 5.5 (~6-7 mL) then QS to 50 mL with dH2O.

1. Prepare 13 mL tube with 2 mL of LB + 2 uL Amp (stock 100 mg/mL).

2. Pick colony with sterile yellow pipette tip, drop tip into tube and mix.

3. Place tube at 37°C at ~300 rpms, overnight.

4. The next day transfer 1.5 mL of media to a 1.5 mL microfuge tube and centrifuge at 14,000 rpms for 2 minutes.

5. Discard the resulting supernatant.

6. Resuspend the pellet in 100 uL of ice cold P1 Buffer and place on ice 5-10 minutes.

7. Add 100 uL of P2 Buffer and incubate @RT for 5-10 minutes.

8. Add 100 uL of P3 Buffer and incubate on ice for 5-10 minutes.

9. Centrifuge at 14,000 rpms for 30 minutes @ 4°C.

10. Transfer the supernatant to fresh 1.5 mL microfuge tube.

11. Add 900 uL ice cold 100% EtOH, vortex briefly.

12. Incubate at -20°C for 10 minutes.

13. Centrifuge at 14,000 rpms for 30 minutes at 4°C.

14. Discard the resulting supernatant, wash the pellet with 500 uL of 70% EtOH.

15. Centrifuge at 14,000 rpms for 5 minutes.

16. Discard the resulting supernatant and air-dry pellet for 20-30 minutes.

17. Resuspend the pellet in 50 uL TE – Rnase (1u/100uL TE) and incubate for 15 minutes @ 37°C.