Mini prep protocol

Buffers:

P1 (keep in 4°C)

- 50 mM Tris pH 8
- 10 mM EDTA
- 100 mg/L RNaseA
- 43 mg/L Thymophthalein (make a 1:1000 stock in 100% ethanol)

P2 (lysis buffer)

- 200 mM NaOH
- 1% SDS

N3 (neutralization buffer)

- 4.2M guanidine hydrochloride (Gu-HCL)
- 0.9M potassium acetate pH 4.8 (titrate using acetic acid)

PB

- 5M guanidine hydrochloride (Gu-HCL)
- 30% 2-propanol

PE

- 10 mM Tris pH 7.5
- 80% ethanol

Protocol

1. Grow culture overnight at 37°C
2. Harvest culture by centrifugation 4000g, 10 min
3. Discard supernatant
4. Resuspend pellet with 250 µl P1 buffer and move to an eppendorf tube
5. Add 250 µl P2 buffer and rotate the tube a few times. Solution should turn blue and be clear and homogenous
6. Add 350 µl N3 buffer and rotate tube a few times. A white precipitate should form. Continue turning until the blue color disappears completely
7. Centrifuge at 10,000g, 10 min
8. Use a P1000 pipet to carefully move the supernatant to a spin column
9. Centrifuge 10000g, 1 min and discard waste
10. Add 500 µl PB buffer, centrifuge 10000g, 1 min and discard waste
11. Add 750 µl PE buffer, centrifuge 10000g, 1 min and discard waste
12. To dry the spin column centrifuge 10000g, 2 min
13. Transfer the spin column to a properly labeled eppendorf
14. Apply 50 µl of either nuclease-free water, EB, or TE buffer
15. Elute by centrifuging 10000g, 1 min
16. Measure concentration using the nanodrop, blanking with the same buffer used for elution