"Dirty" *E. coli* minipreps

1. Pick single colony to 3 mL LB+amp (or other antibiotic)

2. Grow O/N with shaking at 37°C

3. Add 1.5 mL of culture to an eppendorf tube, spin 1 min to pellet cells

4. Pour off media. (Yield may be greater if you freeze the pellet at -20°C at this step).

5. Resuspend pellet in 200 µL cold buffer P1 by pipetting up and down.

6. Add 200 µL room temp P2, mix by inversion. Don't let the cells sit in P2 for more than a minute of two before going to step 7.

7. Add 200 µL P3, mix by inversion

8. Spin at max speed for 10 min, label tubes for next step.

9. Pipette the supernatant into a new tube, avoid the white stuff.

10. Add 600 µL isopropanol and mix by inversion.

11. Spin at max speed for 15 min.

12. Wash DNA pellet with 500 µL 70% EtOH by dribbling it down the side of the tube opposite the pellet. Carefully pour off the EtOH.

13. Air dry for ~5 min, resuspend the pellet in 25-50 µL TE.

Buffer recipes
These are the buffers that come with Qiagen minipreps kits, but we can make our own too:

P1 (resuspension)
1. Dissolve 1.21 g Tris and 0.774 g Na_2EDTA in 160 mL dH_2O
2. pH to 8.0 with HCl
3. Bring to 200 mL with dH_2O
4. (optional but recommended: add RNaseA to 100 µg/mL to small aliquots)

P2 (lysis) – you should make this fresh
1 mL 20% SDS
400 µL 10 N NaOH
18.6 mL dH_2O

P3 (neutralization)
1. Dissolve 58.9 g KOAc in 100 mL dH_2O
2. pH to 5.5 with glacial acetic acid (~22 mL)
3. Bring to 200 mL with dH_2O