Tsai Lab Plasmid MiniPrep Protocol

- Don't over-grow bacteria: **8 h/3 ml** (preferred, daytime culture) or 12-16 h/5 ml with vigorous shaking (overnight culture, shorter is better). DO NOT grow longer than 16 h. Bacterial pellets can be stored at -20°C until use.
- Use 1-1.5 ml cultures for high copy-number plasmids; do "double load" for low-copy plasmids (e.g., binary vectors).

Buffers and Solutions:

P1 = 50 mM Tris-HCl, pH 8; 10 mM EDTA; 50 µg/ml RNaseA, store at 4°C
P2 = 200 mM NaOH; 1% SDS, **make fresh from stock solutions each time (good for a week).**
N3 = 4.2M Guanidine-HCl; 0.9 M KOAc, pH 4.8, store at 4°C (Note: make Gu-HCl stock in 8M)
PB = 5M Guanidine-HCl; 30% isopropanol
PE = 10 mM Tris-HCl, pH 7.5; 80% ethanol (pH is important for elution)

Protocol:

1. Resuspend bacterial pellet in 250 µl ice-cold P1 by pipetting up and down.
2. Add 250 µl P2 buffer, gently invert the tube 3-5 times to mix. **No incubation**.
3. Add 350 µl N3 buffer, gently invert the tube 3-5 times to mix. **No incubation**.
4. Centrifuge at top speed for 5 min at room temp.
5. Transfer 750 µl supernatant to a DNA column, spin 1 min.
6. Wash with 750 µl PB, spin 1 min. *
7. Wash with 750 µl PE, spin 1 min. Repeat this step for "double-load" samples. *

* Let buffer stand 1-2 min before centrifugation will remove excess salt during all washing steps.

8. Spin the empty column in an emptied collection tube 2 min to **remove residual ethanol**. **

** Do 1 min with the hinge to the outside of the rotor and another min with hinge to the inside of the rotor.

9. Place the column in a new tube and elute in 30-50 µl warm (50-60°C) H_2O, incubate 5-10 min before spinning (E1).
10. Repeat the elution (E2).
11. Nanodrop and Qubit both (E1 and E2) fractions. Nanodrop readings higher than 500 should be diluted and re-measured.
12. Make an aliquot of 100 ng/µl (~30 µl) for gel QC and sequencing and save the rest as is in concentrated form (DO NOT dilute the entire sample).
13. QC with either 50 or 100 ng on a gel, along with 100 ng of appropriate DNA markers. **DO NOT LOAD MORE THAN 100ng ON GEL.**
14. For binary vector sequencing, submit 100 ng/µl of sample for sequencing.