Pam's STET Plasmid Prep Protocol
(Boiling Prep a la Birmboin and Doly)

Modified 4/23/98; last modified 9/28/10

Reagents

STET Buffer

8% sucrose
50 mM TRIS-HCl, pH 8.0
50 mM EDTA
5% Triton X-100

Make up 100 ml STET Buffer at a time. *Autoclave*. NOTE: The Triton-X 100 often separates out after autoclaving; therefore, re-mix the phases well once solution has cooled down.

10 mg/mL lysozyme in STET buffer, FRESHLY-MADE – *Lately, I've been making a 20 mg/mL stock, but still adding 20 uL per sample (Step 4).*

Isopropanol

70% ethanol

TE

Protocol

1. Grow liquid cultures to saturation overnight at 37°C in 3-5 mL LB+Ampicllin or LB+Carbenicillin liquid (or patch out colony on 1/4 of LB+Amp or LB+Carb plate). Spin down ~1.5 mL of culture in a microfuge tube, 15 seconds at maximum speed. *If you want more DNA you can spin down a second aliquot of 1.5 mL in the same tube.*

2. Heat water bath to boiling (or make sure 100°C heating block is on).

3. Resuspend cells in 200 µL **STET buffer**, by gently pipetting up and down (or by "zotzing" and vortexing). *Lately, I've been adding 250 uL here.*

NOTE: I put tubes on TOMY shaker to loosen pellets before adding STET buffer; in this case, pipeting up and down usually is unnecessary.

4. Add 20 µL **lysozyme** solution. Mix briefly by quick vortex or inversion.

5. **Immediately** place at 100°C for 3 minutes.

NOTE: If processing a lot of samples, it might be easier to resuspend loosened cell pellets directly in STET buffer containing lysozyme—with minimal vortexing—then immediately place in

boiling water bath.

6. Remove promptly. Let tubes cool down for a few minutes on bench top. Spin tubes at top speed for 20 minutes.

7. Remove the gooey precipitate/pellet with a wooden toothpick and discard.

8. Add an equal volume of **100% isopropanol** to each tube (~250-300 µL). Mix well. Put on ice or at 4°C for at least 30 minutes (RT is okay, too). If you are in a hurry this incubation can be shortened; however, the yield might be reduced. Also, this can be a stopping point.

9. Spin down precipitated nucleic acids (DNA+RNA) at top speed for 15 or 20 minutes. (The temperature is not important—pellets will be bigger at 4°C, but I think this is mostly due to salt.)

10. Decant or aspirate the supernatant and discard. Wash pellets with **70% ethanol**; vortex and respin for a few minutes.

11. Aspirate ethanol and dry pellet.

NOTE: It is not necessary to dry the pellet, provided you aspirate all the ethanol.

12. Resuspend pellet in 50-200 µL **TE** (let sit at room temperature or on ice for a while).

13. Use 1-5 µL per restriction digest. Don't forget to add RNAse to digests or loading dye, as these preps will have RNA in them.

14. Store miniprep DNA at -20°C.