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Ed’s alkaline lysis MiniPrep protocol

1. Grow 2 mL overnight culture in Falcon 2059 tube and transfer ~ 1.5 mL to microfuge tube-spin 30 seconds.

2. Decant supernatant and resuspend pellet in 100 µL solution I (50 mM glucose, 25 mM Tris, pH 8, 10 mM EDTA).

3. Add 200 µL freshly made solution II (0.2 N NaOH, 1 % SDS), mix by inverting or gently shaking.

4. Add 150 µL ice-cold solution III (29.5 g K-Ac, 11.5 mL H-Ac in 100 mL ddH2O)-mix by vortexing.

5. Spin in microfuge for 5 min. Transfer supernatant to new microfuge tube (avoid flocculent ppt).

6. Precipitate DNA by adding 1 mL 95% ethanol, vortexing and spinning in microfuge for 5 min.

7. Remove all traces of ethanol, allowing to air dry or in vacuum chamber for a few minutes. To aid in this, you can pour off ethanol, spin again and pipette off remaining ethanol. I have left out the 70% ethanol rinse because of pellet loss and still get good restriction digests.

8. Depending on pellet size, resuspend in 50-100 µL TE containing 20 µg/mL RNAse A. Use 1 µL for restriction digest.

Notes🡪To make DNAse-free RNAse A, prepare a 10 mg/mL stock in TE and boil 10 min, and cool to room temperature before freezing aliquots at -20. The quality of this plasmid DNA is fine for restriction digests, PCR or transformations. I have gotten mixed results for sequencing and the yield is not sufficient for large scale digest for insert purification. For positive clones, I will take an aliquot of the bugs from step 1 and inoculate a 100 mL flask for Qiagen or cesium mid-scale preparation.