Alkaline Lysis Miniprep

1. Spin down 1.5mls bacteria in a microcentrifuge at 15,000rpms. Aspirate off broth.
2. Resuspend pellet extremely well in QIAGEN resuspension solution-100uls.
3. Add 200ul of QIAGEN lysis buffer. Place on ice for 5min.
4. Add 1/2 volume –150uls 7.5M Ammonium Acetate (pH 7.4). Mix well and place on ice for 5 mins.
5. Spin down for 5min at 15,000rpms. Recover supernatant being extremely careful not to include any SDS or contaminating agents. Add 0.7vol –300uls 2-propanol. Mix well and precipitate at 15,000rpms for 15mins.
6. Dry pellet, resuspend in 400uls dH20 and place at 65^{o}-3min. Add 100uls 10M Ammonium Acetate. Mix well and place on ice for 5mins.
7. Spin down protein debris for 6mins at 15,000rpms. Recover supernatant. Add 0.7vol –350uls Isopropanol. Mix well and precipitate for 15mins.
8. Dry pellet, resuspend in 20-50uls TE plus RNase.

*1.5ml culture=8+ug DNA

Alternative Boiling Method

1. Inoculate 5 ml of LB (Luria-Bertani) broth or TB (Terrific Broth) plus the appropriate antibiotic with an isolated colony of interest. Shake the sample(s) overnight at 37° C.
2. Transfer 750 ml of each overnight culture to a 1.5 ml Eppendorf tube. Add 15 ml Triton X-100 and 7.5 ml of a 10mg/ml lysozyme solution (from Maniatis). Vortex briefly. Place on ice for one minute.
3. Pierce each lid with a needle. Place in boiling water for one minute. *Alternatively, can put tubes in 100°C heat block for two minutes.* Immediately spin in microcentrifuge at maximum rpm for 8 minutes.
4. Transfer the supernatant phase to a fresh 1.5 ml Eppendorf tube. Add an equal volume amount of isopropanol. Vortex thoroughly and place on ice for two minutes.
5. Spin in a microfuge at maximum rpm for 10 minutes. Pour off supernatant. Dry pellet in speed-vac for 5-10 minutes.
6. Resuspend in 30ul of TE plus 1ul RNase. Use 5-10ul for each restriction digest.

Notes: - This prep works well for DH5a and XL1Blue; mixed results may be obtained with TG1 and MV1190. Reference is Rajeevan, M. S. and Bassett, C. L. (1994) BioTechniques ***16,*** *376-380.*