SMALL-SCALE ISOLATION OF PLASMID DNA
(alkaline lysis method)

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REAGENT:

TEG: 25mM tris-HCl, pH8.0/ 10mM EDTA/ 50mM glucose.

5M KOAc: 29.44g KOAc, 11.5ml acetic acid, water to 100ml.

RNase/TE: 50ml TE + 100μl RNase (5mg/ ml).

PEG/NaCl: 20% polyethyleneglycol/ 2.5M NaCl.

0.2N NaOH/ 1% SDS

PROCEDURE:

1. Mix a single bacterial colony with 1.5ml of LB+ampicilin medium. Incubate at 37°C overnight.
2. Transfer the culture into an Eppendorf tube.
3. Spin at 6K for 5min. Remove the supernatant with an aspirator.
4. Suspend the bacteria in 100μl of TEG.
5. Add 0.2ml of NaOH/ SDS and mix. Leave on ice for 5min.
6. Add 150μl of cold 5M KOAc. Mix gently by inversion. Leave on ice for 5min.
7. Spin at 12K for 10 min at 4°C. Transfer the supernatant to a new tube.
8. Add 2volumes of EtOH. After 3min, spin at 12K for 5min at r.t.
9. Remove the supernatant wit an aspirater. Dry DNA pellet *in vacuo*.
10. Dissolve the plasmid DNA in 100μl of RNase/TE. Incubate at 37°C for 15min.
11. Add 60μl of PEG/NaCl. Mix well. Leave on ice for 1hr.
12. Spin at 12K for 10min at 4°C. Aspirate off the supernatant.
13. Add 100μl of TE. Dissolve the DNA pellet.
14. Add 100μl of phenol/$CHCl_3$ and mix. Spin at 12K for 5min at r.t.
15. Transfer the supernatant to a new tube. Add 300μl of EtOH/ Na acetate. Store at -70°C.
16. Spin at 12K for 10 min at 4°C. Aspirate off the supernatant. Rinse the ppt with 80% EtOH. Dry the DNA pellet i*n vacuo*.

17 Dissolve the ppt in 50μl of TE.