

DIFFERENTIAL PRECIPITATION OF PLASMID DNA (for 1 liter of cells)
(Large Scale, without cesium)

This procedure is essential when DNA must be totally free of nicks (i.e. in-vitro transcriptions). Typical yield from 1 liter of cells is approximately 1 mg of DNA for clones with the pGEM vectors.

Materials:

- Solid NH₄OAc
- 5 M NaCl
- 30% PEG 6000 in 1.5M NaCl
- 0.5M NaCl in TE
- dOH sevag equilibrated in 0.5M NaCl/TE
- Lots of Corex tubes and ice
- HB 4 rotor

Procedure:

1. Starting with alkaline lysis maxi-prep DNA (CSHCM; pp 90,91) resuspend final pellet from 1 liter of cells in 10 ml TE.
2. Add solid NH₄ OAc to 2.5 M (1.93 g/10ml); 0°C, 30'.
3. Spin at 8K, 10', HB-4 rotor 4°C.
4. Transfer supernatant to new Corex tube (discard pellet).
5. Add 2 volumes abs. EtOH ; 0°C, 30'.
6. Spin at 8K, 10', HB-4 rotor 4°C; pour off sup., damp dry.
7. Dissolve pellets in 5 ml TE (completely).
8. Add RNase A to 10 µg/ml; 37°C, 30'.
9. Add 5 M NaCl to 1.5 M final (2.1 ml).
10. Add 0.25 vol. (1.8 ml) 30% PEG 6000 in 1.5 M NaCl; 0°C, 30'.
11. Spin at 8K, 10', HB-4 rotor 4°C (don't worry if pellet is small).
12. Dissolve pellet in 5 ml 0.5 M NaCl in TE.
13. Extract 1 x w/phenol:Sevag equilibrated with 0.5 M NaCl + TE.
14. Add 2 vol. EtOH to sup.; -20°C, 1 hr.
15. Spin at 8K, 10', HB-4 rotor 4°C; pour off sup.
16. Rinse pellet 1 x with R.T. 70% EtOH; damp dry.
17. Resuspend pellet in 1 ml TE/liter.
18. Determine DNA concentration as usual.

Reference:

We obtained this protocol from the Engel lab.