Mini-Plasmid Preparation

Protocol of D. Grant McFadden

Solution 1: 25 mM Tris pH 8.0, 50 mM glucose, 10 mM EDTA
Solution 2: 1% SDS, 0.2 M NaOH (prepare fresh and keep at r.t.)
Solution 3: 5 M potassium acetate (see Maniatus for recipe)

1. Grow 1.5 ml overnight cultures in Luria Broth.

2. Transfer 1.5 ml of bacteria to microfuge tube (save 0.5 ml for later use) and microcentrifuge for 30 sec at r.t.

3. Remove supernatant with aspirator, cool pellet on ice.

4. Resuspend pellet in 100 ul solution 1-GTE, vortex vigorously to resuspend, incubate on ice 5 min.

5. Add 200 ul solution 2-NaOH/SDS, invert tube several times (do not vortex), incubate on ice 5 min.

6. Add 150 ul solution 3-KOAc, invert tube several times, incubate on ice 5 min.

7. Microfuge sample for 4 min at r.t.

8. Pour all supernatant (avoid the white pellet) to a fresh tube.

9. Extract the supernatant with 0.45 ml phenol/chloroform, vortex, microfuge 1–2 min.

10. Remove all (@0.45 ml) aqueous phase to a fresh tube.

11. Add 0.90 ml ethanol, incubate 1–2 min at r.t. (do not incubate any longer otherwise protein will precipitate along with the DNA).

12. Microfuge 4 min at r.t. , aspirate supernatant under low vacuum.

13. Add 1 ml 70% ethanol, invert tube several times, microfuge 1 min.

14. Remove ethanol slowly with aspirator (it should be possible to see a small white pellet). Dry pellet under vacuum for 10 min.

15. Resuspend plasmid DNA in 20 ul 10mM Tris pH 8.0, 50ug/ml RNase A, incubate at r.t., 5 min.

16. Set up restriction enzyme digestion, 2 ul plasmid digested in a total volume of 10 ul, incubate 1 hr, analyze on agarose gel.

17. If you do not need to restriction enzyme digest the miniprep (i.e., for fast miniscreen for inserts that are fairly large), add 100 mg/ml RNase into solution 1 at step 4 and run directly on an agarose gel omitting sets 11–16.