Miniprep Protocol & Hints

Here is a suggested protocol; the yield of the plasmid should be approxima tely 0.2-0.3ug/ul. The bolded should be noticed for a nice miniprep.

Procedures:

1. Incubate 5ml LB medium (containing antibiotic) with a bacterial clone, culture with vigorous shaking at 37 degree for 12-16 hrs.

2. Harvest bacteria by spinning at 13000rpm (~12000g) for 1 min. Aspirate supernatant. Add additional 750ul culture media, respin and aspirate supernatant forseveral times .

3. Resuspend bacterial pellet by complete **vortexing** in 250ml resuspension buffer(RB, with 10ul RnaseA in it). The bacteria should be completely resuspended – no clumps should be visible.

4. Add 250ul freshly lysis buffer (LB) and mix **gently** by inverting 5-10 times at roomtemperature. The mixture should appear translucent and mucous-like. The time of lysis will never be longer than 5 min.

5. Add 350ul neutralization buffer (NB) and mix **gently** by inverting 5-10 times, The mixture should contain flocculent white precipitate at this point.

6. Remove bacterial debris by centrifugation at 13000rpm for 10 min; pour supernatant to a fresh adsorption column which can avoid the transfer of precipitate to the new column causing the precipitate "sticy".

7. Add 500ul buffer DWI to get rid of the protein before centrifugation at 9000 rpm for 30 sec. Pour off the liquid into beaker.

8. Add 500 ul washing buffer (WB) before centrifugation at 9000 rpm for 1 min. Pour off the liq uid into beaker.

9. repeat 8 once.

10. Centrifuge the empty column and tube at 9000 rpm for 1min.

11. Put the column into a fresh EP tube. Add 30-50 ul elution buffer (EB) to elute the DNA. Incubate at room temperature for **1-2 min**.

12. Store the purified DNA at -20° C.