

Miniprep Protocol & Hints

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

Procedures:

1. Inoculate 5ml LB medium (containing antibiotic) with a bacterial clone, culture with vigorous shaking at 37 degree for 12-16 hrs.
2. Put EB (elution buffer) at **65 degree** water bathing.
3. Harvest bacteria by spinning at 13000rpm (~12000g) for 1 min. Aspirate supernatant. Add additional 750 ul culture media, respin and aspirate supernatant for several times.
4. 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.
5. Add 250ul freshly lysis buffer (LB) and mix **gently** by inverting 5-6 times at room temperature. The mixture should appear translucent and mucous-like. The time of lysis will never be longer than 5 min.
6. Add 350ul neutralization buffer (NB) and mix **gently** by inverting 5-6 times, incubate at room temperature for 3 min. The mixture should contain flocculent white precipitate at this point.
7. 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 is "sticky".
8. Centrifuge at 13000rpm for 1 min. Pour off the liquid in the collection tube. **For critical samples**, repeat the operation above.
9. Add 600 ul washing buffer (WB) before centrifugation at 13000 rpm for 1 min. Pour off the liquid into beaker.
10. **Centrifuge at 13000rpm for 10 min** to spin the ethanol down.
11. Put the column into a fresh EP tube. Air dries DNA for **10 min**.
12. Add 30-50 ul elution buffer (EB) to elute the DNA.

Notes:

1. Typical yield of high-copy-number plasmids, such as PSB1AK3, prepared by this method is about 0.2-0.3 ug of DNA per ul of original bacterial culture, and 0.1 ug of DNA per ul for low-copy-number plasmids such as PSB3T5.
2. To analyze the DNA by cleavage with restriction enzyme(s) remove 2 μ l of the DNA solution and add it to fresh microfuge tube that contains 5 μ l of water. Add 1 μ l of the appropriate 10 x restriction enzyme(s). Incubate the reaction for 2 hr at the appropriate temperature. Store the remainder of the DNA preparation at -20 degree. Analyze the DNA fragments in the restriction digest by gel electrophoresis.
3. For tetracycline, notice its photolysis.
4. Resuspension buffer (RB) should be stored in the refrigerator. RNase should be in the -20 degree freezer.

References:

*Current protocols in molecular biology