HiSpeed Midiprep - 8/28/07

Bacterial culture, harvest, and lysis

1. Inoculate a 10 ml LB tube with cells and antibiotic. If ampicillin is used, add 20 µl of ampicillin, and cells. If chlorophenocol is used, add 14.7 µ of 34 mg/ml chlorophenocol and cells to the LB. Incubate at 37°C for 8 hours. If desired use 100 µl of 20% w/v glucose. Use these cells to inoculate a larger flask of 100 ml / 400 ml LB.
2. Inoculate a 100 ml (for high copy plasmid)/ 400 ml (low copy plasmid) LB flask with cells and antibiotic. If ampicillin is used, add 200 µl / 800 µl of ampicillin and 1/4 ml cells. If chlorophenocol is used, add 147 µl / 588 µl of 34 mg/ml chlorophenocol and 1/4 ml cells to the LB. If desired add 1ml/4ml 20% w/v glucose to the flasks. Incubate at 37°C for 12-16 hours.
3. Pellet 100 ml (high copy) or 400 ml (low copy) overnight LB culture at 6000 x g for 15 min at 4°C.
4. Homogeneously resuspend the bacterial pellet in 6 ml Buffer P1. Transfer to a 50 ml centrifuge tube.
5. Add 6 ml Buffer P2, mix thoroughly by vigorously inverting 4-6 times, and incubate at room temperature for 4 min. Do not let reaction continue for more than 4 min.
6. Add 6 ml of chilled Buffer P3, mix thoroughly by vigorously inverting 4-6 times.

Bacterial lysate clearing

7. Centrifuge the lysate for 30 min at 20,000 x g. A white pellet should form.
8. Prepare the QIAfilter Cartridge during spin. Screw the cap onto the outlet nozzle of the QIAfilter Midi Cartridge. Place the QIAfilter Cartridge into a convenient tube.
9. Equilibriate a HiSpeed Midi Tip by applying 4 ml of Buffer QBT and allow the column to empty into a waste tube by gravity flow.
10. Decant the supernatant of the centrifuged lysate into the barrel of the QIAfilter Cartridge.
11. Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Midi Cartridge and filter the cell lysate into the previously equilibrated HiSpeed Tip.

Bind, wash and elute plasmid DNA on HiSpeed Tip

12. Allow the cleared lysate to enter the resin by gravity flow. Discard flow-through.
13. Wash the HiSpeed tip with 20 ml Buffer QC.
14. Elute DNA into a 15 ml tube with 5 ml Buffer QF.

Precipitate, wash, and redissolve plasmid DNA

15. Precipitate DNA by adding 3.5 ml of room-temperature isopropanol to the eluted DNA. Mix and incubate at room temperature for 5 min.
16. During incubation remove the plunger from a 20 ml syringe and attach the QIAprecipitator Midi Module onto the outlet nozzle.
17. Place the QIAprecipitator over a waste bottle, transfer the eluate/isopropanol mixture into the 20 ml syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.
18. Remove the QIAprecipitator from the 20 ml syringe and pull out the plunger. Reattach the QIAprecipitator and add 2 ml 70% ethanol to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitator using constant pressure.
19. Remove the QIAprecipitator from the 20 ml syringe and pull out the plunger. Attach the QIAprecipitator to the 20 ml syringe again, insert the plunger, and dry the membrane by pressing air through the QIAprecipitator quickly and forcefully. Repeat this step until the membrane is dry.
20. Dry the outlet nozzle of the QIAprecipitator with Kimwipes to prevent ethanol carryover.
21. Remove the plunger from a new 5 ml syringe and attach the QIAprecipitator onto the outlet nozzle. Hold the outlet of the QIAprecipitator over a 1.5 ml collection tube. Add 500 µl of DI water to the center of the 5 ml syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.
22. Remove the QIAprecipitator from the 5 ml syringe, pull out the plunger, and reattach the QIAprecipitator to the 5 ml syringe.
23. Add the eluted 500 µl to the center of the 5 ml syringe and elute for a second time into the same 1.5 ml tube.