HiSpeed Maxiprep

Bacterial culture, harvest, and lysis

1. Inoculate a 10 ml LB tube with cells and antibiotic. If desired add 20% w/v glucose to final concentration of 0.2%. Incubate at 37°C for 8 hours. Use these cells to inoculate a larger flask of 400 ml / 500 ml LB.
2. Inoculate a 400 ml / 500 ml LB flask with cells and antibiotic. If desired add 20% w/v glucose to final concentration of 0.2%. Incubate at 37°C for 12-16 hours.
3. Pellet 400 ml (high copy) or 500 ml (low copy) overnight LB culture at 6000 x g for 15 min at 4°C.
4. Homogeneously resuspend the bacterial pellet in 10 ml Buffer P1.
5. Add 10 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 10 ml of chilled Buffer P3, mix thoroughly by vigorously inverting 4-6 times.

Bacterial lysate clearing

7. Prepare the QIAfilter Cartridge during incubation. Screw the cap onto the outlet nozzle of the QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge into a convenient tube.
8. Centrifuge the lysate for 30 min at 20,000 x g. A white pellet should form.
9. Equilibriate a HiSpeed Maxi Tip by applying 10 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 Maxi 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 60 ml Buffer QC.
14. Elute DNA into a 50 ml tube with 15 ml Buffer QF.

Precipitate, wash, and redissolve plasmid DNA

15. Precipitate DNA by adding 10.5 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 30 ml syringe and attach the QIAprecipitator Maxi Module onto the outlet nozzle.
17. Place the QIAprecipitator over a waste bottle, transfer the eluate/isopropanol mixture into the 30 ml syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.
18. Remove the QIAprecipitator from the 30 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 30 ml syringe and pull out the plunger. Attach the QIAprecipitator to the 30 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 another 500 µl of DI water to the center of the 5 ml syringe and elute for a second time into the same 1.5 ml tube.