Plasmid or Cosmid DNA Purification Using QIAGEN HiSpeed Plasmid Midi Kits

This protocol is for preparation of up to 200µg of high- or low-copy plasmid or cosmid DNA using the QIAGEN HiSpeed Plasmid Midi Kit.

A final DNA concentration of up to 0.4µg/µL can be expected, if eluting a high-copy plasmid with 500µL of Buffer TE.

Things to do before starting:

Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100µg/mL.

Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.

Pre-chill Buffer P3 at 4°C.

Pre-warm the adequate volume of Buffer QF and Buffer TE at approx. 50°C.

Grow bacterial culture during approximately 16 hours, which typically is the transition from logarithmic into stationary growth phase.

Optional: Add LyseBlue reagent to Buffer P1.

Procedure

1. Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2-5mL LB medium containing the appropriate selective antibiotic. Incubate for approx. 8h at 37°C with vigorous shaking (approx. 300 rpm).

2. Dilute the starter 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate 50mL medium. Grow at 37°C for 12-16h with vigorous shaking (approx. 300 rpm).

3. Harvest the bacterial cells by centrifugation at 6000 x g for 15 min at 4°C.

4. Resuspend the bacterial pellet in 6 mL Buffer P1.

5. Add 6 mL Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4-6 times, and incubate at room temperature (15-25°C) for 5 min. During the incubation prepare the QIAfilter Cartridge. Screw the cap onto the outlet nozzle of the QIAfilter Midi Cartridge. Place the filter into a convenient tube or a QIArack.

6. Add 6mL of chilled Buffer P3 to the lysate, and mix immediately and thoroughly by vigorously inverting 4-6 times. Do not incubate the lysate on ice.

7. Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature for 10 min. Do not insert the plunger !

8. Equilibrate a HiSpeed Midi Tip by applying 4 mL Buffer QBT and allow the column to empty by gravity flow.

9. Remove the cap from the QIAfilter outlet nozzle. Gently insert the plunger into the QIAfilter Midi Cartridge and filter the cell lysate into the previously equilibrated HiSpeed Tip.

10. Allow the cleared lysate to enter the resin by gravity flow.

11. Wash the HiSpeed Midi Tip with 20 mL of Buffer QC.

12. Elute DNA with 5 mL of Buffer QF.

13. Precipitate DNA by adding 3,5mL of room-temperature isopropanol to the eluted DNA. Mix and incubate at room-temperature during 5 min.

14. During the incubation remove the plunger from a 20 mL syringe and attach the QIAprecipitator Midi Module onto the outlet nozzle.

15. 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 QIA precipitator using constant pressure.

16. Remove the QIAprecipitator from the 20 mL syringe and pull

out the plunger. Re-attach the QIAprecipitator and add 2mL 70% ethanol to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitaor using constant pressure.

17. 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 QIAprecipitaot quickly and forcefully. Repeat this step.

18. Dry the outlet nozzle of the QIAprecipitator with absorbent paper to prevent ethanol carryover.

19. 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 1 mL of Buffer TE to the 5 mL syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.

20. Remove the QIAprecipitator from the 5 mL syringe, pull out the plunger and reattach the QIAprecipitator to the 5 mL syringe.

21. Transfer the eluate from step 19 to the 5 mL syringe and eluate for a second time into the seme 1,5 mL tube.

Determination of yield.

Agarose gel analysis.

This protocol is extracted from "HiSpeed Plasmid Purification Handbook" by **QIAGEN**