Purpose: Extract plasmid DNA (larger quantity)

QIAGEN Plasmid Midi Kit Protocol

1. Separate 100 mL of bacterial overnight culture into 5 separate 50 mL falcon tubes and centrifuge at 6,000 rpm for 15 minutes at 4°C.
2. Decant supernatant.
3. Add 4 mL of Buffer P1 to one tube, pipet mix, and transfer to another tube. Mix and transfer contents to the next tube with pelleted cells. Repeat until all tubes are combined.
4. Add 4 mL of Buffer P2 to the tube containing 4 mL of Buffer P1 and the combined resuspended pelleted cells and vigorously invert 6 times.
5. Incubate at room temperature for 3 minutes.
6. Add 4 mL of Buffer P3 and vigorously invert 10 times.
7. Incubate on ice for 15 minutes.
8. Centrifuge at 20,000 x g at 4°C for 30 minutes.
9. Once centrifuged, transfer clear supernatant to another centrifuge tube while avoiding all of the flakes on the sides and in the solution.
10. Centrifuge the tube again at 20,000 x g at 4°C for 15 minutes
11. While this runs, equilibrate the QIAGEN-tip by adding 4 mL of QBT to the QIAGEN-tip.
12. Add the clear solution (from step 10) to the QIAGEN-tip and allow it to enter the resin by gravity flow
13. Next, add 10 mL of Buffer QC to the QIAGEN-tip and allow to gravity drip.
14. Once that passes through, add 10 mL more of Buffer QC and allow to flow through.
15. Then, add 5 mL of Buffer QF and allow to flow through.
16. Add 3.5 mL of room temperature isopropanol to elute the DNA and mix. Then centrifuge at 15,000 x g for 30 minutes at 4°C.
17. Carefully remove the supernatant making sure not to disrupt the clear pellet.
18. Add 2 mL of room-temperature 70% ethanol and centrifuge for 10 minutes at 15,000 x g at 4°C. Discard the supernatant leaving as little liquid behind as possible, careful not to disrupt the clear pellet.
19. Air-dry the pellet for 20 minutes in the vent hood and redissolve in 100 µL of Buffer EB.