Qiagen Miniprep

Isolation of the DNA from the bacteria.

Estimated bench time: 30 min
Estimated total time: 1 hours

MATERIALS

- Small culture
- Minispin centrifuge
- From the Qiagen miniprep kit:
  - P1 buffer
  - P2 buffer
  - N3 buffer
  - Qiaprep 2.0 spin column
  - PE buffer
- MilliQ
- Clean DNAse-free 1.5 mL Eppendorf tube
- Nanodrop

SETUP & PROTOCOL

1. Collect the small culture from the incubator.
2. Centrifuge the bacteria at 4000 rpm RT for 10 minutes.
3. Discard the supernatant in the autoclave waste.
4. Re-suspend the pellet in 250 µL buffer P1 and transfer to a clean DNAse-free 1.5 mL Eppendorf tube.
5. Add 250 µL buffer P2. Mix by inverting 4-6 times. The solution should turn blue, due to a pH indicator in buffer P1. It should also become viscous due to the lysis. NB: Continue immediately with step 6 (Wait no longer than 15 minutes).
6. Add 350 µL buffer N3 and mix well by inverting the tube 4-6 times. NB: Continue immediately with step 7.
7. Place the tubes in a microcentrifuge with the hinge point of the lid facing the outside and centrifuge at max speed for 10 minutes. Afterward, continue immediately with step 8. Centrifuge longer if it is not fully precipitated!
8. Apply the 800 µL supernatant from step 7 to a Qiaprep 2.0 spin column by pouring or pipetting.
9. Centrifuge at max speed for 1 minute. Discard the flow-through.

The protocol continues on the next page

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SETUP & PROTOCOL

10. If you have endA+ strain: Wash with 0.5 mL buffer PB and centrifuge at max speed for 1 min.
11. Wash with 0.75 mL buffer PE and centrifuge at max speed for 1 minute. Discard the flow-through.
12. Centrifuge for 2 minutes to dry the membrane.
13. Place the spin column into a clean, DNAse-free 1.5 mL Eppendorf tube.
14. Apply 40 µL MilliQ to the center of the membrane. Incubate for 1 minute at room temperature. For higher concentration, you could also add 30 microliters MilliQ.
15. Centrifuge at max speed for 1 minute.
16. Measure the DNA concentration with the Nanodrop.
17. For a higher concentration, you can transfer the DNA back to the right spin column, place it into a clean DNAse-free Eppendorf tube, incubate for 1 min at room temperature and repeat steps 15 and 16.
18. Store the DNA at -20 ºC.