Qiagen DNeasy DNA extraction protocol for bacterial cultures

Adapted from QIAgen DNeasy handbook, July, 2006.

Procedure:

1. Appropriately label a 1.5 ml tube for each sample.

2. Add 1.75 ml of bacterial culture to a labeled 2 ml tube.

3. Spin tubes at 20,000 x g for 5 minutes in centrifuge. Decant liquid.

4. Add 180 ul of enzymatic lysis buffer to you tube and vortex 10-20 s.

5. Incubate at 37° C for 30 min.

6. Add 25 ul of proteinase K to the tube

7. Add 200 ul of Buffer AL to the tube.

8. Vortex the tube briefly.

9. Incubate at 56° C for 30 min. *Now is a good time to label all the tubes you need for the rest of the protocol.*

10. Add 200 ul of 100% ethanol to the tube

11. Vortex briefly.

12. Using a micropipette, transfer entire contents (~600 ul) of tube to labeled spin column.

13. Centrifuge column at 10,000 x g for 1 min.

14. Remove column from collection tube. Place column in new collection tube.

15. Add 500 ul of buffer AW1 to the column and centrifuge at 10,000 x g for 1 minute.

16. Remove column from collection tube. Place column in new collection tube.

17. Add 500 ul of buffer AW2 to the column and centrifuge at 20,000 x g for 3 minute.

18. Carefully remove tubes from centrifuge, do not let flow-through contact column. If this happens, spin tube again for 1 min at 20,000 x g.

19. Transfer the column to a 1.5 ml tube and add 200 ul of buffer AE to the column. Let column stand at room temperature for 1 minute.

20. Centrifuge at 10,000 x g for 1 minute. Discard the column and store the DNA appropriately (4° C for short term, -20° C for long term).

Materials required

Qiagen DNeasy Blood and Tissue kit
200 and 1000 ul pipette tips
1.5 ml microcentrifuge tubes
2.0 ml microcentrifuge tubes
Overnight bacterial cultures

Equipment required

Bench top centrifuge capable of 20,000 x g
200 ul micropipette
1000 ul micropipette
vortexer