SOP

10.3.3 DNA extraction from pure bacterial cultures

Objectives and scope

This SOP describes the various steps to follow to obtain nucleic acid (DNA) from pure bacterial cultures using the Qiagen DNeasy® UltraClean® Microbial Kit.

This SOP is intended for Mini-Lab laboratory technicians.

Principle

This SOP describes the steps included in the Quiagen Handbook for the DNeasy® UltraClean® Microbial Kit to obtain high quality and quantity DNA from pure bacterial cultures.

In this kit, microbial cells are resuspended in a bead solution and added to a bead beating tube containing beads. Then, lysis solution is added. The microorganisms are lysed by a combination of heat, detergent and mechanical force against specialized beads. The cellular components are lysed by mechanical action using a designed Vortex Adapter on a standard vortex. The DNA released from the lysed cells is bound to a silica spin filter. The spin filter is washed, and the DNA is recovered in DNA-free buffer (Diagram1).

Safety and environment

DO NOT add bleach or acidic solutions directly to the sample preparation waste --> PowerBead Solution and Solution SB contain *guanidine salts*, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

- Wear your PPE for the duration of this technique: lab coat, gloves;
- All biological samples should be considered as potentially infectious and handled with the usual precautions;
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

Sample

- Type of material:
  - Pure bacterial cultures

Equipment

Common Name	*Associated SOP*
Freezer -20°C	POS-7.7-REFCON
Microcentrifuge (10,000 x g)	

Vortex Vortex Adapter for 24 (1.5–2.0 ml) tubes

Consumables

*Common Name**	*Storage conditions*
Gloves	*N/A*
10-µL inoculation loop	*N/A*
Transfer pipettes (50–200 µl; 100–1000 µl)	*N/A*
DNeasy UltraClean Microbial Kit	*15–25°C*
Water tube for inoculum 3 mL (Beckman Coulter)	*15-30°C*
Cryosystem BALL TUBE	*2–8°C*

DNeasy UltraClean Microbial kit Content

Common Name	*Quantity*	*Safety Information*
PowerBead Tubes (Garnet 100)	*50*	*N/A*
PowerBead Solution	*16.5 mL*	*Not hazardous. Do not bleach!*
MB Spin Columns	*50*	*N/A*
Solution SL	*2x1.5 mL*	*Not hazardous*
Solution IRS	*15 mL*	*Not hazardous*
Solution SB	*50 mL*	*Flammable, skin and eye irritation. Do not bleach!*
Solution CB	*30 mL*	*Flammable, eye irritation. Contains alcohol.*
Solution EB	*9 mL*	*Not hazardous*
Collection Tube (2mL)	*4 x 50*	*N/A*

Quality Control (see SOP "8.1 Internal Quality Control")

As quality control, a blank water tube as negative can be used extraction control

Procedure

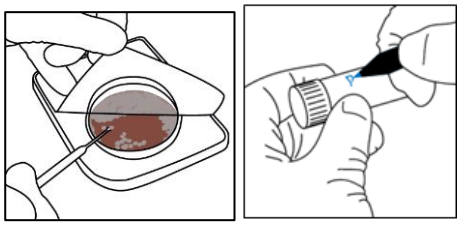
Important points before starting

1. Check the **Solution SL** by looking through the bottle against a light source. If you see visible particles at the bottom, warm in the incubator for 5-10 mins
2. Shake to mix **Solution SB** before use

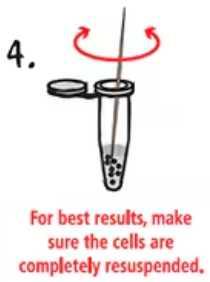
On how to obtain pure cultures on the InTray cassettes see SOP "10.3.2 Subculturing for pure cultures"

Preparation of the pellet

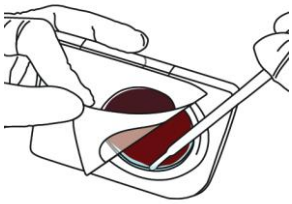
3. With a transfer pipette, transfer 1.8 ml of water from the Water tube for inoculum 3 mL to a 2 ml Collection Tube (provided).
4. Label them with the culture names
5. With a 10µL loop pick up as much bacterial culture as possible from each InTray– the loop should be full of material.



6. Insert the loop into the water tube and swirl in the water or rub it against the inner walls to release the cells. Your suspension should look cloudy.



Note: if there is not enough bacterial culture on the InTray agar, you could resuspend it all by pipetting directly 0.5 mL of resuspension water on the surface of the InTray agar and delicately turning the water around with a sterile 10 ul loop. Very carefully collect all the liquid by pipetting from the side of the agar cassette, as done when preparing the cassette (SOP 10.3- Subculture:Inoculation)



7. Discard the loop in the red benchtop bin.

8. Perform the same steps for each bacterial culture.

9. Insert all the tubes containing the resuspended cultures in a microcentrifuge (or as many as it fits, otherwise repeat for the other tubes).
10. Centrifuge at 10,000 x g for 30 s.
11. Gently tilt the tube in the red benchtop bin to remove the supernatant, being careful not to lose the pellet
12. Centrifuge the tubes again at 10,000 x g for 30 s. Completely remove the supernatant with a pipette.

Note: Depending on the type of microbial culture, it may be necessary to centrifuge longer than 30 s. This step concentrates and pellets the microbial cells. It is important to pellet the cells completely.

DNA extraction procedure

13. Label and prepare in advance per sample:
    a. 100 ul Solution IRS in 2 ml Collection Tube
    b. 900 ul Solution SB in 2 ml Collection Tube
    c. Powerbead Tube (label)
    d. Spin Columns (label)

14. Thaw the pellets at RT (if needed), Code the samples 1-.....

15. Pipette 300 ul of Power Bead solution in the tube with the pellet and resuspend the pellet by pipetting. Transfer resuspended cells to a PowerBead Tube.

16. Add 50 µl of Solution SL to the PowerBead Tube.

17. Put the PowerBead Tubes horizontally on the Vortex Adapter and vortex at maximum speed for 10 min.

18. Centrifuge the tubes at a maximum of 10,000 x g for 30 s.
    Note: Make sure the 2 ml PowerBead Tubes rotate freely in the centrifuge without rubbing.

19. Transfer the supernatant into the Collection tube containing the 100 µl of Solution IRS and vortex for 5 s. Incubate at 4°C (in the fridge) for 5 min.

20. Centrifuge the tubes at 10,000 x g for 1 min.
21. Using a transfer micropipette, collect the entire volume of supernatant by tilting the tube on the side and pointing the tip to the side of the internal wall of the tube, without touching the pellet. Transfer the liquid to a 2 ml Collection Tube already prepared with 900 ul Solution SB and labelled, and vortex briefly. Be careful to avoid the pellet!

22. Using a 1000uL transfer pipette, load 650 µl into a MB Spin Column and centrifuge at 10,000 x g for 30 s.

23. Discard the liquid underneath the filter in the red benchtop bin, add the remaining supernatant to the MB Spin Column, and centrifuge again at 10,000 x g for 30 s.

24. Repeat this step until all the liquid is loaded in the MB Spin Column.

25. Using a 1000uL transfer pipette, add 300 µl of Solution CB and centrifuge at 10,000 x g for 30 s.

26. Discard the liquid underneath the filter in the red benchtop bin, centrifuge again at 10,000 x g for 1 min.

27. Prepare and label as many clean 2 ml Collection Tube as many samples processed

28. Place the corresponding MB Spin Column in a new labelled 2 ml Collection Tube.

29. Using a 100uL transfer pipette, add 50 µl of Solution EB to the center of the white filter membrane, being careful not to touch the filter!
    Note: if there was a large pellet at the beginning of the extraction you can add 100µl of Solution EB instead of 50µl (using a 200 µL pipette)

30. Centrifuge at 10,000 x g for 30 s.

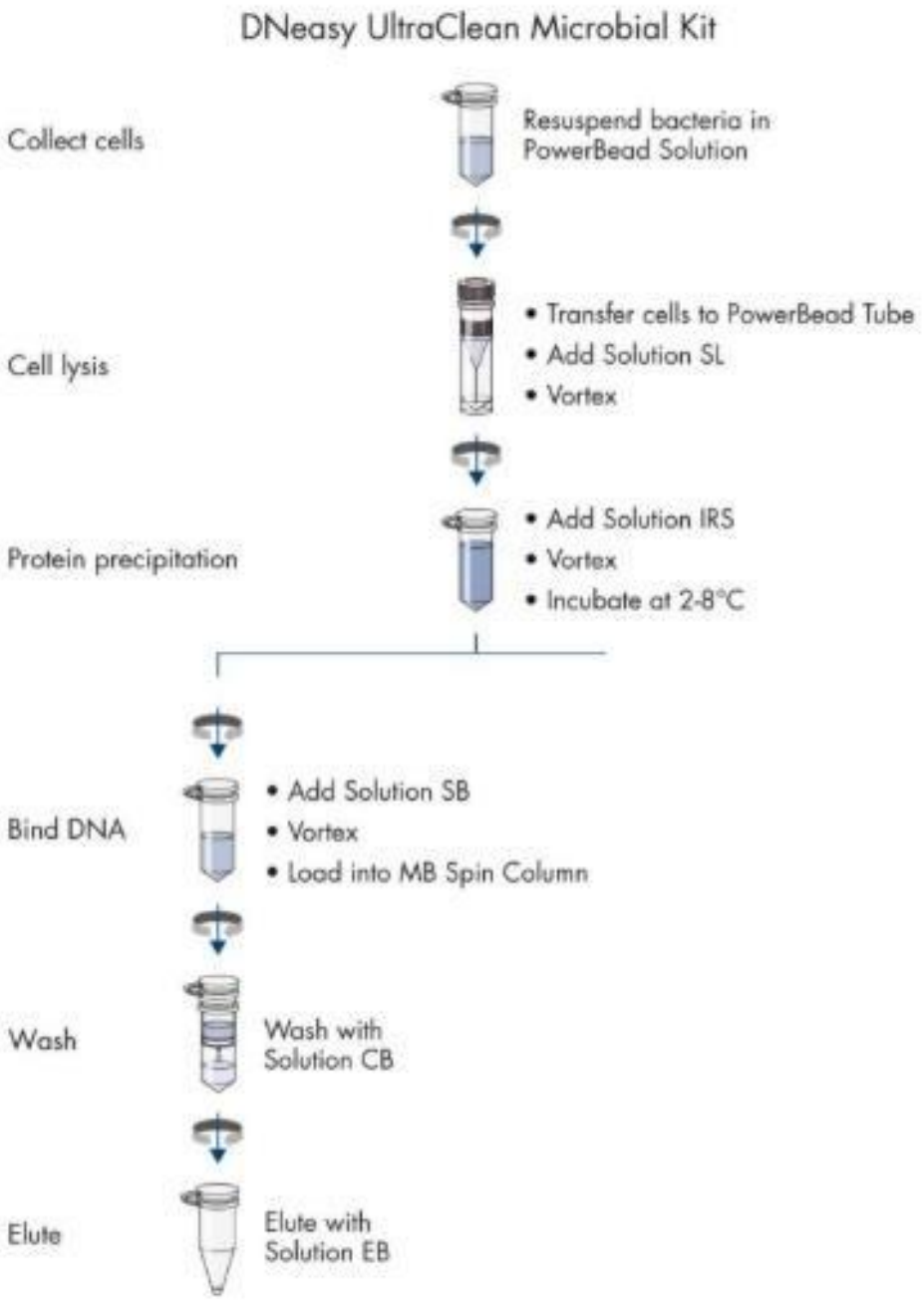
31. Discard the MB Spin Column. Throw it in the red benchtop bin.

32. The DNA is in the collection tubes and is now ready for downstream applications.

DNA STORAGE

It is recommended to store the DNA in the fridge up to 3 days or in the freezer (–20°C or –80°C) as Solution EB does not contain EDTA.

Diagram 1. DNA purification steps using the DNeasy UltraClean microbial kit.



Related documents

- Qiagen DNeasy® UltraClean® Microbial Kit Handbook (https://www.qiagen.com/gi/resources/download.aspx?id=a733f3a9-5a8b-4003-b28e-55bb050bad1e&lang=en)
- DNeasy Microbial Kit Safety Data Sheet (https://www.qiagen.com/gi/knowledge-and-support/product-and-technical-support/quality-and-safety-data/sds-search?l=GB&q=DNeasy%20UltraClean%20Microbial%20kit%20)
- SOP-10.3-SCENSE: 10.3 Subculture: Seeding
- SOP-10.3.1: TECHREP
- SOP 10.3.2 : SUBPUR
- SOP 10.3.4: DNAQNT
- SOP-11.3-SOUENV
- DOC-6.8-DECHINT: 6.8 Internal waste management
- POS-8.1-CQI: 8.1 Internal Quality Control
- FM-9-SCENSE: 9 Subculture: Seeding
- IFU InTray Chocolate and Colorex Cassettes