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Genomic DNA Extraction Protocol using DNeasy® UltraClean® Microbial Kit

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We use this protocol and it's working

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Disclaimer

This protocol has been validated under controlled laboratory conditions. Users must follow biosafety regulations when handling bacterial cultures and ensure compliance with institutional safety guidelines. The authors are not responsiblefor procedural errors or misuse.

Abstract

This protocol describes the extraction of high-quality genomic DNA from *Pseudomans* sp. using mechanical and chemical lysis, followed by column-based purification. The extracted DNA is suitable for PCR, sequencing, and other downstream applications. This protocol follows strict contamination control measures to ensure sample integrity and avoid DNA degradationduring extraction.

Guidelines

- If Solution SL has precipitated, heat at 55°C for 5–10 minutes before use.
- Shake Solution SB well before use.
- Perform all centrifugation steps at room temperature (15–25°C).
- Use a clean workspace and filter pipette tips to prevent DNA contamination.
- Ensure the PowerBead® tubes rotate freely in the centrifuge to prevent breakage.
- Store extracted DNA at -80°C to maintain integrity for long-term use.



Materials

Reagents & Solutions

- PowerBead® Solution – Homogenization buffer for cell dispersion
- Solution SL – SDS-based lysis buffer
- Inhibitor Removal Solution (IRS) – Removes non-DNA contaminants
- SB Solution – DNA binding buffer (high-salt)
- Column Binding (CB) Solution – Removes salt and contaminants
- Solution EB – Elution buffer for final DNA recovery

Consumables & Equipment

- PowerBead® Tubes – For mechanical disruption
- 2 mL Collection Tubes – For DNA binding and purification
- MB Spin Columns – Silica-based DNA binding columns
- Vortex Mixer with Adapter – For bead beating and lysis
- Microcentrifuge – Capable of 10,000 x g
- Pipettes & Sterile Tips – For precise liquid handling
- Water Bath (55°C) – For dissolving SDS precipitates
- Ice Bath – For cooling during inhibitor removal
- -80°C Freezer – For long-term DNA storage

Safety warnings

- ! ▪ Always use gloves and pipette carefully to avoid contamination.
- Ensure proper disposal of biological waste according to institutional guidelines.
- Do not skip inhibitor removal to prevent downstream PCR inhibition.

Ethics statement

This protocol involves bacterial DNA extraction and does not involve animal or human subjects. However, researchers must follow institutional biosafety regulations when handling microbial cultures and ensure compliance with laboratory safety guidelines.

If this protocol is adapted for DNA extraction from animal or human sources, prior approval must be obtained from the Institutional Animal Care and Use Committee (IACUC) or an equivalent ethics committee. Researchers must comply with internationally accepted ethical standards and include any relevant permit numbers and committee approvals where applicable.

Proper biosafety precautions should be followed to prevent contamination and ensure safe handling of all biological materials.

Before start

- **Very important:** Before beginning the DNA extraction process, ensure that all required materials and equipment are prepared and readily available. This helps prevent contamination, reduces workflow interruptions, and ensures smooth execution.

Check and recheck the following before starting:

Reagents & Solutions:

- ✓ PowerBead® Solution – Homogenization buffer
- ✓ Solution SL – Lysis buffer (pre-warm at 55°C if precipitated)
- ✓ Inhibitor Removal Solution (IRS) – Removes contaminants
- ✓ SB Solution – DNA binding buffer
- ✓ Column Binding (CB) Solution – Removes salt and inhibitors
- ✓ Solution EB – Elution buffer

Equipment & Consumables:

- ✓ 2 mL Collection Tubes – For processing samples
- ✓ MB Spin Columns – For DNA binding
- ✓ PowerBead® Tubes – For mechanical lysis
- ✓ Tube Holder or Rack – Keeps tubes organized
- ✓ Vortex Mixer with Adapter – For bead beating
- ✓ Microcentrifuge (capable of 10,000 x g) – For sample processing
- ✓ Pipettes & Filtered Tips (P20, P200, P1000) – For precise liquid handling

Cleaning & Contamination Control:

- ✓ KimWipes® – For wiping and decontaminating surfaces
- ✓ Ethanol Spray Bottle – For workspace sterilization
- ✓ Disposable Gloves – To prevent contamination
- ✓ Biohazard Waste Bin – For proper disposal of contaminated materials
- ✓ Bleach Solution (if needed) – For disinfecting used consumables

Storage & Temperature Control:

- ✓ Ice Bath – For cooling IRS-treated samples
- ✓ 55°C Water Bath – For dissolving SDS precipitates
- ✓ -80°C Freezer – For storing extracted DNA

Final Check:

- Confirm all solutions are properly mixed and at the correct temperature.
- Ensure the workspace is clean and free from contamination.
- Label all tubes and prepare a log sheet for tracking samples.

- Preheat water bath at 55°C to warm up solution if needed.
- Pre-warm Solution SL (if precipitated) at 55°C before use.
- Check all buffers and reagents to ensure proper mixing and availability.
- Label all tubes properly to avoid mix-ups between samples.
- Prepare a clean and contamination-free workspace.

Procedures

1 Bacterial Cell Collection

1. Measure the optical density (OD600) of the culture, ensuring it is around 0.7 for optimal DNA yield.
2. Transfer 1.8 mL of bacterial culture (grown in LB broth for 24 hours) into a 2 mL collection tube.
3. Centrifuge at 10,000 x g for 30 seconds.
4. Carefully decant the supernatant.
5. Repeat centrifugation (10,000 x g, 30 seconds) and remove the remaining supernatant using a pipette.

Note 1: Freshly grown 24-hour LB culture (OD600 ~0.7) provides optimal DNA yield and purity.

Note 2: Longer centrifugation times may be required to achieve an adequate pellet.

Note 3: It is recommended to perform this step inside a biosafety cabinet to maintain sterility and prevent contamination.

2 Cell Lysis

1. Resuspend the cell pellet in 300 µL of PowerBead® Solution and vortex gently.
2. Transfer the resuspended cells to a PowerBead® Tube.
3. Add 50 µL of Solution SL to the PowerBead® Tube.
 - If precipitated, preheat Solution SL at 55°C before use.

Note: Solution SL contains SDS and disruption agents to break and open bacterial cells.

4. Secure the PowerBead® Tubes horizontally in the vortex adapter and vortex at maximum speed for 10 minutes.

Note: This step ensures efficient mechanical and chemical lysis of cells.

3 Removing Cell Debris

1. Ensure PowerBead® tubes rotate freely in the centrifuge to prevent damage.
2. Centrifuge at 10,000 x g for 30 seconds.
3. Transfer the supernatant (~300–350 µL) to a clean 2 mL collection tube.

Note: The pellet contains cell debris, while the DNA remains in the supernatant.

4 Inhibitor Removal

1. Add 100 µL of Inhibitor Removal Solution (IRS) to the supernatant.
2. Vortex for 5 seconds.
3. Incubate at 4°C for 5 minutes.
4. Centrifuge at 10,000 x g for 1 minute.
5. Carefully transfer the supernatant to a new 2 mL collection tube, avoiding the pellet.

Note: The pellet contains non-DNA organic and inorganic contaminants.

5 DNA Binding

1. Add 900 µL of SB solution to the supernatant and vortex for 5 seconds.

Note: SB solution creates high-salt conditions for DNA binding to the spin column.

1. Load 700 µL of the mixture into an MB Spin Column.
2. Centrifuge at 10,000 x g for 30 seconds.
3. Discard the flow-through.
4. Repeat steps 18–20 until the entire sample is processed (usually 2–3 loads).

6 **Washing & Purification**

1. Add 300 µL of Column Binding (CB) Solution to the MB Spin Column.
2. Centrifuge at 10,000 x g for 30 seconds.

Note: CB solution removes residual salts and contaminants.

3. Discard the flow-through and centrifuge again for 1 minute at 10,000 x g to remove residual CB solution.

7 **DNA Elution**

1. Transfer the MB Spin Column to a new 2 mL collection tube.
2. Add 50 µL of autoclaved Double DI (DDI) water directly to the center of the white filter membrane instead of Solution EB.
3. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
4. Discard the MB Spin Column and store the extracted DNA at -80°C.

Note 1: The eluted DNA is ready for PCR, sequencing, or other molecular applications.

Note 2: Autoclaved Double DI (DDI) water was used instead of Solution EB to elute the DNA, ensuring a nuclease-free environment while avoiding potential salt or EDTA interference in downstream applications.

8 **Expected Results**

High-quality, purified DNA suitable for molecular biology applications such as PCR, sequencing, and cloning.

1. **DNA concentration and purity** can be measured using:

- NanoDrop™ spectrophotometer – for absorbance-based quantification and purity assessment (A260/A280 ratio should be ~1.8–2.0 for high-purity DNA).
- Qubit™ fluorometer – for precise quantification using fluorescent dye-based detection.

2. **DNA integrity can be confirmed via agarose gel electrophoresis:**

- Run 5 µL of extracted DNA on a 0.8% agarose gel stained with EtBr or SYBR Safe.
- High-quality genomic DNA should appear as a sharp, high-molecular-weight band with minimal smearing.
- Smearing or degraded bands may indicate DNA fragmentation due to poor handling or degradation.

Note: If DNA is degraded or of low yield, consider optimizing lysis conditions, increasing sample input, or improving elution efficiency.



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