BECKMAN COULTER Life Sciences

Extraction of DNA from Mucoid Bacteria using GenFind V3

Researchers working on mucoid bacteria (i.e. Pseudomonas aeruginosa), bacteria producing excessive exopolysaccharides, or cystic fibrosis infection models may use this protocol.

Please reference current GenFind V3 IFU for product information (Part Number: C34880, C34881)

Purpose

Several bacterial species make extensive amounts of exopolysaccharide, which can significantly increase the viscosity of samples and also lead to coelution of polysaccharides with DNA. This increased viscosity can clog columns and prevent bead settling if not addressed in the DNA purification process. This protocol reduces viscosity due to the presence of exopolysaccharides and decreases the amount of polysaccharide in the final eluate.

Materials Used

Material	Part Number	Supplier
1.2 mL 96-well plate	AB1127	Thermo Fisher Scientific
Nuclease-free water (Molecular Grade)	AM9932	Thermo Fisher Scientific
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Microcentrifuge tubes 1.5 mL	357448	Beckman Coulter Life Sciences
EDTA	AB00502	American Bio
PEG 8000	25322-68-3	Sigma-Aldrich
LB Broth	AB08041	American Bio
RNase A	B85620	Beckman Coulter Life Sciences

Protocol

1. **Sample Preparation**
    a. Grow cells up overnight in LB broth
    b. Transfer **1 mL** of **overnight culture** to a microcentrifuge tube
    c. Pellet cells by spinning for **5 minutes** at **3,000 x *g***
    d. Remove and discard the supernatant without disrupting the cell pellet
    e. Resuspend the cells in **800 µL** of **PEG Wash solution**
        i. **PEG Wash solution** is prepared as follows:
            a. **80 µL of (50% w/v) PEG 8000**
            b. **400 µL of 0.5 M EDTA**
            c. **360 µL of nuclease-free water**
    f. Pellet cells by spinning for **5 minutes** at **3,000 x *g***
    g. Remove and discard the supernatant without disrupting the cell pellet

2. **Lysis**

    a. Add **200 µL** of **Lysis (LBB)** to cell pellet
    b. Add **200 µL** of **0.5 M EDTA**
    c. Add **30 µL** of **Proteinase K (PK)**
    d. Add **1 µL** of **RNase**
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. Transfer resuspension to a 1.2 mL 96-well plate
    g. **Incubate** the plate for **30 minutes** at **55°C**

3. **Bind**

    a. Vortex to fully resuspend the **Bind (BBB)**
    b. Add **300 µL** of **Bind (BBB)** to the plate
    c. **Incubate** the plate for **5 minutes** at **room temperature**
    d. Place the plate on a **magnet** for **15 minutes** (or until the supernatant is clear)
    e. Remove and discard the supernatant without disrupting the beads
    f. Remove the plate from the magnet

4. **Wash**

    a. Add **800 µL** of **Wash (WBC)** to each sample well
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **3 minutes** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Add **800 µL** of **Wash (WBB)** to each sample well
    g. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    h. Place the plate on a **magnet** for **6 minutes** (or until the supernatant is clear)
    i. Remove and discard the supernatant without disrupting the beads
    j. Remove the plate from the magnet
    k. Repeat steps 3.a-3.e for a total of **2 washes**
    l. Add **800 µL** of **Wash (WBC)** to each sample well
    m. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    n. Place the plate on a **magnet** for **3 minutes** (or until the supernatant is clear)
    o. Remove and discard the supernatant without disrupting the beads
    p. Leave the plate on a **magnet** for **1 minute** (or until no visible liquid is present)
    q. Remove the plate from the magnet

5. **Elute**

    a. Add **40 µL** of **nuclease-free water** to each sample well
    b. **Incubate** the plate for **2 minutes** at **room temperature**
    c. Place the plate on a **magnet** for **2 minutes** (or until the supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Data shown resulted from isolated genomic DNA from a dense overnight culture of *P. aeruginosa*. Genomic DNA (gDNA) isolated from *P. aeruginosa* assessed for concentration and purity by NanoDrop (Thermo Fisher Scientific) (Table 1). Total yield averaged 440ng and A260/A280 average 1.73. The gDNA integrity was assessed on an Agilent Genomic DNA Screen Tape; the DIN score was 8.5 (Figure 1).

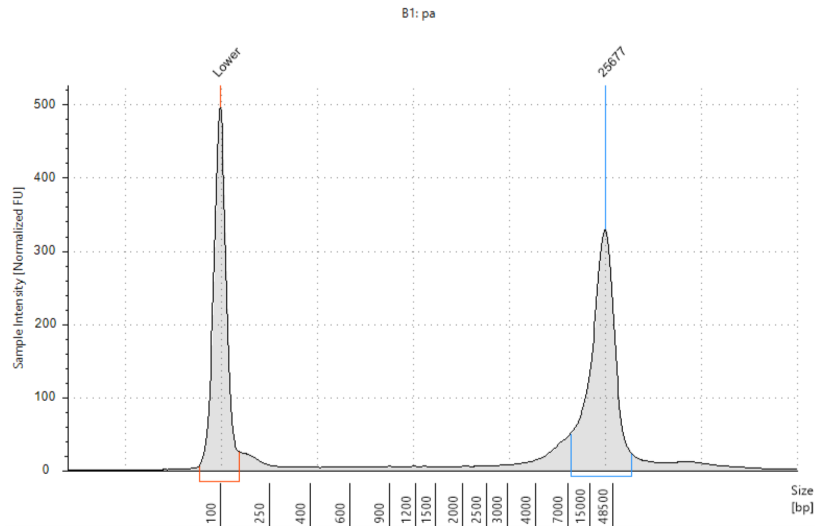

Figure 1. An Agilent Genomic DNA Screen Tape of DNA extracted from P. aeruginosa. The electropherogram of one of the replicates is shown above. The DIN score for this sample was 8.5 indicating intact genomic DNA.

BECKMAN COULTER Life Sciences

Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this protocol, including but not limited to warranties of fitness for a particular purpose or merchantability or that the protocol is non-infringing. All warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter. Not intended or validated for use in the diagnosis of disease or other conditions. This protocol is for demonstration only, and is not validated by Beckman Coulter.

© 2024 Beckman Coulter, Inc. All rights reserved. Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

2023-GBL-EN-102534-v2

danaher