BECKMAN COULTER
Life Sciences

DNA Extraction from Bacteria using GenFind V3

Researchers working on bacteria who want to extract DNA may use this protocol.

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

Purpose

The cell wall structure of bacteria differs from eukaryotes and requires lysis optimization. This protocol provides a method for extracting DNA from bacteria with data showing its effectiveness with *Escherichia coli, Staphylococcus aureus, Salmonella, Corynebacterium and Mycobacterium*, a difficult to work with bacteria.

Materials Used

Material	Part Number	Supplier
1.2 mL 96-well plate	AB1127	Thermo Fisher Scientific
Lysozyme from chicken egg white	L6876	Sigma-Aldrich
100% Ethanol (Molecular Grade)	AB00138	American Bio
2xYT	AB15063-01000	American Bio
PBS, pH 7.4	10010023	Thermo Fisher Scientific
Nuclease-free water (Molecular Grade)	AM9932	Thermo Fisher Scientific
100 % Isopropanol (Molecular Grade)	AB07015-01000	American Bio
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Microcentrifuge tubes 1.5 mL	357448	Beckman
RNase A	B85620	Beckman

Protocol

1. **Lysis**
    a. Transfer **500 µL of overnight culture** to a 1.5 mL microcentrifuge tube
        i. If the overnight culture is not greater than an OD of 1 then a larger volume of culture can be transferred.
    b. Pellet overnight culture by spinning for **10 minutes** at **5,000 rpm** (adjust as needed for specific bacteria)
    c. Resuspend pellet in **200 µL** of **PBS**
    d. Transfer **200 µL** of **Culture** in **PBS** to 1.2 mL 96-well plate
    e. Add **12 µL** of **lysozyme (100 mg/mL)** to plate
    f. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    g. **Incubate** the plate for **30 minutes** at **37 °C**
    h. Add **500 µL** of **Lysis (LBB)** to plate
    i. Add **30 µL** of **Proteinase K** to plate
    j. Add **1 µL** of **RNase A (100 mg/mL)** to plate
    k. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    l. **Incubate** the plate for **2 hours** at **65 °C**

2. **Bind**
    a. Vortex to fully resuspend the **Bind (BBB)**
    b. Add **300 µL** of **Bind (BBB)** to the plate
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** the plate for **5 minutes** at **room temperature**
    e. Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
    f. Remove and discard the supernatant without disrupting the beads
    g. Remove the plate from the magnet
3. **Wash**
    a. Add **800 µL** of **Wash 1 (WBB)** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat steps 3.a-3.e for a total of **2 washes**
    g. Add **800 µL** of **Wash 2 (WBC)** to plate
    h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    i. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    j. Remove and discard the supernatant without disrupting the beads
    k. Remove the plate from the magnet
    l. Repeat steps 3.g-3.l for a total of **2 washes**
4. **Elute**
    a. Add **40 µL** of **nuclease-free** water to plate
    b. **Incubate** the plate for **2 minutes** at **room temperature**
    c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Data shown below is the result of isolating genomic DNA from a dense overnight culture of *E. coli, S. aureus, Salmonella, Corynebacterium and Mycobacterium*. Genomic DNA (gDNA) isolated from *E. coli* and *S.* aureus was quantified and purity was assessed by NanoDrop (Thermo Fisher Scientific) (Table 1). The gDNA integrity was assessed on an Agilent Genomic DNA Screen Tape; the DIN scores were 8.9 for E. coli and 9.6 for S. aureus (Figure 1). These high DIN scores indicate low levels of gDNA degradation.

Bacteria	Conc. (ng/µL)	Yield (µg)	$A_{260/280}$
E. coli	57.3	2.29	1.86
S. aureus	80.5	3.22	1.86

Table 1. DNA concentration, yield and purity of DNA extracted from S. aureus (gram positive) and E. coli (gram negative) measured on a NanoDrop (Thermo Fisher Scientific)

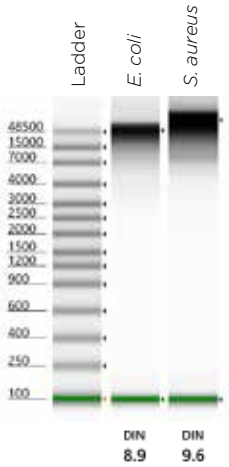

Figure 1. An Agilent Genomic DNA Screen Tape of DNA extracted from *E. coli* and *S. aureus*.

The yields of gDNA isolated from *Salmonella, Corynebacterium* and *Mycobacterium* were quantified by Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) (Table 2). As expected, DNA yields from *Mycobacterium* were much lower than that of the other two bacteria due to the low cellular input common with *Mycobacterium*.

Genus	Average DNA concentration (ng/µL)
Salmonella	62.2
Corynebacterium	42.2
Mycobacterium	1.3

Table 2. The DNA concentration extracted from *Salmonella, Corynebacterium* and *Mycobacterium* as measured by Qubit dsDNA HS Assay Kit at customer sites

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AAG-4752SP02.19