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**

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

**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.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Conc. (ng/µL)</th>
<th>Yield (µg)</th>
<th>A260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>57.3</td>
<td>2.29</td>
<td>1.86</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>80.5</td>
<td>3.22</td>
<td>1.86</td>
</tr>
</tbody>
</table>

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*. 

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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*.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Average DNA concentration (ng/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>62.2</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>42.2</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>1.3</td>
</tr>
</tbody>
</table>

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