User-Developed Protocol:

Isolation of bacterial DNA from soil using the QIAamp® DNA Stool Mini Kit and QIAamp DNA Blood Midi Kit

This procedure has been adapted by customers from the QIAamp® DNA Stool Mini Kit Protocols and is for use with the QIAamp DNA Stool Mini Kit and QIAamp DNA Blood Midi Kit. It has not been thoroughly tested and optimized by QIAGEN.

As starting material, 5 g soil was mixed with different amounts of *Bacillus subtilis* cells. Sensitivity was $5 \times 10^3$ cells/5g soil.

Please be sure to read the *QIAmp DNA Stool Mini Kit Handbook* carefully before beginning this procedure.

Procedure

DNA isolation

1. Weigh up to 5 g soil in a 50 ml BD Falcon™ tube.
2. Add 2–5 ml distilled water to the tube, and mix for 5 min on a shaker.
3. Incubate for 10 min at 95°C.
4. Centrifuge at 3000 rpm for 5 min. Transfer the supernatant to a new tube.
5. Add 7 volumes of Buffer ASL to the supernatant, and mix well.
6. Add 1 InhibitEX™ tablet to the tube and incubate for 1 min at room temperature (15–25°C) on a shaker.
7. Centrifuge sample at 5000 x g for 5 min. Transfer the supernatant into a new tube.
8. Add 1 volume of Buffer AL to the supernatant, and mix well.
9. Add 1 volume of ethanol (96–100%).
10. Place a QIAamp Midi Spin Column on the QIAvac 24 vacuum manifold.
11. Apply the sample lysate onto the QIAamp Midi Spin Column. Apply maximum vacuum.
12. Wash the column once with 1 ml Buffer AW1.
13. Wash the column once with 1 ml Buffer AW2.
14. Place the QIAamp Midi Spin Column in a 15 ml tube (provided), and centrifuge at 5000 rpm for 15 min to dry the membrane.
15. Place the QIAamp Midi Spin Column in a clean 15 ml tube. To elute the DNA, add 300 µl Buffer AE, and centrifuge at 5000 rpm for 5 min.

16. Reload the eluate onto the membrane of the QIAamp Midi Spin Column, and centrifuge at 5000 rpm for 5 min.

**Amplification of a 528 bp atpase gene fragment from B. subtilis**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′–3′</th>
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<tbody>
<tr>
<td>UEB1</td>
<td>GTGTGATTGTTTTATATTGATTGC</td>
</tr>
<tr>
<td>UEB2</td>
<td>GTACCGACAAGACCGAGAGC</td>
</tr>
</tbody>
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**PCR mix**

- 25 µl 10x HotStarTaq™ Master Mix
- 1 µl primer UEB1, 10 µM
- 1 µl primer UEB2, 10 µM
- 22 µl water
- 1 µl DNA (eluate)

**Amplification conditions**

- 95°C for 15 min
- 94°C for 1 min; 52°C for 1 min 45 s; 72°C for 3 min 50x
- 72°C for 10 min 1x

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Trademarks: QIAGEN®, QIAamp®, QiAsec, HotStarTaq™; InhibitEX™ (QIAGEN); BD Falcon™ (Becton, Dickinson and Company).

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