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 x 10^3 cells/5g soil.

Please be sure to read the *QIAamp 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*

Primer	**Sequence 5'–3'**
UEB1	GTGTGATTGTTTTATTGATTGC
UEB2	GTACCGACAAGACCGAGAGC

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