DNA Extraction Protocol for Cockroach Gut Microbiota (Adapted from Omega Bio-Tek's E.Z.N.A. Bacterial DNA Kit)

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Abstract

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Guidelines

This protocol uses an adapted version of Omega Bio-Tek's E.Z.N.A. Bacterial DNA Kit. In new versions of the kit BTL Buffer has been renamed TL Buffer, BDL Buffer has been renamed BL Buffer, and HB Buffer has been renamed HBC Buffer.

Materials

EZNA Bacterial DNA Kit D3350 by Omega Biotek

Protocol

Step 1.

Remove samples, usually *P. americana* gut lumen stored in 100µL RNALater, from -80°C freezer and set them on ice to thaw. Remove lysozyme solution from the -20°C freezer and set it on ice to thaw. Preheat Thermomixer to 37°C.

Step 2.

Remove 50µL sample preserved in RNAlater. Place aliquot in a new 1.5mL microcentrifuge tube.

Step 3.

Add 100µL Balanced Salt Solution (2.5 g K2HPO4, 1 g KH2PO4, 1.6 g KCl, 1.4 g NaCl, and 10 ml of 1 M NaHCO3 per liter, pH 7.2) to the new microcentrifuge tube. Spin microcentrifuge tube 10 minutes at 5,000xg. Discard supernatant.

Step 4.

Add 100µL 1xTE (10 nM Tris, 1mM EDTA [pH 8]) and vortex to completely resuspend the pellet. Add

10µL of lysozyme solution. Incubate 30 minutes at 37°C. Add 25mg glass beads to new 1.5mL microcentrifuge tube.

Step 5.
Preheat Thermomixer to 55°C. Add sample to the glass beads. Vortex at maximum speed for 5 minutes.

Step 6.
Let sample stand to allow the beads to settle. Transfer supernatant to a new 1.5mL microcentrifuge tube.

Step 7.
Add 100µL BTL Buffer and 20µL Proteinase K Solution. Vortex to mix thoroughly.

Step 8.
Incubate at 55°C, shaking at 600rpm for 60 minutes.

Step 9.
Centrifuge at 10,000xg for 2 minutes to pellet any undigested material. Preheat Thermomixer to 65°C. Place Elution Buffer in the hybridization oven and preheat to 65°C.

Step 10.
Transfer the supernatant to a new 1.5mL microcentrifuge tube. Do not disturb the pellet.

Step 11.
Add 220µL BDL Buffer to the new microcentrifuge tube with the supernatant. Vortex to mix.

Step 12.
Incubate at 65°C for 10 minutes.

Step 13.
Add 220µL ethanol. Vortex for 20 seconds at maximum speed to mix thoroughly. Note: If any precipitate can be seen at this point, break the precipitate by pipetting up and down 10 times.

Step 14.
Insert a HiBind DNA Mini Column into a 2mL Collection Tube.

Step 15.
Transfer the entire sample to the HiBind DNA Mini Column, including any precipitate that may have formed.

Step 16.
Centrifuge at 10,000xg for 1 minute.

Step 17.
Discard the filtrate and the collection tube.

Step 18.
Insert the HiBind DNA Mini Column into a new 2mL Collection Tube

Step 19.
Add 500µL HB Buffer.

Step 20.
Centrifuge at 10,000xg for 1 minute.

Step 21.
Discard filtrate and reuse the collection tube.

Step 22.
Add 700µL DNA Wash Buffer. Centrifuge at 10,000xg for 1 minute.

Step 23.
Discard the filtrate and reuse the collection tube.

Step 24.
Add 700µL DNA Wash Buffer. Centrifuge at 10,000xg for 1 minute.

Step 25.
Discard the filtrate.

Step 26.
Centrifuge the empty HiBind DNA Mini Column at maximum speed for 2 minutes to dry the column.

Step 27.
Insert HiBind DNA Mini Column into a new nuclease-free 1.5mL microcentrifuge tube.

Step 28.
Add 50µL preheated Elution Buffer to the HiBind DNA Mini Column. Make sure to add the elution buffer to the center of the HiBind matrix.

Step 29.
Incubate the HiBind DNA Column for 5 minutes at 65°C.

Step 30.
Centrifuge at 10,000xg for 1 minute to elute the DNA.

Step 31.
Measure DNA concentration on the NanoDrop and store eluted DNA at -20°C.