RNA Extraction from Cockroach Gut Microbiota

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Abstract

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Materials

EZNA HP Total RNA Kit R6812 by Omega Biotek

EZNA MicroElute RNA Clean Up Kit R6247 by Omega Biotek

Turbo DNA-free Kit AM1907 by Invitrogen - Thermo Fisher

Superase-In RNase Inhibitor AM2694 by Thermofisher

Protocol

Step 1.

Remove samples, cockroach gut lumen stored in 100µl RNALater, from -80°C and thaw on ice. Remove half of the sample for RNA extraction and then return the rest of the sample to the -80°C. Preheat thermomixer to 30°C. Prepare stocks and other materials, if necessary:

• GTC Lysis Buffer (from HP Total RNA Kit): Add 20µl B-mercapoethanol per 1mL of GTC Lysis Buffer. Can store up to 2 weeks at room temperature.

• RNase-free Lysozyme: 30 mg lysozyme per 1 mL of TE buffer. Store aliquots at -20°C

• Add 25-40mg glass beads to new tubes

• QVL Lysis Buffer (from MicroElute RNA Clean Up Kit): Add 20µl B-mercapoethanol per 1mL of QVL Lysis Buffer. Can store up to 1 week at room temperature.

Warning: Using the full gut lumen sample is too much--it will not purify.

Step 2.

Add 200µl PBS to each sample. Centrifuge at 5,000 x rcf for 5 minutes.

Step 3.

Remove and discard supernatant. Resuspend cockroach samples in 50µl lysozyme/TE buffer. Add 4µl Superase-In RNase inhibitor to each sample and vortex for 30s before incubating at 30°C for 10 minutes at 300rpm.

Warning: Using less Superase-In RNase inhibitor will result in degradation.

Step 4.

Add 700µl GTC Lysis Buffer to each incubated tube. Mix by pipetting. Transfer mixture to a new tube with beads and vortex for 30 seconds, then place on ice for 30 seconds. Repeat 3 times (so bead beating occurs for a total of 2 minutes). Centrifuge on short. Continue with HP Total RNA kit protocol (December 2010 Version), starting at step 5:

Step 5.
Transfer lysate to a DNA clearance column placed in a collection tube. Centrifuge at 13,000 x rcf for 1 minute. Discard the DNA clearance column and save the flow through.

Step 6.
Add an equal volume (700µl) of 70% ethanol to the lysate and mix by pipetting. Do not centrifuge.

Step 7.

Apply the sample to a HiBind RNA column inserted into a collection tube. Centrifuge at 10, 000 x rcf for 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step. Place DEPC water in 70°C hybridization oven. Preheat thermomixer to 37°C.

Note: Maximum capacity of the HiBind RNA spin column is 700 µL, so you will need to repeat this step twice (adding half of the volume to the RNA spin column each time).

Step 8.
Add 500µl of RNA Wash Buffer I to the HiBind RNA column. Centrifuge at 10,000 x rcf for 60 seconds. Discard the flow-through and reuse the collection tube in the next step.

Step 9.
Add 500µl of RNA Wash Buffer II by pipetting directly onto the HiBind column. Centrifuge at 10,000 x rcf for 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.

Step 10.
Repeat step 9 for a second wash. Discard flow-through and place the HiBind RNA column in a new 2mL collection tube.

Step 11.
Centrifuge the HiBind RNA column for 2 minutes at maximum speed.

Step 12.

Transfer the column into a clean 1.5 mL centrifuge tube and add 50µl of DEPC water. Incubate at

room temperature for 5 minutes. Centrifuge for 2 minutes at maximum speed.

Step 13.

Follow Turbo DNA-free procedure for routine DNase treatment (Step 1 & 2): Add 0.1 volume 10X Turbo DNase Buffer (5µl) and 1µl TURBO DNase to the RNA and mix gently. Incubate at 37°C for 20-30 minutes.

Step 14.

Follow Omega Bio-Tek's E.Z.N.A. MicroElute RNA Clean Up Kit (July 2014). Adjust the sample volume to 100µl (add 44µl of DEPC water).

Step 15.
Add 350µl of QVL Lysis buffer and mix by vortexing. Add 250µl of 100% Ethanol and mix thoroughly by vortexing.

Step 16.
Apply sample to MicroElute LE RNA Mini Column inserted in a 2mL collection tube. Centrifuge at 10,000 x rcf for 15 seconds. Discard the flow-through.

Step 17.
Add 500µl of RNA Wash Buffer II. Centrifuge at 10,000 x rcf for 30 seconds. Discard the flow-through.

Step 18.
Add 500µl of RNA Wash Buffer II. Centrifuge at 13,000 x rcf for 2 minutes (to dry the membrane). Discard the flow-through and collection tube.

Step 19.
Transfer the column into a new collection tube. Open the cap of the column and centrifuge at maximum speed for 5 minutes. Discard the flow-through and collection tube.

Step 20.

Transfer the column to a clean 1.5mL microfuge tube and pipette 30µL DEPC water into the column. Centrifuge for 1 minute at max speed to elute RNA.