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Extration of Scapharca broughtonii genomic DNA

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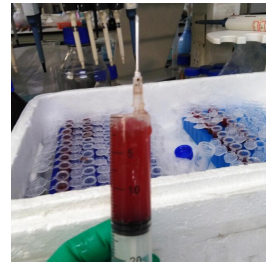
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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is used to eliminate excessive polysaccharide in *Scapharca broughtonii* tissues during DNA extraction.

Attachments



工作簿13.xlsx

9KB

Troubleshooting

- 1 Collect 1 mL haemocyte into a 1.5 mL microcentrifuge tube and centrifuge at 1000 g for 10 min at 4°C, then discard the supernatant and resuspend the pellet with 200 µL PBS.
- 2 Add 200 µL Buffer AL* and 20µL Rnase A, mix thoroughly by vortexing and put the tube at room temperature for 3-5 min.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)

- 3 Add 20 µl proteinase K*. Mix thoroughly by vortexing, and incubate at 56 °C for 15 min. Then add 10% SDS (Sodium dodecyl sulfate) and continue to incubate at 56 °C for 15 min.

Note

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- 4 Add the same volume of cooled Chloroform at 4°C and mix by inverting, then put the tube at room temperature for 10 min.
- 5 Centrifuge at 12,000 g for 10 min at 4°C and then transfer the supernatant aqueous phase in a new tube.
- 6 Add 1/3 volume of absolute ethanol, and then mix by inverting.
- 7 Pipet 700 µl mixture from step 6 into the DNeasy Mini spin column* placed in a 2 ml collection tube*. Centrifuge at 12,000 g for 30 sec at 4°C. Discard flow-through and collection tube.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)

- 8 Place the DNeasy Mini spin column* in a new 2 ml collection tube*, add 500 µl Buffer AW1*, and centrifuge at 12,000 g for 30 sec at 4°C. Discard flow-through and collection

tube.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)

- 9 Place the DNeasy Mini spin column* in a new 2 ml collection tube*, add 500 µl Buffer AW2*, and centrifuge at 12,000 g for 30 sec at 4°C. Discard flow-through and collection tube.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)

- 10 Repeat the step 9.

- 11 Place the DNeasy Mini spin column* in a new 2 ml collection tube*, centrifuge at 12,000 g for 2 min at 4°C, and then put the spin column at room temperature for 10 min to dry the DNeasy membrane.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)

- 12 Place the DNeasy Mini spin column* in a clean 1.5 ml microcentrifuge tube and pipet 50 µl Buffer AE* directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 12,000 g to elute.

Note

*: these items were provided by Qiagen DNeasy® Blood & Tissue kit (Catalog no. 69504)