Nov 06, 2020

DNA Extraction for Beetle DNA with Qiagen DNeasy

Demian F Gomez[1], Caroline Storer[1]

[1]University of Florida

1 Works for me dx.doi.org/10.17504/protocols.io.bnu9mez6

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to extract DNA from beetles using the DNeasy protocol with additional notes to the manufacturer guidelines.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

DOI

dx.doi.org/10.17504/protocols.io.bnu9mez6

DOCUMENT CITATION

Demian F Gomez, Caroline Storer 2020. DNA Extraction for Beetle DNA with Qiagen DNeasy. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnu9mez6

CREATED

Oct 23, 2020

LAST MODIFIED

Nov 06, 2020

DOCUMENT INTEGER ID

43649

ABSTRACT

The purpose of this protocol is to extract DNA from beetles using the DNeasy protocol with additional notes to the manufacturer guidelines.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

General note: Kit Qiagen DNeasy works better for degraded specimens.

Materials:

Sterile Falcon tube

200 ml absolute ethanol (brown and violet glass bottle)

1 spin column per individual
2 collecting tubes (with no caps) per individual
2 eppendorf vial per individual
trash container

Manufacturer Protocol:

1. Add 180 µl Buffer ATL. Add 20 µl proteinase K, mix by vortexing and incubate at 56°C until completely lysed. Vortex occasionally during incubation. Vortex 15 s directly before proceeding to step 2.
2. Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.
3. Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing.
4. Pipet the mixture into a DNeasy Mini spin column placed in a 2m collection tube. Centrifuge
5. at ≥6000 x g (8000 rpm) for 1 min. Discard the flow-through and collection tube.
6. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge
7. for 1 min at ≥6000 x g. Discard the flow-through and collection tube.
8. Place the spin column in a 2ml Collection Tube, add 500 µl Buffer AW2 and centrifuge
9. for 3 min at 20,000 x g (14,000 rpm). Discard the flow-through and collection tube.
10. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
11. Elute the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x g.

Additional notes of protocol:
Turn on heating block - 56 Celsius
Turn on Hood (UV for at least 15 minutes)

Excel sheet - DNA extractions (see template)
Add it to Database of Extractions
Print sheet to add notes, like bad quality individual
Gloves on
Label each vial with pen (Extraction number)
Label cap and vial for the final eppendorf vial
Order of the grid:
Cryovial with specimen
Eppendorf
Spin column
Collecting tube
Collecting tube
Final Eppendorf (2 labels)
Prepare specimens and put them in the eppendorf vials
Wait until ethanol is dry
Go to hood - prepare tips (filtered 200 uL), 200 pipet (yellow), ATL, proteinase K (do not touch red cap).

Add 180 uL ATL
Add 20 uL proteinase K
Move every vial one position down to keep track

Check temperature of heating block
Use vortex to mix proteinase K (10 seconds)
Incubation 3 hours in heating block - check every hour and use vortex for 2 seconds.
Final vortex for 15 seconds. Incubate more time and vortex more times if DNA quality is bad.

Clean hood with ethanol

Turn heat block off

Add 200 uL AL Buffer (200 pipet)
Vortex 15 seconds

Add 200 uL Absolute ethanol

Citation: Demian F Gomez, Caroline Storer (11/06/2020). DNA Extraction for Beetle DNA with Qiagen DNeasy. https://dx.doi.org/10.17504/protocols.io.bnu9mez6

Vortex 15 seconds
Grab 1000 pipet (blue + big tips) and set 550 - take liquid out to the column and leave only the beetle. Repeat or go down and up if necessary. Add ethanol to the vial (later to store in database).
Centrifuge 8000 rpm for 1 minute
Change column to next vial, discard liquid in special container, and discard the collecting tube

Add 500 uL of AW1 (use big tips)
Centrifuge 8000 rpm for 1 minute
Change column to next vial, discard liquid in special container, and discard the collecting tube

Add 500 uL of AW2 (use big tips)
Centrifuge 14.4 rpm for 3 minutes
Put column in last eppendorf - discard collecting tube

Add 50 uL Buffer AE (200 uL pipet) - be very careful to put in in the middle (check open bottle)
Let the vials sit for 10 minutes - use time to put insects away. Can be more:15 or 20 minutes. But the longer you leave it, there will be more degradation. It is a compromise between getting more and getting more complete DNA.

Centrifuge 8000 rpm 1 min
Get rid of column - if the samples are bad quality then use the column again with the same vial and centrifuge last step again
Store DNA extraction to strip tube in the freezer.

Citation: Demian F Gomez, Caroline Storer (11/06/2020). DNA Extraction for Beetle DNA with Qiagen DNeasy. https://dx.doi.org/10.17504/protocols.io.bnu9mez6