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# Non-destructive DNA extraction protocol for minute insects

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**We use this protocol and it's working**

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## Abstract

This is a modified protocol for Qiagen's DNEasy Blood and Tissue kit for non-destructive DNA extraction of minute insects and other organisms. It was optimized for parasitic wasps (0.5-2mm size).

## Materials

MATERIALS

- Proteinase K, 2mL **Qiagen Catalog #**19131
- 1.5 mL Eppendorf tubes
- Ethanol 100%
- DNeasy Blood & Tissue Kit, QIAGEN **Catalog #**Cat No./ID: 69504
- KimWipes **Fischer Scientific**
- 20-200 uL Sterile Pipette Tips
- 100-1000 uL Sterile Pipette Tips

## Before starting

1 Confirm that there is at least 1 set of standard pictures of the specimens that will go through DNA extraction. Check that the following equipments are available: hot bath or oven, racks, vortex mixer, centrifuge. If the insects are in alcohol and kept in the freezer, let the samples get to room temperature.

2 Check the DNA extraction kit:
If necessary, redissolve precipitates in buffers ATL and AL.
Make sure ethanol has been added to buffers AW1 and AW2.
There must be at least 1 set (1 set = number of insect samples) of spin columns 1 extra set of collecting tubes and 3 sets of microcentrifuge tubes.

## Day 1

3 Label and sterilize (expose to UV) 3 sets of microcentrifuge tubes and turn on the hot bath or oven to stabilize at 56°C.

4 Put 90uL buffer ATL in each microcentrifuge tube.

5 If applicable, blot alcohol from specimen on a kimwipe (may need to let specimen dry a little). Put the entire insect in a microcentrifuge tube with buffer ATL. If the specimens are too small, it may be necessary to perform this step under stereomicroscope.

> Note
>
> If the specimen has alcohol, buffer ATL is likely to form crystals which will affect the quality of the voucher slide.

6 Add 10uL proteinase K to each microcentrifuge tube, for a total of 100uL (1/2 regular protocol).

7 Make sure the specimen is in contact with the solution. If the specimen is stuck to the wall of the tube, tap it gently on the bench to force it down. Do NOT use the centrifuge, especially for insects that are weakly sclerotized.

8 Incubate at 56ºC overnight (at least 8 hours).

## Day 2

9 Take the second set of sterile eppendorf tubes. Label a set of spin columns from the kit.

10 With a pipette set to approximately 150uL, transfer all the fluid (make sure the “gunk” is pulled) to a new eppendorf tube. The insect is left in the tube where it incubated. Be careful not to pipette the insect as weakly sclerotized ones may have become transparent.

11 Separate the set of eppendorf tubes with the insects. Add some distilled water. Leave insect in water while the remaining steps are concluded.

12 Get the eppendorf tubes with the extracts. Vortex for 15 seconds, then add 100uL buffer AL and vortex again.

13 Add 100uL ethanol 100%, vortex.

14 Set a pipette to 350-400uL to transfer the mixture to a DNeasy Mini spin column in collection tube. Centrifuge at 8000rpm for 1min.

15 Discard collection tube with flow-through, place the spin column in a new 2mL collection tube.

16 Add 500uL buffer AW1 to each spin column. Centrifuge 8000rpm for 1min, discard flow-through (the liquid must not touch the bottom of the spin column).

17 Add 500uL buffer AW2 to each spin column, centrifuge 14,000rpm (or maximum possible if centrifuge doesn't reach 14,000rpm) for 3 minutes.

18 Discard collection tube and transfer spin columns to the labeled 1.5uL microcentrifuge tubes.

19 Add 100uL buffer AE to each spin column. Wait for 1-2 minutes to incubate at room temp.

20 Centrifuge at 8,000rpm for 1 minute. Repeat.

Note

Place microcentrifuge tubes so that tops are towards the center of the centrifuge. The original DNeasy protocol recommends this step is repeated, but if the insect is too small do not add more elution (AE) buffer, or the extract becomes too diluted.

21 Store extracts in -20°C freezer.

## Day 2 - insect

22 To finish recovering and mounting the voucher specimen, ATL+proteinase K needs to be washed from the specimen before exposing it to ethanol. Take the specimen to observe under a stereomicroscope. Use a pipette to remove it from the water. Place it in the small plates or tubes with water where dehydration series will be done and check for specimen integrity (specimen should be complete, although more transparent or faded). The ethanol dehydration must start with pure water and gently increase the amount of ethanol (30%, 50%, 75%, 90%, 100%) until reaching absolute alcohol for next stage of mounting (card-mount or slide-mount). Weakly sclerotized specimens should be moved to slides (see separate protocol for slide preparation).