

DNA Extraction From Archival Museum Insect Specimens

Modified from Qiagen® DNeasy Blood & Tissue Kit

Samuel Crane, July 2011

Designed for digestion of intact, whole beetle heads and prothorax

This DNA extraction protocol uses the Qiagen DNeasy Blood & Tissue kits to process pinned insect specimens from natural history museum collections without the destruction of external morphological characters that may be useful in downstream applications. The protocol was designed to work on beetle specimens but has been used successfully with other small insect groups.

For beetles, the protocol requires separating the specimens into two halves, soaking one half in the lysis buffer, and then returning the processed half to the pin. The dried internal tissues are lysed and the chitinous external structures are unharmed, even fine setae. Using the head and thorax minimizes the potential for symbiont contamination and preserves internal reproductive structures in the abdomen. The amount of DNA recovered will likely be less than with fresh tissue. Thus, the protocol recommends reducing the elution buffer volume by half (to 100 µl). Amount of starting tissue will also affect yield, so adjust accordingly. The Qiagen protocol recommends up to 50 mg of tissue.

ATL is a lysis buffer. It contains SDS, which denatures proteins, and EDTA, which is an enzyme stabilizer and sequesters metal ions such as Ca²⁺. Buffer AL is also a lysis buffer; it contains guanidinium chloride (GndCl), which is a protein denaturant, and a detergent to rupture plasma and nuclear membranes. Qiagen recommends using ATL buffer when lysing tissue (versus blood), so the protocol starts with ATL and proteinase K. Buffer AL is also used to complete the lysis and help bind the DNA to the spin column silica membrane.

Citations

- Gilbert MTP, Moore W, Melchior L, Worobey M (2007) DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS ONE* 2: e272. doi:10.1371/journal.pone.0000272.
- Mandrioli M (2008) Insect collections and DNA analyses: how to manage collections? *Museum Management and Curatorship* 23: 193–199. doi:10.1080/09647770802012375.
- Qiagen FAQ Topic 633 <http://bit.ly/17ZVLva>
- Qiagen Supplementary Protocols: Purification of total DNA from insects using the DNeasy® Blood & Tissue Kit - (EN). Available online: <http://bit.ly/12j9Pa7>
- Rohland N, Siedel H, Hofreiter M (2004) Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. *BioTechniques* 36: 814–816, 818–821.
- Rowley D, Coddington J, Gates M, Norrbom A, Ochoa R, et al. (2007) Vouchering DNA-barcoded specimens: test of a nondestructive extraction protocol for terrestrial arthropods. *Mol Ecol Notes* 7: 915–924.
- Suarez AV, Tsutsui ND (2004) The value of museum collections for research and society. *BioScience* 54: 66–74.
- Thomsen PF, Elias S, Gilbert MTP, Haile J, Munch K, et al. (2009) Non-destructive sampling of ancient insect DNA. *PLoS ONE* 4: e5048. doi:10.1371/journal.pone.0005048.t003.
- Whitfield JB (1999) Destructive sampling and information management in molecular systematic research: an entomological perspective. *Managing the modern herbarium: An interdisciplinary approach*: 301–314.

Museum Extraction Protocol

This is a cheat sheet. For the full set of directions, see the documentation that came with the Qiagen kit or the Supplementary Protocols document: Purification of total DNA from insects using the DNeasy® Blood & Tissue Kit. The following steps are similar to the manufacturer's directions but with slight modifications.

1. Add **60µl PBS** to labeled microcentrifuge tubes.
2. Remove the head and prothorax from each specimen by inserting a forceps tip at the mesoscutellum (the joint of the pronotum and elytra) and place in the corresponding labeled 1.5ml microcentrifuge tube. *Do not homogenize the tissue.*
3. Add **40µl proteinase K** to the microcentrifuge tube.
4. Add **200µl Buffer ATL** (without added ethanol) to the microcentrifuge tube. Mix gently by vortexing.
5. Incubate with gentle agitation for several hours. Typically, 4 hours is sufficient though lysis can be carried out overnight as well without noticeable damage to the specimen (for beetles). Lysis completion can be confirmed by observation of digested internal tissue as well as increased color (brownish) and opacity of the lysate solution.
6. Add **200µl Buffer AL** to the digestion buffer (lysate) and vortex.
7. Add **200µl 100% ethanol** to the digestion buffer (lysate) and vortex.
8. Pipet the lysate mixture from step 7 (including any precipitate, should be 700µl) into the DNeasy Mini spin column placed in a 2 ml collection tube.
 - a. Add **1mL 100% ethanol** to completely submerge the head and prothorax left behind in the microcentrifuge tube. Drain off and replace with another 1mL ethanol after extraction.
9. Centrifuge lysate at $\geq 6000 \times g$ (8000 rpm) for 1 min. Discard flow-through and collection tube.
10. Place the DNeasy Mini spin column from step 9 into a new 2 ml collection tube, add **500 µl Buffer AW1**, and centrifuge for 1 min at $\geq 6000 \times g$ (8000 rpm). Discard flow-through and collection tube.
11. Place the DNeasy Mini spin column in a new 2 ml collection tube, add **500 µl Buffer AW2**, and centrifuge for 3 min at $20,000 \times g$ (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
12. Place the DNeasy Mini spin column in a clean, labeled 1.5 ml microcentrifuge tube and pipet **100 µl Buffer AE** warmed to 70 °C directly onto the DNeasy membrane. Incubate at room temperature for **10min**, and then centrifuge for 1 min at $\geq 6000 \times g$ (8000 rpm) to elute.
13. Repeat elution step 12 with a new microcentrifuge tube, incubating with warm Buffer AE for **30min**.