Jan 12, 2025

Chelex-based DNA extraction protocol for insect museum vouchers

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6×54rlqe/v1

Morgan Brown[1], Sara Ottati[2], Valeria Trivellone[1]

[1]University of Illinois at Urbana-Champaign, United States of America;
[2]Institute for Sustainable Plant Protection (IPSP), National Research Council, Italy

Valeria Trivellone
University of Illinois

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6x54rlqe/v1

Protocol Citation: Morgan Brown, Sara Ottati, Valeria Trivellone 2025. Chelex-based DNA extraction protocol for insect museum vouchers. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6x54rlqe/v1

Protocol status: Working
We use this protocol and it's working

Created: January 31, 2024

Last Modified: January 12, 2025

Protocol Integer ID: 94500

Keywords: microorganisms, voucher, museum, acid nucleic extraction, DNA, next-generation sequencing, phytoplasma, bacteria, leafhoppers, bleaching, chelex, Column-based extraction, qPCR, insect, insect museum vouchers museum biorepository, insect museum, voucher specimen, taxonomic status, dna extraction, borne pathogens detection, taxonomic status of the associate, based dna extraction protocol, microbiome association, pathogens detection, museum specimen, tracking insect, insect, diverse community of microorganism, specimen, biodiversity, species, species on earth, phylogenetics, pathogen, high quality nucleic acid, dna, microorganism, described species, inferring evolutionary pathway, documenting association, nucleic acid, catalog record

Funders Acknowledgements:
U.S. National Science Foundation
Grant ID: DEB-2244871

Abstract

Museum biorepositories preserve precious material for scientific research in several fields such as biodiversity, phylogenetics, population genetics, toxicology, environmental monitoring and pathology.
About half of the 8.7 million described species on Earth are insects and harbor a diverse community of microorganisms representing different association types, such as parasitic, mutualistic, and commensal.
Voucher specimens may play an important role in tracking insect-microbiome associations.
Catalog records and voucher specimens are the primary source for: (1) tracking past associations and inferring evolutionary pathways; (2) documenting associations in no longer accessible collection sites; (3) revising the taxonomic status of the associates; (4) resampling the associations over time when new technologies become available.
Here we present a non-destructive, fast, inexpensive, non-toxic protocol for DNA extraction from insect museum specimens with the aim to obtain high quality nucleic acid and sufficient yield for vector-borne pathogens detection, quantification and characterization.

Image Attribution

Image source: Christopher H. Dietrich (University of Illinois at Urbana-Champaign)

Materials

- UltraPure™ SDS Solution, 10% **Thermo Fisher Catalog #**24730020
- Chelex-100 resin **Bio-Rad Laboratories**
- 95% EtOH
- 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025
- EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G

Insect specimen selection and preparation

9m

1 Transfer the the ethanol-preserved insect specimen on a piece of paper towel to allow the ethanol to evaporate for a few minutes. 2m

Note

For dry insect voucher, re-hydrate the insect specimen in a humidifying chamber Overnight

2 Place insect specimen in a 1.5 mL centrifuge tube containing 500 µL ethanol [M] 90 % (v/v) for 00:02:00 . 2m *

2.1 Discard the ethanol and add 500 µL bleach [M] 2.5 % (v/v) (NaOCl - The Clorox Co., Oakland, CA, USA) for 00:05:00 at 7.5 °C . 5m

Note

The insect body must be fully immersed in the bleach solution.

2.2 Discard bleach solution and rinse 3 X with 250 µL of sterile water, discard the water each time. 10m

DNA Extraction

19h 19m

3 Place the insect specimen into a fresh 1.5 mL centrifuge tube. 5m

4 Pipet 50 µL TES buffer and 2 µL Proteinase K ([M] 10 mg/mL , Qiagen) into the tube. 2m

Note

Prepare TES buffer (Tris-HCl [M] 20 millimolar (mM) pH 8.0 , EDTA [M] 10 millimolar (mM) , SDS [M] 0.5 % (v/v))
For 10 mL TES add 0.2 mL EDTA [M] 0.5 Molarity (M) , 0.5 mL SDS [M] 10 % (v/v) , 0.2 mL Tris-HCl [M] 1 Molarity (M) , 9.1 mL water (MilliQ)

5 Incubate 55 °C Overnight ; 18h

6 Centrifugate the sample for a few seconds to spin down the liquid. With a pipet, transfer 50 µL of the liquid sample into a fresh tube. 2m
Store the insect specimen as appropriate for further morphological studies.

7 Prepare [M] 25 Mass / % volume Chelex 100 suspension. 15m

Note

Add 2.5 g Chelex 100 resin into 10 mL water (MilliQ or NANOpure)

8 Place a flask containing a [M] 25 Mass / % volume Chelex 100 suspension on a magnetic agitator and mix. 2m

9 Pipet 40 µL of Chelex directly from the agitator into the tube with the sample. 2m

10 Incubate the tube at 55 °C for 00:30:00 . Do not shake the tube. 30m

11 Gently invert the tube 5 times. 2m

12 Allow the solution to cool down to Room temperature . 15m

13 Centrifugate at 14000 rpm for 00:01:00 . 2m

14 Transfer the supernatant into a fresh 1.5 mL centrifuge tube. 2m

Safety information

The present protocol do not include toxic chemicals

Protocol references

1. Bleaching step
Arora, A. K., Pesko, K. N., Quintero-Hernández, V., Possani, L. D., Miller, T. A., & Durvasula, R. V. (2018). A paratransgenic strategy to block transmission of Xylella fastidiosa from the glassy-winged sharpshooter Homalodisca vitripennis. *BMC biotechnology*, *18*, 1-10.
Greenstone, M. H., Weber, D. C., Coudron, T. A., Payton, M. E., & Hu, J. S. (2012). Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. *Molecular Ecology Resources*, *12*(3), 464-469.
2. Chelex protocol modified from
Casquet, J., Thebaud, C., & Gillespie, R. G. (2012). Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular ecology resources*, *12*(1), 136-141.
Lienhard, A., & Schäffer, S. (2019). Extracting the invisible: obtaining high quality DNA is a challenging task in small arthropods. *PeerJ*, *7*, e6753.