Supplementary material to: Djernæs M., Bras A., Simonsen T.J., Jensen M.R., Bechsgaard J.S., Pedersen J.B., Mowery A.A., Bilde T. & Thomsen P.F. 2026: Optimization of DNA extraction for insect museomics substantially increases DNA yield. — *Eur. J. Entomol*. **123**: 48–60.

Extraction protocol, QIAamp DNA Micro Kit

Protocol from Twort et al. (2021).

Place each leg in a clean 1.5 ml Eppendorf tube and add **180 µl buffer ATL** (room temperature).

Add **20 µl proteinase K** and mix thoroughly by pipetting.

Incubate at 56° C overnight in a thermomixer at 300 rpm.

Briefly centrifuge to remove drops from the lid.

Transfer liquid to a clean 1.5 ml Eppendorf tube, keep legs for return to museum collections, continue extraction with liquid.

Add **200 µl Buffer AL**, pulse-vortex for 15 seconds.

Add **200 µl ethanol** (96-100%), pulse-vortex for 15 seconds.

Incubate at room temperature for 5 minutes.

Briefly centrifuge to remove drops from the lid.

Carefully transfer the entire lysate to the QIAamp MinElute column in a 2 ml collection tube, without wetting the rim. Close the lid and centrifuge at 6,000 xg (8,000 rpm) for 1 minute.

Place column in a clean collection tube, discarding the collection tube containing the flow-through.

Carefully open the column and add **500 µl Buffer AW1**, without wetting the rim. Close lid, and centrifuge at 6,000 xg (8,000 rpm) for 1 min. Place column in a new collection tube, discard collection tube containing flow-through.

Add **500 µl of Buffer AW2** to the column without wetting the rim, close lid and centrifuge at 6,000 xg (8,000 rpm) for 1 min. Place in a new collection tube, discarding collection tube containing flow-through.

Centrifuge at full speed (14,000 rpm / 20,000 xg) for 3 minutes to dry the membrane completely.

Place in a clean Eppendorf tube, discarding collection tube containing flow-through.

Carefully open the lid and apply **25 µl Buffer AE** to the center of the membrane, close the lid.

Incubate at room temperate for 20 minutes, before centrifuging at 14,000 rpm / 20,000 xg for 1 minute.

Open the lid and apply an additional **25 µl buffer AE** to the center of the membrane, close the lid and incubate 20 minutes at room temperature, before centrifuging at 14,000 rpm / 20,000 xg for 1 minute.

Transfer the eluate to a new, labelled low bind Eppendorf tube.

Extraction protocol, Monarch PCR & DNA Cleanup Kit

Non-destructive, modified from Patzold et al., 2020, dx.doi.org/10.17504/protocols.io.8m9hu96.

This is the selected version of the protocol.

Place each leg in a clean 1.5 ml Eppendorf tube. Briefly centrifuge to ensure that the sample is at the bottom of the tube.

Add **135 µl buffer ATL** (room temperature) to each 1.5 ml Eppendorf tube.

Add **15 µl proteinase K** and mix thoroughly by shaking by hand.

Briefly centrifuge to remove drops from the lid and to ensure that the femur is immersed in the lysis solution.

Incubate at 42° C overnight (ca. 20 hours) in a thermomixer at 400 rpm.

Briefly centrifuge to remove drops from the lid.

Transfer liquid to a clean 2 ml Eppendorf tube, keep legs for return to museum collections, continue extraction with liquid. Add 150-200 µl 70% ethanol[i] to tubes containing insect legs.

Add **300 µl DNA Cleanup Binding Buffer** to the 150 µl sample. Mix by pipetting. Note: lysate + binding buffer can form a coagulate, this can form so fast that the mixture gets stuck inside the pipette tip when pipetting up and down. If this is a problem, do not mix lysate + binding buffer by pipetting here, just mix really well when ethanol has been added.

Add **900 µl ethanol** (96-100%). Mix by pipetting.

Carefully transfer half of the lysate (675 µl) to the spin column in a 2 ml collection tube, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper (enough paper to ensure that the surface underneath is not contaminated), re-insert spin column in collection tube.

Carefully open the column and transfer the remaining half of the lysate to the spin column in. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper, re-insert spin column in collection tube.

Carefully open the column and add **500 µl DNA Wash Buffer**, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper, re-insert spin column in collection tube.

Carefully open the column and add **500 µl DNA Wash Buffer**, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute

Place in a clean Eppendorf tube, discarding collection tube containing flow-through. Take care that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to the next step.

Carefully open the lid and apply **20 µl Elution Buffer** to the center of the membrane, close the lid.

Incubate at room temperate for 10 minutes, before centrifuging at 16,000 xg (13,000 rpm) for 1 minute.

Open the lid and apply an additional **20 µl Elution Buffer** to the center of the membrane, close the lid and incubate 10 minutes at room temperature, before centrifuging at 16,000 xg (13,000 rpm) for 1 minute.

Transfer the eluate to a new, labelled low bind Eppendorf tube.

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[i] 70% percent ethanol was used in order to preserve the morphological integrity (prevent breakage of legs or spines on the legs) of the legs. Insect cuticle becomes very brittle when stored in high concentrations of ethanol (e.g. 95-100% ethanol) and can then easily break when handled.

Extraction protocol, Monarch Spin PCR & DNA Cleanup Kit

Very similar to the protocol used with the Monarch PCR & DNA Cleanup Kit.

Place each leg in a clean 1.5 ml Eppendorf tube. Briefly centrifuge to ensure that the sample is at the bottom of the tube.

Add **135 µl buffer ATL** (room temperature) to each 1.5 ml Eppendorf tube.

Add **15 µl proteinase K** and mix thoroughly by shaking by hand.

Briefly centrifuge to remove drops from the lid and to ensure that the femur is immersed in the lysis solution.

Incubate at 42° C overnight (ca. 20 hours) in a thermomixer at 400 rpm.

Briefly centrifuge to remove drops from the lid.

Transfer liquid to a clean 2 ml Eppendorf tube, keep legs for return to museum collections, continue extraction with liquid. Add 150-200 µl 70% ethanol[i] to tubes containing insect legs.

Add **300 µl Monarch Buffer BZ** to the 150 µl sample. Mix by pipetting. (Note: lysate + binding buffer can form a coagulate, this can form so fast that the mixture gets stuck inside the pipette tip when pipetting up and down. If this is a problem, do not mix lysate + binding buffer by pipetting here, just mix really well when isopropanol has been added.)

Add **900 µl isopropanol**. Mix by pipetting.

Carefully transfer half of the lysate (675 µl) to the spin column in a 2 ml collection tube, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper or use an extra collection tube (NEB-T2118L), (re-)insert spin column in collection tube.

Carefully open the column and transfer the remaining half of the lysate to the spin column in. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper or use an extra collection tube, (re-)insert spin column in collection tube.

Carefully open the column and add **500 µl Monarch Buffer WZ**, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute. Discard flow-through, dap column dry on tissue paper or use an extra collection tube, (re-)insert spin column in collection tube.

Carefully open the column and add **500 µl Monarch Buffer WZ**, without wetting the rim. Close the lid and centrifuge at 16,000 xg (13,000 rpm) for 1 minute

Place in a clean Eppendorf tube, discarding collection tube containing flow-through. Take care that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to the next step.

Carefully open the lid and apply **20 µl Monarch Buffer EY** to the center of the membrane, close the lid.

Incubate at room temperature for 10 minutes, before centrifuging at 16,000 xg (13,000 rpm) for 1 minute.

Open the lid and apply an additional **20 µl Monarch Buffer EY** to the center of the membrane, close the lid and incubate 10 minutes at room temperature, before centrifuging at 16,000 xg (13,000 rpm) for 1 minute.

Transfer the eluate to a new, labelled low bind Eppendorf tube.

[i] 70% percent ethanol was used in order to preserve the morphological integrity (prevent breakage of legs or spines on the legs) of the legs. Insect cuticle becomes very brittle when stored in high concentrations of ethanol (e.g. 95-100% ethanol) and can then easily break when handled.