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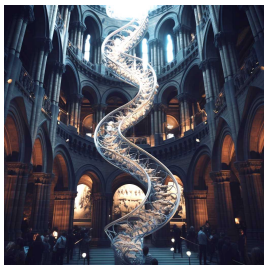
Archival DNA extraction protocol for insect specimens from museum collections V.2



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We use this protocol and it's working

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Abstract

This protocol can be used to dry specimens from natural history collections to obtain DNA for UCE-seq.

Safety warnings

Use Nitrile gloves in the steps you need to directly touch 2-mercaptoethanol. This chemical is harmful! Do not forget to conduct steps that require 2-mercaptoethanol in a fume hood.
Change your gloves as quickly as possible and how many times you judge necessary.

Before start

- **The protocol can be performed in two days**
- **On the first day, you prepare your samples for overnight lysis and prepare plastic ware for overnight UV bath**
- **The following day is used for filtering, washing, and elution steps**
- **Buffers must be prepared beforehand (check item 3 carefully). Some buffers can be stored for long times, others are just for one-time use**

More tips below:

- *Tween-20: Stock concentration (100%) is impossible to pipette. Make a 10% solution before starting using a small becker. 20-50 ml is a good amount to keep in the lab if you use it with some frequency. Avoid the light, since the 100% tween-20 comes inside an amber bottle.*

- *Non-destructive buffer: 1 sample = 1 ml of non-destructive buffer*
- *Always add one extra sample to the calculation to give you flexibility while setting up your buffer!*
- *2-mercaptoethanol must be added just prior to use! in a fume hood. Gently invert it 3 times to mix.*
- Use Nitrile gloves in the steps you need to directly touch 2-mercaptoethanol. This chemical is harmful! Do not forget to conduct steps that require 2-mercaptoethanol in a fume hood.
- Change your gloves as quickly as possible and how many times you judge necessary.

- *Set the oven* to *37* °C. Your rotator must fit inside the oven. Our DNA lab has this brand: Boekel Scientific Mini Tube Rotator, 260750

Fundamental reading before starting

1 **From museum drawers to a tree: phylogenomics of historical DNA sheds light on the systematics of rare dung beetles (Coleoptera: Scarabaeinae) from museum collections**

Authors: Fernando Lopes, Nicole Gunter, Giulio Montanaro, Michele Rossini, Federica Losacco, Conrad P.D.T. Gillett, Gimo Daniel, Nicolas Straube and Sergei Tarasov

This protocol follows the guanidine treatment protocol by Straube et al. (2021), based on Dabney et al. 2013 and Rohland et al. 2004.

If applying this protocol cite and read the references mentioned above and in the manuscript.

We have successfully extracted and sequenced Ultra Conserved Elements (UCEs) from specimens deposited in museum collections for more than 40 years.

The adapted part of this protocol is described in steps 4 and 5.

List of Lab Equipment & Reagents

2 **Checklist of consumables:**

	A	B	C
	Products used	Company	Catalogue number
	10-200 ul filter tips	Sartorius	Z757764-960EA; Z757799-960EA
	100-5,000 ul tips	Sartorius	780304; 780300
	Axygen Tube 2.0 ml	Axygen	12659585
	Axygen Tube 1.5 ml	Axygen	11351904
	Zymo column	BioSite	C1016-50
	Weighing Boats	Fisherbrand	11344125
	Single use scalpels	Any	
	1.5 ml Low Retention Tube	ThermoScientific	11569914
	Falcon Tube 15 and 50 ml	Fisher	E1450-0200; E1415-0200
	Nitril Gloves	Ansell TouchNTuff	11726584
	LLG-Spoon spatulas, 18	Labnet	9150800
	Ethanol 70-96%	Any	
	Bleach 10% for cleaning bench and equipment	Any	

	A	B	C
	DNA Away surface decontaminant	Thermo Scientific	10223471

Checklist of reagents:

	A	B	C
	Reagent used	Company	Catalog number
	Guanidine thiocyanate (GuSCN)	FisherScientific	10503345
	NaCl 5M	FisherScientific	10609823
	Tris-HCL 1M pH 8.0	Invitrogen	15568-025
	EDTA 0.5M pH 8.0	FisherScientific	10135423
	Tween-20 100%	Bio RAD	1706531
	HPLC water	Fisher	10367171
	Guanidine hydrochloride	Fisher	10543325
	Isopropanol	Any	
	Sodium Acetate 3M	Fisher	J61928
	MiniElute Kit (containing PE buffer)	Qiagen	28006

Preparation for laboratory work

3 The protocol should be conducted in a dedicated room for archival DNA.
Clean the bench and all instruments with DNA contamination removal solution and UV light before the experiment. Prepare buffers.

3.1 **BUFFERS**

TET Buffer
Can be kept indefinitely.

TIPS: Suggested volume 50ml
UV irradiate before use

	A	B	C
	Reagents	Final concentration	Amount for 50ml
	Tris-HCL 1 M ph 8.0	10 mM	500 ul
	EDTA 0.5 M pH 8.0	1 mM	100 ul
	10% Tween-20	0.05 %	250 ul
	ddH2O	-	up to 50 ml

3.2 **Biding buffer**

It can be stored for up to 1 month

TIPS: Guanidine is hard to dissolve completely. It will take 5 minutes or more to dissolve all the guanidine. Do it by inverting by hand gently in a Falcon tube or small bottle. Try to avoid lumps of guanidine present in the stock bottle. Lumps can make the guanidine even harder to dissolve.

Think about the amount of binding buffer you will use in an interval of 1 month.
It takes some time to set up the buffer. Good to do it beforehand.

	A	B	C
	Reagents	Final concentration	Amount for 50 ml
	Guanidine hydrochloride (MW 95.53)	5 M	23.9 g
	Isopropanol	40%	20 ml
	10% Tween-20	0.05%	250 ul
	Sodium Acetate 3 M	90 mM	1.5 ml
	ddH2O	-	up to 50 ml

3.3 Preparation of buffers for the guanidine protocol

IMPORTANT NOTE: The extraction buffer contains 2-mercaptoethanol. Steps 4.1 and 4.5 must be performed in a fume hood and corresponding safety measures should be met. The 2-mercaptoethanol and Guanidine thiocyanate buffer waste should be discarded appropriately.

Non-destructive buffer

One-time use buffer

Guanidinium thiocyanate buffer is adapted from Rohland et al. (2004) but DTT is replaced with 2-mercaptoethanol and Triton X100 with Tween-20.
Prepare a fresh buffer each time.

TIPS:

- *1 sample = 1 ml of non-destructive buffer*
- *Always add one extra sample to the calculation to give you flexibility while setting up your buffer! Also, remember to add 2 samples as the negative controls. One is to be placed at the beginning of the row and the other is to be added at the end of the row.* ***Negative controls and the additional sample will totalize 3 extra samples.***
- *2-mercaptoethanol must be added just prior to use! Gently invert it 3 times to mix.*
- *We recommend starting with a few samples until you get experienced.*

	A	B	C
	Reagents	Final concentration	Amount for 1 sample (1 ml)
	GuSCN powder pure	5 M	0.59 g
	NaCl 5M	25 mM	5 ul
	Tris 1M ph 8.0	50 mM	50 ul
	EDTA 0.5 M pH 8.0	20 mM	44.4 ul
	10% Tween-20	1%	100 ul
	ddH2O	-	up to 1 ml
	2-mercaptoethanol	1%	10 ul (in the fume hood)

3.4 Binding apparatus

- Zymo V column extension reservoir (without column)
- Qiagen MinElute silica spin column
- 50 ml Falcom tube
- Pen marker

1. Remove Zymo spin column from the reservoir and submerge the reservoir in bleach for over 20 min. Rinse it well with ddH2O. Dry it out naturally, preferably in a fume hood. Zymo spin column can be discarded.

2. Cut off the caps of Qiagen MinElute columns and keep the collection tube and caps for the next steps. The caps can be placed on the top of the collection tube to help to maintain sterility.

3. Assemble the binding apparatus by forcibly attaching the Qiagen MinElute (without caps) column in the place reserved for the Zymo spin column. This step can require some force. Do not pressure the region close to the silica membrane.

4. Label the side and top of the Falcon tube to ensure that samples will not be mixed up.

5. UV irradiate it overnight with the equipment described in step 5.

Binding apparatus containing MinElute column and 2 ml collection tube with lid.

Note: Zymo extended reservoir has been replaced by Rohland et al. 2018 [(Preassembled silica spin columns and collection tubes (High Pure Viral Nucleic Acid Large Volume Kit; Roche, cat. no. 5114403001)]. This information was included by a peer reviewer's request and was not tested by the authors of the manuscript.

Lysis (protocol's adaptation)

4 **The following steps will guide you through the lysis of tissues. This part is adapted from the mentioned references for dry specimens of museum collections.**

4.1 UV irradiate for 30 min:

- Pieces of parafilm to wrap up caps of 2 ml tubes
- Scissors
- A rack for 1.5 - 2 ml tubes
- 1 15 ml Falcon tube for non-destructive buffer
- Tip Boxes 10, 100/200, and 1,000 ul
- Pipettes
- Pen marker
- Rotator's carousel

4.2 Clean the bench with bleach or DNA contamination removal solution

4.3 For the lysis, you can proceed as below described. We have extracted good amounts of DNA from different sources. However, the yield can vary a lot and sometimes the concentration of DNA can be lower than the required for library preparation protocols.

1. Leg or legs of specimens: Depending on the size and structure of the leg(s) and the amount of muscles exposed you can keep the legs intact. In this case, the efficiency (amount of DNA retrieved) might be lower; or you can macerate the leg(s) or part of the legs with a sterile lab stick. More than one leg of the same individual can be used to increase the total amount of DNA extracted.

After the extraction, clean well the body parts with ddH2O. Remember that body parts will stay overnight in buffer containing 2-mercaptoethanol, a harmful chemical. Then remember to rinse the parts in a fume hood with a sink by also wearing nitrile gloves. Discard gloves after touching the reagent. With the support of a drying tissue paper and/or naturally dry out the parts. Parts then will be ready to be pinned back.

2. Body parts: With the support of sterile/disposable scalpels, pipette tips or pins you can also use detached body parts. You can leave body parts on lysis in the non-destructive buffer overnight. For dung beetles, we have used different body parts (prothorax, head, abdomen, and legs), that fit 2 ml tubes, with success without causing any damage to external structures, even microstructures. Keep in mind that some body parts can contain more sensible parts like antennae. Try to avoid body parts that can get stuck in the tube. This can result in lower efficiency or can damage body parts when removing them from the tubes.

After the extraction, clean well the body parts with ddH2O. Remember that body parts will stay overnight in buffer containing 2-mercaptoethanol, a harmful chemical. Then remember to rinse the parts in a fume hood with a sink by also using nitrile gloves. Discard gloves after touching the reagent. With the support of a drying tissue paper and/or naturally dry out the parts. Parts then will be ready to be pinned back.

3. Internal content: Depending on the size of your specimen you can remove internal content for DNA extraction. Again, with the support of sterile/disposable scalpels, pipette tips or pins you can detach your specimen and extract internal tissues. Keep in mind that in this case you will be also extracting DNA from the microbiota, which can bias the DNA concentration estimations and sequencing.

TIPS: We do recommend trials and adaptations based on the type of material you will be extracting DNA from. Use different sizes of body parts to have a general idea of how much DNA you can extract from your specimens.

Label 2 ml tubes accordingly in the caps and walls.

4.4 The overnight lysis

- Now that you have selected the best tissues for your batch of extractions and before submerging your material in the non-destructive buffer, rinse them with ethanol (except for the internal content) and put them into a 2 ml tube. Leave the caps open and cover the tubes with drying tissue paper to dry out the parts naturally. Tubes containing internal content can be kept closed.

- While your samples are drying, prepare the non-destructive buffer in a Falcon tube of 15 ml as described in step 3.3 for your amount of samples + 2 negative controls and the extra sample. **DO NOT ADD** 2-mercaptoethanol now. The step before adding 2-mercaptoethanol might be done on a regular bench.

- *NOTE: This buffer must be made each time and the others should be prepared beforehand.*

- Move the material described in step 4.1 and the non-destructive buffer to the fume hood.

- Put on nitrile gloves and close the lid of the fume hood in a way you can still work, but as closed as possible.

- Carefully add up the 2-mercaptoethanol (10 ul per sample) to the 15 ml Falcon tube containing the non-destructive buffer. You will see a (soft) chemical reaction when both liquids get in touch. Close the Falcon tube and the bottle containing the 2-mercaptoethanol immediately. Invert the tube gently 3 times to mix both buffer and reagent.

- Open the 2 ml tubes containing the negative controls and samples and add 1 ml of non-destructive buffer containing 2-mercaptoethanol to each tube. Close the tubes and seal them with parafilm to avoid leakage.

- Set the tubes evenly in the rotator carousel and place the rotor inside the oven at 37ºC for the overnight step. The rotator must turn around gently. The cable is ok pinched in the door in most cases.

- Change your gloves

5 UV irradiate overnight:

NOTE: LABEL ALL TUBES (LID AND WALLS) BEFORE TURNING THE UV LIGHT ON.

- 15 ml tubes with 13 ml binding buffer
- MinElute column tubes
- Binding apparatus in 50 ml Falcon tubes (see above)
- 1.5 ml low retention tubes for final extracts

- An aliquot of TET buffer (suggestion 1.5 ml in a 2 ml tube)
- Extra MinElute collection tubes with caps on the top

You can also UV irradiate other equipment. It is up to you!

- Pipettes
- Racks

Example of a set up for overnight UV.

Filtering and washing

6 **The next day: after overnight lysis and UV on plastic ware:**

6.1 *Put on nitrile gloves and work in the fume hood in the following steps.* Change nitrile gloves as much as needed. For the samples macerated with sterile sticks or internal tissues removed from specimens, centrifuge for two minutes at maximum speed (~15,600 g) to pellet tissues. For entire detached parts, skip to the next step (6.2).

6.2 Carefully, add the supernatant to the respective labeled Falcon tube containing the binding buffer, close the lid, and mix by inverting the tube 3 times. Pour the buffer with the pellet into the apparatus reservoir. Tape the lids onto the binding apparatus. Repeat the procedure for all the samples.

6.3 Centrifuge the binding apparatus for 4 min at 940 g (1,500 rpm). Remember to tape the lids on. Rotate 90º and centrifuge again at the same speed and time. You can repeat the procedure a couple of times if there is still liquid in the reservoir (the part above or in the column). You can increase the speed a bit, but go slowly! Otherwise, you can detach the MinElute column from the reservoir by releasing it in the already-filtered pellet. Worse: you can break the 50 ml tube and spoil harmful liquid inside the centrifuge. If after 3 or 4 times of centrifugation you still find some liquid inside you can discard the liquid in the proper waste and proceed with the protocol.

6.4 Prepare 3 new rows of collection tubes (2 ml collection tubes) in the same rack with the collection tubes+lids from the previous day for the next steps.

6.5 Take the filtered material to the fume hood. Detach the MinElute column from the binding apparatus, put them in the original 2 ml collection tubes, and cover them with the lids (already labeled). You must have retained them from earlier. Discard the waste properly. Dry spin for 1 min at 3,300 g (6,000 rpm). For some samples, a higher speed can be necessary if the liquid does not pass through the column.

6.6 Change the columns to a new row of collection tubes. Discard the flow through properly. Change your gloves!

6.7 From now on you can work on the regular bench. Wash the silica membrane by adding 650 ul of PE buffer that came with MinElute columns. Centrifuge the tubes at 900 g (3,300 rpm).

6.8 Repeat 6.7 once.

6.9 Dry spin columns at maximum speed (~15,600 g) for 1 min. Place the columns in clean and labeled 1.5 ml low-retention tubes.

6.10 Elute by adding 12.5 ul TET buffer to the center of the silica membrane. **Incubate for 10 min,** and centrifuge at maximum speed for 30 sec. Repeat to give a total of 25 ul DNA extract. You must incubate for 10 min in the repetition too.

Concentration and purity measurements

7 We advise the use of Qubit High-Sensitive reagents and 1-2 ul of extract for concentration estimations. Nanodrop can give you purity rates.

Protocol references

Straube, N., Lyra, M. L., Paijmans, J. L., Preick, M., Basler, N., Penner, J., ... & Hofreiter, M. (2021). Successful application of ancient DNA extraction and library construction protocols to museum wet collection specimens. *Molecular Ecology Resources*, *21*(7), 2299-2315.

Basler, N., Xenikoudakis, G., Westbury, M. V., Song, L., Sheng, G., & Barlow, A. (2017). Reduction of the contaminant fraction of DNA obtained from an ancient giant panda bone. BMC Research Notes, 10(1), 754. https://doi.org/10.1186/s13104-017-3061-3.

Dabney, J., Knapp, M., Glock, I., Gansauge, M., Weihmann, A., Nickel, B., Valdioserad, C., García, N., Pääbo, S., Arsuag, J. & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. PNAS, 110(39), 15758-15763.

Rohland, N., Siedel, H., & Hofreiter, M. (2004). Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. Biotechniques, 36(5), 814-821.
Rohland, N., Hofreiter, M. (2007). Comparison and optimization of ancient DNA extraction. Biotechniques, 42(3), 343–352.

Rohland, N., Hofreiter, M. (2007). Ancient DNA extraction from bones and teeth. Nature Protocols, 2, 1756–1762. https://doi.org/10.1038/nprot.2007.247

Rohland, N., Siedel, H., & Hofreiter, M. (2010). A rapid column-based ancient DNA extraction method for increased sample throughput. Molecular Ecology Resources, 10, 677–683.

Rohland, N., Glocke, I., Aximu-Petri, A., & Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. Nature Protocols, 13(11), 2447-2461. doi: 10.1038/s41596-018-0050-5.