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DNA extraction protocol for historical toe pad samples from birds

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lutgendave

Museums hold collections of specimens from vast taxonomic and geographic ranges that constitute rich resources for research into the origins, evolution, and maintenance of biodiversity on earth. However, specimens are often not prepared for specific research purposes. Especially the isolation of adequate quantities of DNA for genomic research can be challenging. Here, we report a modified protocol of a commercially available kit optimized to reliably extract adequate quantities of DNA from bird toepads and dried skin from sutures of museum specimens for whole-genome resequencing.

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Birds, Museomics, Illumina sequencing, historical DNA, Toe pads, whole-genome resequencing

protocol ,

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Material

QiAmp DNA Micro kit **Qiagen Catalog #56304** Step 1

Micropestle **Sigma Aldrich Catalog #BAF199230001** Step 2

1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** In 5 steps

Digestion

1. **40 µL 2x20µl** Proteinase K, 2mL **Qiagen Catalog #19131** In 3 steps
2. **360 µL** Buffer ATL **Qiagen Catalog #19076** Step 6
3. **4 µL** RNase A **Qiagen Catalog #19101** In 2 steps
4. **400 µL** AL lysis buffer **Qiagen Catalog #19075** In 4 steps

Extraction

Buffer AW1, Wash buffer (1), concentrate **Qiagen Catalog #19081** Step 22

Buffer AW2, Wash buffer (2), concentrate **Qiagen Catalog #19072** Step 23

AE buffer **Qiagen Catalog #19077** In 3 steps

Ultrapure Distilled, Nuclease Free Water **Contributed by users** In 2 steps

Quality control

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Qubit® 3.0 Fluorometer **Thermo Fisher Scientific Catalog #Q33216**

NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**

TapeStation 4200 **Agilent Technologies Catalog #G2991BA**

D1000 Screen Tape **Agilent Technologies**

Preparation

1 ⚠

This protocol uses the QiAmp DNA Micro kit **Qiagen Catalog #56304** with 2x the amount/volumes of Proteinase K, 2mL **Qiagen Catalog #19131** Buffer ATL (tissue lysis buffer) **Qiagen Catalog #19076** AL lysis buffer **Qiagen Catalog #19075** RNase A **Qiagen Catalog #19101** .

2 Micropestle **Sigma Aldrich Catalog #BAF199230001** per sample.

Prepare a 1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** to store the micropestle separately for each sample.

Preheat AL lysis buffer **Qiagen Catalog #19075** at **56 °C** . Adding preheated AL lysis buffer **Qiagen Catalog #19075** prevents the formation of potential precipitates.

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Tissue preparation

3 Put toe pad/breast skin tissue (2-3mg) into ⊠1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** (use scalpel tip to transfer).

4 Museum toe pad samples might have been treated with chemicals to better conserve them, therefore, we will rince the tissue before starting the digestion

4.1 Add **1000 µL** of ⊠Ultrapure Distilled, Nuclease Free Water **Contributed by users** to wash down chemicals on the toe pad. Incubate at **1000 rpm, 56°C, 01:00:00** Discard all ⊠Ultrapure Distilled, Nuclease Free Water **Contributed by users** . Make sure no ⊠Ultrapure Distilled, Nuclease Free Water **Contributed by users** is left.

5 Mince the tissue with a pointed scalpel blade. Use a new blade for each sample!

Digestion

6 To make sure no tissue is left on the blade, pipet **80 µL** of ⊠Buffer ATL **Qiagen Catalog #19076** over the scalpel blade, then remove it and ensure all tissue remains in the digestion.

7 ⊠Proteinase K, Add **20 µL** of 2mL **Qiagen Catalog #19131** and start crushing the tissue with a micropestle. Keep the pestle in the tube.

8 ⊠Buffer ATL (tissue lysis Pipet **100 µL** of buffer) **Qiagen Catalog #19076** over the micropestle to ensure all tissue remains in the digestion. Transfer the pestle into the prepared

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1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** for storage.

9 Mix well by vortexing. Incubate tubes at **900 rpm, 56°C, 06:00:00** . Make sure the timer is on infinity.

10 After **06:00:00** , centrifuge the tubes and add **20 µL** of Proteinase K, 2mL **Qiagen Catalog #19131** to the remaining tissue. Use the pestle to further crush tissue parts. Pipet a second volume of **180 µL** Buffer ATL (tissue lysis buffer) **Qiagen Catalog #19076** over the pestle to ensure all tissue remains in the digestion.

6h

11

Vortex well. Make sure all pieces of tissue are in solution and continue digestion overnight at **56 °C** . Leave to digest **900 rpm, 56°C, 40:00:00** . (Note: Tests with extraction after 24 and 40 hours show that DNA amount doubles within the additional 16 hours).

Pre-extraction preparations

12 Preheat AL lysis buffer **Qiagen Catalog #19075** at **56 °C** . This step prevents the formation of precipitates when AL lysis buffer **Qiagen Catalog #19075** is added to the digestion.

13 Prepare a 1:1 dilution of Ultrapure Distilled, Nuclease Free Water **Contributed by users** and AE buffer **Qiagen Catalog #19077** . Preheat at **37 °C** .

14 Prepare and label 1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** for each

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extraction (2 for 2 elutions)

Digestion

15 5m

Once all tissue is properly digested, in case there are undigested parts (bones, feathers, etc), centrifuge the lysate at max speed for **00:05:00** , and transfer the lysate into a new tube. Else, continue with the next step.

15.1 Note: it is possible that with increased digestion time bigger pieces of tissue are becoming visible. This is likely due to increasing hydration/swelling of tissue pieces with prolonged digestion, and exactly what you want)

16 10m

Add **4 µL** RNase A **Qiagen Catalog #19101** . Mix by vortexing, and incubate at **Room temperature** for **00:10:00** .

17 15s

Vortex for **00:00:15** . Add **400 µL** of preheated **56 °C** AL lysis buffer **Qiagen Catalog #19075** . Mix very quickly. A coagulate will form. Dissolve this in the heat block at **900 rpm, 56°C, 00:10:00**

Extraction 8m

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Turn temperature on the heat block down to **37 °C** . Preheat 1:1 diluted Buffer AE at **37 °C** .

19

Add **400 µL** ice-cold (**-20 °C**) Ethanol, absolute 99.8% **Contributed by users Catalog #10342652** . Mix very well by vortexing. Incubate at room temperature for 10 min.

20 1m

Apply **650 µL** of the lysate to the column. Centrifuge at **8000 rpm, 00:01:00** . Discard flow-through, dry the collection tube, and place the column back into the collection tube.

21 1m

Apply the rest of the lysate. Centrifuge at **8000 rpm, 00:01:00** . Discard flow-through and

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collection tube.

22 Place the column into a new collection tube. Add **500 µL** Buffer AW1, Wash buffer (1), concentrate **Qiagen Catalog #19081** . Centrifuge at **8000 rpm, 00:01:00** . Discard flow-through and collection tube. 1m

23 Place the column into a new collection tube. Add **500 µL** Buffer AW2, Wash buffer (2), concentrate **Qiagen Catalog #19072** . Centrifuge at **8000 rpm, 00:01:00** . Discard flow-through, dry collection tube, and place column back into it. 1m

24 Centrifuge at **14000 rpm, 00:02:00** to dry the membrane. Make sure no ethanol is left anywhere in or on the column. Discard the collection tube. 2m

25 Place the column into a labeled 1.5 mL Safe Lock Tube **Eppendorf Catalog #0030120086** . Apply **20 µL** of AE preheated (**37 °C**) 1:1 diluted buffer **Qiagen Catalog #19077** to the column.

26 Put the column into the thermoshaker **0 rpm, 37°C, 00:07:00** Then spin at **8000 rpm, 00:02:00** . 2m

27

If you expect high(er) amounts of DNA, repeat steps **go to step #25** and **go to step #26** with another **20 µL** of 1:1 diluted AE buffer **Qiagen Catalog #19077** .

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Results

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Individual	Collection year	Toe pad	
		ng/mg	Average size (bp)
OENLRSSCH_BMNH-KE-1902-1965.M.12161	1902	528	282
Museum_43	1904	612	279
Museum_58	1913	340	278
OENPICCAP_ZMUC-AF-HAI-1949-29495	1949	621	344
OENPICOPI_ZMUC-AF-MAI-1949-29578	-	808	327
Museum_48	1952	1033	237
OENPHI_YPM-SO-TOG-1954-ORN035210	1954	709	313
OENLRSVAU_AMNH-SML-ERI-1954-461151	-	443	239
MYRTHO_YPM-AN-BUN-1958-ORN95640	1958	791	321
Museum_46	1959	1854	359
Museum_44	1960	886	325
Museum_45	-	891	307
Museum_47	-	646	264
Museum_59	-	459	322
Museum_60	-	990	363

Examples of DNA yield per milligram input tissue for samples of varying age.

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