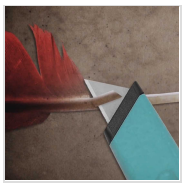

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DNA extraction from feathers

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dx.doi.org/10.17504/protocols.io.bzu2p6ye

EBL_ANU

George Olah
Australian National University, Conservation Genomics, Wildl...

This protocol describes a DNA extraction method from feathers collected non-invasively in the wild.

DOI

dx.doi.org/10.17504/protocols.io.bzu2p6ye

https://doi.org/10.1007/s12686-016-0573-4

George Olah 2021. DNA extraction from feathers. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bzu2p6ye

protocol

Olah G, Heinsohn RG, Brightsmith DJ, Espinoza JR, Peakall R (2016) Validation of non-invasive genetic tagging in two large macaw species (Ara macao and A. chloropterus) of the Peruvian Amazon. Conservation Genetics Resources 8(4):499–509. doi:10.1007/s12686-016-0573-4

birds, feathers, non-invasive, DNA, extraction

protocol ,

Screenshot from the documentary "The Macaw Kingdom" (https://youtu.be/3ieppWouPxk).

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54906

Set up the extraction on a clean lab bench in a PCR-free area. Always use a negative control during each batch of extractions.

Gloves **Contributed by users** In 2 steps

70% Ethanol **Contributed by users** Step 5

100% Ethanol **Contributed by users** Step 23

scalpel blades **Contributed by users** In 3 steps

Proteinase K, 2mL **Qiagen Catalog #19131** Step 12

DTT (1,4-Dithiothreitol) **Sigma Aldrich Catalog #10708984001** Step 13

Eppendorf Safe-Lock Tubes 1.5 mL PCR clean colorless 500 tubes **Eppendorf Catalog #022363212** Step 2

1.5 mL Eppendorf tubes **Contributed by users** Step 32

Microtubes 1.5ml with screw caps **Contributed by users** In 2 steps

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** Step 18

Be careful when cutting feathers with the surgical blades, as the shafts of larger feathers are very hard and blades can slip. Aim to cut through with the pointy tip of the blades first.

Collection & Preparation 30m

1 When collecting in the field, avoid touching the tip of the feathers. If wet, dry the feather on paper towel before storing it. 15m

Place each feather sample in a separate paper envelope, labelling: individual sample ID, collection date, location, species, collector's name, etc.

Store the envelopes with samples in an airtight dry-box with Silica gel crystals at **Room temperature** .

2 For each feather sample, prepare and label an Eppendorf Safe-Lock Tubes 1.5 mL PCR clean colorless 500 tubes **Eppendorf Catalog #022363212** with the unique sample ID. 15m

3 For each feather sample, get: 1m

- a clean A4 paper sheet
- a pair of Gloves **Contributed by users**
- a sterile scalpel blades **Contributed by users** .

Isolation 1h

4 Put on the Gloves **Contributed by users** , remove the feather sample from the envelope and place it 1m

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on the clean A4 paper sheet.

5 Carefully clean the surface of the feather with 70% Ethanol **Contributed by users** and a paper towel. 1m

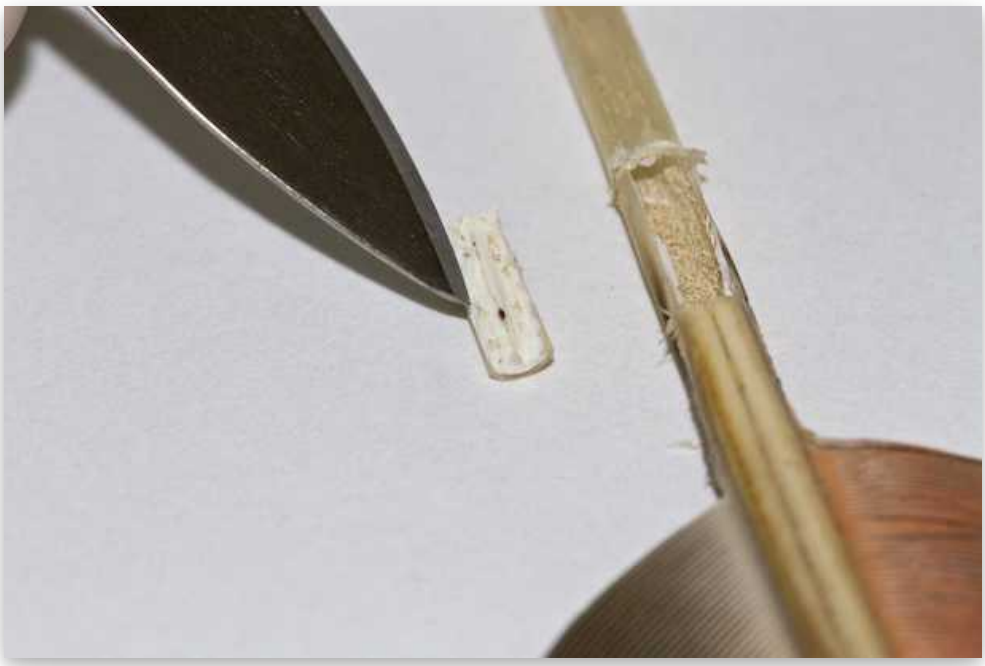
6 ⚠ 2m

Remove a scalpel blades **Contributed by users** from the sterile packaging.

Large feathers: carefully cut out a window around the blood clot from the superior umbilicus of the feather (this can usually be seen just below the vane).
See details in:

> Horváth, M.B.; Martínez-Cruz, B.; Negro, J.J.; Kalmár, L.; Godoy, J.A. (2004). An overlooked DNA source for non-invasive genetic analysis in birds. Journal of Avian Biology.
> http://dx.doi.org/10.1111/j.0908-8857.2005.03370.x

Small feathers: Chop up the entire shaft of small feathers (<20 mm).



Dry blood clot from the superior umbilicus of a large macaw feather.

7 Place the isolated sample into the corresponding Eppendorf tube with the correct sample ID. 1m
Dispose the scalpel blades **Contributed by users** to a yellow sharps container.

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8 Put the remaining part of the feather back to its envelope. Dispose the A4 paper and the gloves. 1m

9 go to step #4 and repeat the steps for each feather sample, avoiding cross-contamination.

10 Negative control: label an empty tube as negative control for the subsequent steps. 1m

Lysis 1d

11 Add 180 µl of Buffer ATL (tissue lysis buffer) Qiagen Catalog #19076 to each tube. 1m

12 Add 20 µl of Proteinase K, 2mL Qiagen Catalog #19131 [M]20 mg/ml to each tube. 1m

13 Add 10 µl 1M of DTT (1,4-Dithiothreitol) Sigma Aldrich Catalog #10708984001 to dissolve Creatine. 1m

14 Vortex each tube for 00:00:20 . 20s

15 Incubate 01:00:00 at 56 °C on a block heater. 1h

16 Vortex each tube for 00:00:20 . 20s

17 Incubate Overnight at 56 °C on a block heater. 15h

Purification 2h

18 Unpack appropriate number of spin column + collection tubes (according to the number of samples you are extracting) from QIAgen DNeasy Blood and Tissue Kit, 50 rxn Qiagen Catalog #69504 and label 10m

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them with the sample IDs accordingly.

19 Vortex each Eppendorf tube with the samples for **00:00:15** . 1m
Spin down briefly.

20 Buffer AL, Lysis 1m
Add **200 µl** of buffer **Qiagen Catalog #19076** to each Eppendorf tube.

21 Vortex for **00:00:15** . 1m
Spin down briefly.

22 Incubate **00:45:00** at **70 °C** on a block heater. 45m

23 Spin down briefly and add **210 µl** of 100% Ethanol **Contributed by users** to each Eppendorf tube. 1m

24 Vortex and incubate for **00:05:00** at **Room temperature** . 5m

25  2m

Spin down briefly and pipette liquid from the Eppendorf tubes to the correspondingly labelled spin columns.
Use 1 ml pipette tips **Contributed by users** at this step.

26 Centrifuge **10000 rpm, 00:01:00** . 1m

27 Discard collection tube with the filtrate and place the spin column in a clean collection tube. 1m

28 Buffer 2m
Add **500 µl** of AW1 **Qiagen Catalog #19081** and centrifuge **10000 rpm, 00:01:00** .

29 Discard collection tube with the filtrate and place the spin column in a clean collection tube. 1m

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30 4m

Buffer

Add **500 µl** of AW2 **Qiagen Catalog #19072** and centrifuge **12000 rpm, 00:03:00** .

Recovery of DNA 30m

31 3m

Buffer

Preheat appropriate volume of AE **Qiagen Catalog #19077** (**100 µl** x number of samples + a little extra) to **70 °C** .

32 3m

Prepare as many 1.5 mL Eppendorf tubes **Contributed by users** as samples in your batch, and cut off their lids.

33 1m

Discard collection tube with the filtrate and place spin column into a clean 1.5 mL Eppendorf tube (with lid cut off).

34 5m

Buffer

Add **100 µl** of preheated AE **Qiagen Catalog #19077** and incubate at **70 °C** for **00:05:00** on a block heater.

35 5m

Prepare Microtubes 1.5ml with screw caps **Contributed by users** and label them for final storage (species, sample ID, date of extraction, amount in µL).

36 1m

Centrifuge incubated samples **10000 rpm, 00:01:00** .

37 2m

⚠

Carefully lift the spin column, pipette up the eluted sample from the Eppendorf tube (~100 uL), place back to the spin column into the Eppendorf tube, and pipette the solution back to the center of the filter.

This helps to increase the concentration of DNA in the extraction.

38 1m

Incubate at **70 °C** for **00:01:00** .

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39 Centrifuge **10000 rpm, 00:01:00** . 1m

40 Transfer filtrate to labeled Microtubes 1.5ml with screw caps **Contributed by users** . 1m

41 Store screw cap tubes in fridge at **4 °C** for short-term or in a freezer at **-20 °C** for long-term storage.

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