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Ethanol extraction protocol

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1 *Works for me* This protocol is published without a DOI.

IBA

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ABSTRACT

Developed for IBA at the NRM DNA lab.

PROTOCOL CITATION

Nannie 2021. Ethanol extraction protocol. **protocols.io** https://protocols.io/view/ethanol-extraction-protocol-btz8np9w

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MATERIALS TEXT

Samples: Catches from Malaise traps (insects soaked in ethanol)
Filters: Sterivex
Extraction kit: Qiagen DNeasy Blood and Tissue kit

1 Decant the ethanol through a mesh and add new ethanol to the insects.

2 Filter the ethanol: method 2.1 or 2.2

Sterivex filter attached to the large syringe.

2.1 Vacuum pump

Put the Sterivex filters on the vacuum pump with the large syringe attached to the filter. Turn on the pump and pour the ethanol into the syringe.

The filtered ethanol will not be saved.

2.2 Manually

Filter the ethanol manually through the large syringe.

The filtered ethanol can be saved.

3 Add 540 µl Buffer ATL from the extraction kit to the filter.

Add 60 µl Proteinase K from the extraction kit to the filter.

Put the filters in a shaking incubator overnight at 56°C.
Remember to screw the caps on properly, otherwise the buffer will evaporate!

4 Extract the DNA

Remove the buffer from the filter using the small syringe and put the liquid in a 1.5 ml Eppendorf tube.

4.1 Qiagen Blood and Tissue kit protocol:

Vortex 15 seconds.

Add 200 µl Buffer AL. Mix thoroughly by vortexing.

Incubate at 56°C for 10 min.

Add 200 µl ethanol (96-100%). Mix thoroughly by vortexing.

Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard the flow-through and collection tube.

Place the spin column into a new collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min. at 8000 rpm. Discard the flow-through and collection tube.

Place the spin column into a new collection tube. Add 500 µl Buffer AW2. Centrifuge for 3 min. at

20,000 x g (14,000 rpm). Discard the flow-through and collection tube.

Transfer the spin column to a new 1.5 ml Eppendorf tube.

Elute the DNA by adding 100 µl Buffer AE to the center of the column membrane. Incubate for 1 min. at room temperature (15-25°C). Centrifuge for 1 min. at 8000 rpm.

Store the extracted DNA in freezer.

5 PCR for COI

Primers F:
COIBF3_P5_ins1, COIBF3_P5b_ins2, COIBF3_P5b_ins3, COIBF3_P5
ACACTCTTTCYCCHCG

Primers R:
COIBR2_P7_sub1, COIBR2_P7_ins1, COIBR2_P7_ins2, COIBR2_P7
GRGACTGGAGRAAYCA

Dilute and mix primers to 10 µM (stock is 100 µM).

5.1 PCR mix for 20 µl total volume/sample:

A	B
2X Qiagen Master Mix	10 µl
10 µM primer mix F	2 µl
10 µM primer mix R	2 µl
ddH2O	2 µl
DNA template	4 µl

5.2 PCR program

A	B	C	D
Step	**Temp [°C]**	**Time [s]**	**Number of cycles**
Taq activation	95	900	1
Denaturation	94	30	35
Annealing	50	90	35
Extension	72	90	35
Final extension	72	600	1
End	8	forever	

6 Electrophoresis

6.1 Mix a 1% agarose gel (buffer, agarose, gelgreen).
Remember to put a black box on top to keep it from the light.

Dilute Blue loading dye 1:2 ddH_2O.

Use 1 µl Ladder 100 bp (per row).

Mix 1 µl loading dye with 4 µl PCR product and add to wells.

Run at 80-90 V.

Visualize the gel on the UV plate and take a picture with the camera.