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DNA extraction protocol

PLOS One

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1 *Works for me* dx.doi.org/10.17504/protocols.io.2ptgdnn

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Baroja U, Garin I, Aihartza J, Arrizabalaga-Escudero A, Vallejo N, Aldasoro M, Goiti U (2019) Pest consumption in a vineyard system by the lesser horseshoe bat (*Rhinolophus hipposideros*). PLoS ONE 14(7): e0219265. doi: 10.1371/journal.pone.0219265

BEFORE STARTING

(N = number of samples)

Set the heatblock at 65 °C

Set the oven at 37 °C

Heat C1 solution at 65 °C in the heatblock (aliquoted in a 1.5 ml tube)

Preparte pippet tips (200 ul and 1000 ul)

Label N Dry Bead Tubes with sample numbers/names

1 Add the pellets to sterilised weigh trays and weigh the sample (note down number of pellets + total weight)

2 Add the pellets to the labelled **Dry Bead Tubes with Bead Solution** and give a quick spin

3 Add 60 µl of preheated **C1 solution** to each **Dry Bead Tube** and vortex thoroughly (15 sec)

4 Heat the tubes for 15 minutes at 65 °C in the heatblock

Meanwhile add 250 µl **C2 solution** to empty 1.5 ml tubes and label them (for step 7)

Meanwhile add 200 µl **C3 solution** to empty 1.5 ml tubes and label them (for step 10)

5 **Tissuelyse** the tubes 10 minutes at freq 20 (2x20) in ***Precellys Tissue Homogenizer***.

6 **Centrifuge** the tubes 13,000g for 3 minutes

7 Transfer the supernatant (450 µl) to the 1.5 ml tubes containing **C2 solution** and vortex briefly

8 **Incubate** for 5 minutes at 4 °C

Meanwhile label N spin filters and 2N collection tubes

9 **Centrifuge** the tubes 13,000g for 1 minute

10 Transfer the supernatant (up to 600 µl) to the 1.5 ml tubes containing **C3 solution** and vortex briefly

11 **Incubate** for 5 minutes at 4 °C

Meanwhile label N low-binding 1.5 ml tubes with sample names/numbers

12 **Centrifuge** the tubes 13,000g for 1 minute

13 Transfer the supernatant (up to 750 µl) to the 2 ml Collection Tubes

14 **Shake** the **C4 solution**, add **1100 µl** (550+550) to the supernatant and vortex briefly

15 **Load** **650 µl** onto a Spin Filter, centrifuge 8,000g for 1 minute and discard the flowthrough to a 50ml falcon tube

15.1 Repeat the ***step 15*** until loading all the mix

16 Add **500 µl** of **C5 solution** and centrifuge at 13,000g for 1 minute. Check if the liquid spun through and discard the flowthrough

Avoid splashing C5 solution onto the Spin Filter!

17 **Centrifuge** again at 13,000g for 1 minute and place the Spin Filter in a clean 2 ml Collection Tube

18 Add 50 ul **C6 solution** to the center of the Spin Filter and incubate at **37 °C** 15 minutes

19 **Centrifuge** 13,000g for 1 minute and discard the Spin Filter

20 Transfer the extract to 1.5 low bind tubes and distribute the extracts in PCR strips