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DNA extraction protocol

PLOS One

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BEFORE START INSTRUCTIONS

(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)

Prepate pipette tips (200 ul and 1000 ul)

Label N Dry Bead Tubes with sample numbers/names

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- 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

Note

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

Note

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

Note

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

Note

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
- Note**
- 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