DNA extraction protocol

Unai Baroja1, Inazio Garin1, Joxerra Aihartza1, Aitor Arrizabalaga-Escudero1, Nerea Vallejo1, Miren Aldasoro1, Urtzi Goiti1

1Department of Zoology and Animal Cell Biology, University of the Basque Country, UPV/EHU, 48940 Leioa, Basque Country

WORKS FOR ME
dx.doi.org/10.17504/protocols.io.2ptgdnn

EXTERNAL LINK
https://doi.org/10.1371/journal.pone.0219265

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION


DOI
dx.doi.org/10.17504/protocols.io.2ptgdnn

EXTERNAL LINK
https://doi.org/10.1371/journal.pone.0219265

PROTOCOL CITATION

Unai Baroja, Inazio Garin, Joxerra Aihartza, Aitor Arrizabalaga-Escudero, Nerea Vallejo, Miren Aldasoro, Urtzi Goiti 2019. DNA extraction protocol. protocols.io
https://dx.doi.org/10.17504/protocols.io.2ptgdnn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol


LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED
May 08, 2019

LAST MODIFIED
Jul 19, 2019

PROTOCOL INTEGER ID
22995

BEFORE STARTING
(N = number of samples)
Set the heatblock at \( 65 \, ^\circ\text{C} \)
Set the oven at 37 °C

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

Prepare pipette 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

---

**Citation:** Unai Baroja, Inazio Garin, Joxerra Aihartza, Aitor Arrizabalaga-Escudero, Nerea Vallejo, Miren Aldasoro, Urtzi Goiti (07/19/2019). DNA extraction protocol. https://dx.doi.org/10.17504/protocols.io.2ptgdnn

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 µl **C6 solution** to the center of the Spin Filter and incubate at 37 °C for 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.