Jarquín-Díaz, Víctor Hugo; Balard, Alice, 2017

DNA extraction protocols for *Eimeria* spp. molecular detection studies

Ecology and Evolution of Molecular Parasite-Host Interactions

Genomic DNA (gDNA) from faeces or colon content was extracted using a NucleoSpin®Soil (MACHEREY-NAGEL GmbH & Co. KG, Germany) following the manufacturer's protocol with some modifications:

Before starting the preparation, check Lysis Buffer **SL2** for precipitated SDS. In case of precipitation, warm the buffer until solubilization of the crystals **(NOT microwave it!)**.

Sample lysis:

1. Transfer the material to a 1.5mL tube. Add **700µL Buffer SL2**.
    a. For colon content: Vortex 5sec the fresh sample material, then transfer **350µL**
    b. For faces: Use **100mg** or minimum **4 pellets**
2. *Adjust lysis conditions*: Add **75µL Enhancer SX** and close the cap. (32.5µL at round 2)
3. **Mechanical lysis:** The samples are mechanically disrupted at **room temperature:** a. **@IZW:** This was performed in the high-speed benchtop homogenizer Precellys®24 (Bertin Technologies, France) with a lysis program including two cycles of disruption at 6000 rpm for 30 s with 15s delay between cycles. b. **@HU:** Performed with the horizontal Mill Benchtop Mixer MM 2000 (Retsch GmbH, Haan, Germany) at 80% frequency for 5min.

Precipitate contaminants:

4. Centrifuge for 2 min at 11,000xg to eliminate the foam caused by the detergent 5. Collect the clear supernatant in a new collection tube with lid **(keep remaining sample 2nd round)**
6. Add 150µL Buffer SL3 (75µL at round 2) and vortex for 5 s. Incubate for 5 min at 0 to 4°C (fridge)
7. **Redo the first steps with the saved remaining sample**
8. Centrifuge 1min 11,000xg

Filter lysate:

9. Place a NucleoSpin® Inhibitor Removal Column **(red ring)** in a 2mL Collection Tube with lid
10. Load **up to 700µL** clear supernatant of the previous step onto the filter. 11. Centrifuge for **1 min at 11,000xg**. Keep the column for the parallel round. If a pellet is visible in the flow-through, transfer the clear supernatant to a new collection tube.

Bind DNA:

12. *Adjust binding conditions:* Add **250µL Buffer SB** (adapt at round 2) and close the lid. Vortex for 5 s
13. **Collapse your 2 parallel tubes together**
14. Place a NucleoSpin® Soil Column **(green ring)** in a 2 mL Collection Tube no lid. Load 600µL sample
15. Centrifuge for 1 min at 11,000xg
16. Discard flow-through and place the column back into the collection tube 17. **Load**

the remaining sample onto the column → Centrifuge for **1 min at 11,000xg** 18. Discard flow-through and place the column back into the collection tube

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Wash and dry silica membrane:

19. *First wash***:** Add **500µL Buffer SB** to the NucleoSpin® Soil Column → Centrifuge for 30 s at 11,000xg. Discard flow-through and place the column back into the collection tube.(use a tissue to dry column by tapping, avoid to spread the liquid in the area)
20. *Second wash***:** Add **500µL Buffer SW1** to the NucleoSpin® Soil Column → Centrifuge for 30 s at 11,000xg. Discard flow-through and place the column back into the collection tube.(use a tissue to dry column by tapping, avoid to spread the liquid in the area)
21. *Third wash***:** Add **700µL Buffer SW2** to the NucleoSpin® Soil Column →**vortex 2sec**→Centrifuge for 30 s at 11,000g. Discard flow-through and place the column back into the collection tube.(use a tissue to dry column by tapping, avoid to spread the liquid in the area)
22. *Fourth wash***:** Add **700µL Buffer SW2** to the NucleoSpin® Soil Column →**vortex 2sec**→Centrifuge for 30 s at 11,000xg. Discard flow-through and place the column back into the collection tube.(use a tissue to dry column by tapping, avoid to spread the liquid in the area)
23. ***Dry silica membrane:*** Centrifuge for **2 min at 11,000xg**.
24. **Elute DNA:** Place the column into 1.5mL autoclaved eppendorf. Add **40µL Buffer SE** to the column

→ Do not close the lid and **incubate for 5 min at room temperature** → Close the lid + centri **30s 11,000xg**.
→ annotate your tube well

25. **Quality and integrity of the DNA** were assessed using a NanoDrop 2000c (Thermo Fisher Scientific, USA). Concentrations of double-stranded DNA were quantified using a Qubit® Fluorometer and the dsDNA BR (Broad-range) Assay Kit (Thermo Fisher Scientific, USA). DNA extracts were adjusted to a final concentration of 50 ng/µL with nuclease-free water (Carl-Roth, Germany) and stored at -80°C until further processing.