EZNA DNA extraction from *Frogs*

Preparation the day before the extraction

Materials needed

- E.Z.N.A DNA extraction kit
- Heat block
- Water bath
- Dilute HBC with 100% isopropanol before use.
- Dilute DNA Wash Buffer with 100% ethanol before use.
- 1 falcon tube per family/clutch.
- 1.5 ml eppendorfs, 1/sample for final elution, 1/sample for Buffer BL, 1 per sample for prot K.
- Metal spoon/net to transfer embryos
- Scapel and glass surface to cut tissue
- 0.2% MS222 if old embryos
- Tissue to wipe scapel between samples
- 100% molecular grade ethanol, for samples
- 70% low quality ethanol, for cleaning

Protocol

1. Turn on heat block to 56ºC.
2. Turn on water bath at 70°C.
3. If old embryos, label MS222 contained per family/ clutch with name.
4. Label 1 eppendorf per sample and add 200 µl **Buffer TL**[1]
5. Transfer embryos to MS222.
6. After they stop moving, wash each in H2O, cut in pieces and transfer in labelled microcentrifuge tubes.
7. Add 25 µl proteinase K to each tube and vortex well.
8. Incubate at 55°C for 3h (vortex every 20-30 minutes).
9. Label 1 eppendorf per sample and add 220 µl **Buffer BL**.
10. Prepare rack with collection tubes.
11. After 3 h, centrifuge at max speed (≥10,000 ×g) for 5 minutes.
12. Transfer the supernatant to the labelled microcentrifuge tube[2].
13. Incubate at 70°C for 10 min.
14. Add 220 µL **100% ethanol**.
15. Place a HiBind® DNA Mini Column into a 2 mL Collection tube (provided) for each sample and transfer the entire sample into the Mini Column.
16. Centrifuge at max speed for 1 min.
17. Discard the filtrate and reuse the collection tube.
18. Add 500 µL **Buffer HBC**.
19. Centrifuge at max speed for 30 sec.
20. Discard the filtrate and the collection tube. Place the Mini Column into prepared Collection tube.
21. Add 700 µL **DNA Wash Buffer**.
22. Centrifuge at max speed for 30 sec.
23. Discard the filtrate and reuse the collection tube.
24. Repeat steps 21-23 for a second DNA Wash.
25. Centrifuge the empty Mini Column at max speed for 2 min to dry the column.
26. Label 1 nuclease-free microcentrifuge tube per sample.
27. Transfer the Mini Column into labelled nuclease-free microcentrifuge tube.
28. Add 100 µL **Elution Buffer** heated to 70°C.
29. Let it sit at room temperature for 2 min.
30. Centrifuge at max speed for 1 min.
31. Store at -20°C.

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[1] All buffers can be disposed in sink.

[2] Don't transfer the insoluble pellet.