DNA extraction from *Frogs*

Preparation the day before the extraction

Materials needed

- Qiagen DNA extraction kit (blood and tissue)
- Heat block
- 1.5 ml eppendorfs, 1/sample for final elution and 1/ sample for mixing ethanol and AL.
- Metal spoon 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
- Old plastic bottle for wash buffer waste

Setup

1. Turn on heat block to 56ºC.
2. Clean the bench, trays, pipetes, scoop and centrifuge with 70% ethanol.
3. Fill 1 labelled eppendorf per sample with 180 µl **ATL**. Add 20 µl **proteinase K**.
4. Collect one embryo per sample, cut[1] in pieces (≤25 µg) and transfer to ATL + Prot K tube.
5. Incubate for 3 h at 56ºC[2]. Mix Every 30 min if possible. Do a final 15 µl mix before using.
6. Prepare 1 tray with labeled 1 white DNEasy mini spin column per sample.
7. Prepare 1 tray with labelled 1 eppendorf per sample, same names as above.
8. Prepare 2 trays with 1 empty collection tube per sample.
9. After 3h, prepare 1 tray with 1 eppendorf per sample [3]with 200 µl **100% Ethanol** and 200 µl **AL**.
10. Transfer lysate to prepared AL + Ethanol eppendorf, avoiding big pieces but including any white precipitate. Vortex well.
11. Transfer to pre-labelled DNeasy Mini spin column, already in 2 ml collection tube.
12. Centrifuge at 6,000 rcf[4] for 1 min.
13. Discard flow through in old plastic bottle, discard bottom collection tube and place column in new collection tube from step 8.
14. Add 500 µl **AW1**, invert/roll tubes.
15. Centrifuge at 6,000 rcf for 1 min.
16. Discard flow through in old plastic bottle, discard bottom collection tube and place column in new collection tube from step 8.
17. Add 500 µl AW2, invert/roll tubes.
18. Centrifuge at 14,000 rcf for 3 min to dry the DNeasy membrane.
19. Discard flow through in old plastic bottle, and place column in pre-labelled eppendorf (frop step 7)with lids open.
20. Add 200 µl **AE** to each sample. Close lids and let stand for 1 min.
21. Wipe centrifuge including lid with 70% ethanol.
22. Centrifuge at 6,000 rpm for 1 min to elute DNA[5].
23. Discard spin column, store samples at -20ºC.

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[1] If the animal is old enough to feed, first place in 0.2% MS222 and dissect when has stopped moving.

[2] Longer (overnight) incubation is an alternative, but need to start the previous evening.

[3] These do not need to be labelled as long as their order is not mixed.

[4] RCF = G ≠ RPM

[5] Early embryos have some black dye that transfers over to elution making it appear slightly black. This results in high 230 absorption but does not inhibit PCR.