Protocol: DNA-Extraction

This protocol was designed for about 50 adult female *Daphnia magna*, it should also work for 1-150 animals but with adjusted reagent volumes*.* For achieving HMW DNA consider using red additions. For maximizing yield when preparing samples for Illumina consider gray additions.

Materials:

* Purgene Core Kit A (or alike)
* Proteinase K c[20 mg/ml]
* RNAse A c[20 mg/ml]
* Isopropanol
* Glycogen
* Ethanol 70 %
* SRE Kit (Depletion of DNA <25 kb)

Equipment:

* Pestle for 1.5 ml tube
* pluriStrainer Mini 70 μm

Cookbook:

1. Add snap-frozen animals and 200 μl cold Cell lysis solution to cold 1.5 ml tube.
2. Grind animals with bleached cold pestle by moving the pestle up and down 10 times.
3. Add 300 μl Cell lysis solution and vortex shortly.
4. Add 20 μl ProtK and invert 25 times.
5. Incubate at 55 °C while shaking at 400 rpm overnight (overnight incubation increases yield dramatically).
6. Put sample on ice, add 20 μl RNAse A to the cooled sample, and invert 25 times.
7. Incubate at 37 °C while shaking at 400 rpm for 1 h.
8. Put sample on ice for 1 min.
9. Add 300 μl Protein precipitation solution and vortex for 15 s.
10. Centrifuge for 4 min at 16,000 g. (If pellet is not tight, put on ice for 5 min and repeat.)
11. Pipette supernatant (800 – 1,000 μl) to a 2 ml tube through 70 μm mesh. Discard tissue.
12. Add the same amount of cold isopropanol (800 – 1,000 μl) and 2 μl of glycogen to the supernatant and invert 25 (50) times. Then, put the sample in the freezer for 1 h.
13. Centrifuge for 3 min at 16,000 g.
14. Discard supernatant, add 500 μl cold 70 % ethanol, and invert until the pellet dislodges.
15. Centrifuge for 1 min at 16,000 g.
16. Discard supernatant.
17. Apply SRE Kit for HMW DNA and repeat step 14.-16. twice for purification.
18. Put the open tube in the vacuum centrifuge for 15 min.
19. Add 80 μl DNA Hydration solution and incubate in the dark overnight. If fewer animals are being used or less DNA yield is expected, add less (20-50 μl)