High-molecular weight DNA extraction by phenol-chloroform

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This is based on a protocol gently shared by Marta Puig (Universitat Autònoma de Barcelona), and modified to be applied on flies, instead of human tissues.

1 Sample preparation

1. Put a frozen fly into a 1.5 ml microtube, previously cooled. Keep in ice.
2. With the sample still frozen, add 370 μl of **extraction buffer**, and crush the tissue using a sterile micropistile. Optionally, add a tiny bit of sand. Keep it cool while processing.
3. Add 370 μl more of extraction buffer and homogenize it.
4. Add 2.5 μl of **RNase coctail**[1] (per 750 μl of extraction buffer used) and incubate 1 h at 37°C in a hybridization oven with rotation (2 rpm).
5. Add 3.75 μl of **proteinase K**[2].
6. Incubate overnight at 50°C in the hybridization oven with rotation (2 rpm).

2 DNA isolation

1. Check that the solution is clear and viscous after the overnight incubation. If not, add more proteinase K and incubate again to achieve this. No visible pieces of the cell pellet should be present at this stage.
2. If you used sand, now it's a good time to remove it. Centrifuge for 2 min at 8000 g at 20°C, and transfer the supernatant to a new tube. Discard the sand.

[1] Let's try with RNase A.

[2] This assumes the proteinase K is at 20 mg/ml; it should end up at 100 μg/ml. The proteinase K from NEB is at 20 mg/ml. But the proteinase K from the DNeasy Blood & Tissue kit seems to be at 15 mg/ml, so I would add 5 μl instead, if using this one

3. Add 1 volume (750 μl) of **TE-equilibrated phenol pH 7.9**[3] and agitate in the orbital rotator (40 rpm) for 15 min until the two phases are mixed. Alternatively, swirl gently by hand for 15-20 min.

4. Centrifuge 15 min at 5000 g at room temperature.

5. Recover the aqueous phase (upper phase) with wide-bore 1 ml tips and transfer it to a fresh 1.5 or 2 ml tube. Pipette slowly to avoid breaking DNA. Try to recover the maximum amount of aqueous phase possible without disturbing the white interphase. This can be difficult in the first phenol step because the aqueous phase is very viscous.

6. Repeat steps 3 to 5

7. Add 1 volum (750 μl) of **Phenol:Chloroform:IAA ph 7.9**[4] and agitate in the orbital rotator (40 rpm) for 15 min until the two phases are mixed. Alternatively, swirl gently by hand for 15-20 min.

8. Centrifuge 10 min at 5000 g at room temperature.

9. Since PCR amplification or other applications could be inhibited by phenol contamination, recover the aqueous phase (upper phase) with wide-bore 1 ml tips and transfer it to a fresh 2 ml tube. Add 1 volume (750 μl) of Chloroform:IAA and swirl gently by hand until the complete emulsion of the two phases is achieved.

10. Centrifuge 10 min at 5000 g at room temperatue.

11. Recover the aqueous phase (upper phase) with wide-bore tips and transfer it to a 15 ml tube. Add 0.1 volumes of 3M Sodium Acetate (NaOAc) and 2 volumes of absolute EtOH at room temperature. **Imporant:** Correct the volume depending of the aqueous phase recovered from the last chloroform pass to avoid salt precipitates.

12. Mix slowly by inversion until the DNA precipitates (a transparent/white mucus forms in the solution).

13. Transfer part of the liquid to a fresh 15 ml tube until the volume left can fit into a 1.5 ml eppendorf tube, and then transfer the DNA precipitate together with the rest of the solution carefully to a 1.5 ml low-binding eppendorf tube.

14. Centrifuge 1 min at 5000 g at room temperature to form a pellet of DNA.

[3] Atention: Adjust the pH of the phenol solution to 7.9 (appropriate for DNA) by adding the buffer supplied with the phenol following the instructions of the manufacturer. This step should be performed prior to starting the DNA isolation to allow the complete separation of the two phases in the phenol solution before using it for the first time. This pH adjustment only needs to be done when a new bottle of phenol is opened, not every time the phenol is used.

[4] See previous footnote.

15. Eliminate supernatant and add 1 volume (750 μl) of 70% EtOH. Separate the pellet from the bottom of the tube and wash it by moving it around the 70% EtOH.

16. Centrifuge 1 min at 5000 g at room temperature and repeat steps 15–16 once more.

17. Eliminate the maximum amount of EtOH 70% without touching the white DNA pellet after the final wash. Leave the eppendorf open to air dry for a few minutes. Important: Do not let the DNA pellet dry too much because then it can be difficult to resuspend. Wait until there are no drops of EtOH 70% left on the tube walls. It is possible that in this process the DNA pellet turns transparent and becomes more difficult to see.

18. Resuspend the DNA in 150-300 μl milliQ water or TE. Be aware that EDTA from the TE buffer could inhibit some reactions (for example, those that use Mg^{2+}).

19. Leave the DNA to dissolve overnight at 4°C. Do not freeze the DNA sample because freezing/thawing cycles can fragment the DNA.

3 List of reagents

Extraction Buffer 10 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 150 mM NaCl, 0.5% SDS. Autoclave before adding SDS. For 50 ml: 0.5 ml Tris-HCl 1 M, 1 ml EDTA 0.5 M, 5 ml NaCl 1.5 M, 43.5 ml H_2O.

RNase Cocktail Cat # AM2286 AMBION.

Proteinase K Cat # AM2546 AMBION.

Phenol solution Equilibrated with 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, molecular biology grade. Cat # P4557-100ML SIGMA.

Phenol:Chloroform:IAA Molecular biology grade, cat # AM9730 AMBION.

Chloroform:IAA Molecular biology grade, cat # C0549 SIGMA.

Sodium acetate buffer solution pH 5.2. S7899-100ML SIGMA.

Ethanol .

4 List of devices

Centrifuge There is one available in the Evolutionary Ecology lab.

Autoclave Available.

Hybridization oven Not available yet.

pH-meter There is one available.

Orbital rotor Not available yet.

Precision scale Available, I think, at the Evol. Ecol. lab.