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Phenol-chloroform DNA extraction protocol for biodiversity V.1

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DNA extraction Animals

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We use this protocol and it's working

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Abstract

This protocol is optimized for DNA extraction in 1.5 mL tubes, but it can be adapted for larger tubes without depending strictly on volume ratios. It was developed for extracting DNA from a wide range of biodiversity sample types.

Before start

Preheat the Lysis Solution in a water bath at 55 °C. Mammalian blood: Preheat Tris NH4Cl at 37 °C.

DIGESTION

1 Transfer a small piece of tissue or blood into 1.5 mL tubes.
Muscle tissue (10–25 mg); Liver (5–10 mg).
Mammalian blood (20–100 µL); Avian blood (2–10 µL).
Avian eggshell membrane (25–40 mg – target blood vessels).
Coagulated blood: Heat the sample at **55 °C for 10 minutes,** then immediately place it on ice. Invert or gently shake to disperse the clot.

1.1 Rigid tissues: Add Milli-Q H2O and incubate in a **55 °C water bath for 10–20 min. D**iscard the supernatant and proceed to the next step.

2 Thoroughly mince the tissue using a micro-scissor and/or pestle.

2.1 Mammalian blood: Add 1 mL pre-warmed Tris NH4Cl (37 °C). Gently mix by inversion, incubate for 5 minutes at room temperature, and centrifuge at 7,000 rpm for 5 minutes. Discard the supernatant and proceed.

3 Add 1 mL saline solution (0.85% NaCl).
For blood: Preferably use 1 mL PBS instead of saline.

4 Briefly vortex three times and centrifuge at **7,000 rpm for 5 minutes**.

5 Discard the supernatant and retain the pellet.

6 Repeat steps 3–5.

7 Add 400 µL Lysis Solution 1 (100 mM Tris-HCl, 10 mM EDTA, 100 mM NaCl, 1% SDS), pre-warmed at 55 °C.

8 Add 5–15 µL Proteinase K (20 mg/mL).

9 Briefly vortex five times.

10 Incubate in a water bath or Thermomixer at **55 °C** with moderate rotation (~550 rpm) for 2–12h, or until complete digestion.
If digestion is incomplete after overnight incubation, add an additional 10 µL Proteinase K and incubate for at least 2 more hours.
Rigid tissues: digestion time may be extended up to 24 h.

CLEAN UP AND PRECIPITATION (ABSOLUTE ETHANOL)

11 Briefly vortex the lysed sample.

12 Add 10 µL RNase A (10 mg/mL).

13 Incubate for 3 minutes at room temperature.

14 Add 120 µL Milli-Q H2O.

15 In a fume hood, add 1 volume Phenol:Chloroform:Isoamyl Alcohol (25:24:1).

16 Mix vigorously for 30 seconds.

17 Centrifuge at 14,000 rpm for 3 minutes.

18 Transfer the supernatant to a new tube.
For tissues rich in structural proteins or incomplete lysis: repeat steps 5–8.

19 Add 1 volume Chloroform:Isoamyl Alcohol (24:1).

20 Mix vigorously for 30 seconds.

21 Centrifuge at 14,000 rpm for 3 minutes.

22 Transfer 400 µL of the supernatant to a new tube.
If the aqueous phase is turbid or yellowish, repeat steps 9–12.

23 Change gloves.

24 Outside the fume hood, add 100 µL Ammonium Acetate 10 M (final concentration 2 M).

25 Add 1 mL ice-cold Absolute Ethanol, then mix well by inversion (20 times).

26 Store the sample at **-80 °C for at least 1 h, or overnight at -20 °C,** to stimulate DNA precipitation.
DNA can be indefinitely stored in ethanolic solutions at 0 °C or -20 °C. Invert the tube a few times before proceeding.

27 Centrifuge at 14,000 rpm for 20 minutes at 0 °C.

28 Carefully discard the supernatant with a pipette without disturbing the pellet.

29 Add 1 mL 70% Ethanol and gently invert the tube 3 times.

30 Centrifuge at 14,000 rpm for 5 minutes at 4 °C.
Recommended: Repeat steps 18–20.

31 Carefully remove the supernatant with a pipette without disturbing the pellet, and let it air dry.
If necessary, use pipettes with progressively smaller volumes until all ethanol is removed.
Do not let the pellet dry completely – it should remain moist before elution.

32 Dissolve the pellet in an appropriate volume of Low TE or ultrapure water (20–100 µL).
Preheating Low TE aliquots at 55 °C is recommended for better elution.

33 Centrifuge at 14,000 rpm for 1 minute.

34 Incubate in a Water Bath or Thermomixer with minimal rotation at 55 °C for 1–2 h.

To remove ethanol traces, keep tubes open for 10 min at 55 °C.
Alternatively, incubate overnight at room temperature after a brief pre-heating at 55 °C.

35 Store the samples in the refrigerator or freezer.

CLEAN-UP AND PRECIPITATION (ISOPROPANOL)

36 Briefly vortex the lysed sample.

37 Add 10 µL RNase A (10 mg/mL).

38 Incubate for 3 minutes at room temperature.

39 Add 300 µL Milli-Q H2O.

40 In a fume hood, add 1 volume Phenol:Chloroform:Isoamyl Alcohol (25:24:1).

41 Mix vigorously for 30 seconds.

42 Centrifuge at 14,000 rpm for 3 minutes.

43 Transfer the supernatant to a new tube.
For tissues rich in structural proteins or incomplete lysis: repeat steps 5–8.

44 Add 1 volume Chloroform:Isoamyl Alcohol (24:1).

45 Mix vigorously for 30 seconds.

46 Centrifuge at 14,000 rpm for 3 minutes.

47 Transfer the supernatant to a new tube.
If the aqueous phase is turbid or yellowish, repeat steps 9–12.

48 Change gloves.

49 Outside the fume hood, add Ammonium Acetate 10 M to a final concentration of 2–2.5 M.
Tip: For a final concentration of 2 M, add 1/4 of the recovered volume of 10 M A.A.

50 Add 0.7–1 volume of room-temperature Isopropanol, then mix well by inversion (20 times).

51 Incubate for 3 minutes at room temperature, inverting occasionally.

52 Centrifuge at 14,000 rpm for 25 minutes at 4 °C.

53 Carefully discard the supernatant with a pipette without disturbing the pellet.

54 Add 1 mL 70% Ethanol at room temperature and gently invert the tube 3 times.

55 Centrifuge at 14,000 rpm for 5–10 minutes at 4 °C.

56 Repeat steps 18–20.

57 Carefully remove the supernatant with a pipette without disturbing the pellet, and let it air dry.
If necessary, use pipettes with progressively smaller volumes until all ethanol is removed.
Do not let the pellet dry completely – it should remain moist before elution.

58 Dissolve the pellet in an appropriate volume of Low TE or ultrapure water (20–100 µL).
Preheating Low TE aliquots at 55 °C is recommended for better elution.

59 Centrifuge at 14,000 rpm for 1 minute.

60 Incubate in a Water Bath or Thermomixer with minimal rotation at 55 °C for 1–2 h.
To remove ethanol traces, keep tubes open for 10 min at 55 °C.
Alternatively, incubate overnight at room temperature after a brief pre-heating at 55 °C.

61 Store the samples in the refrigerator or freezer.

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