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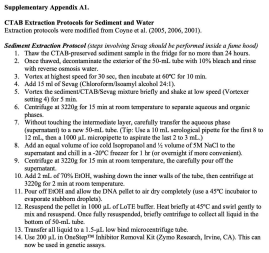
# CTAB Extraction Protocol for Sediment



Supplementary Appendix A1.

**CTAB Extraction Protocols for Sediment and Water**

Extraction protocols were modified from Coyne et al. (2005, 2006, 2001).

***Sediment Extraction Protocol*** *(steps involving Sevag should be performed inside a fume hood)*

1. Thaw the CTAB-preserved sediment sample in the fridge for no more than 24 hours.
2. Once thawed, decontaminate the exterior of the 50-mL tube with 10% bleach and rinse with reverse osmosis water.
3. Vortex at highest speed for 30 sec, then incubate at 60°C for 10 min.
4. Add 15 ml of Sevag (Chloroform:Isoamyl alcohol 24:1).
5. Vortex the sediment/CTAB/Sevag mixture briefly and shake at low speed (Vortexer setting 4) for 5 min.
6. Centrifuge at 3220g for 15 min at room temperature to separate aqueous and organic phases.
7. Without touching the intermediate layer, carefully transfer the aqueous phase (supernatant) to a new 50-mL tube. (Tip: Use a 10 mL serological pipette for the first 8 to 12 mL, then a 1000 µL micropipette to aspirate the last 2 to 3 mL).
8. Add an equal volume of ice cold Isopropanol and ½ volume of 5M NaCl to the supernatant and chill in a -20°C freezer for 1 hr (or overnight if more convenient).
9. Centrifuge at 3220g for 15 min at room temperature, the carefully pour off the supernatant.
10. Add 2 mL of 70% EtOH, washing down the inner walls of the tube, then centrifuge at 3220g for 2 min at room temperature.
11. Pour off EtOH and allow the DNA pellet to air dry completely (use a 45°C incubator to evaporate stubborn droplets).
12. Resuspend the pellet in 1000 µL of LoTE buffer. Heat briefly at 45°C and swirl gently to mix and resuspend. Once fully resuspended, briefly centrifuge to collect all liquid in the bottom of 50-mL tube.
13. Transfer all liquid to a 1.5-µL low bind microcentrifuge tube.
14. Use 200 µL in OneStep™ Inhibitor Removal Kit (Zymo Research, Irvine, CA). This can now be used in genetic assays.

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**Protocol status:** Working
**Successfully used by Turner et al. (2015) to detect carp sedDNA**

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## Abstract

Successfully used by Turner et al., 2015 to detect bigheaded Asian carp surface sedimentary DNA from experimental ponds and natural rivers

https://www.sciencedirect.com/science/article/pii/S000632071400442X

## Materials

**Materials**

[M] 100 millimolar (mM) Tris-HCL pH 8

[M] 1.4 Molarity (M) NaCl

[M] 1 Mass / % volume Polyvinylpyrrolidone pH 8

[M] 2 Mass / % volume Cetyl trimethyl ammonium bromide (CTAB)

[M] 20 millimolar (mM) EDTA pH 8

## Troubleshooting

## Safety warnings

Steps involving Sevag should be performed inside a fume hood.

## Sample preparation

1d 1h 47m 30s

1 **THAW** the CTAB-preserved sediment sample in the fridge for no more than 24:00:00 — 1d

**DECONTAMINATE** the exterior of the sample tube with 10 % bleach solution and rinse with reverse osmosis water

2 **VORTEX** at max speed for 00:00:30 — 10m 30s

**INCUBATE** at 60 °C for 00:10:00

## Chloroform extraction

1d 1h 47m 30s

3 **ADD** 15 mL of Sevag (Chloroform/Isoamyl alcohol 24:1)

> Note
>
> Steps involving Sevag should be performed inside a fume hood.

4 **VORTEX** the sediment/CTAB/Sevag mixture briefly — 5m

**SHAKE** at low speed (Vortexer setting 4) for 00:05:00

5 **CENTRIFUGE** at 3220 x g for 00:15:00 at Room temperature — 15m

**CAREFULLY** transfer the supernatant to a new 50 mL tube

## Ethanol precipitation

1d 1h 47m 30s

6 **ADD** an equal volume of ice-cold Isopropanol to the supernatant — 1h

**ADD** ½ volume of 5M NaCl to the supernatant

**INCUBATE** at -20 °C for 01:00:00 (or overnight if more convenient)

7 **CENTRIFUGE** at 3220 x g for 00:15:00 at Room temperature — 15m

**CAREFULLY** pour off the supernatant

8 **ADD** 2 mL of 70% EtOH, washing down the inner walls of the tube 2m

**CENTRIFUGE** at 3220 x g for 00:02:00 at Room temperature

**POUR** off EtOH and allow the DNA pellet to air dry completely (a 45 °C incubator can be used to evaporate stubborn droplets)

## DNA resuspension 1d 1h 47m 30s

9 RESUSPEND the pellet in 1000 µL of LoTE buffer. Heat briefly at 45 °C and swirl gently to mix and resuspend. Once fully resuspended, briefly centrifuge to collect all liquid in the bottom of 50-mL tube.

10 Transfer all liquid to a 1.5-µL low-bind microcentrifuge tube

11 Use 200 µL in OneStepTM Inhibitor Removal Kit (Zymo Research, Irvine, CA)

## Protocol references

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation, 183, 93-102. https://doi.org/10.1016/j.biocon.2014.11.017