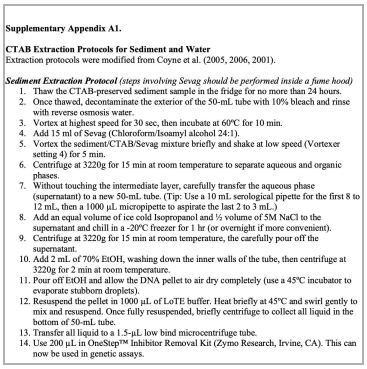

Supplementary Appendix A1.

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.

APR 07, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ewov1ozkolr2/v1

Protocol Citation: Cameron R. Turner 2023. CTAB Extraction Protocol for Sediment. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1ozkolr2/v1

MANUSCRIPT CITATION:
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

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

Created: Jan 13, 2023

CTAB Extraction Protocol for Sediment

Cameron R. Turner[1]

[1]Department of Biological Sciences, University of Notre Dame

Yuanyu Cheng
McGill University

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

SAFETY WARNINGS

Steps involving Sevag should be performed inside a fume hood.

Last Modified: Apr 07, 2023

PROTOCOL integer ID:
75293

Keywords: Sedimentary
DNA, SedDNA

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 1⁄2 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)