APR 17, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvr4n8ogmk/v1

Protocol Citation: Masayuki K. Sakata 2023. Sakata et al. Fish SedDNA Extraction Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr4n8ogmk/v1

Sakata et al. Fish SedDNA Extraction Protocol

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ABSTRACT

Variations of this standardised protocol have been used by Masayuki K. Sakata and colleagues to successfully extract fish eDNA from modern and historic Japanese lake and river sediments.

The method has been used to recover fish species composition data from modern surface sediments from a lake (Sakata et al., 2020) and a small, natural river (Sakata et al., 2021). It has also been applied to detect target fish species in lake sediments up to 100 years old (Sakata et al., 2022).

This extraction method represents a consolidation of the methods applied in the following publications:

- Sakata, M. K., Yamamoto, S., Gotoh, R. O., Miya, M., Yamanaka, H., & Minamoto, T. (2020). Sedimentary eDNA provides
different information on timescale and fish species composition compared with aqueous eDNA. Environmental DNA, 2(4),
505– 518. https://doi.org/10.1002/edn3.75
- Sakata, M. K., Watanabe, T., Maki, N., Ikeda, K., Kosuge, T., Okada, H., … Minamoto, T. (2021). Determining an effective
sampling method for eDNA metabarcoding: a case study for fish biodiversity monitoring in a small, natural river.
Limnology, 22(2), 221–235. https://doi.org/10.1007/s10201-020-00645-9
- Sakata, M.K., Tsugeki, N., Kuwae, M., Ochi, N., Hayami, K., Osawa, R., Morimoto, T., Yasashimoto, T., Takeshita, D.,
Doi, H., & Minamoto, T. (2022). Fish environmental DNA in lake sediment overcomes the gap of reconstructing past fauna
in lake ecosystems. bioRxiv, https://doi.org/10.1101/2022.06.16.496507

MANUSCRIPT CITATION:

- Sakata, M. K., Yamamoto, S., Gotoh, R. O., Miya, M., Yamanaka, H., & Minamoto, T. (2020). Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA. Environmental DNA, 2(4), 505– 518. https://doi.org/10.1002/edn3.75
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- Sakata, M.K., Tsugeki, N., Kuwae, M., Ochi, N., Hayami, K., Osawa, R., Morimoto, T., Yasashimoto, T., Takeshita, D., Doi, H., & Minamoto, T. (2022). Fish environmental DNA in lake sediment overcomes the gap of reconstructing past fauna in lake ecosystems. bioRxiv, https://doi.org/10.1101/2022.06.16.496507

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Protocol status: Working
We use this protocol and it's working

Created: Apr 10, 2023

Last Modified: Apr 17, 2023

MATERIALS

Reagents:

- NaOH (0.33M)
- TE buffer (pH 6.7) (Prepared with Tris-HCl buffer: 10 mM, EDTA: 1 mM)
- Tris-HCl (1M, pH 6.7)
- NaAc (3M, pH 5.2),
- Ethanol (99.5%),
- G2 enhancer (AMPLIQON, liquid type)

Kit: DNeasy PowerSoil Kit (QIAGEN)

Required equipment:

- Thermostatic bath
- Centrifuge (capable of centrifuging 50mL, 15mL, 1.5mL tubes)
- Vortex
- Vortex adapter (adapter to fix tube)
- Lo-Bind tubes for storage (recommended)

PROTOCOL integer ID:
80234

Alkaline extraction

50m

1 **PLACE** 9.0 g of sediment sample into a 50 mL tube

ADD 6 mL NaOH

ADD 3 mL TE buffer

ADD 500 µL G2 enhancer

Note

Sediment volume can be adjusted but reagent volumes should remain the same

2 **VORTEX** samples to mix

50m

INCUBATE samples at 94 °C for 00:50:00

3 **ALLOW** samples to cool to Room temperature

30s

CENTRIFUGE at 5000 x g for 00:00:30

4 **TRANSFER** 7.5 mL of supernatant to a new 50 mL tube

NEUTRALIZE by adding 7.5 mL Tris-HCL (1M)

Ethanol precipitation

1h 20m

5 **ADD** 1.5 mL sodium acetate

VORTEX well to mix

6 **ADD** 30 mL of ethanol

VORTEX well to mix

7 **INCUBATE** at -20 °C for at least 01:00:00 1h

Note

Incubation at -20 °C Overnight is recommended

8 **WIPE** off condensation from around the 50 mL tube and centrifuge at 5,350xG for 20 min 20m

CENTRIFUGE at 5350 x g for 00:20:00

9 **DISCARD** supernatant and leave the tube mouth down for a few minutes

TRANSFER the precipitate to a PowerBead Tube (Qiagen Kit)

DISSOLVE the remaining precipitate with 100 µL of DW then transfer to the PowerBead Tube

DNeasy PowerSoil extraction 22m

10 **ADD** 60 µL of Solution C1 10m 30s

VORTEX at max speed for 00:10:00

CENTRIFUGE at 10000 x g for 00:00:30

Note

Vortex for 15-20 minutes if you have more than 12 samples

11 **TRANSFER** supernatant to a new 2 mL tube

Note

Expect ~700ul of supernatant, but the more supernatant transferred, the better

12 **ADD** 250 µL of Solution C2 6m

VORTEX briefly to mix

INCUBATE at 4 °C for 00:05:00

CENTRIFUGE at 10000 x g 00:01:00

13 **TRANSFER** 600 µL of supernatant to a new 2 mL tube 6m

ADD 200 µL of Solution C3

VORTEX briefly to mix

INCUBATE at 4 °C for 00:05:00

CENTRIFUGE at 10000 x g for 00:01:00

14 **TRANSFER** 750 µL of supernatant to a new 2 mL tube 6m

ADD 1.2 mL of Solution C4

VORTEX well to mix

15 **TRANSFER** 675 µL to a Spin Filter 1m

CENTRIFUGE at 10000 x g for 00:01:00

DISCARD the liquid filtrate

16 **REPEAT** the above step until all liquid has passed through the Spin Filter

17 **ADD** 500 µL of Solution C5 to the Spin Filter 30s

CENTRIFUGE at 10000 x g for 00:00:30

DISCARD the liquid filtrate

18 **TRANSFER** the Spin Filter to a new 1.5 mL tube 1m 30s

ADD 100 µL of Solution C6 to the Spin Filter

LET stand for 00:01:00

CENTRIFUGE at 10000 x g for 00:00:30

19 **DISCARD** the Spin Filter

DNA is now ready for downstream applications