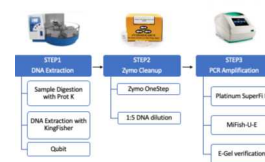


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Automated eDNA Extraction from Estuarine Samples Using Magnetic Beads

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Fouad El Baidouri¹, Muriel Kelly², Heather L. Gilbert¹, Alison Watts¹

¹Department of Civil & Environmental Engineering, University of New Hampshire, Durham, NH, USA.;

²Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH, USA



Fouad El Baidouri

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We use this protocol and it's working

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Abstract

This method presents an optimized protocol for the automated extraction of environmental DNA (eDNA) from estuarine samples using magnetic beads. The addition of Proteinase K to the lysis solution facilitates DNA solubilization at 56°C and mechanical rotation ensures thorough lysis. The DNA is then purified using a double ethanol wash and magnetic bead-based extraction on a KingFisher system. The method is designed for filters preserved in Longmire's buffer. If a different preservation is used the first step should be modified.

Guidelines

Follow Personal Protective Equipment (PPE) procedures.

- **Clean workspace:** Thoroughly clean all work surfaces including the benchtop and equipment exteriors using a 5%-10% bleach solution followed by a distilled water rinse. Wipe down pipettes, pipette tip boxes, sharpies, pens and any other tools that will be used during the protocol to avoid cross contamination.
- **Incubator check:** Verify that the incubator is operational and set to the correct temperature before use (56°C).
- **Handling:** Use aseptic techniques to avoid introducing contaminants into sample preparations. Include extraction blanks to check for cross contamination from adjacent wells.
- **Storage:** If immediate DNA extraction is not possible, samples can be stored in Longmire's buffer at room temperature for up to 50 days (minimize exposure to light), or frozen at -20°C or -80°C for long-term storage.

Notes

- Illumina Tune beads (CAT: 20060057) performed better than Ampure XP and are cheaper.

Materials

Protective gear

- Gloves: to eliminate/reduce contamination when handling samples
- Lab coat

Cleaning supplies

- 5%-10% bleach solution: for cleaning benches and equipment
- Distilled water: for rinsing

Consumables

- Deep Well Plates (DWP) (97002540)
- 200 µl plates: for elution (97002540)
- Tip comb for DW magnets (97002534)
- Pipettes and pipette tips: 1,000 µl, 200 µl, 20 µl
- Reagent troughs/reservoirs
- Tape: for securing during incubation

Reagents

- Longmire's buffer (see [References](#)): prepare using the following components to make a total of 500 mL:
 - 50 mL of 1 M Tris-HCl, pH 8.0 (for buffering the solution)
 - 100 mL of 0.5 M EDTA, pH 8.0 (chelates divalent cations and prevents degradation by nucleases)
 - 1 mL of 5 M NaCl (provides ionic strength to the buffer)
 - 12.5 mL of 20% SDS (w/v) (a detergent that aids in cell lysis and protein solubilizing)
 - 336.5 mL of PCR grade water (used to bring the solution up to the final volume and ensure purity)
- Proteinase K: from Promega (CAT: PRMC5005)
- 80% ethanol: used for wash steps
- Tris-HCl (10 mM): used in the elution plate
- Illumina Tune Beads (CAT: 20060057): for magnetic bead based DNA extraction

Equipment

Incubator with a rotator: set at 56°C for incubation

Multichannel pipette (i.e. Integra): for efficient handling of multiple samples

Kingfisher Flex Purification System

Qubit reagents or HTS Microplate Reader: for quantifying DNA concentration

Nanodrop analyzer: for assessing DNA quality (optional)

Troubleshooting



Safety warnings

- ⚠ Ensure all surfaces and equipment are thoroughly cleaned with 5%-10% bleach and rinsed with distilled water to prevent contamination. Include extraction blanks to check for sample cross contamination.

Before start

Longmire's buffer preparation: ensure that Longmire's buffer is prepared using PCR grade water.

Sample Preparation

- 1 Perform the following steps in a biosafety cabinet under aseptic conditions
For samples collected on filters stored in Longmire's buffer (wet filters)
 - Pipette out all of the Longmire's buffer from the sample tube into a separate Eppendorf tube (leave the sample filter in the sample tube)
 - Pipette 490 μl *per filter* of the original Longmire's buffer back into the sample tube
 - Add 10 μl of Proteinase K *per filter* into the sample tube (total volume 500 μl)
 - Using the pipette tip, mix the reagents thoroughly
 - Using the same pipette tip, ensure the filter(s) are completely submerged in the Longmire's buffer/Proteinase K solution**For dry filters, add 1000 μl of Longmire's solution to the tube, ensuring the filter is completely submerged - Incubate for 90 min to overnight.**
 - Pipette out all of the Longmire's buffer from the sample tube into a separate Eppendorf tube (leave the sample filter in the sample tube)
 - Pipette 490 μl *per filter* of the original Longmire's buffer back into the sample tube
 - Add 10 μl of Proteinase K *per filter* into the sample tube (total volume 500 μl)
 - Using the pipette tip, mix the reagents thoroughly
 - Using the same pipette tip, ensure the filter(s) are completely submerged in the Longmire's buffer/Proteinase K solution

Sample Incubation & Lysis

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 - Place tubes in tube rack on a rotating or gently shaking surface in a 56°C incubator
 - Secure rack with tape
 - Turn on rotator
 - Incubate for 90 minutes or overnight
 - After incubation proceed with steps below. Alternatively samples can be kept at 4°C overnight for a later extraction.
- 2.1 **Washing and Elution Plates Preparation**
 - During the 90 minute incubation step, prepare two wash plates by pipetting 500 μl freshly prepared 80% ethanol into the wells of two DWPs
 - Prepare an elution plate by pipetting 100 μL of 10 mM Tris-HCl into the wells of a 200 μl plate



Magnetic Beads Addition

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 - After incubation, transfer 400 μl of the supernatant lysis to a new DWP (this is the sample plate). Bubble formation sometimes occurs due to the presence of SDS. Ensure the tubes are tightly sealed to prevent evaporation during the 56°C incubation period



- Add 320 µl of Illumina Tune beads to the sample plate wells (1:8 sample to beads ratio to remove small DNA fragments < 200bp)

KingFisher Extraction (DNA Purification)

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 - Place the sample plate (containing the 720 µl of sample + beads), wash plates 1 and 2 and the elution plate into the KingFisher and run the program as follows
 - The KingFisher is optimized for the volumes stated in this protocol

4.1 KingFisher Program

1. Pick up:

- Tip comb plate

2. Binding:

- Mix for 5 minutes at medium speed
- Collect beads, count: 5
- Move to sample plate

3. 1st Wash:

- Release beads
- Bottom mix and medium speed, each for 10 seconds, loop count 2
- Collect beads, count: 3; collect time: 5 seconds
- Transfer to wash plate 1

4. 2nd Wash:

- Release beads for 20 seconds
- Bottom mix and medium speed, each for 10 seconds, loop count 2
- Collect beads, count: 4; collect time: 5 seconds
- Transfer to wash plate 2

5. Dry:

- Use wash plate 2

6. Elution:

- Preheat to 60°C
- Bottom mix for 15 seconds, medium speed for 45 seconds, loop count: 6
- Collect beads, count: 1
- Transfer to elution plate

7. Collection of Beads:

- 2 minutes at slow speed

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 - After the program ends, immediately proceed with the Zymo cleanup steps using the elution plate, or store the plate at -20°C for future use. Dispose of chemical reagents according to Lab Hazardous Waste Management Plan.



Protocol references

Sanches, T.M. and Schreier, A.D., 2020. Optimizing an eDNA protocol for estuarine environments: Balancing sensitivity, cost and time. *PLoS One*, 15(5), p.e0233522.

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