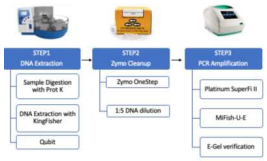
Jun 13, 2024

Automated eDNA Extraction from Estuarine Samples Using Magnetic Beads



DOI
dx.doi.org/10.17504/protocols.io.5jyl82jn9l2w/v1

Fouad El Baidouri[1], Muriel Kelly[2], Heather L. Gilbert[1], Alison Watts[1]

[1]Department of Civil & Environmental Engineering, University of New Hampshire, Durham, NH, USA.;
[2]Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH, USA

Fouad El Baidouri
UNH

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl82jn9l2w/v1

Protocol Citation: Fouad El Baidouri , Muriel Kelly, Heather L. Gilbert, Alison Watts 2024. Automated eDNA Extraction from Estuarine Samples Using Magnetic Beads. **protocols.io https://dx.doi.org/10.17504/protocols.io.5jyl82jn9l2w/v1**

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

Created: May 13, 2024

Last Modified: June 13, 2024

Protocol Integer ID: 99676

Keywords: eDNA, Environmental DNA, Automation, PCR inhibitor removal, DNA extraction, Magnetic beads, Environmental biomonitoring, Automated extraction, Zymo clean up, KingFisher instrument

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)

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

2

- 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

3

- 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)

4
- 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

5
- 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.

Longmire, J.L., Maltbie, M. and Baker, R.J., 1997. Use of" lysis buffer" in DNA isolation and its implication for museum collections.