Feb 22, 2024

eDNA extraction from water samples filtered through 47 mm diameter filters (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL).

DOI
dx.doi.org/10.17504/protocols.io.j8nlkox7dv5r/v1

Marine Vautier[1], Cecile Chardon[1], Cyrielle GALIEGUE[1], Isabelle Domaizon[1]

[1]INRAE - Univ. Savoie Mont Blanc - UMR CARRTEL - Pole R&D Ecla

CARRTEL

Marine Vautier
INRAe

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkox7dv5r/v1

Protocol Citation: Marine Vautier, Cecile Chardon, Cyrielle GALIEGUE, Isabelle Domaizon 2024. eDNA extraction from water samples filtered through 47 mm diameter filters (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL).. **protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlkox7dv5r/v1**

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

Created: February 22, 2024

Last Modified: February 22, 2024

Protocol Integer ID: 95601

Keywords: eDNA, water, Sterivex, DNA, extraction, filter, rare DNA, magnetapure 32 nucleic acid purification system, dna extraction, nucleic acid purification system, nucleic acid purification, reversible adsorption of nucleic acid, extraction from water sample, rna water kit, environmental dna, nucleomag dna, nucleic acid, nucleomagdna, extraction, water sample, dna, purification, rna, molecular biology analysis

Abstract

The objective of this protocol is the **environmental DNA (eDNA) extraction from water samples filtered through 47 mm diameter filters.** This protocol can be performed from filters preserved in tubes prefilled or not with preservation buffer.

DNA extraction is performed using a **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) and with the **NucleoMagDNA/RNA Water Kit** (Macherey Nagel).

The procedure is based on **reversible adsorption of nucleic acids to paramagnetic beads** under appropriate buffer conditions.

The benefits of using the **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) are **increased productivity and repeatability, as well as eliminating human error and the pain of repetitive work**.

This protocol is used **prior to molecular biology analysis** (e.g. qPCR, metabarcoding, ddPCR) to specifically target **both macro- and micro-organisms eDNA extracted from water samples.**

This protocol is optimised for rare eDNA and the suggested **elution volumes are therefore low** (between 50 and 65 µL), but can be increased if targets are more abundant.

Image Attribution

Marine Vautier

Guidelines

The main steps of the protocol are:

- Material preparation
- Plate preparation
- Sample lysis
- Extraction with the MagnetaPure 32 System (Dutscher)
- DNA elution

Materials

- **Materials:**

- 1000 µL pipette
- 100 µL pipette
- Scissors
- Vortex + benchtop centrifuge for 5 mL tubes
- Horizontal vortex with 5 mL tube holder (15 mL tube holders)
- Centrifuge for 2 mL tubes (relative centrifugal force needed: 11,000 x g)
- MagnetaPure 32 Nucleic Acid Purification System (Dutscher)
- Specific DNA-workstation (sterile area equipped with air filtration and UV systems)

- **Consumables:**

All tubes and tips must be sterile

- 1000 µL tips with filter
- 100 µL tips with filter
- 50 mL tubes: 4 to prepare aliquots and 3 for scissors decontamination
- 5 mL tubes: 1 per 8 samples to prepare NucleoMag B-Beads and MWA2 mix
- 2 mL tubes: 1 per sample to transfer lysate + 2 to prepare aliquots
- 1.5 mL tubes: 1 per sample to transfer eluted DNA
- 96-well plate with 2 mL deep-wells, U-Bottom (Macherey Nagel - 746032.DEEP): 1 per 16 samples
- Magnetic rod coverfor MagnetaPure 32 (Macherey Nagel – 747032.TC): 1 per 8 samples
- Plastic film to protect the 96-well plate
- Gloves

For any manipulation in a rare DNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Reagents:**

- NucleoMag DNA/RNA Water kit (Macherey Nagel)
  Note: shelf life of reagents for 24 months from production

- To clean the scissors:
  - Solution to remove DNA (e.g.*DNA-off, DNA away*)
  - Ethanol 96% for molecular biology
  - Molecular biology grade water

- **Samples to be extracted:**

- Filters in 5 mL tubes prefilled with MWA1 buffer or not

Troubleshooting

Before start

- **Filtration and preservation of the water samples through filters**

This protocol can be performed from filters preserved in tubes prefilled or not with preservation buffer (e.g. MWA1). If the filter is not prefilled with buffer, it should be frozen immediately after water filtration and until DNA extraction.

Note: If the filters are preserved with MWA1 buffer, the extraction can be performed directly, but for other preservation buffers such as Longmire and CTAB, the protocol proposed here will work, but upstream precipitation is recommended to increase the extraction efficiency.

This protocole is suitable for the DNA extraction from different types of 47 mm diameter filters:

	Filter Type
	Polyethersulfone (PES)
	Polycarbonate (PC)
	Cellulose nitrate (NC)
	Cellulose mixed esters (CM)
	Cellulose acetate (CA)

The filters should be preserved in 5 mL tubes for this DNA extraction protocol.

- **The following precautions must be applied:**

- Wear gloves throughout the extraction process
- Clean the bench with a DNA-removing solution (e.g. DNA-off, DNA away).
- Use tips with filters to avoid contaminations
- All steps have to be performed under a specific DNA-work station (sterile area equipped with air filtration and UV systems)

For any manipulation in a rare DNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Pre and post extraction equipment decontamination:**

  - Clean a specific DNA work station and apply UV

- MagnetaPure 32 System (Dutscher): Visual check for residues to be removed and UV decontamination – *see instrument manual*

- **Scissors *decontamination*** *(t*o be done before starting the protocol, and between each filter cutting)

- Prepare :
1. *one 50 mL tube with* DNA-removing solution (e.g. DNA-off, DNA away)
2. *one 50 mL tube with molecular biology grade water*
3. *one 50 mL tube with ethanol*

*- S*uccessively dip the scissors into each tube, opening and closing the scissors in each tube.

Note: The cutting of filters is not a requirement, but it does have an impact on the extraction efficiency.

Material preparation

1

- *To limit contamination of the kit buffers, it is recommended to aliquote them:*

- Into 50 mL tubes for MWA1, MWA2, MWA3 and MWA4
- Into 2 mL tubes for NucleoMag B-Beads solution and DNase-free H_2O

- *Tubes annotation*

- one 2 mL tube per sample for lysate collection
- one 1.5 mL tube per sample for DNA collection
- one 5 mL tube per 8 samples for the NucleoMag B-Beads and MWA2 mix preparation

- Scissors *decontamination (*to be done before starting the protocole, and between each filter cutting)

- Prepare :
1. *one 50 mL tube with* DNA-removing solution (e.g. DNA-off, DNA away)
2. *one 50 mL tube with molecular biology grade water*
3. *one 50 mL tube with ethanol*

- Successively dip the scissors into each tube, opening and closing the scissors in each tube.

Note: The cutting of filters is not a requirement, but it does have an impact on the extraction efficiency.

Plate preparation 1/2

2 *In this step, the buffers provided by the kit are distributed in a 96-well plate.*
For DNA extraction from filtered water samples, the 12 columns of the plate are divided into 2 sections of 6 columns each, allowing up to 16 samples to be extracted per plate.

30m

- Annotate the 96-well plate as recommended below:

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	NucleoMag B-Beads and MWA2 mix + Lysate	MWA3	MWA3	MWA4	x	DNase-free H_2O	NucleoMag B-Beads and MWA2 mix + Lysate	MWA3	MWA3	MWA4	x	DNase-free H_2O
Column name	L	3	3	4	x	E	L	3	3	4	x	E
	Samples between 1 to 8						Samples between 9 to 12					

Table 1: Recommended plaque annotation

Note: It is useful to mark the dividing line between columns 6 and 7 with a marker pen to provide a visual cue for filling the plate.

- Add the appropriate buffers into the appropriate wells of the plate.

1st column / 7th column: *Will be filled during plate preparation 2/2*
2nd column / 8th column: 850 µL of **MWA3** *(1st wash)*
3rd column / 9th column: 850 µL of **MWA3** *(2nd wash)*
4th column /10th column: 850 µL of **MWA4** *(3rd wash and bead drying)*
5th column / 11th column: *not used*
6th column / 12th column: 50 µL or 65 µL of **DNase-free H_2O** *(DNA elution)*

Note: The choice of elution volume is based on the expected eDNA amount. The smaller the amount, the smaller the elution volume in order to obtain more concentrated DNA.

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	Plate preparation 2/2	MWA3	MWA3	MWA4	x	DNase-free H_2O	Plate preparation 2/2	MWA3	MWA3	MWA4	x	DNase-free H_2O
Volume (µL)		850	850	850	x	50 or 65		850	850	850	x	50 or 65
Column name		3	3	4	x	E		3	3	4	x	E
		Samples between 1 to 8						Samples between 9 to 12				

Table 2: Plate preparation 1/2

- Film and reserve the plate at Room temperature

3 - Preparation of the NucleoMag B-Beads and MWA2 mix: 5m
- Prepare one 5 mL tube / maximum 8 samples
- Add **MWA2** only, the NucleoMag B-beads will be added during plate preparation 2/2
(allow a margin of one sample for the mix preparation. For example: plan a mix for 9 samples if 8 samples are to be extracted)

Sample Number	Volume NucleoMag B-Beads (µL)	Volume MWA2 (µL)
1	25	475
2	50	950
3	75	1425
4	100	1900
5	125	2375
6	150	2850
7	175	3325
8	200	3800
9	225	4275

Table 3: Volume required to prepare NucleoMag B-beads and MWA2 mix (*no margin*)

Sample Lysis

4 *During this step, a mechanical and chemical lysis of the sample is performed.* 10m 20s

- **For samples prefilled with buffer:**

- Collect the 5 mL tubes containing the filters

Note: If the tubes containing the filters are frozen, defrost them 00:10:00 *at* Room temperature

- Cut the filter into small pieces directly into the tube using decontaminated scissors

Note: Scissors must be decontaminated between each sample (see section 1 "material preparation")

- Place the tubes on the vertical vortex 00:00:05 at median speed

- Place the tubes on the horizontal vortex 00:00:05 at maximum speed

- Place the tubes into the benchtop centrifuge

- Pipette the lysate from the tube and transfer it into a 2 mL tube

- Centrifuge at 11000 x g, 00:00:30

- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the 96-well plate

- **For samples without buffer:**

- Collect the 5mL tubes containing the filters from the freezer and place On ice

- Add immediately 750 µL of **MWA1** buffer into each tube

- Cut the filter into small pieces directly into each tube using decontaminated scissors

Note: Scissors must be decontaminated between each sample (see section 1 "material preparation")

- Place the tubes on the vertical vortex 00:00:05 at median speed
- Place the tubes on the horizontal vortex 00:00:05 at maximum speed
- Place the 5 mL tubes into the benchtop centrifuge
- Pipette the lysate from the tube (approximately 650 µL) and transfer it into a 2 mL tube
- Centrifuge at 11000 x g, 00:00:30
- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the 96-well plate

Plate preparation 2/2

5 15m

▪ Preparation of the NucleoMag B-Beads and MWA2 mix

NucleoMag B-Beads and MWA2 mix sediment quickly, vortex between each samples to ensure homogeneity

- Vigorously vortex **NucleoMag B-Beads** tube
- For each tube containing MWA2 buffer (previously prepared), add the appropriate volume of **NucleoMag B-Beads** (*see Table 3 above*)
- Vortex

▪ NucleoMag B-Beads - MWA2 mix and lysate distribution

- Remove the film from the plate
- Add the appropriate solution into each well of the plate

1st column / 7th column:

500 µL of **NucleoMag B-Beads and MWA2 mix**

450 µL of **Lysate** (supernatant from the 2 mL tubes)

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	NucleoMag B-Beads and MWA2 mix	Plate preparation 1/2	Plate preparation 1/2	Plate preparation 1/2	x	Plate preparation 1/2	NucleoMag B-Beads and MWA2 mix	Plate preparation 1/2	Plate preparation 1/2	Plate preparation 1/2	x	Plate preparation 1/2
Volume (µL)	500				x		500				x	
Solution	Lysate				x		Lysate				x	
Volume (µL)	450				x		450				x	
Column name	L				x		L				x	
	Samples between 1 to 8						Samples between 9 to 12					

Table 4: Plate preparation 2/2

Extraction step performed in the MagnetaPure 32 System

6 ▪ Place the plate into the MagnetaPure 32 System and insert the magnetic rod coverfor – *see instrument manual* 40m

- Select the appropriate program to the chosen elution volume and elution temperature

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µL)	Mix speed	Temp (°C)
1	1	Binding	8	10	0	950	8	OFF
2	2	MWA3	2,3	5	0	850	8	OFF
3	3	MWA3	2,3	5	0	850	8	OFF
4	4	MWA4	2,3	5	15	850	8	OFF
5	6	Elution	5	20	0	50 or 65	8	56°C or OFF
6	4	Release	0.5	0	0	850	10	OFF

Table 5: MagnetaPure 32 System program for NucleoMag DNA/RNA Water Kit DNA extraction

Note: Heating to 56°C during elution gives a higher yield of DNA, but there is a risk of evaporation which reduces the volume of eluted DNA recovered

- Start the run *(The run lasts approximately* 00:40:00 *)*

Transfer of DNA extracts 30m

7 ▪ At the end of the run, remove the plate and place it into the DNA-workstation

- Remove the magnetic rod cover and start UV for decontamination – *see instrument manual*
- In the DNA-workstation, transfer each **DNA extract** into a 1.5 mL tube previously annotated

Note: DNA concentration and quality can be measured at this step (e.g. Nanodrop)

- Store DNA extracts at 4 °C for immediate use, or at -20 °C or -80 °C for long-term preservation

Protocol references

NucleoMag DNA/RNA Water kit (Macherey Nagel) manual : https://www.mn-net.com/media/pdf/ce/b5/38/Instruction-NucleoMag-DNA-RNA-Water.pdf

MagnetaPure 32 Nucleic Acid Purification System (Dutscher) manual : https://pdf.dutscher.com/doc/255743/255743_MEen.pdf