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

### Optimized QIAGEN DNeasy Blood & Tissue kit Protocol for Environmental DNA Extraction

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#### **ABSTRACT**

This document aims at providing a transparent method and detailing mandatory steps to produce reproducible 1) preparation of an eDNA filter, and 2) environmental DNA extraction.

### **GUIDELINES**

The extraction of environmental DNA (eDNA) should be processed in the dedicated room of the ultraclean laboratory (i.e., with filtered air and positive air pressure) of the Maurice Lamontagne Institute, Fisheries and Oceans Canada. All samples, consumables, and material entering the room are cleaned with a 0.6% sodium hypochlorite solution. Laboratory users are trained to work in clean conditions (i.e., specific instructions about when to wear and change sterile gloves, coats, mobcaps, chirurgical masks, and overshoe protections) according to the SOP 020901\_VXX. The extraction room is decontaminated between projects or every week (i.e., if a room is used for a project longer than a week) with a 0.6% sodium hypochlorite solution.

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**MATERIALS** 

### **Equipment:**

- 1. Tyvex lab coat (VWR #80200-600)
- 2. Disposable hair caps
- 3. Surgical mask
- 4. Scissors
- 5. Tweezers
- 6. Nacelles
- 7. Tube opener
- 8. -20 °C freezer
- 9. Pipettes 200 µL (Eppendorf P200)
- 10. Pipettes 1000 µL (Eppendorf P1000)
- 11. Racks
- 12. Vortexer
- 13. Thermomixer with 2 mL block adaptor (Eppendorf ThermoMixer Model C)
- 14. Microcentrifuge with rotor for 2 mL tubes (Eppendorf Model 5430)
- 15. Safety wash bottle of ethanol 96-100%
- 16. Safety wash bottle of Milli-Q water
- 17. Safety wash bottle of sodium hypochlorite 0.6 %
- 18. Solid trash
- 19. Liquid trash

### Reagents:

- 1. Qiagen DNeasy Blood & Tissue kit (QIAGEN #69506)
- 2. EB Buffer (QIAGEN #19086)
- 3. AL Buffer (QIAGEN #19075)
- 4. ATL Buffer (QIAGEN #19076)
- 5. Proteinase K (QIAGEN #19133)
- 6. Ethanol 96-100%
- 7. Commercial sodium hypochlorite 6%

#### **Consumables:**

- 1. 2 mL microtubes (Ultident #87-B200-C)
- 2. 2 mL Eppendorf Safe-Lock microtubes (VWR #CA20901-505)
- 3. Lyse&Spin baskets with associated collection tubes (QIAGEN #19598)
- 4. Collection tubes (QIAGEN #19201)
- Pipette tips with filter for P200 (VWR #CA89092-968) and P1000 (VWR #CA76416-026)
- 6. Kimwipes
- 7. Nitrile gloves
- 8. Filters: 1.2 or 1.5  $\mu$ m GF, 47 or 25 mm (Millipore, Cat no. 1822-047, 1822-025, 1827-047 et 1827-025), and other possibilities.

SAFETY WARNINGS

### From the QIAGEN DNeasy Blood and Tissue Handbook (version 2022):

Safety Information (p. 7)

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.
- DO NOT add bleach or acidic solutions directly to the sample preparation waste.
- Buffers AL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

## From the safety data sheet of the DNeasy Blood and Tissue Kit (50, revision date 25.08.2023):

■ Buffer AL contains guanidine hydrochloride: harmful, irritant.

Hazard statements:

H302 + H332 Harmful if swallowed or if inhaled.

H315 Causes skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

Precautionary statements:

Prevention:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

*P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.* 

Buffer AW1 contains guanidine hydrochloride: harmful, irritant.

Hazard statements:

H302 + H332 Harmful if swallowed or if inhaled.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Precautionary statements:

Prevention:

P271 Use only outdoors or in a well-ventilated area.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Disposal:

P501 Dispose of contents/ container to an approved waste disposal plant.

■ Proteinase K contains proteinase K: sensitizer, irritant. Risk and safety phrases: *R36/37/38-42/43*, *S23-24-26-36/37*.

Hazard statements:

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements:

Prevention:

P261 Avoid breathing mist or vapours.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P284 Wear respiratory protection.

Response:

P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.

P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor (Québec: 1-800-463-5060).

# From the safety data sheet of the Ethanol Solution 96% from ThermoFisher Scientific (version 24.12.2021):

 Ethanol solution 96%: flammable liquid, serious eye damage/irritation.

Hazard statements:

Highly flammable liquid and vapor Causes serious eye irritation

Precautionary statements:

Prevention:

Use personal protective equipment as required
Wash face, hands and any exposed skin thoroughly after handling

Wear eye/face protection

Do not breathe dust/fume/gas/mist/vapors/spray

Use only outdoors or in a well-ventilated area

Keep away from heat/sparks/open flames/hot surfaces. - No smoking

Keep container tightly closed

Ground/bond container and receiving equipment

Use explosion-proof electrical/ventilating/lighting equipment

Use only non-sparking tools

Take precautionary measures against static discharge

eep cool

### Response

IF exposed or concerned: Get medical attention/advice

IF INHALED: Remove victim to fresh air and keep at rest in a position

comfortable for breathing

IF ON SKIN (or hair): Take off immediately all contaminated clothing.

Rinse skin with water/shower

IF IN EYES: Rinse cautiously with water for several minutes. Remove

contact lenses, if present and easy to do. Continue rinsing

If eye irritation persists: Get medical advice/attention

In case of fire: Use CO2, dry chemical, or foam for extinction

## From the safety data sheet of the sodium hypochlorite solution (10-15%) from ThermoFisher Scientific (version 13.10.2023):

 Sodium hypochlorite: corrosive to metals, respiratory irritation, skin burns and eye damage, toxic gas when in contact with acids.

### Hazard statements:

May be corrosive to metals

Causes severe skin burns and eye damage

May cause respiratory irritation

Contact with acids liberates toxic gas

### Precautionary statements:

Prevention

Take any precaution to avoid mixing with acids

Do not breathe dust/fumes/gas/mist/vapours/spray

Wear respiratory protection

Wash face, hands and any exposed skin thoroughly after handling

Keep only in original container

Use only outdoors or in a well-ventilated area

Wear protective gloves/protective clothing/eye protection/face

protection

Response

IF INHALED: Remove person to fresh air and keep comfortable for breathing.

Immediately call a POISON CENTER/doctor (Québec: 1-800-463-5060)

IF SWALLOWED: Rinse mouth. Do NOT induce vomiting

IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing Wash contaminated clothing before reuse

Absorb spillage to prevent material damage

#### **BEFORE START INSTRUCTIONS**

Buffer AL may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has fully dissolved.

Buffer AW1 and Buffer AW2 are supplied as concentrated solutions. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Preheat a thermomixer at 56°C.

## Filter Preparation

6m

1

- Clean bench with water (i.e., Milli-Q water, hereafter water for cleaning procedures, to remove QIAGEN solutions with guanidine salts prior bleaching; see warnings for details), 0.6% sodium hypochlorite solution (i.e., to degrade DNA), and water (i.e. to rinse traces of sodium hypochlorite) before use.
- 2 Install all the material on the benchtop including 2 mL microtubes with Lyse&Spin baskets for extraction, pre-identified 2 mL Eppendorf Safe-Lock for back up, tweezers, scissors, nacelles, waste beaker, microtube opener, and gloves.
- Above a clean nacelle, take a filter with clean tweezers, unfold it, and cut it in half with clean scissors.
  - Note: Attemps to divide the filtrate equally on each half.
- Roll the filter half (filtrate inside) and put it in the extraction microtube (2 mL microtube with a Lyse&Spin basket) using clean tweezers. If DNA extraction will occur a subsequent day, put the

30s

1m



filter half into a 2 mL microtube and store it at <u>\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\\$\. If DNA extraction will occur on the same day, keep it at room temperature on the bench.</u>

5

Roll the second half with tweezers, put it in a Eppendorf Safe-Lock 2 mL previously identified, and store it as a back up at  $$^{\circ}$  -80  ${^{\circ}}$ C.

30s

6

Rinse tweezers, scissors, and nacelle with water, 0.6% hypochlorite bleach, water, and ethanol 96-100% (i.e., to dry equipment and avoid rust; hereafter, ethanol for cleaning procedures). Change gloves. Repeat steps 3 to 6 for each filter.

2m

7

Clean bench and material with water, 0.6% sodium hypochlorite solution, and water after use.



### **Critical Notes for DNA Extraction**

8 DO NOT TOUCH microtube edges with gloves while opening. Always use a microtube opener. In case of doubt for contamination, change gloves.

DO NOT OPEN more than one microtube at a time to limit contamination.

ALWAYS do a quick spin before microtube opening to limit aerosols. In case of doubt for contamination, rinse the microtube opener with water, 0.6% sodium hypochlorite, and water.

ALWAYS change the pipette tip between microtubes when adding a solution, even if the same solution is added.

### **DNA Extraction**

2h 24m 15s

Clean bench with water, 0.6% sodium hypochlorite solution, and water before use.



10

Clean pipettes and centrifuges by wiping them down with water, 0.6% sodium hypochlorite solution, water, and ethanol.

- 11 Install all the material on the benchtop including microtubes, reagents and collection tubes from the QIAGEN DNeasy Blood and Tissue Kit, and racks.
- 12 Prepare an extraction negative control for each extraction day.

  Note 1: Use a filter of material and porosity identical to those from the project.

  Note 2: If filters were frozen (i.e., usually moisten), then humidify the filter with Milli-Q water and proceed with steps 3 and 4. If filters were in silica beads then proceed with steps 3 and 4 without humidifying.
- 13 Change gloves and work under extractor hood or arm for all subsequent steps because of proteinase K (i.e., may cause breathing difficulties if inhaled).
- 14 Add  $\triangle$  450  $\mu$ L of ATL solution to each microtube.
- 15 Add Д 50 µL of proteinase K to each microtube.
- Mix microtubes by inversion for few seconds. Make sure that the filter stays immerged in the
- Place microtubes in the thermomixer. Incubate for a minimum of 02:00:00 at 56 °C with shaking at 900 rpm for lysis of the filtrate.

  Note: GF filters should not digest.
- Centrifuge microtubes at 18,000 g during 00:01:00.

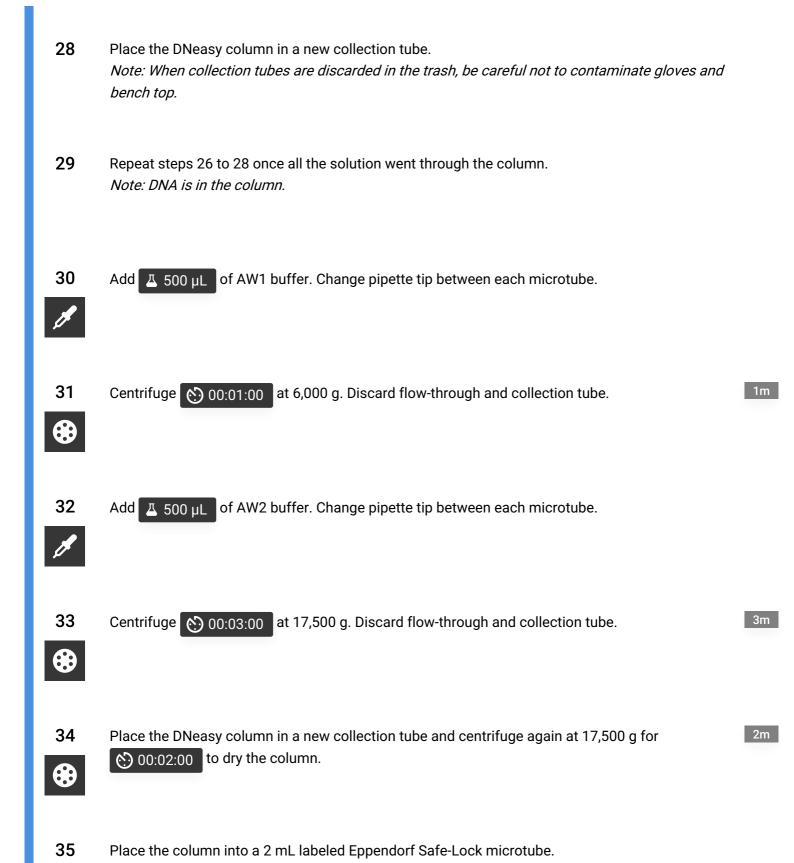
  Note: If lysis solution is still present in the column after centrifugation, redo step 18 until all the
- 19 Transfer the eluate to a new labeled 2 mL microtube.

solution went through the column. Make note in the excel sheet.

solution.

1m





- Add  $\underline{\mathbb{Z}}$  100  $\mu$ L of EB buffer directly in the center of the membrane.
- Note: We use EB buffer instead of the AE buffer provided in the Blood and Tissue kit as EB buffer is a TRIS buffer without EDTA. For rare species detection, we observed qPCR inhibition due to this small concentration of EDTA in the past.
  - 37 Incubate at Room temperature for 00:05:00

5m

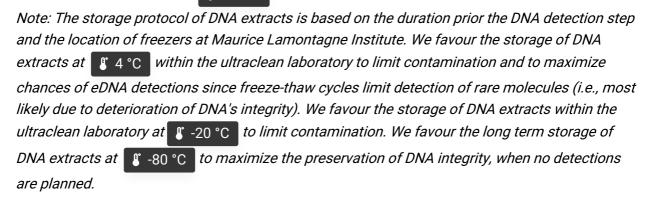


38 Centrifuge 00:01:00 at 6,000 g.

1m



- 39 Discard the column and store the 2 mL labeled Eppendorf Safe-Lock microtube with the eluate at
  - Weeks and 3 months, and at \$\frac{1}{3} \cdot -80 \cdot \cdo



- Throw liquid bench top trash into the liquid trash of the laboratory.
- - Rinse the liquid bench top trash with water, discard this water in the liquid trash of the laboratory, then clean it with water, 0.6% sodium hypochlorite solution, and water.
  - 42 Clean bench with water, 0.6% sodium hypochlorite solution, and water.

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Rinse microtube racks by spraying them with 0.6% sodium hypochlorite solution, and rinsing them with water.