Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

U of S-ETL-EDNA-13

Isolating eDNA from Sediments

Version 1, April 2018

Yuwei Xie, Ph.D. and John P. Giesy, Ph.D., FRSC, FSETAC

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 966-2096
Facsimile: (306) 966-4796

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: Yuwei Xie and John P. Giesy Date: 04/12/2018

Supervisor Review By: ________________________________ Date: 04/30/2018__

Reviewed By: ________________________________ Date: ____________
(QA Coordinator)

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

DEFINITIONS AND ACRONYMS

ETL Environmental Toxicology Laboratory (University of Saskatchewan)

DQO Data Quality Objective

DHSE Department of Health Safety and Environment (University of Saskatchewan)

QA Quality Assurance

QAPP Quality Assurance Project Plan

SOP Standard Operating Procedure

GWF Global Water Futures

eDNA Environmental DNA

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

TABLE OF CONTENTS

Section	Heading	Page
1.0	**PURPOSE**	**5**
2.0	**SCOPE AND APPLICATION**	**5**
3.0	**SAFETY CONSIDERATIONS**	**5**
	3.1 PERSONAL PROTECTIVE EQUIPMENT	**5**
4.0	**EQUIPMENT, MATERIALS, AND REAGENTS**	**5**
5.0	**METHOD, PROCEDURES, AND REQUIREMENTS**	**6**
	5.1 Mobilization and Training	**6**
	5.2 **Decontamination of instruments and laboratory areas before and after isolation**	**6**
	5.3 Isolating eDNA from sediment samples using Qiagen DNeasy PowerSoil kit	**7**
	5.4 Optional: Isolating eDNA from sediment samples (10 g) using Qiagen DNeasy PowerMax Soil Kit	**8**
	5.5 Quality control of isolated eDNA	**9**
6.0	**RECORDS, DOCUMENTATION, AND QC REQUIREMENTS**	**10**
7.0	**RESPONSIBILITIES**	**10**
8.0	**REFERENCES**	**11**

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

1.0 PURPOSE

The primary purpose of this SOP is to extract, purify and quantify environmental DNA (eDNA) from environmental sediment samples.

2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for eDNA isolation from sediment samples from the Global Water Futures (GWF) program titled "Next generation solutions to ensure healthy water resources for future generations" (eDNA project).

3.0 SAFETY CONSIDERATIONS

Biosafety training, laboratory training and medical monitoring requirements are described in the Health and Safety Plan for eDNA project of the GWF program. In addition, there are various safety concerns regarding the preparing, storage, and disposal of sample containers at the ETL. If biological spill happens, the appropriate personnel will be immediately notified and the Department of Health, Safety and Environment (DHSE) will be called in order to assess the hazard. DHSE will also be contacted in the case of chemical spills and will be responsible for the disposal of hazardous wastes.

3.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE), consisting of nitrile gloves will be worn at all times when handling samples.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

- Freezer (-20 ºC, -80 ºC)
- Permanent markers
- Chain of custody forms
- Kim wipes
- Nitrile gloves
- Absolute ethanol
- Centrifuge for 50-ml tubes (≤ 4000 x g)
- Microcentrifuge (20,000 x g)

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

- Electronic scales (maximum measure: 1000 g) for balance of centrifuge
- 50-mL sterile Graduated Conical-Bottom Tubes with rack
- 2-mL sterile microcentrifuge tubes
- 1.5-mL sterile microcentrifuge tubes
- Adjustable Pipettes (1000-µl, 200-µl and 10-µl)
- Disposable aerosol filter pipette tips (1000-µl, 200-µl and 10-µl)
- Qiagen DNeasy® PowerSoil Kit
- Qiagen DNeasy® DNeasy PowerMax Soil Kit
- Vortex Adaptor for 24 tubes
- Vortex Adapter for two 50-ml tubes
- Two Heater
- Biosafety cabinet
- Nanodrop
- Qubit V4
- Qubit® dsDNA HS Assay kit
- Qubit® assay tubes (500 tubes, Life Technologies, Cat. no. Q32856)
- Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- Spray bottle with 70% ethanol
- Spray bottle with 10% bleach
- Spray bottle with di Water

5.0 METHOD, PROCEDURES, AND REQUIREMENTS

The following procedures describe methods for isolating eDNA in a stepwise fashion and explain the reasoning behind the techniques. Detailed below are the preparatory procedures, molecular techniques, and procedures for sample documentation and preservation.

5.1 Mobilization and Training

This section describes requirements for mobilization for the necessary lab-work, as well as the necessary safety training. The objective of this section is to ensure that all of the necessary preparatory work has been conducted to enable the successful completion of the overall project. Mobilization for the necessary lab-work entails maintenance and decontamination of equipment and biosafety training in accordance with the site Health and Safety Plan (HASP).

5.2 **Decontamination of instruments and laboratory areas before and after isolation**

A. Wipe down surfaces of all points of contact, including bench tops, pipettes, and fridge/freezer handles, using 10% bleach. Let bleach remain on surfaces for 15 minutes.

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

B. After 15 minutes, use a DI water-dampened paper towel to remove bleach residue.
C. Use a 70% alcohol dampened paper towel to help quickly dry the surfaces. **Note**: **For PCR PRE-station, DO NOT use buffers containing ethanol to clean the clear acrylic surface.**
D. If necessary, repeat the cleaning procedure from step B.
E. For biosafety cabinet and PCR PRE-station, turn on the UV-light for at least 15 min.

F. Decontaminate laboratory equipment during work using RNase AWAY™ Surface Decontaminant (Thermo Scientific™)
G. Wipe down surfaces of bench tops, pipettes, and racks using RNase AWAY™ Surface Decontaminant, then wipe dry with dust-free paper or rinse clean.

5.3 Isolating eDNA from sediment samples using Qiagen DNeasy PowerSoil kit

Notes before starting

- Equilibrate frozen sample to room temperature.
- Perform all centrifuge steps at room temperature (15-25 °C).
- If Solution C1 has precipitated, heat at 60 °C until precipitate dissolved.
- Preheat Solution C6 at 60 °C.
- Preheat an incubator to 60 °C.
- Preheat a water bath to 70 °C.

1) Add 0.25 g of sediment sample to the PowerBead Tube provided. Gently vortex to mix.
2) Add 60 µl Solution C1 and invert several times or vortex briefly.
3) Secure PowerBead Tubes horizontally using a Vortex Adaptor for 24 tubes.
4) Vortex at maximum speed for 10 min. Note: if using the 24-place Vortex Adaptor for more than 12 preps, increase the vortex time by 5-10 min.
5) Centrifuge tubes at 10,000 x g for 1 min.
6) Transfer the supernatant (expected 400-500 µl) to a clean 2 mL Collection Tube provided.
7) Add 250 µl Solution C2 and vortex for 5 s. Incubate at 2-8 °C for 5 min.
8) Centrifuge tubes at 10,000 x g for 1 min.
9) Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 mL Collection Tube.
10) Add 200 µl Solution C3 and vortex briefly. Incubate at 2-8 °C for 5 min.
11) Centrifuge tubes at 10,000 x g for 1 min.
12) Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 mL Collection Tube.
13) Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5s.

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

14) Load 675 µl onto an MB Spin Column and centrifuge tubes at 10,000 x g for 1 min. Discard flow-through.
15) Repeat step 14) twice, until all of the sample has been proceeded.
16) Add 500 µl of Solution C5 and centrifuge tubes at 10,000 x g for 1 min. Discard flow-through.
17) Centrifuge again for 2 min at 10,000 x g to dry the column.
18) Carefully transfer the spin column to a new 2 mL microcentrifuge tube. Avoid splashing any Solution C5 onto the column.
19) Elute the DNA by adding 50 µl of preheated Solution C6 to the center of the spin column membrane. Incubate for 2 min at room temperature (15–25°C). Centrifuge for 1 min at 10,000 x g. Note: Solution C6 is free of EDTA.
20) Repeat step 10 with another 50 µl of preheated Solution C6 for increased DNA yield.

5.4 Optional: Isolating eDNA from sediment samples (10 g) using Qiagen DNeasy PowerMax Soil Kit

21) Add 15 ml of PowerBead Solution to a PowerMax Bead Tube.
22) Add up to 10 g of sediment sample to the PowerMax Bead Tube containing PowerBead Solution. Vortex vigorously for 1 min.
23) Add 1.2 ml of Solution C1 to the PowerMax Bead Tube and vortex vigorously for 30 s.
24) Place the PowerMax Bead Tube on a 50 mL-vortex adapter and vortex for 10 min at the highest speed. Alternatively, place the tube in a shaking water bath set at 65°C and shake at maximum speed for 30 min.
25) Centrifuge at 2500 x g for 3 min at room temperature.
26) Transfer supernatant to a clean Collection Tube (provided).
27) Add 5 ml of Solution C2. Invert twice to mix. Incubate at 2–8°C for 10 min.
28) Centrifuge at 2500 x g for 4 min at room temperature.
29) Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided).
30) Add 4 ml of Solution C3 and invert twice to mix. Incubate at 2–8°C for 10 min.
31) Centrifuge at 2500 x g for 4 min at room temperature.
32) Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided).
33) Shake to mix Solution C4. Add 30 ml of Solution C4 to supernatant and invert twice.
34) Fill an MB Maxi Spin Column with the solution from Step 13.
35) Centrifuge at 2500 x g for 2 min at room temperature. Discard the flow-through and add a second volume of supernatant to the same MB Maxi Spin Column and centrifuge again at 2500 x g for 2 min at room temperature. Discard the flow-through. Repeat until the entire volume has been processed. This will take up to 4 total spins.
36) Add 10 ml of Solution C5. Centrifuge at 2500 x g for 3 min at room temperature. Discard the flow-through.
37) Centrifuge at 2500 x g for 5 min at room temperature.

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

38) Carefully place the MB Maxi Spin Column in a new Collection Tube (provided). Avoid splashing Solution C5 onto the column.
39) Add 5 ml of sterile Solution C6 to the center of MB Maxi Spin Column membrane and centrifuge at 2500 x g for 3 min at room temperature.

5.5 Quality control of isolated eDNA

40) Purity check and quantify the isolated eDNA using NanoDrop 1000.
    a. Turn on NanoDrop computer and open ND-1000 software.
    b. Wipe NanoDrop pedestal with a clean Kimwipe.
    c. In NanoDrop software, select Nucleic Acid.
    d. Pipette 1.5 µl of sterile DNase-free water onto the pedestal of NanoDrop. Press OK. After calibrating, wipe NanoDrop pedestal with a clean Kimwipe.
    e. Load 1.5 µl of elution buffer onto the pedestal of NanoDrop. Select "Blank". Waiting for Blank to be read (about 10 s).
    f. Wipe NanoDrop pedestal with a clean Kimwipe.
    g. Enter first sample and name. Apply 1.5 µl of eDNA sample onto the pedestal and select "Measure".
    h. Repeat f-g for each sample.
    i. Save file to an appropriate folder on the computer and print report.
    j. Reapply 1.5 µl of sterile DNase-free water onto the pedestal and wipe pedestal with a clean Kimwipe.
    k. Close NanoDrop.
41) Quantify the eDNA with low concentration (< 10 ng/µl) with Qubit® dsDNA HS Assay Kits.
    a. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® dsDNA HS Assay requires 2 standards.
    b. Label the tube lids.
    c. Prepare the Qubit® working solution by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. Note: Do not mix the working solution in a glass container.
    d. Add 190 µl of Qubit® working solution to each of the tubes used for standards.
    e. Add 10 µl of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.
    f. Add 198 µl Qubit® working solution to individual assay tubes.
    g. Add 2 µl of each eDNA sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µl.
    h. Allow all tubes to incubate at room temperature for 2 minutes.
    i. Measure the concentration of eDNA samples using Qubit® 4.0 Fluorometer.

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

j. Transfer data file to an appropriate folder on the computer and print report.

6.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

Preserve isolated eDNA sample under -80 ºC for long-term storage. Sample management will also follow a scripted procedure (ETL-SOP-EDNA-09: Management of eDNA sample: Receiving, Preservation, Storage, Documentation, Decontamination, and Disposal) to ensure samples will not be lost or destroyed. The results of quality control of eDNA samples should be archived properly (hard-copy and electric copy).

7.0 RESPONSIBILITIES

Project Director — Will oversee and approve all project activities.

Project Manager — Will oversee and approve all project activities; review QA reports; approve final project QA needs; authorize necessary actions and adjustments to accomplish program QA objectives; and act as liaison between agencies, field staff.

Quality Assurance (QA) Manager — Will oversee all QA activities to ensure compliance with contract specifications; initiate audits on work completed by project personnel and subcontractors, including analytical laboratories and independent data validation contractors; review program QA activities, quality problems, and quality-related requests. In response to field and analytical findings, this person will approve the corrective actions. This person will report quality non-conformances to the Project Manager and review all pertinent portions of the deliverables before they are transmitted to ensure conformance with QA/QC procedures and quality work product.

Data Manager — Will oversee data management for this project. This person is responsible for the structure, organization, format, implementation, and operation of the project database.

Field Team Leader — Will oversee field activities and supervise the field crews. This person will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed. This person will inform the Project QA Manager when field problems occur, and will communicate and document corrective actions taken. The Field Team Leader will discuss field activities with the Project Manager.

Laboratory Project Manager — The laboratory project manager for this project is the person responsible for assuring that the analysis of all samples is performed in accordance with the QAPP and the laboratory's quality assurance manual. In addition,

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.

the Laboratory Project Manager performs the final laboratory review of project data packages for completeness and compliance with project requirements.

8.0 REFERENCES

Qiagen. DNeasy® PowerMax® Soil Kit Handbook. September 2017.

Qiagen. DNeasy® PowerSoil® Kit Handbook. May 2017.
Thermo Scientific. NanoDrop 1000 Spectrophotometer User's Manual. Rev. V3.8
Thermo Scientific. Qubit™ 4 Fluorometer manual. Pub. No. MAN0017209. Rev. B.0.
Thermo Scientific. Qubit® dsDNA HS Assay Kits manual. Pub. No. MAN0002326. Rev. B.0.

©Copyright 2018, University of Saskatchewan, Environmental Toxicology Laboratory. Do not copy or forward without permission.