Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-08

Field eDNA Sampling Protocol for Biomonitoring of Remote Sites Using the eDNA Sampling Kit

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APPROVAL PAGE

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

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Supervisor Review By: _____ Date: 04/30/2018

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(QA Coordinator)

DEFINITIONS AND ACRONYMS

eDNA Environmental DNA

GWF Global Waters Futures

ETL Environmental Toxicology Laboratory (University of Saskatchewan)

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

PPE Personal Protective Equipment

QA Quality Assurance

QC Quality Control

QAPP Quality Assurance Project Plan

SOP Standard Operating Procedure

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Section Heading Page

1.0 PURPOSE

This protocol describes procedures for collecting water and sediment samples for environmental DNA (eDNA) analysis. eDNA is genetic material left by organisms in their surrounding environment, and which can be obtained by collecting environmental matrices such as soil, sediment, water, etc. without the source organisms having to be visually present. eDNA may originate from any tissue or biological material left behind by an organism such as cells, tissues, urine, hair, skin, mucus and dead individuals leaking genetic material. Samples collected can be subjected to two types of eDNA analyses: 1) Targeted DNA analysis for individual species of interest (e.g. endangered or invasive species); this analysis is based on specific genetic primers/probes that enable detection of the presence of species of interest using quantitative real-time PCR (qPCR); and 2) Non-target biodiversity analysis for monitoring biodiversity; this approach makes use of next-generation sequencing based metabarcoding technology (sequence-by-synthesis).

The eDNA Sampling Kit is designed for field sampling of eDNA from waters and sediments in remote sites. Note: After water samples are collected, they need to be immediately preserved using the DNA preservation buffer (670 mL of absolute ethanol and 30 ml of sodium acetate (3M) per sampling bottle; provided with the kit). Sediment samples need to be frozen after collection, and stored at -20^{o}C until arrived at the laboratory. One blank water sample and one blank sediment sample are also included in the eDNA Sampling Kit as field blanks for each site.

2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for water samples supplied for the GWF project: “Next generation solutions to ensure healthy water resources for future generations”.

3.0 SAFETY CONSIDERATIONS

Safety training and medical monitoring requirements are also consistent among all protocols for field studies, and are described in the Health and Safety Plan for the GWF field studies. Personnel should review before collecting samples. The trip leader should hold a tailgate safety briefing before collecting samples. Identify risks and potential mitigative procedures to minimize those risks. For instance, if collecting while wearing waders always wear a belt on the outside of the waders to minimize potential for the waders to fill with water.

3.1 PERSONAL PROTECTIVE EQUIPMENT

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

5.0 EQUIPMENT, MATERIALS, AND REAGENTS (USE CHECKLIST ATTACHED)

- Global Positioning Unit (GPS)
- Calibrated probes to measure DO, temperature, pH, conductivity
- Turbidity meter
- Sediment corer or grabber
- Camera
- Bucket
- Ladle
- 12V DC Portable Freezer with 110V plug
- Ice bags (-80 C chilled in Styrofoam box)
- 2 Large coolers
- 1 1200 watt inverter
- 20 foot extension cord
- DNA free Sandwich bags
- Parafilm
- Notebook
- Pencil
- 3 Permanent markers (1 thick, 2 thin)
- Chain of custody forms
- 1 Field Record Sheet per site
- Field collection sheets printed on Rite in the Rain® paper
- Bleach water and rag for wiping down equipment
- Paper towels
- Kim wipes
- Nitrile gloves
- Absolute ethanol
- Non-denatured absolute ethanol (molecular biology level, 200 proof)
- 10% bleach water (bleach, RO water)
- 70% ethanol (absolute ethanol, RO water)
- Sterile DO water
- Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- Spray bottle with 70% ethanol
- Spray bottle with 10% bleach

- 3 250 mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure
- 4 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure (one for the blank water sample)

6.0 METHOD, PROCEDURES, AND REQUIREMENTS

The following field procedures describe methods for collection of samples in a stepwise fashion and explain the reasoning behind the sampling design and techniques. Detailed below are the preparatory procedures, sampling design, sampling techniques, and procedures for sample documentation, preservation, and shipping.

6.1 Mobilization and Training

This section describes requirements for mobilization for the necessary fieldwork, 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 fieldwork entails procuring and packing equipment and training of field personnel in accordance with the site Health and Safety Plan (HASP).

The project manager will assemble and pack all equipment specified in the Section above. If any items need to be purchased, they will be ordered well in advance to ensure that the schedule is not impacted by equipment needs. Pre-cleaned sample containers will be purchased to minimize the potential for contamination.

6.2 Sampling Objectives

The primary objective of the field-sampling portion of a project is to collect eDNA from water and sediment samples to ultimately be used in monitoring programs. The sample should contain eDNA from prokaryotic and eukaryotic species; therefore, prevention of contamination of samples with exogenous DNA is paramount. Detection of false positives due to contamination of samples will limit conclusions that might be drawn from the sample.

6.3 Sampling Locations

Samples should be collected from various locations within a study area as defined in the work plan. Once field samples are collected, each location will be plotted and numbered on the most recent maps of the area.

6.4 Sampling Methodology

This section details the overall sampling methodology, equipment, and techniques to be employed in this sampling effort.

6.4.1 Record geographic information

Utilize a GPS unit to determine precise site locations and document the coordinates.

6.4.2 Measure and record environmental variables

Measure and record environmental variables of interest (DO, temperature, pH, conductivity, turbidity) and observations. Be sure to record the serial number of the probes.

All instruments used for measurements in the field need to be calibrates and the date of calibration logged in the log book for that piece of equipment (See other SOPs for measurements and calibration of instruments).

6.4.3 Prepare sampling kit

- Chill the ice bags in -80 C freezer and store them in Styrofoam boxes.
- Clean, sterile and dry the Nalgene PP bottles for field sampling.
- Prepare sampling kit

The eDNA water Sampling Kit is designed for field sampling of eDNA from waters in remote sites. Preparing 1 L tubes containing the DNA preservative buffer in the hood in the Biochemistry & Molecular Biology Room (Room 261 in ETL) before field sampling. Using the Cryogenic permanent marker which is alcohol resistant and water resistant, write sample information (station number, replicate number, and date) on caps and tubes and a Ziplock bag for storage.

Note: After water samples are collected, they need to be immediately preserved using the DNA preservation buffer (670 mL of absolute ethanol and 30 ml of sodium acetate (3M) per sampling bottle; provided with the kit). One blank water sample is also included in the eDNA Sampling Kit as field blanks for each site.

Table 1. Content of the eDNA Sampling Kit for each sampling site

	Content	Number per site
Field Sample	Water sampling kit	
	Sterile 1 L small mouth Nalgene bottle containing DNA preservation buffer;	3
	Sterile 250 mL sampling bottle for filling 1 L Nalgene bottle, maximum volume: ~300 mL	3
	Sediment sampling kit	

	Sterile empty 50 mL tube	3
	sterile disposable spatula	3
Blank sample	Blank water sampling bottle	
	Sterile 1 L small mouth Nalgene bottle with the DNA preservation buffer and 300 mL diH2O	1
	Blank sediment sampling tube	
	Sterile empty 50 mL tube with 10 mL sterile di-water, 0.2 mL 0.1 mm and 0.2 mL 0.5 mm glass beads	1

- Clean, sterile and dry the sediment corer or grabber before and after each use.
  - Clean corer with tap water.
  - Spray corer with 50% bleach. Let bleach remain on surfaces for 15 minutes.
  - After 15 minutes, use a DI water-dampened Kim Paper to remove bleach residue.
  - Spray corer again with 70% ethanol.
  - Decontaminate the corer with burner for 10 seconds burning. Cool them using sterile RO water.
  - Spray RNase Away to remove RNase and residual DNA.

6.4.4 Sampling water for eDNA

1) Water should be sampled prior to sediment sampling.
2) In lotic systems, samples should be collected consecutively in an upstream direction where possible, to avoid re-sampling of water. However, depending on logistics this may not be possible in all cases. According documentation must be made in the field note-book.
3) Samples should be collected with the surveyor not entering the water body where possible (from a boat, through the ice, standing on the bank/shore) to avoid possible contamination from the surveyor boots, or by stirring up sediment.
4) Sampling process:
   A. Don a pair of gloves (included with your kit). **Note:** Wear a new pair of clean gloves for each sampling site!
   B. Open the field blank water sampling bottle during water sampling and place next to sampling site (on bank/shore or next to hole during sampling through ice).
   C. Use the second smaller sterile 250 mL sampling bottle provided within each kit that does not contain any liquid for scooping up water to fill the water sampling

bottle to the 1 L marker highlighted on the Nalgene bottle. Proceed in the same way with replicate sample 2 for each site.

D. Cap and label the bottle properly (include site name, replicate #, date, initials of person sampling, matrix samples [water]). Mix thoroughly by inverting the bottle ten times. The water sample can be stored and shipped at ambient temperature.

E. Cap the field blank water sampling bottle after the three water samples were taken. Label the field blank bottle with the site name, date and initials).

5) Store and ship samples on ice (recommended) or at ambient temperature (within one week).

6.4.5 Sampling sediment for eDNA

1) Sediment sampling should proceed after water sampling.
2) Samples should be collected with the surveyor not entering the water body where possible (from a boat, through the ice, standing on the bank/shore) to avoid possible contamination from the surveyor boots, or by stirring up sediment.
3) Don a new pair of clean gloves (included with your kit). **Note:** Wear a new pair of clean gloves for each sediment sampling!
4) Sampling process:
   A. Open the field blank sediment sampling tube during sediment sampling and place next to sampling site (on bank/shore or next to hole during sampling through ice).
   B. Use a sediment corer (Please read SOP ETL 4012: Standard Method for Field Collection of Sediments for Chemical Analyses) to collect sample and then use the sterile spatula provided within each kit to collect the top 1-2 cm of the surface sediment (avoid collecting from the edges where the sediment had contact with the 50 mL sampling tube) and transfer approximately 5 mL to a sterile sediment sampling tube provided with the kit. Proceed in the same way with replicate samples 2 and 3 for each site.
   C. Cap and label the sediment sampling tube properly (include site name, replicate #, date, initials of person sampling, matrix samples [sediment]).

      Note: For eRNA analysis, the sediment samples should be preserved using LifeGuard Solution, three volumes (15 mL) LifeGuard Solution for one volume sediment (5 mL).

      The sediment sample should be frozen at or below -20^{o}C shortly after sampling, and then be stored and shipped at -20^{o}C.

   D. Cap the field blank sediment sampling tube after all replicate samples were taken. Label the field blank sediment sampling tube with the site name, date and initials.

E. The 'corer negative control' consisted of 5 mL of water collected below an internal/external rinsing of a decontaminated and reassembled corer (corer, liner tube, nosepiece) and added to 10 mL of CTAB in a 50-mL centrifuge tube. The corer negative control is treated as sample.

4. Store and ship samples at or below -20^{o}C.

7.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

Field personnel will document all sampling activities in accordance with the work plan. Before exiting each site, the following information will be recorded on the field record sheet (Appendix A): (a) site name; (b) date and time of collection; (c) the analyses requested (i.e. water quality parameters); (d) the method of preservation; and (e) initials of personnel taking the sample. If any additional biological samples such as fish or invertebrates are taken from that site, those should be noted as well.

8.0 SAMPLE PRESERVATION

All samples should be temporally stored and shipped on dry ice to ETL, and stored at -20 ^{o}C or -80 ^{o}C until sample preparation. Note: Analyses should be undertaken within 1 month of sample collection. A chain-of-custody form (UofS-ETL-SOP-EDNA-07: Standard Operating Procedure for Maintenance of Sample Chain-of-Custody) will be completed for each sample and be archived. A copy of the form will accompany all samples when transported, relinquished or split. 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.

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

9.0 REFERRENCES

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology letters*, *4*(4), 423-425.

THOMSEN, P., Kielgast, J. O. S., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular ecology*, *21*(11), 2565-2573.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... & Williams, P. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). *Biological Conservation*, *183*, 19-28.

APPENDIX A: FIELD RECORD SHEET

Field Record Sheet

Book No: ETL-eDNA-FRS Page No.: _____

Project:		
Sample Identification:		
Date:		Time:
Location:	Latitude:	Longitude:
Description of Sample Location/ Observations:		
Conditions:		
Principal Scientist:		
Crew Members:		
Water Depth (m):		Water Temp (°C):
Specific Conductivity (µmoh):		Air Temp (°C):
Dissolved Oxygen(mg/L):		pH:
Container Preparation/Storage:		
Samples Collected and Preserved:		
Sample Distribution:		

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APPENDIX B: CHECKLIST FOR EDNA SAMPLING FROM REMOTE SITE

Statue Number Item

- ☐ _____ Global Positioning Unit (GPS)
- ☐ _____ Calibrated probes to measure DO, temperature, pH, conductivity
- ☐ _____ Turbidity meter
- ☐ _____ Camera
- ☐ _____ Bucket
- ☐ _____ Ladle
- ☐ _____ Sediment corer or grabber
- ☐ _____ 12V DC Portable Freezer with 110V plug
- ☐ _____ Ice bags (-80 C chilled in Styrofoam box)
- ☐ _____ 2 Large coolers
- ☐ _____ 1 1200 watt inverter
- ☐ _____ 20 foot extension cord
- ☐ _____ DNA free Sandwich bags
- ☐ _____ Parafilm
- ☐ _____ Notebook
- ☐ _____ Pencil
- ☐ _____ 3 Permanent markers (1 thick, 2 thin)
- ☐ _____ Chain of custody forms
- ☐ _____ 1 Field Record Sheet per site
- ☐ _____ Field collection sheets printed on Rite in the Rain® paper
- ☐ _____ Bleach water and rag for wiping down equipment
- ☐ _____ Paper towels
- ☐ _____ Kim wipes
- ☐ _____ Nitrile gloves
- ☐ _____ Absolute ethanol
- ☐ _____ Non-denatured absolute ethanol (molecular biology level, 200 proof)
- ☐ _____ 10% bleach water (bleach, RO water)
- ☐ _____ 70% ethanol (absolute ethanol, RO water)
- ☐ _____ Sterile DO water
- ☐ _____ Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- ☐ _____ Spray bottle with 70% ethanol
- ☐ _____ Spray bottle with 10% bleach
- ☐ _____ Three 250 mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure per site
- ☐ _____ Three 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure with preservation buffer (670 mL of absolute ethanol and 30 ml of 3M sodium acetate per sampling bottle) per site

- _____ One Blank water sampling bottle (Sterile 1 L small mouth Nalgene bottle with the DNA preservation buffer and 300 mL diH2O) per site
- _____ One Blank sediment sampling tube (Sterile empty 50 mL tube with 10 mL sterile di-water, 0.2 mL 0.1 mm and 0.2 mL 0.5 mm glass beads) per site