Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

UofS-ETL-EDNA-01

Water Sample Collection for eDNA: Filtration Method

Version 2, April 2018

Yuwei Xie, Ph.D., Abigail DeBofsky, M.Sc., Jenna Zee, M.Sc., Tim Jardine, Ph.D., Markus Hecker, 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

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:

Authored By: Yuwei Xie, Abby DeBofsky, Jenna Zee, Tim Jardine, Markus Hecker and John P. Giesy Date: 04/04/2018

Supervisor Review By: John P. Giesy Date: 04/30/2018

Reviewed By: _____ Date: _____
(QA Coordinator)

DEFINITIONS AND ACRONYMS

eDNA Environmental DNA

eRNA Environmental RNA

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

GWF Global Waters Futures

HASP Health and Safety Plan

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 (use checklist attached) 5

5.0 METHOD, PROCEDURES, AND REQUIREMENTS 7

5.1 Mobilization and Training 7

5.2 Sampling Objectives 8

5.3 Sampling Locations 8

5.4 Sampling Methodology 8

5.4.1 Record geographic information 8

5.4.2 Measure and record environmental variables 8

5.4.3 Collection of water for eDNA 9

6.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS 12

7.0 Sample Preservation 12

8.0 RESPONSIBILITIES 12

APPENDIX A: Field Record Sheet 14

APPENDIX B: Checklist for Water Sample Collection for eDNA (Filtration Method) 15

1.0 PURPOSE

This protocol describes procedures for collecting and filtering water for environmental DNA (eDNA) based aquatic taxa identification and community composition characterization. Sampling collection for environmental RNA (eRNA) is also included in this protocol. eDNA is genetic material left by organisms in their surrounding environment, which can be obtained by collecting environmental matrices such as soil, sediment, water, *etc*. without the source organisms having to be visually present. eDNA can 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).

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 the 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.

4.0 EQUIPMENT, MATERIALS AND REAGENTS (<u>USE CHECKLIST ATTACHED</u>)

- Global Positioning Unit (GPS)
- Calibrated probes to measure DO, temperature, pH, conductivity

- Turbidity meter
- Camera
- 1 set MultiVac 300-MB-T, Vacuum Filtration System, 110V (Rocker 400 Vacuum Pump, Manifold, Burner, Waste Bottle, Filters and 2m Silicone Tubing)
- 2 Nalgene Repairable Hand-Operated PVC Vacuum Pumps with Gauge (backup)
- 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
- Two 15mL tube rack
- Sharps container
- Box of scalpel blades
- DNA free Sandwich bags
- Notebook
- Pencil
- Three Permanent markers (one thick and two thin)
- Chain of custody forms
- 1 Field Record Sheet per site
- Field collection sheets printed on Rite in the Rain® paper
- Burner
- Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING.)
- 2-mL Sterile DNase-free and RNase-free tubes
- 2-mL Greiner Bio-One™ Cryo.s™ Round Bottom 2mL Polypropylene Tubes with Internal Thread Cap (Cat. No.: 122261*)
- 5-mL Axygen™ Screw Cap Transport Tubes (SCT-5ML-S)
- EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size 0.2 µm, diam. 47 mm (Cat. No.: GVWP04700)
- EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size 0.45 µm, diam. 47 mm (Cat. No.: HVLP04700)
- EMD Millipore™ MF-Millipore™ Mixed Cellulose Ester Membranes, pore size, 1.2 µm, diameter, 47 mm (Cat. No.: RAWP04700)
- 50-mL sterile Graduated Conical-Bottom Tubes
- Disposable 1250 ul plastic pipette tips
- One 1000 ul adjustable pipette
- 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)
- Qiagen™ LifeGuard Soil Preservation Solution®
- 10% bleach water (bleach, RO water)
- 50% 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
- Spray bottle with 50% bleach
- Three Sets of sterile forceps per site
- Three Clean scalpels per site
- Three 500 mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for samples of targeting eDNA
- Four Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with CN Membrane (09-740-2B), 0.45µm, 250mL for samples of targeting eDNA
- Four 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure (or 42-oz Whirl-Pak™ Stand-up Sample Bags, 1242 mL) for samples of non-targeting eDNA of each site
- Four 1 L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure (or 42-oz Whirl-Pak™ Stand-up Sample Bags, 1242 mL) for samples of non-targeting eRNA of each site

5.0 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.

5.1 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.

5.2 Sampling Objectives

The primary objective of the field-sampling portion of a project is to collect eDNA from water samples to ultimately be used in monitoring programs. The sample should contain eDNA from prokaryotic (bacteria) 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.

5.3 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.

5.4 Sampling Methodology

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

5.4.1 Record geographic information

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

5.4.2 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 calibrated and the date of calibration logged in the log book for that piece of equipment (See other SOPs for measurements and calibration of instruments).

5.4.3 Collection of water for eDNA

6.4.3.1 Prepare sampling kit

1) Chill bags of ice in -80 ºC freezer and store them in Styrofoam boxes.

2) Clean, sterilize and dry the Nalgene PP bottles for field sampling.

3) Using the Cryogenic permanent marker which is alcohol resistant and water resistant, write sample information (Location number, replicate number, pore size, preserve method, sampler, and date) on tubes, bottles and a Ziplock bag for storage for each sampling site.

4) Test the MultiVac Vacuum Filtration System, Hand-Operated Vacuum Pumps and portable freezer.

5) Prepare one blank water sampling bottle with 500 mL filtered tap water with 0.2 μm membrane filter for each sampling location in the hood in the Biochemistry & Molecular Biology Room (Room 261 in ETL) before field sampling. The blank sample is treated as a sample.

6.4.3.2 Collecting water

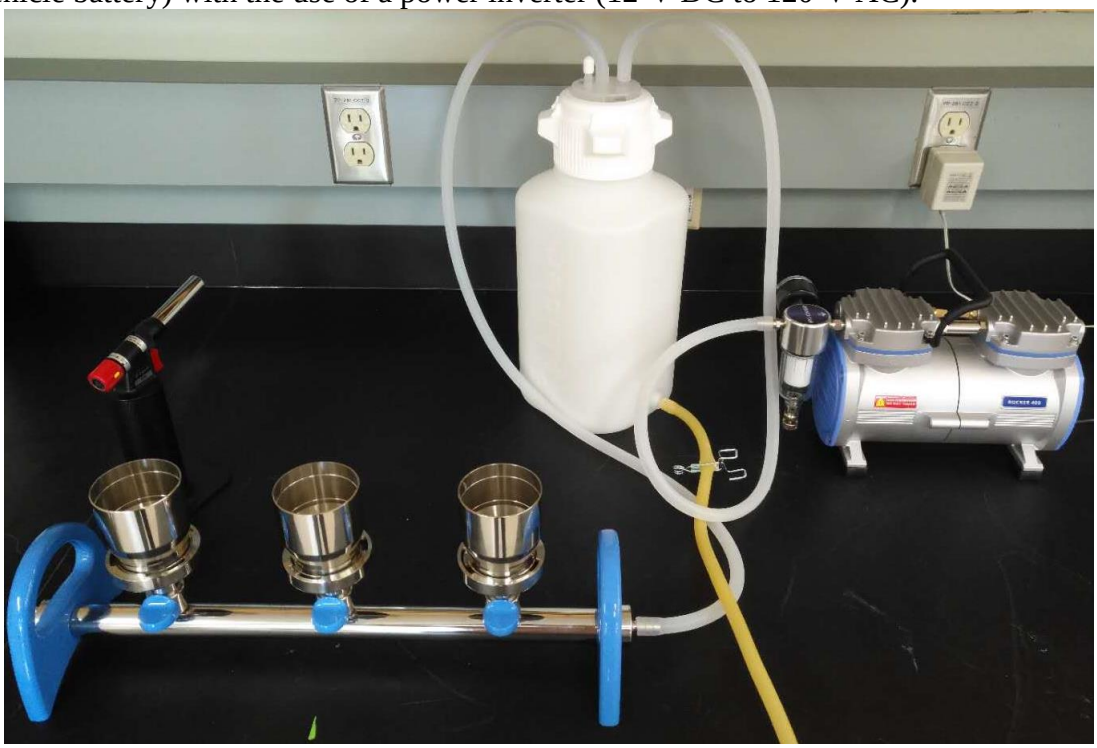
1) Water should be collected before collection of any synoptic samples of sediment.
2) In lotic systems, samples should be collected consecutively in an upstream direction where possible, to avoid re-sampling of water. However, depending on logistics this may not be possible in all cases. According documentation must be made in the field notebook.
3) To avoid possible contamination from the surveyor boots, or by stirring up sediment, 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) or from a bridge.
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 container during water sampling and place next to sampling site (on bank/shore or next to hole during sampling through ice).
    C. Submerge Sterile Nalgene™ collection bottle in water to collect the desired volume of water. Proceed in the same way with replicate sample 2 for each site.
    D. Cap and label the bottle properly (include site name, replicate # and date).
    E. Cap the field blank water sampling bottle after the three water samples will be taken. Label the field blank bottle with the site name and date).

F. To minimize degradation of DNA, filter the water samples as soon as possible. The water sample can be stored on ice in the cooler for short-term storage (within 12 hours) for delivery to sample preparing base or ETL. Note: DO NOT FREEZE.

Note: If use Whirl-Pak™ Stand-up Sample Bag instead of Nalgene PP bottle to collet water, please follow APPENDIX C to seal the bag properly.

5) Assembling pump

Connect pump head, manifold, Sterile Disposable Filter Units, and Waste Bottle with 2m Silicone Tubing. Secure filter funnel to adapter stem, creating an airtight seal. Waste Bottle does not need to be sterile (but if it is not decontaminated between sites, discharge water should not be emptied in aquatic environments). Connect pump to 120-V AC power source such as a wall outlet. The pump can also be powered by a 12-V DC battery (such as a vehicle battery) with the use of a power inverter (12-V DC to 120-V AC).



6) Decontaminate funnel, membrane filter holder and forceps before and after each use

A. Clean funnel, adapter stem and forceps with tap water.

B. Spray funnel, adapter stem and forceps with 50% bleach. Let bleach remain on surfaces for 15 minutes.

C. After 15 minutes, use a DI water-dampened Kim Paper to remove bleach residue.

D. Spray funnel, adapter stem and forceps again with 70% ethanol.

E. Decontaminate the surface of the funnel, membrane filter holder and forceps with the burner for 10 seconds burning. Cool them using sterile RO water.

F. Spray RNase Away to remove RNase and residual DNA.

7) Filter assembly and water filtration

A. Assemble proper membrane filter to the funnel on the manifold.

Note: Non-targeting eDNA/eRNA waters: 0.2 µm or 0.45 µm membrane filter depending on the turbidity; If the turbidity is high, use stack filter method (put 1.2 µm membrane filter on the top of 0.45 µm membrane filter); the blank water sample: 0.2 µm membrane filter.

B. Fill funnel with a water sample and check the leak. Turn the vacuum pump on.

Note: If there is a leak, re-assemble the tubes, funnel and adaptor stem.

C. After the water has been passed through the filter, dry the membrane filter as much as possible using a vacuum, then turn the vacuum pump off.

a) For disposable filter units, cut the filter out of the apparatus using a new scalpel blade, along the inside of the rubber seal, about 2 mm from the edge.

b) For manifold funnel, remove the funnel.

D. Use two sets of sterile forceps, pick up the filter membrane at opposite edges and roll the filter into a pre-labeled 5-mL tube. Do not tightly roll or fold the filter membrane. To see a video, please visit https://youtu.be/KUT6nKJPj4s.

8) Preserve the membrane filter during delivery or short-time storage.

A. LifeGuard Solution is recommended for preserving the membrane filters for eRNA analysis. Add 1.6 mL LifeGuard solution into the 5-mL tube. Carefully insert one 2-mL Cryogenic Vial in the hole of rolled membrane filter in the 5-mL tube. Let 5-mL tube sit on ice during delivery and be stored in freezer for long-term analysis. If the cooler is unavailable, the LifeGuard preserved RNA can keep stable for one day under 37 ^{o}C, one week at room temperature, two weeks at 2-8 ^{o}C and one month at -20 ^{o}C.

B. Freezing is recommended for preserving membrane filters for eDNA analysis without any preservation buffers if the -20 ^{o}C or -80 ^{o}C freezer is available.

C. Non-denatured ethanol can be used for the membrane filters for eDNA analysis. Add 5 mL non-denatured ethanol into the 5-mL tube. Let 5-mL tube sit on ice during delivery and be stored in freezer for long-term analysis. If the cooler is unavailable, the ethanol preserved DNA can be delivered under ambient temperature during delivery. Then store the ethanol preserved membrane in freezer for long-term storage. Ensure that the label on 5-mL tube has not washed off with the addition of ethanol.

6.0 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.

7.0 SAMPLE PRESERVATION

Filters containing eDNA will be placed in preservation buffer under -20 ^{o}C or -80 ^{o}C for long-term storage. eRNA should be isolated as soon as possible according to the preservation buffer and storage condition. 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.

8.0 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 a 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 the 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.

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:		

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan
44 Campus Drive, Saskatoon, Saskatchewan, S7N 5B3, Canada
Tel:(306) 966-5062;966-2096;966-4978

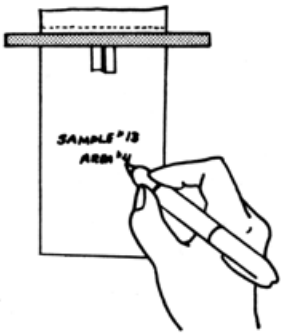
APPENDIX B: CHECKLIST FOR WATER SAMPLE COLLECTION FOR EDNA (FILTRATION METHOD)

Statue Number Item

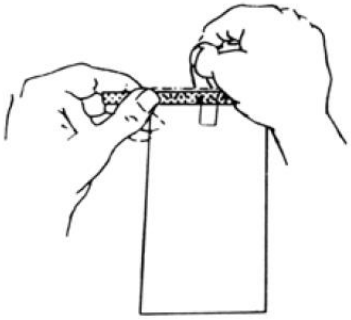
- ☐ _____ Global Positioning Unit (GPS)
- ☐ _____ Calibrated probes to measure DO, temperature, pH, conductivity
- ☐ _____ Turbidity meter
- ☐ _____ Camera
- ☐ _____ One set MultiVac 300-MB-T, Vacuum Filtration System, 110V (Rocker 400 Vacuum Pump, Manifold, Burner, Waste Bottle, Filters and 2m Silicone Tubing)
- ☐ _____ Two Nalgene Repairable Hand-Operated PVC Vacuum Pumps with Gauge (backup)
- ☐ _____ Bucket
- ☐ _____ Ladle
- ☐ _____ 12V DC Portable Freezer with 110V plug
- ☐ _____ Ice bags (-80 C chilled in Styrofoam box)
- ☐ _____ Two Large coolers
- ☐ _____ One 1200 watt inverter
- ☐ _____ 20-foot extension cord
- ☐ _____ Two 15-mL tube rack
- ☐ _____ Sharps container
- ☐ _____ Box of scalpel blades
- ☐ _____ DNA free Sandwich bags
- ☐ _____ Burner
- ☐ _____ Butane gas for decontamination of stainless steel manifold (Caution: EXPLOSIVE, AWAY FROM FLAMMING!!!)
- ☐ _____ 2-mL Sterile DNase-free and RNase-free tubes
- ☐ _____ 2-mL Greiner Bio-One™ Cryo.s™ Freezing Tubes
- ☐ _____ 5-mL Axygen™ Screw Cap Transport Tubes (SCT-5ML-S)
- ☐ _____ EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size 0.2 µm, diam. 47 mm (Cat. No.: GVWP04700)
- ☐ _____ EMD Millipore™ Durapore™ PVDF Membrane Filters, pore size 0.45 µm, diam. 47 mm (Cat. No.: HVLP04700)
- ☐ _____ EMD Millipore™ MF-Millipore™ Mixed Cellulose Ester Membranes, pore size, 1.2µm, diameter, 47mm (Cat. No.: RAWP04700, Fishersci)
- ☐ _____ 50-mL sterile Graduated Conical-Bottom Tubes
- ☐ _____ Disposable 1250 ul plastic pipette tips
- ☐ _____ One 1000 uL adjustable pipette
- ☐ _____ Notebook

- ☐ _____ Pencil
- ☐ _____ Three Permanent markers (1 thick, 2 thin)
- ☐ _____ Chain of custody forms
- ☐ _____ One 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)
- ☐ _____ Qiagen™ LifeGuard Soil Preservation Solution®
- ☐ _____ 10% bleach water (bleach, RO water)
- ☐ _____ 50% 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
- ☐ _____ Spray bottle with 50% bleach
- ☐ _____ Three Sets of sterile forceps per site
- ☐ _____ Three Clean scalpels per site
- ☐ _____ Three 500-mL Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for samples of targeting eDNA
- ☐ _____ Four Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with CN Membrane (09-740-2B), 0.45µm, 250mL for samples of targeting eDNA
- ☐ _____ Four 1-L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for samples of non-targeting eDNA of each site
- ☐ _____ Four 1-L Sterile Nalgene™ Narrow-Mouth PPCO Packaging Bottles with Closure for samples of non-targeting eRNA of each site
- ☐ _____ Or four 42-oz Whirl-Pak™ Stand-up Sample Bags, 1242 mL, for samples of non-targeting eRNA of each site
- ☐ _____ Or four 42-oz Whirl-Pak™ Stand-up Sample Bags, 1242 mL, for samples of non-targeting eRNA of each site

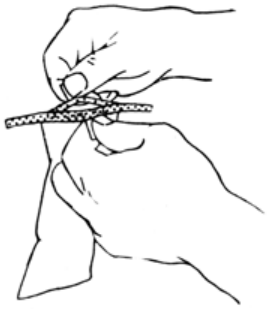
APPENDIX C: INSTRUCTION SHEET FOR Whirl-Pak™ SAMPLE BAGS



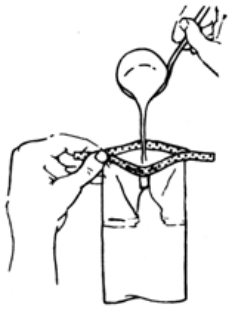
1. Label the bag with sample information if necessary.



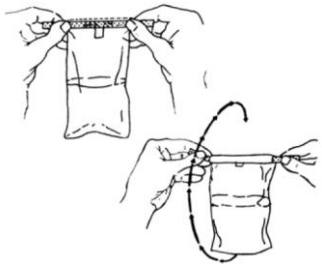
2. Tear off the top of the bag along the perforation.



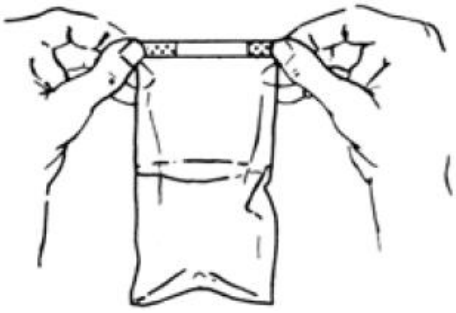
3. Use pull tabs on each side to open the bag. Sometimes a little pull on the bottom of the bag helps open it completely.



4. Put sample, liquid or solid, into bag. Leave enough space at the top for closing and mixing if needed.



5. Pull the ends of the wire to close the bag. Holding the bag by the wire ends, whirl the bag three complete revolutions to form a leakproof seal. Whirling the bag Will form the tightest seal. Larger bags can be closed by "folding" the tab over as tightly as possible.



6. Bend the wire ends over onto the bag to complete the closing.