NEON DNA Extraction Standard Operating Procedure v.4

Prepared for:
Battelle/National Ecological Observatory Network (NEON) Program

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Effective Date: February 1, 2021

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I. Version History

Version 1: Initial release.

Version 2: Disposable polypropylene weighing funnels (that are autoclavable) are now used to weigh soil samples and insert samples into plate wells; revised acceptance criteria.

Version 3: Removed the manual references in Section II; updated materials information in Section III; clarified the use of sterile consumables throughout Section IV.B; revised the concentration level below which the extraction is considered a fail to 1 ng/µL in Section V; updated user manual references.

Version 4: Added a microbial community standard as a positive extraction control. Clarified negative extraction controls used with each sample type. Clarified the frequency of both positive and negative extraction controls. Updated acceptance criteria in Section V to include results of the positive and negative extraction controls.

II. Objective and Overview

Samples from the National Ecological Observatory Network (NEON) program are extracted to isolate DNA for use in subsequent qPCR, marker gene, and/or metagenomic sequencing analyses. DNA is extracted from soil samples using the PowerSoil HTP 96 Kit, and from Sterivex water filters using the PowerWater Sterivex Kit. DNA is extracted from the benthic biofilms of sediment samples (sand and silt) and adherent biofilms that are scraped from plant grab samples with sterile spatulas using the PowerBiofilm Kit. Extraction batches are accompanied by quality control samples as described in Section IV. Kits are detailed in Section III.

III. Recommended Materials

Material	Manufacturer	Catalog #
DNeasy PowerSoil HTP 96 Kit	Qiagen	12955-4
DNeasy PowerBiofilm Kit	Qiagen	24000-50
DNeasy PowerWater Sterivex Kit	Qiagen	14600-50-NF
QuantiFluor ONE dsDNA Kit	Promega	E4870
Disposable Weighing Funnels	TWD Scientific	DDWF-PP-XS
X-Pierce Film	Sigma Aldrich	Z722502-100EA
Spike-in Control I (High Microbial Load)	ZymoBIOMICS	D6320

IV. Procedure

A. Sample Receipt and Storage

Soil and aquatic samples are held in cryostorage before extraction. Upon receipt Battelle will ensure the samples are in good condition and sort them according to post-extraction analysis type. Samples are stored at -60°C to -85°C until extraction.

B. Sample Preparation

Note: all extraction work takes place in a biosafety cabinet (BSC). Prior to beginning work the BSC is wiped down with 10% bleach solution followed by 70% isopropyl alcohol. The germicidal light is turned on for a minimum of 10 minutes. All consumables used during sample processing either come in sterile, unopened bags that are only opened in a BSC (sealing film, collection tubes) or are autoclaved prior to use (disposable weighing funnels).

1. Positive Extraction Control (PEC)

The ZymoBIOMICS Spike-in Control I (High Microbial Load) Catalog # D6320 is stored at -80°C when not in use. In preparation for extraction, thaw the standard completely. Mix thoroughly by vortexing to ensure there is no cellular aggregation. Once the PEC is aliquoted for extraction, return the standard to -80°C.

2. Soil Samples

Disposable weighing funnels and scoopulas are autoclaved for 80 minutes to ensure sterility. In addition, the sealing film that covers the 96-well plate is UV-crosslinked for 5 minutes. Prior to DNA extraction, soil samples are filled into a 96-well plate. A full extraction batch consists of 94 soil samples, one negative extraction control (NEC), and one PEC. For each extraction plate, well A1 is left empty as the NEC. Prior to loading soil into plate wells, 20 µL of the ZymoBIOMICS Spike-in Control I (High Microbial Load) standard is added to well H12 as the PEC. A pierce-able sealing film is then applied to plates to prevent cross contamination. Next, 0.25 +/- 0.03 g of thawed soil sample is weighed and placed into the appropriate well by using a sterilized disposable weighing funnel to pierce the seal. Each well is then individually sealed with a square of adhesive. After all the wells have been filled, the plate may be stored at 2-8°C for up to 48 hours if not immediately proceeding to Section C.

3. Aquatic Samples

3.1. **Biofilm**. Biofilm samples from sediment and from plant tissues (epiphyton, epipsammon, and epipelon sample collections) are extracted in tubes. Up to 24 biofilm samples are included in the same extraction batch. One NEC and one PEC will be prepared with each PowerBiofilm Kit (each kit can extract approximately 50 samples). This results in one NEC and one PEC with approximately every two extraction batches using the same

PowerBiofilm Kit. Each extraction batch will contain up to 22 biofilm samples when both a PEC and NEC accompany the batch. For sediment biofilm material, 0.10-0.25 g of the material is weighed in a 2 mL collection tube with an appropriate label. For plant biofilm material, a sterile spatula is used to scrape 0.10-0.25 g of the material into a 2 mL collection tube with an appropriate label. The biofilm PEC will consist of 20 µL of the ZymoBIOMICS Spike-in Control I (High Microbial Load) standard added to a 2 mL collection tube. The biofilm NEC will consist of an empty 2 mL collection tube. Biofilm samples are extracted the day they are prepared.

3.2. **Filters**. For surface water and benthic filter samples (originating from epixylon and epilithon biofilm sample collections), samples will be extracted as-is with no additional preparation steps. Up to 24 filter samples are included in the same extraction batch. One PEC and one NEC will be prepared with each PowerWater Sterivex Kit (each kit can extract approximately 50 samples). This results in one PEC and one NEC with approximately every two extraction batches using the same PowerWater Sterivex Kit. Each extraction batch will contain up to 22 filter samples when both a PEC and NEC accompany the batch. The filter PEC will consist of 20 µL of ZymoBIOMICS Spike-in Control I (High Microbial Load) standard added directly to a Sterivex filter unit. The filter NEC will consist of an empty Sterivex filter unit. Filter samples are extracted the day they are prepared.

C. DNA Extraction and Isolation

1. Soil Samples

Genomic DNA (gDNA) is extracted from soil samples using the Qiagen DNeasy PowerSoil HTP 96 Kit (cat #12955-4) according to the manufacturer's instructions (Manual: Qiagen_HB-2258-003_PowerSoil).

2. Biofilm Samples

Genomic DNA (gDNA) from biofilm samples (epiphyton, epipsammon, epipelon) is extracted using the Qiagen DNeasy PowerBiofilm Kit (cat #24000-50) according to the manufacturer's instructions (Qiagen_HB-2274-002_PowerBiofilm).

3. Sterivex Samples

Genomic DNA (gDNA) from aquatic samples in Sterivex filter units (including epilithon and epixylon biofilm samples) is extracted using the Qiagen DNeasy PowerWater Sterivex™ Kit (cat #14600-50-NF) according to the manufacturer's instructions (Manual: Qiagen_HB-2266-002_PowerWater).

D. Quantus dsDNA Assay

After extraction and isolation, the gDNA of each sample, including the PECs and NECs, is quantified using a Promega Quantus Fluorometer with a QuantiFluor ONE dsDNA Kit (#E4870) according to the manufacturer's instructions (Manual: Quantus_FluorometerManual_TM396_ rev012020).

V. Quality Review

Internal quality review is conducted by qualified personnel not involved in conducting the work under review.

Batch-level quality control criteria are outlined in Table 1 and sample-level quality control criteria are outlined in Table 2.

Table 1. Batch- level Quality Control and Acceptance Criteria

QA/QC measurement	Frequency	Requirement	Corrective Action
DNA concentration in Negative Extraction Control (NEC) (no DNA control)	Soil – one per 96-well extraction plate Aquatic – one per DNA extraction kit lot #	< 1 ng/µL	Suspected batch-level contamination. Halt processing the batch and contact NEON immediately to evaluate batch-level resolution. NEON must approve the resolution. Potential outcomes of evaluation are: continue with analysis as normal; continue with analysis with additional quality flags; re-extract batch of samples; replacement of reagents/kits before proceeding with more DNA extractions.
DNA concentration in Positive Extraction Control (PEC)	Soil – one per 96-well extraction plate Aquatic – one per DNA extraction kit lot #	DNA concentration that is at least 20% of manufacturer's expected yield (e.g. obtained yield of at least 20 ng if the manufacturer's expected yield is 100 ng)	Suspected DNA extraction method failure. Halt processing the batch and contact NEON immediately to evaluate batch-level resolution. NEON must approve the resolution. Follow up with manufacturer if commercial standard fails to generate high-quality DNA of sufficient concentration to meet the NEON requirements.

Table 2. Sample-level Quality Control and Acceptance Criteria

QA/QC measurement	Frequency	Requirement	Corrective Action
DNA concentration in each NEON field sample extract (does not include PECs or NECs, which are evaluated using Table 1)	Per sample	≥1 ng/µL	If <1 ng/µL on initial extraction, re-extract one time. After 2 unsuccessful attempts (initial and one repeat), notify NEON of sample-level failure. NEON may instruct laboratory to continue downstream analyses (e.g. marker gene sequencing) with a failed sample DNA extract or to report the extract as a fail in the extraction ingest.