

NEON DNA Extraction Standard Operating Procedure v.1

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I. Version History

None. This is the first version of this SOP.

II. Objective and Overview

DNA from soil and benthic biofilm sediment samples is extracted using the PowerSoil HTP kit (Manual: v. 02112014) or PowerBiofilm Kit (Manual: v. 11062013), respectively, and from Sterivex water filters using the PowerSterivex DNA extraction kit (Manual: v. 01162013). Adherent biofilms are scraped from plant grab samples using sterile spatulas and DNA is extracted using the PowerBiofilm kit.

III. Recommended Materials

| Material | Manufacturer | Catalog # |
|---------------------------|--------------|-------------|
| DNeasy PowerSoil HTP Kit | Qiagen | 12955-4 |
| DNeasy PowerBiofilm Kit | Qiagen | 24000-50 |
| PowerWater Sterivex DNA | Qiagen | 14600-50-NF |
| QuantiFluor ONE dsDNA Kit | Promega | E4870 |

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 -80°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 followed by 70% isopropyl alcohol. The germicidal light is turned on for a minimum of 10 minutes.

1. Soil Samples

Prior to DNA extraction soil samples are filled into a 96-well plate. A full processing run consists of 94 soil samples. Between 0.25-0.28 g of thawed soil sample is weighed out and placed into a well. The appropriate well number is labeled on the original bag holding the soil sample. Prior to loading soil into plate wells, a pierce-able sealing film is applied to plates to prevent cross contamination. Soil is placed into each well using a paper funnel to pierce the seal. Each well is then individually sealed with a square of adhesive. For each extraction plate the wells A1 and

H12 are left empty as negative controls. After all the wells have been filled, the plate is placed into a refrigerator at 2-8°C for up to 96 hours.

2. Aquatic Samples

For biofilm material, 0.10-0.25g of the material is weighed out in a 2-ml collection tube with an appropriate label. A full processing run consists of 8 surface water/benthic filter/grab samples.

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.

2. Biofilm Samples

Genomic DNA (gDNA) from membrane-filtered water samples is extracted using the MO Bio PowerBiofilm DNA Isolation Kit (cat #24000-S) according to the manufacturer's instructions.

3. Sterivex Samples

Aquatic samples in Sterivex filter units are extracted using the MO Bio PowerWater Sterivex™ DNA Isolation Kit (cat #14600-S) according to the manufacturer's instructions.

D. Quantus dsDNA Assay

After extraction and isolation, the gDNA of each sample is quantified using a Quantus Fluorometer with a QuantiFluor ONE dsDNA Kit (#E4870, Manual: MAN0002326vB.0) according to the manufacturer's instructions.

V. Quality Review

Internal quality review is conducted by qualified personnel not involved in conducting the work under review. Samples with a concentration lower than 1 ng/μL of DNA according to the Quantus dsDNA Assay are reported as failing the QAQC check and are re-extracted.