Sterivex DNA extraction V.1

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1 Works for me

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ABSTRACT

A mostly automated protocol for extraction of genomic DNA from seawater filtered onto a Sterivex filter (Cat. No. SVGP0150). Reagents come from the Macherey-Nagel NucleoMag Plant Kit for DNA purfication (Cat. No. 744400). Automated liquid handling is performed on an eppendorf EpMotion 5075t with multi-channel pipettes. This protocol assumes that the Sterivex is sealed with tube sealant on the male end and a male-luer lock plug on the female end. Using this protocol, we routinely extract ~80 samples at a time.

The protocol has been modified to increase to an 800 µL starting volume. This normally results in less Binding Buffer MC2 than is needed. Extra binding buffer should be purchased separately.

The kit manual can be found here: https://www.mn-net.com/media/pdf/09/46/a0/Instruction-NucleoMag-Plant.pdf

The epMotion program is attached here.

ATTACHMENTS

Application_JCVI MN DNA Aplaqua 800_200225_173356.export6

MATERIALS

NAME	CATALOG #	VENDOR
80% Ethanol		
MAGNUM EX Universal Magnet Plate	A000380	Alpaqua
Masterblock 96 Deep Well Plate	780286	greiner bio-one
Flexible Tube Cutter	97642	
Vortex Adapter for 5mL tubes	13000-V1-5	Mobio

BEFORE STARTING

- Prepare extraction sheet listing sample name, extraction number (starting from #1), and plate position (e.g. A1). We normally randomly add a few blank samples (no liquid in starting deep well plate) scattered throughout the positions.
- Clean all surfaces and tools with 70% EtOh.
- Set shaker to 56C

Lysis

1 Prepare DNA lysis buffer (N = number of samples plus extra for pipetting)

1.1 In sterile container, add 800 µL x N of Lysis Buffer MC1

1.2 Add 10 µL x N RNase A

1.3 (optional) Add 1 µL of each internal standard

1.4 Mix well.

2 Keep sterivex on dry ice. In each, remove luer plug and pipette the per sample volume prepared in step 1 into each. For example, if 800 µL MC1 + 10 µL RNase A + 2 µL internal standards were used, add 812 µL. Dispense quickly as the lysis buffer will freeze upon contact. Replug with luer lock. Place luer-side down into 1.5 mL tube rack.

3 Incubate at **56 °C** and shake gently for **00:30:00** .

4 Vortex for **00:05:00** using adapter for 5 mL tubes.

5 Transfer lysate to sterile 1.5 mL tubes labeled with extraction numbers.

5.1 Label each sterviex with its extraction number.

5.2 Pop the lid with the male end off the end of the sterivex using the tube cutters. The tube cutters will fit in the lip between the lid and the rest of the unit. Apply pressure at an upward angle and it should release. Continue to cut around the top if needed.

5.3 Rub the filter on the inside of the sterviex to release as much of the lysis buffer and material as possible. Then pipette as much liquid as possible from inside the sterviex into the corresponding 1.5 mL tube.

6 Clear lysate by centrifugaion at **5600 x g 00:20:00** .

This is a good time to prepare your buffers. Start the program on the epMotion which will calculate your buffer volumes depending on the number of samples. Add a few mL more than is requested and enter that as the volume supplied. We premix the beads and MC2 at a ratio of 30 µL beads:770 µL MC2.

7 Transfer to another set of numbered 1.5 mL tubes. Remove as much supernatent as possible without disturbing the pellet.

8 Align 1.5 mL tubes in rack in plate position. Set adjustable spacer multichannel pipette to **800 µl** and transfer into the deep well plate.

9 Transfer deep well plate to epMotion and run program.