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Sterivex DNA extraction V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bc2hiyb6

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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 Lysis Buffer MC1 and 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
Additional notes from the manufacturer on using the kit with the epMotion can be found here: https://www.mn-net.com/media/pdf/95/2c/ef/AN-NucleoMag-Plant-epMotion5075.pdf . Note that we have modifications to their protocol.

The epMotion program is attached here.

ATTACHMENTS

Application_JCVI MN DNA Aplaqua 800_200225_173356.export6

GUIDELINES

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. Importantly, the epMotion must be equipped with a gripper, 1000 uL multichannel, 300 uL multichannel, an integrated themomixer, an a thermomodule. 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 Lysis Buffer MC1 and Binding Buffer MC2 than is needed. MC2 is increased for the bead/MC2 mixture while the beads volume is kept the same. Extra binding buffer is sold in 1L quantities and should be purchased separately.

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
twin.tec® PCR plate 96 LoBind skirted 150 µL PCR clean	Catalog No.	Eppendorf

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 **56 °C**

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** x N 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 Binding Buffer MC2 at a ratio of 30 µL beads:770 µL MC2. Note that this keeps the bead amount the same as specified in the manual while increasing the amount of 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 **no more** than **800 µl** into the deep well plate.

9 Transfer deep well plate to the epMotion and run program. The final elution is always into a twin.tec LoBind PCR plate (see materials).

Reservoir rack layout (left to right):

1. MC2 with beads
2. MC3
3. MC4
4. 80% EtOH
5. MC5
6. MC6

Step 2 in the program stops it. At this point with a P1000 multichannel pipette, throughly pipette up and down to mix the bead solution then continue the run.

10 Once program is complete, run **2.5 µl** of the extracted DNA on a gel. Good samples will show a bright band of high molecular weight DNA. Blank samples should show nothing. Degraded samples will show a smear.

We normally use of a 1:10 dilution of this extracted DNA for our template in PCRs for amplicon library preperation. This is a good time to also make the 1:10 dilution plate (**20 µl** total volume) using the elution buffer MC6 (5 mM Tris/HCl, pH 8.5).