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DNA Extraction from Sterivex Filters

Forked from DNA Extraction from Sterivex Filters

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DISCLAIMER

ABSTRACT

Modified 2015 by the Brazelton Lab from protocols by Rika Anderson, Colleen Kellogg, Julie Huber, and Byron Crump. Incorporated some recommendations from Lever et al. (2015) Frontiers in Microbiology doi: 10.3389/fmicb.2015.00476.

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FORK NOTE

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Forked from DNA Extraction from Sterivex Filters, Gigascience Database

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DISCLAIMER:

Prepare DEB

1 Prepare DNA Extraction Buffer (DEB):

0.1M Tris-HCl (pH 8)	4.5 mL of 1.0 M
0.1M Na-EDTA (pH 8)	9 mL of 0.5M
0.1M KH2PO4 (pH 8)	0.54 g
1.5M NaCl	13.5 mL of 5M
0.8M Guanidine HCl	3.44 g
0.5% Triton-X 100	0.225 mL (225 µL) of 100%

Add above ingredients to 50 mL tube.
Add milli-Q water to ~40 mL
Add NaOH to pH 10 (several drops at a time)
Add milli-Q water to 45 mL
Filter-sterilize to remove possible spores
Autoclave. Slightly loosen lid so that it is not air-tight. Recover from autoclave very soon after the autoclave cycle is completed.
Pour autoclaved solution into fresh 50 mL tube.
Aliquot into 1.5 mL tubes.

Hot Lysis

2 Add 1.4 mL of DEB to each Sterivex with syringe and needle. Position the needle just below the mouth of the Sterivex so that it does not come back out the top. Do not fill to the top – stop when solution covers white filter. Possible Stopping Point. Store at 20ºC

2.1 Possible Stopping Point. Store at 20ºC

3 Place sterivex filter in 50mL tube with holes.

4 Incubate at 65ºC for 30 mins. on Genemate spinning machine.

5 Vortex each sterivex again (inside the Falcon tube) for 30 seconds. Bead Beating:

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Bead Beating

6 Using a syringe, withdraw fluid from each Sterivex and eject into bead tube (glass 0.1 mm for bacteria).

7 Bead beat for 40 s.

8 Centrifuge for 2 min at 5000 g.

9 Add equal volume of phenol / chloroform / isoamyl alcohol (25:24:1, bought premixed with alkaline buffer) to each tube.

Phenol/Chloroform extraction

10 Transfer fluid avoiding beads into fresh Eppendorf tube. Add no more than 900 µL in each tube (or no more than 750 µL if using 1.5 mL tubes). Phenol / chloroform extraction:

11 Gently shake a few times and then centrifuge at 14,000g for 1 minute.

12 Remove supernatant to fresh tube.

13 Add equal volume of chloroform / isoamyl alcohol (24:1) to each tube.

14 Gently shake a few times and centrifuge.

15 Remove supernatant to fresh tube, carefully avoiding the bottom organic layer. Ethanol precipitation:

Ethanol precipitation

16 Redistribute aqueous phase among 3 tubes so that each 2.0 mL tube has 550 µL or less and each 1.6 mL tube has 450 µL or less. For some samples, additional salt is not necessary, and you can skip the sodium acetate. In this case, you can add up to 600 µL in a 2.0 mL tube.

17 Add 0.1 volumes sodium acetate (3M, pH 5.2). (e.g. add 55 µL to 550 µL.)

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18 Add 2 volumes 100% ethanol. (e.g. add 1210 µL to 605 µL.)

19 [optional for low biomass samples] Add 1.2 ul of glycogen (20 ug/ul).

20 Invert a few times to mix.

21 Incubate at 20ºC for at least 1 hr. or overnight. Incubation on ice might work just as well and yield a cleaner pellet.

22 Centrifuge for 40 minutes at 16,000g. (Optional: used cooled centrifuge at 0ºC)

23 Pour out supernatant. Do not completely invert tube; keep at a gentle angle to minimize the chance of the pellet falling out.

24 Add 500 µL of cold 70% ethanol to each tube.

25 Invert the tube to mix. Make sure the pellet is dislodged from the bottom so that it is properly washed.

26 Centrifuge at 16,000g for 10 minutes.

27 Remove liquid again with pipettor. Be careful to avoid pellet.

28 Place tubes with open lids in the Vacufuge. Spin for 7 minutes at 30ºC on the VAL setting. If you can see ethanol in the tube, spin for another 25 minutes. If the pellets become powdery, they are too dry.

29 Resuspend in ~100 µL of low EDTA TE. Heat to 55ºC

Recipe for low EDTA TE: 10 mM TrisHCl 0.1 mM EDTA For 50 ml: 500 µl 1 M TrisHCl (pH 8.0) autoclaved 10 µl 0.5 M EDTA (pH 8.0) autoclaved → to 50 ml with milliQ H2O → filter sterilize with 0.22 µm syringe filter TE is good for DNA storage, but EDTA inhibits PCR. So this low EDTA TE buffer is a good compromise for storing DNA for later PCR amplification. You can also just use EB (10 mM TrisHCl, pH 8 or 8.5).

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for 10 or more minutes to dissolve pellet and store at 4ºC. For longterm storage, place at 20 or 80ºC, but avoid repeated freezing and thawing of the DNA. One strategy is to keep half at 4ºC for the working sample and store the other half at-80ºC as the archive sample.

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