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RNA Extraction from Sterivex Filters

Forked from DNA Extraction from Sterivex Filters

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DISCLAIMER

ABSTRACT

Modified 2020 by H. Lizethe Pendleton from the Brazelton Lab DNA extraction protocol.

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DISCLAIMER:

Prepare DEB

1 This is a *total nucleic acid* extraction protocol that provides the option of obtaining both RNA and DNA in separate aliquots, though it is **optimized for RNA**. We have a separate protocol optimized for DNA.

2 **Nucleic Acid Extraction Buffer (NEB):**

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	225 µL of 100%

Add above ingredients to 50 mL tube.
Add UltraPure water to ~40 mL
Add NaOH to pH ~7 (slowly)
Add UltraPure water to 45 mL
Filter sterilize with 0.22 µm filter
Autoclave with lid loosened. Recover from autoclave soon after the cycle is complete.
Pour autoclaved solution into fresh 50 mL tube.
Aliquot into 1.5 mL tubes.
UV sterilize for 10-30 minutes.

Hot Lysis

3 Add 1.4 mL of NEB 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 the solution covers the filter.

3.1 Possible Stopping Point. Store at 20ºC

4 Incubate at room temperature for 30 minutes.

5 Vortex each sterivex (inside a Falcon tube) for 30 seconds.

Bead Beating

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

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6

7 Bead beat for 40 s.

8 Centrifuge for 2 min at 5000 g.

9 Transfer fluid - avoiding beads - into fresh Eppendorf tube. Add no more than 350 µL in each 1.7 mL tube.

RNA Acidic Phenol/Chloroform Extraction

10 **To obtain RNA from the lysates:**
Add equal volume of acid phenol / chloroform (pH 5.2) to each tube.

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

12 Remove supernatant (300 uL) to fresh tube. Avoid organic phase and interface. Repeat phenol extraction until the interface is clear. Keep the tube with the organic phase-- this is where the DNA (theoretically) should be.

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.

DNA Alkaline Phenol/Chloroform Extraction

16 **To obtain DNA from the same lysates:**
Add 300 µL UltraPure Water to the original tubes, shake gently to mix.

17 Add equal volume (of total volume) of phenol / chloroform (pH 8.0) to each original tube.

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

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19 Remove supernatant to fresh tube.

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

21 Gently shake a few times and centrifuge.

22 Remove supernatant to fresh tube, carefully avoiding the bottom organic layer.

Ethanol precipitation

23 Redistribute aqueous phase among tubes:
RNA: 250 µL in each tube
DNA: 400 µL in each tube

24 Add salt solutions:
RNA: 0.5x volumes of ammonium acetate (7.5 M) (ex: 125 µL to 250 µL)
DNA: 0.1 volumes of sodium acetate (3M, pH 5.2). (ex: 40 µL to 400 µL)

25 Add ethanol:
RNA: 2.5x volumes of 100% ethanol (ex: 937.5 µL to 375 µL)
DNA: 2.0 volumes of 100% ethanol (ex: add 880 µL to 440 µL.)

26 Invert a few times to mix.

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

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

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

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

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

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32 Centrifuge at 16,000g for 10 minutes.

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

34 Place tubes with open lids in the UV hood. Let sit until ethanol has evaporated, but pellets are still damp. If the pellets become powdery, they are too dry.

35 Resuspend nucleic acid pellets:

RNA: 50-100 µL of UltraPure water, pipette to mix. If pellets don't readily dissolve, incubate at 55ºC for 30-60 seconds, but avoid heating for too long as RNA may degrade.

DNA: 50-100 µL of low EDTA TE. Heat to 55ºC for 10 or more minutes to dissolve pellet.

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).

36 Convert **RNA** to cDNA immediately and store remaining RNA in -80ºC freezer. Once converted to cDNA, a working aliquot can be stored at 4ºC for short term purposes, or -20ºC or -80ºC for long term storage. Avoid repeated freezing and thawing of RNA and cDNA.

If RNA does not need to be converted to cDNA in the lab and is being prepped to be sent for RNA sequencing, quantify RNA and DNA present in RNA extract, and immediately continue on to concentrating/cleaning bead step. Avoid freeze/thaw cycles as RNA is highly unstable and will easily degrade.

DNA working aliquots can be stored at 4ºC. For long-term 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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