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Phenol-Chloroform-Isoamyl Alcohol DNA Extraction

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We use this protocol for Oxford Nanopore Technologies sequencing and it's working

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Abstract

This protocol is for an organic isolation method of DNA from environmental DNA samples preserved in filter membranes. This protocol is focused on the acquisition of long fragments. 1L of stream water was collected and filtered immediately with 0.42-µm filtered funnels. Filters can be preserved directly in Longmire Buffer at -20°C or preserved in 96% Ethanol at -20°C.

Materials

The following are the materials required for the protocol:

1. LongMire buffer
2. Proteinase K (20 mg/ml) (Working solution: 1 mg/ml)
3. Phenol-chloroform-isoamyl alcohol (25:24:1)
4. Chloroform-isoamyl alcohol (24:1)
5. Isopropanol (70% 2-propanol)
6. Ethanol (Working solution: 70%)

Filters Preparation

1 Filters preserved in 96% ethanol must be dried and transferred to Longmire Buffer before starting this protocol.

2 Place the filter on a clean glass or plastic Petri dish and pour approximately 500 µl of the liquid (EtOH2) onto the filter. Homogenize thoroughly before collecting the volume.

3 Place the samples in the muffle at 25°C for 1 hour or until the filter is fully dried. Ensure the drying time does not exceed 2 hours to prevent DNA degradation. The heat block should feel warm to the touch but must not exceed the recommended temperature.

4 Soak the filter in the extraction buffer (Longmire Buffer for eDNA samples that will be processed using the Phenol-Chloroform protocol) and store at -20°C.

DNA Extraction

5 Place ½ filter membrane in a new 1.5 Eppendorf tube with 900 µl of Longmire Eppendorf and store the remaining filter for future use.

6 Using sterile scissors, cut the filters that are preserved and submerged in Longmire Buffer into small pieces without removing them from the Eppendorf tube.

7 Perform a heat shock at 95°C for 10 minutes, then allow the samples to cool to room temperature.

8 Vortex the samples for 5–10 seconds to ensure homogenization.

9 Heat-shock at 95°C for 10 minutes and then allow to temperate the samples at room temperature.

10 Enzymatic digestion with proteinase K, adding 9 µl at 20 mg/ml. Final concentration per sample of 0.2 mg/ml.

11 Vortex the samples for 30 seconds to mix thoroughly, repeating this step two times with a 2-minute waiting period between each vortexing.

12 Incubation for 2 hours at 56°C.

13 Add 100 µl of vacuum grease with a syringe onto the wall of the tube of the 2 ml Eppendorf tube. Prepare 2 sets of tubes for each sample.

14 Add 800 µl of 25:24:1 phenol-chloroform-isoamyl pH 8 to the first set of grease tubes.

15 Vortex the samples for 5–10 seconds to mix thoroughly, then homogenize using a pipette and collect 800 µl of the supernatant. Add it to the first grease tube and vortex until complete homogenization is achieved, which will be indicated by a uniform white phase throughout the tube.

16 Centrifuge for 5 minutes at 13300 rpm. It could be done at 4°C or room temperature and then cool down for 5 minutes after centrifugation to improve the process.

17 Add 800 µl of 24:1 chloroform: isoamyl to the second set of grease tubes.

18 After centrifugation, carefully transfer the upper phase from the previous tube into the second grease tube. Ensure that the pipette tip does not touch the layer of grease separating the phases.

19 Centrifuge for 5 minutes at 13300 rpm.

20 Carefully transfer the upper phase from the previous tube and add it to a 1.5 mL Eppendorf tube without transferring grease.

21 Add 800 µl of isopropanol and 34 µl of 5M NaCl.

22 Mix by inversion and incubate overnight at 4°C for precipitation.

23 Centrifuge for 30 minutes at 13300 rpm at room temperature. It could be done at 4°C.

24 Cool down the samples after centrifugation and pour off slowly the aqueous phase.

25 Wash the samples with 800 µl of 80% iced Ethanol and centrifuge for 5 minutes at 13300 rpm. Repeat this step two times. Note: Repeat 1-2 more times the process for samples

that are likely to experience PCR inhibition.

26 Dry the samples. Pour off the liquid slowly and dry at room temperature until dry.

27 Resuspension once dried in 50-75 µl of ultrapure water at 37°C. To maximize elution and DNA recovery, place the samples in a 37°C water bath for 10 minutes.

28 In the case of environmental samples that may suffer from inhibition, we recommend performing the purification process before storing them at low temperatures.

29 Storage at -20°C or less.