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Phenol-Chloroform environmental DNA (eDNA) Extraction Protocol for Okeanos Explorer Specimens

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is used to extract environmental DNA (eDNA) from disc filters through which water samples taken by NOAA Ocean Exporation's NOAA Ship Okeanos Explorer have been filtered.

Attachments

PDF

eDNA_Extraction_Phen..

60KB

Guidelines

- Perform all steps (besides the centrifugations) in the fume hood with UV light in the Pre-PCR lab (lab free of PCR products).
- Wear nitrile gloves at all steps. Wipe down with DNAaway frequently.
- Prior to starting work, wipe the work surface with a Hype-Wipe (contains bleach) and allow it to air dry. Next, wipe the surface with dilute ethanol (70%). Lastly, run the UV lamp in the hood for 15 minutes.
- Wipe pipettes with a DNAaway or a Hype-Wipe, followed by a wipe with a Kim-wipe or paper towel moistened with dilute ethanol. Place the pipettes in the fume hood prior to running the UV light.
- Use only pipette filter tips.
- Chill isopropanol and ethanol on ice prior to using.
- Allow PCI solution (Autogen Buffer R3) to equilibrate to room temperature for at least 30 min before using.

Materials

- MATERIALS
- 200 µL and 1000 µL filtered tips (sterile)
- P200 and P1000 single-channel pipette
- 1.5 mL LoBind Eppendorf tubes (autoclaved, sterile)
- 5.0 mL LoBind Eppendorf tubes (autoclaved, sterile)
- Simport 2.0 mL Cryovial with Silicon washer (T310-2A)
- KimWipes

Reagents needed:

- DNAaway and/or Hype-wipes (with Bleach)
- Freshly made Longmire's buffer (for extraction blanks)
- Proteinase K solution (20 mg/ml) (e.g. Qiagen Proteinase K or Autogen supply)
- Autogen Buffer R3 (Phenol chloroform isoamyl alcohol)
- Isopropanol (100%)
- Ethanol (70%)
- Autogen resuspension buffer (R9)

Acknowledgements

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