Laboratory for Environmental Pathogens Research
Department of Environmental Sciences
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DNA extraction from a bacterial culture

Materials

Extraction buffer (pH 8.0): 50 mM NaCl
50 mM Tris-HCl, pH 7.6
50 mM EDTA
5% SDS
(autoclave to sterilize)

Phenol (pH 8.0)
24:1 chloroform:isoamyl alcohol
Chloroform
100% isopropanol (filter sterilized)
70% ethanol (filter sterilized)
Microcentrifuge tubes (sterile)
For each sample: Two-2 ml tubes with locking lid
One-1.5 ml tube

- Wear gloves throughout the entire protocol.
- Do not cross-contaminate your samples or the solutions. Be aware of your pipette tip.
- Work clean, either on fresh blue bench paper, in the hood, or on a freshly ethanol treated bench top.
- Perform all centrifugations with the hinge of the tube pointing “up”.
- Do not use a vortex at any point in this protocol unless specified.

All volumes in this protocol are approximate unless otherwise noted. Some reagent volumes might need to be adjusted to account for sample characteristics.

The protocol in brief

You will isolate nucleic acids from your sample. This will be accomplished in two steps. First, you will directly extract nucleic acids from the sample by bead beating. The nucleic acids will be purified by phenol/chloroform cleanup followed by precipitation by centrifugation in isopropanol.

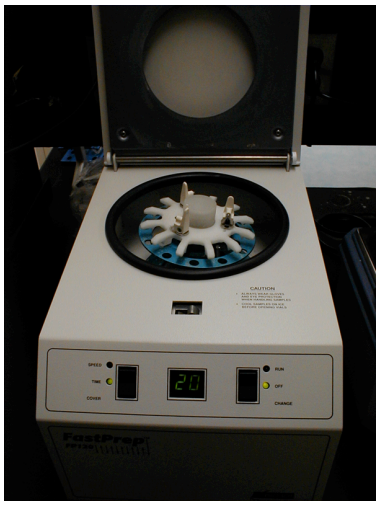
A. Nucleic acid extraction

1. Grow an appropriate volume of bacterial culture to desired OD. Isolating DNA from overgrown cells will result in low yield, therefore, the culture should be in the log phase to facilitate the most efficient extraction.

2. Centrifuge the bacterial suspension for 5 min at 4500 x g to pellet the bacteria. Check to ensure that a sufficiently strong bacterial pellet has formed. Discard the supernatant.

3. Resuspend the pellet in 1 ml of extraction buffer by pipetting up-and-down repeatedly. DO NOT VORTEX, as this will cause considerable foaming and difficulty in transferring the appropriate volume in subsequent steps. Transfer suspension to a sterile 2-ml microcentrifuge tube (with locking lid) containing 0.4-0.5 ml of glass beads (0.10-0.11 mm diameter).

4. Shake with Fast-Prep instrument for **15 s** at **4.0** ms^{-1}. Note: this is a different setting than for the soil DNA extraction.



A bead-beater rapidly shakes microcentrifuge tubes that contain sample and glass beads to lyse cells and release DNA.

5. Centrifuge for 3 minutes at 14,000 x g

B. Nucleic acid purification

6. Add 300 μl of both phenol and chloroform/isoamyl alcohol. Vortex until an emulsion forms and the solution appears milky (5-10 seconds). Centrifuge for 3 min at 14,000 x g or until phases are well separated. The aqueous phase containing the DNA will be the upper phase. With a sterile pipette tip, transfer the aqueous phase to a new 2 ml tube.

7. Extract again with 500 μl of chloroform (not chloroform/isoamyl alcohol) as above. Centrifuge as above, then transfer aqueous phase to a new 1.5 ml tube.

C. Nucleic acid precipitation

8. Determine the volume of your extract. Add exactly 0.1 volumes of 3 M sodium acetate solution and 0.7 volumes of isopropanol. Mix well by inverting the tube several times. Do not vortex.

9. Precipitate the DNA by centrifugation at 14,000 x g for 30 min in the refrigerated centrifuge (10^{o} C, tube hinges pointing up).

10. Carefully discard the supernatant by aspirating the isopropanol. Isopropanol-precipitated pellets may detach from the side of the tube, so be careful not to loosen and/or dislodge the pellet. At this point, **low biomass/DNA samples** may be resuspended in ca. 100 ml of water, pooled, and re-precipitated in order to concentrate them to a manageable concentration.

11. Wash the pellet by adding 0.5 ml ice cold 70% EtOH and inverting the tube gently. Be sure that the EtOH contacts all surfaces inside the tube. Re-pellet the DNA again with centrifugation for 5 min. Optimization of this protocol has shown that centrifugation at cool temperatures (10-15^{o} C) will result in better pellet formation and stability. Thus, the pellets are larger (containing more DNA) and will stick to the sides of the tube, which makes aspirating the alcohol easier. Remove EtOH as above being careful not to aspirate the pellet. Allow pellet to dry for 2-5 minutes.

D. Nucleic acid resuspension and final cleanup

11. Resuspend the pellet by adding exactly 50 μl of DNase/RNase-free water and mixing by flicking the tube with your finger until the pellet dissolves. Do not use nanopure or DI water for this purpose; these water sources are not clean enough.