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Isolation of Genomic DNA from Bacterial Culture

Principle

Isolation of nucleic acids from your sample will be accomplished in two steps. Firstly, direct extraction of nucleic acids from the sample by bead beating and finally. Purification of nucleic acids by phenol/chloroform cleanup method is followed by precipitation in isopropanol.

Materials

1. Extraction buffer (pH 8.0): 50 mM NaCl
2. Tris-HCl, 50 mM, pH 7.6
3. EDTA 50mM
4. SDS 5%
5. Phenol (pH 8.0)
6. Chloroform: isoamyl alcohol : 24:1
7. Chloroform
8. Isopropanol 100%
9. Ethanol 70%
10. Microcentrifuge tubes

Method

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 rpm to pellet the bacteria. 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 the 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 (tissue lyser) instrument for 15 sec. at $4.0ms^{-1}$. 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 rpm.

B. Nucleic acid purification

1. Add 300µl of both phenol and chloroform/ isoamyl alcohol. Vortex until an emulsion forms and the solution appears milky (5-10 sec.). Centrifuge for 3min at 14,000 rpm 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.
2. Extract with 500µl 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

1. 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.

2. Precipitate the DNA by centrifugation at 14,000 rpm for 30 min in the refrigerated centrifuge (10^{0}C, tube hinges pointing up).
3. 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.
4. Wash the pellet by adding 0.5 ml ice cold 70% ethanol and inverting the tube gently. Be sure that the ethanol 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^{0}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 ethanol as above being careful not to aspirate the pellet. Allow pellet to dry for 2-5 minutes.

D. Nucleic acid resuspension and final cleanup

Resuspend the pellet by adding exactly 50µl of DNase/RNase-free water and mixing by flicking the tube with your finger until the pellet dissolves.

Precautions

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