Phenol-chloroform DNA extraction from *Sporosarcina pasteurii*

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Abstract

This protocol is for extraction of genomic DNA from *Sporosarcina pasteurii*. It is based on a standard phenol-chloroform DNA extraction method.

Guidelines

All steps should be performed in a chemical fume hood.

Materials

- Brain-heart infusion (BHI) broth supplemented with 330 mM urea
- Resuspension buffer (50 mM Tris pH 8.0, 10% sucrose)
- Lysis buffer (Tris pH 8.0, 10 mg/mL lysozyme)
- 10% sodium dodecyl sulfate (SDS)
- 100 mg/mL RNase A
- 50 mg/mL proteinase K
- 3.0 M sodium acetate, pH 5.5
- 100% ethanol
- TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)
- Phenol:chloroform:isoamyl alcohol (25:24:1, saturated with 10mM Tris, pH 8.0, 1mM EDTA)
- Chloroform
- 70% ethanol

Protocol

1. Grow *Sporosarcina pasteurii* cells in 150 mL of BHI/330 mM urea at 30°C with 200 rpm shaking to an OD_{600} of 3.5.
2. Concentrate cells by centrifugation at 15,000 x g for 10 min and pour off the supernatant.
3. Resuspend cells in 10 mL of Resuspension Buffer (50 mM Tris pH 8.0, 10% sucrose).
4. Add 2.5 mL Lysis Buffer (Tris pH 8.0, 10 mg/mL lysozyme), 1.5 mL 10% SDS, 5 µL 100 mg/mL RNase A, and 25 µL 50 mg/mL proteinase K to lyse cells and stabilize DNA.
5. Incubate for 1 h at 37°C.

6. Add 1.3 mL of 3.0 M sodium acetate (pH 5.5) and 30 mL of 100% ethanol to separate DNA from other biomacromolecules by precipitation.
7. Spool DNA onto a glass hook and transfer to 20 mL of TE buffer.
8. Dissolve DNA in TE buffer for 1 h at 37°C.
9. Add a 5 mL aliquot of DNA to 7 mL of phenol:chloroform:isoamyl alcohol (25:24:1, Saturated with 10mM Tris, pH 8.0, 1mM EDTA) and mix by inversion.
10. Separate phases by centrifugation at 10,000 x g for 10 min.
11. Pipette off the aqueous phase and add to 5 mL of chloroform. Mix by vortexing.
12. Separate phases by centrifugation at 10,000 x g for 10 min.
13. Pipette off the aqueous phase and add to 30 mL of 100 % ethanol. Mix by inversion.
14. Collect precipitated DNA by centrifugation at 10,000 x g for 10 min.
15. Wash pelleted DNA twice by adding 5 mL of 70% ethanol, mixing by inversion, centrifuging at 10,000 x g for 5 min, and removing the supernatant.
16. Allow the pellet to dry.
17. Dissolve the final pellet in 500 µL of TE buffer at 37°C for 1 h.