MAR 26, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kqdg324eev25/v1

Protocol Citation: Michael S. Carter, Matthew J. Tuttle, Maneesh K. Gupta 2024. Phenol-chloroform DNA extraction from Sporosarcina pasteurii. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg324eev25/v1

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

Phenol-chloroform DNA extraction from Sporosarcina pasteurii

Michael S. Carter[1], Matthew J. Tuttle[1,2], Maneesh K. Gupta[1]

[1]Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Dayton, Ohio, USA;
[2]Biological and Nanoscale Technologies Division, UES Inc., a BlueHalo Company, Dayton, Ohio, USA

Matthew J. Tuttle
Air Force Research Laboratory

ABSTRACT

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

ATTACHMENTS

Phenol-chloroform DNA extraction from Sporosarcina pasteurii.pdf

PROTOCOL REFERENCES

Distribution A. Approved for public release: distribution unlimited. AFRL-2024-1482.

GUIDELINES

All steps should be performed in a chemical fume hood.

Created: Mar 25, 2024

Last Modified: Mar 26, 2024

PROTOCOL integer ID: 97344

Keywords: DNA extraction, Sporosarcina pasteurii

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

DNA extraction

1 Grow *Sporosarcina pasteurii* cells in 150 mL of BHI/330 mM urea at 30°C with 200 rpm shaking to an OD600 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.