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# OPEN ACCESS



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

## 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.

#### **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.

## protocols.io

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#### **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

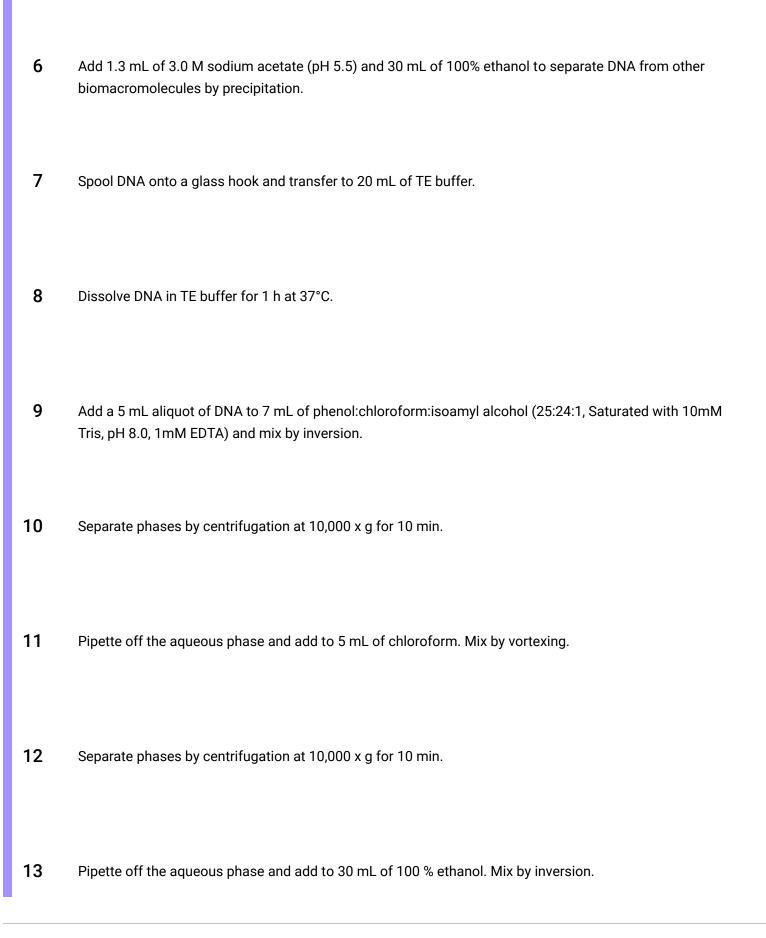
### **DNA** extraction

1

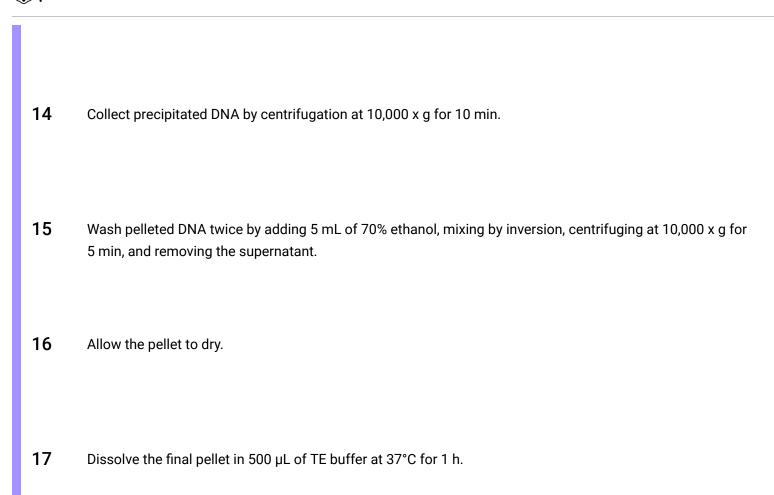
Grow *Sporosarcina pasteurii* cells in 150 mL of BHI/330 mM urea at 30°C with 200 rpm shaking to an OD600 of 3.5.



- 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.



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