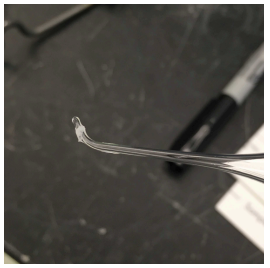
Jul 10, 2023 Version 3

Chromosomal DNA extraction from Gram-positive bacteria, V3 V.3



DOI
dx.doi.org/10.17504/protocols.io.5jyl85119l2w/v3

Anders Kiledal[1], Julia A Maresca[2]

[1]University of Delaware, Department of Biological Sciences;
[2]University of Delaware, Department of Civil and Environmental Engineering

CivilMicroLab

Julia A Maresca
SUNY College of Environmental Science and Forestry

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl85119l2w/v3

Protocol Citation: Anders Kiledal, Julia A Maresca 2023. Chromosomal DNA extraction from Gram-positive bacteria, V3. **protocols.io https://dx.doi.org/10.17504/protocols.io.5jyl85119l2w/v3**Version created by **Julia A Maresca**

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

Created: July 07, 2023

Last Modified: July 10, 2023

Protocol Integer ID: 84620

Keywords: Gram-positive, chromosomal DNA, Firmicutes, Actinobacteria, sequencing, chromosomal dna extraction from gram, chromosomal dna extraction, dna extraction, weight dna from gram, positive bacterial species, positive bacteria, removing surfactant, v3 extraction, extraction, weight dna, dna, gram

Abstract

Extraction of high-molecular-weight DNA from Gram-positive bacterial species, with optional steps for removing surfactants. This DNA is suitable for sequencing and the protocol can be scaled up at least 5-fold. Modified from a protocol by Tina Wecke, **LMU-Munich**.

Image Attribution

Julia Maresca, University of Delaware

Guidelines

Recommend wearing gloves throughout and working in a biosafety cabinet if possible to prevent contamination. This protocol can be scaled up at least 5-fold. If the isopropanol precipitation step is used, subsequent steps do not have to be scaled up unless the culture volume is substantially larger.

Materials

SOLUTIONS

TEN
- 10 mM Tris-HCl, pH 8.0
- 10 mM EDTA
- 150 mM NaCl

*TEN**
- 10 mM Tris-HCL, pH 8.0
- 1 mM EDTA
- 50 mM NaCl

RNAse A
- 20 mg/mL in water

Lysozyme
- 20 mg/mL in water

SDS
- 10% (w/v) in water

Other reagents:
Isopropanol, ethanol (100% and 70%), phenol, chloroform:isoamyl alcohol (24:1), sterile water.

Enzyme solutions should be stored at -20 between uses or prepared freshly. Other solutions can be stored at room temperature.

CONSUMABLES
- microcentrifuge tubes (or larger centrifuge tubes, depending on volume)
- pipetment (P1000, P200, P20)
- pipet tips (P1000, P200, P20)
- Glass Pasteur pipet with tip bent

Troubleshooting

Safety warnings

Phenol and chloroform:isoamyl alcohol should be handled in a fume hood and the liquid waste and contaminated tubes should be disposed of in accordance with the institution's rules for handling organic solvent waste.

Before start

Grow culture to high cell density and prepare all solutions.

Grow culture

1 Inoculate 10 mL rich medium from a fresh overnight culture, and incubate at appropriate temperature on shaker. At OD600 of ~0.8-1.0, harvest cells by centrifugation (10 min., 5000 rpm).

Cell lysis

2 Resuspend cell pellet in 2 mL TEN (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 150 mM NaCl).

3 Add 100 µL lysozyme (20 mg/mL) and incubate for 20 min at 37ºC.

4 Add 20 µL RNAse (10 mg/mL) and incubate for 3 min at 65ºC.

5 Add 40 µl SDS, a small scoop of proteinase K and 550 µl TEN*. Vortex, then incubate at 60°C for 2 hours.

Remove surfactants (optional)

6 IF THE STRAIN PRODUCES A SURFACTANT THAT INTERFERES WITH THE PHASE SEPARATION, Add 0.1 volume 3 M sodium acetate and 1 volume cold isopropanol, mix, and incubate on ice for 20 min. Centrifuge for 10 min at 5000 rpm and decant the supernatant. Then resuspend in 400 µL TEN and 550 µL TEN* and transfer to a microcentrifuge tube.

Phenol & chloroform:isoamyl alcohol extractions

7 Add 900 µL phenol, mix by inversion. Centrifuge for 5 min. at 13000 rpm and transfer the upper phase to a clean microcentrifuge tube.

8 Re-extract once with phenol (1 volume) and twice with chloroform: isoamyl alcohol (24:1 v/v, 1 volume)

DNA precipitation

9 Transfer upper phase to 10 mL cold 100% ethanol.

10 Collect DNA by coiling on the end of a glass Pasteur pipet.

11 Air dry, then resuspend DNA in 100 µL sterile water overnight at 4ºC.