Jul 09, 2021

Chromosomal DNA extraction from Gram-positive bacteria

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

 dx.doi.org/10.17504/protocols.io.bs7knhkw

CivilMicroLab

Julia Maresca

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.

DOI

dx.doi.org/10.17504/protocols.io.bs7knhkw

PROTOCOL CITATION

Anders Kiledal, Julia A Maresca 2021. Chromosomal DNA extraction from Gram-positive bacteria. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bs7knhkw

KEYWORDS

Gram-positive, chromosomal DNA, Firmicutes, Actinobacteria, sequencing

IMAGE ATTRIBUTION

Julia Maresca, University of Delaware

CREATED

Mar 10, 2021

LAST MODIFIED

Jul 09, 2021

PROTOCOL INTEGER ID

48076

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 TEXT

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

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 STARTING

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.

Citation: Anders Kiledal, Julia A Maresca (07/09/2021). Chromosomal DNA extraction from Gram-positive bacteria. https://dx.doi.org/10.17504/protocols.io.bs7knhkw

Remove surfactants (optional)

5 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

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

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

DNA precipitation

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

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

Citation: Anders Kiledal, Julia A Maresca (07/09/2021). Chromosomal DNA extraction from Gram-positive bacteria. https://dx.doi.org/10.17504/protocols.io.bs7knhkw