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Genomic DNA extraction of pure Thermococcales cultures

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Protocol Category: genomic DNA extraction
Model organism(s): Thermococcales (mostly used in our lab on *Thermococcus barophilus*)
Tags: genomic DNA, extraction, purification

Abstract

This protocol is adapted from a lab protocol made by Axel Thiel. The goal of this protocol was to simplify it to faster obtain genomic DNA from cell cultures. This protocol can be used for Thermococcales but could be used for other archaea. The protocol is adapted for small culture volume (1 mL to 20 mL) of cells in the stationary phase of growth; for example, 1mL of culture is enough to obtain genomic DNA for PCR whereas 20 mL is necessary to send sufficient genomic DNA for sequencing. Briefly, after centrifugation, the cellular pellet will be mixed with detergent (Sarkosyl 10% and SDS 10%) and proteinase K. After heating at 55°C, RNAse can be used if genomic DNA will be sequenced. Then a purification by phenol/chloroform/ isoamyl alcohol will be done. After centrifugation, genomic DNA will be washed by ethanol 70% and dry before being resuspended into water or TE buffer.

Background

The protocol is relevant for genomic DNA extraction of pure Thermococcales cultures.

Materials

Where relevant, advise on storage and shelf-life.

Product name	Manufacturer	Catalog number	Notes
Proteinase K 20 mg/ml	SIGMA-ALDRICH	39450-01-6	Use at 20 mg/ml; conserved at -20°C or some weeks at 4°C
Sodium dodecyl sulfate (SDS)	SIGMA-ALDRICH	L4390	Use at 10 %; keep at RT
N-Lauroylsarcosine sodium salt (Sarkosyl)	SIGMA-ALDRICH	L5125	Use at 10 %; keep at RT
Tris HCl	MP	819623	
Ethylenediaminetetraacetic acid (EDTA)	MP	EDTA0500	
NaCl	VWR	27810-364	
Phenol/Chloroform/Isoamyl Alcohol, 25:24:1 (v/v)	SIGMA-ALDRICH	77617	
Chloroform	Carlo Erba	438601	
Isopropanol	Carlo Erba	415154	
Ethanol	Carlo Erba	414607	Use at 70 %; keep at -20°C
RNAse A	Roche	10109142001	Use at 50 µg/ml; conserved at -20°C or some weeks at 4°C

Protocol

A. Cell pellet preparation:

1. Centrifuge 1-20 mL of liquid culture with cells on stationary phase (or around $2x10^8$ cells/mL) using a microtube or conical tube depending on the volume, for 6 min at 8000g at 4°C.
2. Remove supernatant under a chemical hood.
3. Resuspend the pellet with 300 µL TE-Na buffer and transfer the mixture in a new microtube.

B. Cell lysate:

1. Add into the mixture:
   - 30 µL Sarkosyl 10 %.
   - 30 µL SDS 10 % (pay attention, if RT is low, it can precipitate -> microwave for few seconds).
   - 15 µL Proteinase K 20 mg/mL
2. Mix it gently. Do not use vortex.
3. Incubate in a dry or water bath 1h at 55°C (for 1 mL of culture, time can be reduced at 30 min).
4. Optional: If genomic DNA will be used for sequencing add 7 µL of RNAse A 50 µg/mL, incubate 20-30 min at 37°C.

C. Extraction of genomic DNA:

1. Phenol/chloroform/isoamyl alcohol extraction:
   - Add 400 µL (around the same volume of the cell lysate obtained in the previous step) of Phenol/chloroform/isoamyl alcohol, close the tube and shake the tube strongly for a few seconds to obtain an emulsion.
   - Centrifuge for 5 min at 13 000g at 4°C.
   - Take the aqueous phase and transfer it into a new tube.
2. Chloroform extraction.
   - Add 400 µL of Chloroform (around the same volume of aqueous phase obtained in the previous step).
   - Centrifuge the tube 5 min at 13000g at 4°C.
   - Take the aqueous phase and transfer it in a new tube.
   - Add around 300 µL of isopropanol (Isopropanol allows the precipitation of DNA if more than 0.7 volume is added to the aqueous phase, here around 400 µL) and shake the mixture gently.
   - Put the tube at -20°C during 30 min or -80°C during 10 min (it is possible to stop here and let the tube at -20°C overnight or more, and continue the protocol later).
   - Centrifuge for 20 min at 20 000g at 4°C.
   - Remove the supernatant.
   - Add 500 µL of ethanol 70% at -20°C and centrifuge the tube 5 min at 20 000g at 4°C.
   - Remove supernatant carefully.

- Put the tube at 50°C during 5-10 min to remove drop (or longer at RT).
- Add 30µL (if 1mL of culture) to 100 µL (if 20 mL of culture) of water or TE buffer to extract DNA. Resuspend the pellet.

3. Keep at -20°C.

Recipes

TE-Na buffer: For 500 mL, add 50 mL of Tris-HCl 1M, 10 mL of NaCl 5M, 50 mL EDTA 0.5M pH 8 and 390 mL of ultrapure water. Filter at 0.22 µm or autoclave at 121°C 20 min. Keep at RT or 4°C.

SDS 10%: For 100 mL, add 10g of SDS into 100 mL of ultrapure water. To improve dissolution, heat at 68°C. No sterilization. Then, conserve it at room temperature.

Sarkosyl 10%: For 100 mL, add 10g of N-Lauroylsarcosine sodium salt into 100 mL of ultrapure water. No sterilization. Then, conserve it at room temperature.

TE buffer: For 100 mL, add 1mL of Tris-HCl 1M at pH8, 0.2 mL of EDTA 0.5M pH8 and 98.8 mL of ultrapure water.

Proteinase K 20 mg/mL: Dissolve 100 mg of Proteinase K into 5 mL of ultrapure water. Keep at -20°C.

Additional Notes.

For genomic sequencing, use 10-20 mL of cell culture to be sure to have enough quantity of genomic DNA. For PCR, 1 mL of culture is enough.

This protocol template was inspired by the Bio-protocol Manuscript template