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HMW gDNA extraction from prokaryotic cultures and cryo preservation stocks V.1

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Protocol status: Working
We regularly use this protocol to extract gDNA suitable for Nanopore sequencing from archaea and bacteria

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Disclaimer

Abstract

This protocol can be used to extract High Molecular Weight gDNA from bacterial and archaeal cultures and cryo preservations thereof.
The resulting gDNA is usually suitable for long-read sequencing and is regularly used for genome assembly following Nanopore Sequencing.
It has been tested with a variety of mostly archaeal but also bacterial strains, including, but not limited to, *Thermococcales*, *Thermotogales*, *E. coli*, and *Desulfurococcales*.

Protocol materials

- TE Buffer
- Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**L6876
- SDS **Roth Catalog #**CN30.3
- Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #**T3018L
- Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #**P8107S
- Sodium chloride **P212121**
- CTAB (Cetyltrimethylammonium-bromide) **Serva, Germany Catalog #**16530
- Roti-C/I **Carl Roth Catalog #**X984.2
- Roti-Aqua-P/C/I **Carl Roth Catalog #**X985.2
- 1.5 mL LoBind tubes **Eppendorf Catalog #**022431021
- Sodium acetate trihydrate **Carl Roth Catalog #**3856.1
- 2-Propanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**190764
- Ethanol **P212121 Catalog #**BE-BDH1156
- Nuclease-free Water - 25 ml **New England Biolabs Catalog #**B1500S
- Buffer EB **Qiagen Catalog #**19086

Safety warnings

Take appropriate precautions when handling phenol containing solutions!

Prepare cultures or cryopreservation capillaries

1 Transfer the bacteria-/archaea-suspension (~ 50 µL) from one cryo preservation capillary to a 1.5 mL reaction tube or pellet a well-grown culture by centrifugation 15000 rpm, 00:15:00 , discarding the supernatant, and resuspending the cell pellet in ~ 50 µL media

15m

Open up the cells

2 Add 490 µL TE Buffer + 20 µL freshly prepared Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**L6876 solution ([M] 10 mg/mL , in TE Buffer) and vortex briefly

3 incubate 00:30:00 at 37 °C

30m

4 add 5 µL Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #**P8107S ([M] 20 mg/mL) + 10 µL Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #**T3018L ([M] 10 mg/mL) and 15 µL of [M] 20 Mass / % volume SDS **Roth Catalog #**CN30.3 , vortex briefly

5 incubate 01:00:00 at 56 °C

1h

6 freeze at -80 °C for 00:30:00 and thaw at 60 °C for 00:10:00

40m

7 repeat go to step #6 two additional times (total of three cycles)

8 add 100 µL of [M] 5 Mass Percent Sodium chloride **P212121** and mix well

> Note
>
> (this step is crucial as a CTAB–nucleic acid precipitate will form if salt concentration drops below about 0.5 M at room temperature)

9 add 80 µL of [M] 10 Mass / % volume CTAB (Cetyltrimethylammonium-bromide) **Serva, Germany Catalog #**16530 in [M] 700 millimolar (mM) Sodium chloride **P212121**

10 incubate at 65 °C for 00:30:00 30m

Remove non-DNA components 34m

11 add one volume (should be roughly 750 µL) chloroform:isoamyl alcohol (24:1; Roti-C/I **Carl Roth Catalog #**X984.2), shake vigorously, centrifuge full speed (> 12.000 rcf, Room temperature, 00:02:00), transfer top (aqueous) phase to new tube 2m

12 mix aqueous phase with equal volume phenol:chloroform:isoamyl alcohol (25:24:1; Roti-Aqua-P/C/I **Carl Roth Catalog #**X985.2), centrifuge full speed (> 12.000 rcf, Room temperature, 00:02:00) as before, and transfer top (aqueous) phase to new tube 2m

13 mix aqueous phase with equal volume chloroform:isoamyl alcohol (24:1; Roti-C/I **Carl Roth Catalog #**X984.2), centrifuge as before (> 12.000 rcf, Room temperature, 00:02:00), and transfer top (aqueous) phase to new 1.5 mL LoBind tubes **Eppendorf Catalog #**022431021 2m

Pellet and wash gDNA 34m

14 add 0.6 volumes (roughly 360 µL) of cold 100% 2-Propanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**190764 + 0.06 volumes (roughly 36 µL) of [M] 3 Molarity (M) Sodium acetate trihydrate **Carl Roth Catalog #**3856.1 pH 5.2

15 incubate at -20 °C overnight

16 centrifuge 16.000 rcf, Room temperature, 00:20:00 , discard supernatant, air dry briefly 20m

17 add 0.6 volumes cold (-20 °C) [M] 70 % (v/v) Ethanol **P212121 Catalog #**BE-BDH1156 , centrifuge at 16.000 rcf, Room temperature, 00:10:00 , discard supernatant, air dry briefly 10m

18 Optional: repeat go to step #17 once

19 resuspend in 50 µL Nuclease-free Water - 25 ml **New England Biolabs Catalog #**B1500S or Buffer EB **Qiagen Catalog #**19086 or similar (ideally let this rest at 4 °C for several hours)

Protocol references

This Protocol is partially based on methods from the following literature:

Murray, M. G. & Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8, 4321–4326.
Zhou, J., Bruns, M. A., & Tiedje, J. M. 1996. DNA recovery from soils of diverse composition. Appl and env microbiology 62(2):316-322.
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Wilson, K. 2001. Preparation of Genomic DNA from Bacteria. Current Protocols in Molecular Biology 56, 2.4.1-2.4.5.
Moissl-Eichinger, C. 2011. Archaea in artificial environments: their presence in global spacecraft clean rooms and impact on planetary protection. The ISME journal 5(2):209-219.
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