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

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Richard RKS Stöckl¹

¹University of Regensburg



Richard Stöckl

University of Regensburg, Archaea Centre Regensburg

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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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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 ( 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** ( 20 mg/mL) +  10 μL  Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #T3018L** ( 10 mg/mL) and  15 μL of  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  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  10 Mass / % volume  CTAB (Cetyltrimethylammonium-bromide) **Serva, Germany Catalog #16530** in  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**  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) 10m
 Ethanol **P212121 Catalog #BE-BDH1156** , centrifuge at
 16.000 rcf, Room temperature, 00:10:00 , discard supernatant, air dry briefly
- 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:

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