HMW gDNA extraction from prokaryotic cultures and cryo preservation stocks

Richard RKS Stöckl[1]

[1]University of Regensburg

Archaea Center Regensburg

Richard RKS 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

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*.

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Keywords: long-read sequencing, nanopore sequencing, gDNA, DNA extraction, HMW gDNA, HMW DNA, prokaryotic cells, archaea, bacteria

PROTOCOL MATERIALS

1.5 mL LoBind tubes **Eppendorf Catalog #022431021** Step 13

Ethanol **P212121 Catalog #BE-BDH1156** Step 17

Sodium chloride **P212121** In 2 steps

TE Buffer **Contributed by users** In 2 steps

SDS **Roth Catalog #CN30.3** Step 4

Monarch RNase A – 1 ml (2x0.5ml) **New England Biolabs Catalog #T3018L** Step 4

Buffer EB **Qiagen Catalog #19086** Step 19

Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #P8107S** Step 4

CTAB (Cetyltrimethylammonium-bromide) **Serva, Germany Catalog #16530** Step 9

Roti-C/I **Carl Roth Catalog #X984.2** In 2 steps

Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L6876** Step 2

2-Propanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #190764** Step 14

Nuclease-free Water - 25 ml **New England Biolabs Catalog #B1500S** Step 19

Roti-Aqua-P/C/I **Carl Roth Catalog #X985.2** Step 12

Sodium acetate trihydrate **Carl Roth Catalog #3856.1** Step 14

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 **Contributed by users** + 20 µL freshly prepared Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L6876** solution ([M] 10 mg/mL , in TE Buffer **Contributed by users**) 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 (2x0.5ml) **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 Molarity (M) 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; 2m

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**

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) 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)