



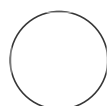
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# HMW gDNA extraction from prokaryotic cultures and cryo preservation stocks

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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*.
















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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 <a href="#">2 steps</a>
 TE Buffer Contributed by users	In <a href="#">2 steps</a>
 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 <a href="#">2 steps</a>
 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  $\mu\text{L}$ ) 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  $\mu\text{L}$  media 15m

## Open up the cells


- 2 Add  490  $\mu\text{L}$   TE Buffer Contributed by users +  20  $\mu\text{L}$  freshly prepared

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


solution ( 10 mg/mL, in  TE Buffer Contributed by users) and vortex briefly

- 3 incubate  00:30:00 at  37 °C 30m








- 4 add  5  $\mu\text{L}$



 Proteinase K, Molecular Biology Grade - 2 ml New England Biolabs Catalog #P8107S (





 20 mg/mL) +  10  $\mu\text{L}$

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


 10 mg/mL) and  15  $\mu\text{L}$  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  $\mu\text{L}$  of  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  $\mu\text{L}$  of  10 Mass / % volume  CTAB (Cetyltrimethylammonium-bromide) Serva, Germany Catalog #16530 in  Sodium chloride P212121  700 millimolar (mM)

10 incubate at  65 °C for  00:30:00 30m



## Remove non-DNA components 34m

11 add one volume (should be roughly  750  $\mu\text{L}$ ) 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 (&gt;

⊗ 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  $\text{360 } \mu\text{L}$ ) of cold 100%

⊗ 2-Propanol Merck MilliporeSigma (Sigma-  
Aldrich) Catalog #190764

+ 0.06 volumes

(roughly  $\text{36 } \mu\text{L}$ ) of  $\text{[M]} \text{ 3 Molarity (m)}$ 

⊗ Sodium acetate trihydrate Carl  
Roth Catalog #3856.1

 $\text{pH } 5.2$ 

15

incubate at  $-20\text{ }^{\circ}\text{C}$  overnight

16

centrifuge  $\text{16.000 rcf, Room temperature, 00:20:00}$ , discard supernatant, air dry briefly

20m

17

add  $\text{0.6 volumes}$  cold ( $-20\text{ }^{\circ}\text{C}$ )  $\text{[M]} \text{ 70 \% (v/v)}$ 

10m

⊗ Ethanol P212121 Catalog #BE-BDH1156

, centrifuge at

⊗  $\text{16.000 rcf, Room temperature, 00:10:00}$ , discard supernatant, air dry briefly

18

Optional: repeat  $\Rightarrow$  go to step #17 once

19

resuspend in  $\text{50 } \mu\text{L}$




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)