



Mar 09, 2023

High-molecular-weight DNA extraction from cheese rind microbial communities

 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzbkj8vx1/v1>

Emily C.P. Weiss¹

¹Arcadia Science

Arcadia Science



Arcadia Science

Arcadia Science

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Protocol Citation: Emily C.P. Weiss 2023. High-molecular-weight DNA extraction from cheese rind microbial communities. protocols.io <https://dx.doi.org/10.17504/protocols.io.rm7vzbkj8vx1/v1>

**Manuscript citation:**

Borges A, Dutton R, McDaniel E, Reiter T, Weiss ECP. (2023). Paired long- and short-read metagenomics of cheese rind microbial communities at multiple time points. <https://doi.org/10.57844/arcadia-0zvp-xz86>

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Protocol status: Working

We use this protocol and it's working

Created: December 12, 2022

Last Modified: January 29, 2024

Protocol Integer ID: 73898

Keywords: HMW DNA, HMW, DNA, genome, genomic, extract, cheese, extraction, rind, microbial community, microbes, metagenomics, long-read, sequencing, genomes, weight dna extraction from cheese rind, weight dna from cheese rind, high molecular weight dna, weight dna extraction, cheese rind, microbial community sample, weight dna, microbial communities this protocol, microbial community, dna, oxford nanopore, suitable for oxford nanopore, read sequencing, nanopore, extraction

Abstract

This protocol describes extracting high-molecular-weight DNA from cheese rind microbial community samples. This DNA is suitable for Oxford Nanopore long-read sequencing.

Materials

Mortar

Pestle

Liquid nitrogen

15 mL conical tubes

Centrifuge that is able to cool to 4 °C

Vortexer

50 °C and 37 °C incubators or water baths

Tris-Cl, pH 8

EDTA, pH 8

SDS

☒ Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #T3018L**

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

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

☒ UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific Catalog #15593031**

Ice-cold isopropanol

3 M sodium acetate

Ice-cold 70% ethanol

Molecular biology grade water



Protocol materials

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

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

⊗ UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific Catalog #15593031**

⊗ Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #T3018L**



Equipment setup

- 1 Cool a centrifuge to 4 °C. It should be able to reach 17,000 × g.

3m

Note

This protocol describes centrifugation steps using 15 mL conical tubes. You may need to adjust volumes or use different tubes depending on the centrifuge you have available.

- 2 Set a water bath or standing incubator to 37 °C and another to 50 °C.

3m

Prepare TLB

1h 16m

- 3 Prepare a minimum of 4 mL of Tris Lysis Buffer (TLB) per extraction sample.

Final concentrations:

- 10 mM Tris-Cl, pH 8
- 100 mM EDTA, pH 8
- 1% SDS
- 10 mg/mL lysozyme



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

- 4 Heat the prepared buffer to 50 °C and vortex periodically until the lysozyme is fully dissolved.

1h

Note

Dissolving lysozyme at this concentration is time-consuming and requires quite a bit of vortexing. While not validated, it is possible that lower levels of lysozyme may be more ideal.

- 5 Cool to room temperature and then add RNase A to a final concentration of 20 µg/mL.

2m



Monarch RNase A – 1 ml (2×0.5ml) **New England Biolabs Catalog #T3018L**



- 6 Filter-sterilize the TLB into a sterile container using a 0.22 μm filter.

5m

Lysis and digestion

3h 15m

- 7 Add 4 mL of prepared TLB to a 15 mL conical tube for each sample being extracted.

5m

Note

This volume is for ~250 mg of cheese rind. Adjust volumes as needed depending on the amount of sample.

- 8 Add a small amount of liquid nitrogen into the mortar and place the pestle into the mortar to pre-chill.

1m

- 9 Add 250 mg of the harvested cheese rind biofilm into the liquid nitrogen in the mortar and slowly use the pestle to grind the rind. Be careful to not splash the liquid nitrogen out of the mortar when first breaking the sample apart.

3m

- 10 Keep adding liquid nitrogen followed by grinding until the sample is a fine powder.

6m

- 11 Transfer the rind powder into the 15 mL conical tube containing 4 mL of TLB and vortex until well mixed.

1m

- 12 Repeat until all samples are in TLB.

Note

Time required will depend on the number of samples you are processing.

- 13 Incubate the tube(s) at 37 °C for 1 h with swirling to mix about every 15 min.

1h

- 14 Add 50 μL of Proteinase K and incubate for 1 h at 50 °C with swirling every 15 min.

1h 10m



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



Extraction

1h 5m

- 15 Add an equal volume (4 mL) of phenol:chloroform:isoamyl alcohol and vortex for five seconds. 5m
-  UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific Catalog #15593031**
- 16 Rotate on a culture wheel or hula mixer for 10 min. 10m
- 17 Centrifuge for 10 min at 8,000 rpm at 4 °C. 10m
- 18 Transfer the upper aqueous layer to a new 15 mL conical tube without disturbing the interphase. 5m
- 19 Add an equal volume of phenol:chloroform:isoamyl alcohol to this aqueous layer. 5m
- 20 Rotate on a culture wheel or hula mixer for 10 min. 10m
- 21 Centrifuge for 10 min at 8,000 rpm at 4 °C. 10m
- 22 Transfer the upper aqueous layer to a new 15 mL conical tube without disturbing the interphase. 5m
- 23 Repeat steps 19–22 as necessary until the aqueous layer is no longer cloudy.
- 24 After obtaining a clear aqueous layer, add an equal volume of chloroform to this aqueous layer. 5m
- 25 Rotate on a culture wheel or hula mixer for 5 min. 5m
- 26 Centrifuge for 5 min at 8,000 rpm at 4 °C. 5m



- 27 Transfer the upper aqueous layer to a new 15 mL conical tube without disturbing the interphase.

5m

Precipitation

50m

- 28 Add an equal volume of ice-cold isopropanol and 0.1 volume of 3 M sodium acetate (sterile) to the aqueous layer.
- 29 Place at $-80\text{ }^{\circ}\text{C}$ for 10 min.
- 30 Centrifuge samples at $17,000 \times g$ for 3 min at $4\text{ }^{\circ}\text{C}$.
- 31 Remove supernatant, taking care not to remove the pelleted DNA.
- 32 Wash pellet with 1 mL ice-cold, 70% ethanol.
- 33 Centrifuge samples at $17,000 \times g$ for 3 min at $4\text{ }^{\circ}\text{C}$.
- 34 Remove ethanol, taking care not to remove the pelleted DNA.
- 35 Do a quick spin to pellet any remaining ethanol and remove residual ethanol by pipetting.
- 36 Leave the tube open to dry for about 15 min or until all ethanol has evaporated.
- 37 Add $250\text{ }\mu\text{L}$ of molecular biology grade water to the DNA pellet and leave at room temperature overnight.

5m

10m

5m

2m

3m

2m

1m

15m

2m

**Note**

To maximize DNA length, it is best to minimize pipetting of the DNA to avoid shearing. When pipetting, using a pipette tip with the end of the tip cut off to make it wider. This can help decrease shearing.

- 38 The following day, make sure the DNA is fully resuspended before performing any desired quality checks. If not using immediately, store DNA at -20°C until needed.

5m

Note

Genomic DNA ScreenTape Analysis on an Agilent Tapestation can help assess quality of genomic DNA. Oxford Nanopore Community pages provide additional recommendations for DNA quality checks to perform prior to ONT sequencing.

 Genomic DNA ScreenTape **Agilent Technologies Catalog #5067-5365**

 Genomic DNA Reagents **Agilent Technologies Catalog #5067-5366**