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We use this protocol and it's working

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

In 1 collection

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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 (2x0.5ml) **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

Keywords: HMW DNA, HMW, DNA, genome, genomic, extract, cheese, extraction, rind, microbial community, microbes, metagenomics, long-read, sequencing, genomes

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. 15m
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 (2x0.5ml) **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. 5m

29 Place at −80 °C for 10 min. 10m

30 Centrifuge samples at 17,000 × g for 3 min at 4 °C.

31 Remove supernatant, taking care not to remove the pelleted DNA. 5m

32 Wash pellet with 1 mL ice-cold, 70% ethanol. 2m

33 Centrifuge samples at 17,000 × g for 3 min at 4 °C. 3m

34 Remove ethanol, taking care not to remove the pelleted DNA. 2m

35 Do a quick spin to pellet any remaining ethanol and remove residual ethanol by pipetting. 1m

36 Leave the tube open to dry for about 15 min or until all ethanol has evaporated. 15m

37 Add 250 µL of molecular biology grade water to the DNA pellet and leave at room temperature overnight. 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**