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DNA extraction for fermented plant based foods

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ABSTRACT

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GUIDELINES

Disinfect mortar and pestle with 70% ethanol in between samples
If the goal is metagenomic sequencing, we recommend bypassing the mortar and pestle step. Simply suspend the samples in 1 ml of ZymoBIOMICS™ Lysis Solution, shake vigorously or vortex, and transfer 750 uL of the supernatant to a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) and proceed to Step 2.

MATERIALS TEXT

ZymoBIOMICS DNA Extraction Kit
Fecal samples
Mortar and pestle
Liquid nitrogen
Ice box with dry ice
1.5 ml microcentrifuge tubes
Vortex
Omni Bead Ruptor Elite bead beater
Centrifuge

SAFETY WARNINGS

Handle liquid nitrogen with proper training and PPE

BEFORE STARTING

- Label the following:
  ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)
  ZymoSpin™ III-F Filter in Collection Tube
  Collection Tube
  ZymoSpin™ II-CR Column in Collection Tube
  ZymoSpin™ III-HRC Filter in Collection Tube
  2 Sets of 1.5 ml microcentrifuge tubes (not provided with kit)
- Spin the labelled ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) for 10 seconds in a mini-centrifuge to ensure that the beads have settled at the bottom
- Include 1 control per batch and assign its position randomly.

1 Take **250 mg** of food sample while on dry ice and transfer to mortar. Add liquid nitrogen until food sample is completely immersed. Using pestle vigorously homogenize sample until a powder forms. Transfer all of the sample to a *ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)*. Add **750 µL** ZymoBIOMICS™ Lysis Solution to the tube. Cap tightly.

2 Secure in a Omni Bead Ruptor Elite bead beater fitted with a 2 ml tube holder assembly and process at max speed (30 m/s) for **00:05:00** . Rest for **00:05:00** . 10m

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3 Centrifuge the *ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)* in a microcentrifuge at ≥ **10000 x g, 00:01:00** . 1m

4 Transfer up to **600 µL** supernatant to the *Zymo-Spin™ III-F Filter* in *the labelled Collection Tube* and centrifuge at **8.000 x g, 00:01:00** . Discard the Zymo-Spin™ III-F Filter. 1m

5 Add **600 µL** of ZymoBIOMICS™ DNA Binding Buffer to the filtrate in *the labelled Collection Tube* from Step 4. Mix well.

5.1 Repeat Step 5 so that the final volume of ZymoBIOMICS™ DNA Binding Buffer added is **1200 µL** .

6 Transfer **800 µL** of the mixture from Step 5 to a *Zymo-Spin™ IICR Column* in a Collection Tube and centrifuge at **10.000 x g, 00:01:00** . 1m

6.1 Discard the flow through from the Collection Tube and repeat Step 6.

7 Add **400 µL** ZymoBIOMICS™ DNA Wash Buffer 1 to the *Zymo-Spin™ IICR Column* in a *new Collection Tube* and centrifuge at **10.000 x g, 00:01:00** . Discard the flow-through. 1m

8 Add **700 µL** ZymoBIOMICS™ DNA Wash Buffer 2 to the *Zymo-Spin™ IICR Column* in a Collection Tube and centrifuge at **10.000 x g, 00:01:00** . Discard the flow-through. 1m

9 Repeat with **200 µL** ZymoBIOMICS™ DNA Wash Buffer 2 to the *Zymo-Spin™ IICR Column* in a Collection Tube and centrifuge at **10.000 x g, 00:01:00** . 1m

10 Transfer the *Zymo-Spin™ IICR Column* to a clean 1.5 ml microcentrifuge tube and add **100 µL** (50 µl minimum) ZymoBIOMICS™ DNase/RNase Free Water directly to the column 6m

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matrix and incubate for **00:05:00** . Centrifuge at **10.000 x g, 00:01:00** to elute the DNA

11 Place a *Zymo-Spin™ III-HRC Filter* in *a new Collection Tube* and add **600 µL** ZymoBIOMICS™ HRC Prep Solution. Centrifuge at **8.000 x g, 00:03:00** . 3m

12 Transfer the eluted DNA (Step 10) to a prepared *Zymo-Spin™ III-HRC Filter* in *a clean 1.5 ml microcentrifuge tube* and centrifuge at exactly **16.000 x g, 00:03:00** . 3m

13 The filtered DNA is now suitable for PCR and other downstream applications. Eluted DNA should be frozen (–30 to –15°C or –90 to –65°C)

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