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DNA extraction from fecal samples

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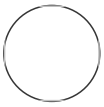

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ABSTRACT

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GUIDELINES

Sample Type	Maximum Input
Feces	200 mg
Soil	250 mg
Liquid Samples[1] and Swab Collections[2]	250 µl
Cells (isotonic buffer, *e.g.* PBS)	50-100 mg (wet weight) (10^9 bacterial and 10^8 yeast cells)
Samples in DNA/RNA Shield™[,3]	≤ 1 ml

Table 1. Sample type and maximum input for DNA extraction using this protocol

MATERIALS TEXT

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

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

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ensure that the beads have settled at the bottom

- Include 1 control per batch and assign its position randomly.

1 Transfer fecal sample to *ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)* and add ZymoBIOMICS™ Lysis Solution:

10s

1.1
- **Flash frozen fecal samples** will be solid and *an aliquot (* 15 mg *) of feces* is expected in a 2mL container (Table 1). Add 250 µL of ZymoBIOMICS™ Lysis Solution to the fecal sample and homogenize evenly using the homogenizing pestle while submerged in liquid nitrogen (or on dry ice). Once the sample is homogenized, add another 500 µL of ZymoBIOMICS™ Lysis Solution such that the final volume is 750 µL. Mix well by vortexing.
- Spin the sample container for 00:00:10 in a minicentrifuge.
- Transfer the entire 750 µL of the sample mix into the *ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)* and proceed to Step 2.

1.2 **Fecal samples collected using Zymo DNA/RNA Shield™** will exist as a solution (Table 1). Mix the sample by shaking well or vortexing. Add up to 250 µL of such sample to a *ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)*. Adjust final volume to 1 mL with ZymoBIOMICS™ Lysis Solution. Cap the tube tightly and proceed to Step 2.

1.3 **Fecal samples collected using other stabilization kits** may exist as a solution (Table 1). Mix the sample by shaking well or vortexing. Add 250 µL of such sample to a *ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)*. Add 750 µL ZymoBIOMICS™ Lysis Solution to the tube. Cap tightly and proceed to Step 2.

10m

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 .

1m

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.

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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 so that the final volume of ZymoBIOMICS™ DNA Binding Buffer added is 1200 µL .

6 1m
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 .

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

7 1m
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.

8 1m
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.

9 1m
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 .

10 6m
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 matrix and incubate for 00:05:00 . Centrifuge at 10.000 x g, 00:01:00 to elute the DNA

11 3m
Place a *Zymo-Spin™ III-HRC Filter* in *a new Collection Tube* and add 600 µL ZymoBIOMICS™

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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 .

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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