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DNA extraction from fecal samples V.3

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ABSTRACT

DNA extraction method for metagenomic sequencing of the gut microbiota

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MATERIALS TEXT

Reagents/Kits

- Phosphate-buffered saline (PBS)
- EZ-Beads (Promega/AMR, AMR76813M)
- Maxwell RSC Blood DNA Kit (Promega, AS1400)

Equipment

- Vortex mixer
- High-speed microcentrifuge
- Block heater
- Micro Smash Beads Cell Disrupter (TOMY Digital Biology, MS-100)
- Maxwell RSC instrument (Promega, AS4500)

Preparation of fecal samples

1 Place **50-100 mg** of fecal sample into tube.

2 

Add **1 mL** of PBS per **100 mg** of feces.

3 

Mix thoroughly by vortexing.

4 

2m

Allow the sample to stand for **00:02:00** to sediment large debris.

5 

Transfer **300 µL** of the suspension to 1.5 mL tube.

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6 3m

Centrifuge at **10000 x g, 00:03:00** .

7

Discard the supernatant.

8

Resuspend the pellet (~ **30 mg** of feces) in **300 µL** of PBS.

9 10m

Incubate at **70 °C** for **00:10:00** on the block heater.

10 Cool to **Room temperature** .

Mechanical cell disruption by bead beating

11

Transfer **300 µL** of the suspension to EZ-beads tube.

> The EZ-Beads tube contains zirconium oxide beads of two different sizes (0.2 mm spheres and a large 5 mm bead) that can facilitate efficient cell lysis by bead beating.

12

Lyse cells either by using disruption device (12.1) or vortex mixer (12.2).

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12.1 Place the EZ-beads tube in Micro Smash instrument and disrupt cells by shaking at **2500 rpm, 00:02:00** .

Micro Smash Beads Cell Disrupter
TOMY Digital Biology **MS-100**

Caution: Avoid using a disruption device with a high-speed linear reciprocating motion, as this may potentially result in breakage of the EZ-Beads tubes.

12.2 Place the EZ-beads tube on MN Bead Tube Holder attached to Vortex-Genie mixer and vortex for **00:05:00** at maximum speed. 5m

MN Bead Tube Holder
Rubber-foam adapter for processing bead tubes with Vortex-Genie instrument
MACHEREY-NAGEL **740469**

13

Briefly spin the tube to collect contents.

Automated DNA extraction using Maxwell RSC Blood DNA Kit 23m

14

Add **300 µL** of Lysis Buffer and **30 µL** of Proteinase K Solution to the sample in EZ-beads tube.

15

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Mix by inverting the tube.

16 

Briefly spin the tube.

17 

20m

Incubate at **56 °C** for **00:20:00** on the block heater.

18 

Briefly spin the tube.

19 

Transfer the supernatant (~ **500 µL**) to 1.5 mL tube.

20 

3m

Centrifuge at **18000 x g, 00:03:00** .

21 

Transfer the cleared lysate to Maxwell RSC Cartridge.

22 

Add **50 µL** of Elution Buffer to elution tube.

23 Start the extraction run following the manufacturer's instructions.

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Maxwell RSC instrument

Automated nucleic acid purification platform

Promega **AS4500**

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