DNA extraction from fecal samples V.2

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ABSTRACT

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Materials

1
- Phosphate-buffered saline (PBS)
- EZ-beads (Promega, AMR76813M): 2 mL tube containing 0.2 mm ceramic (zirconium dioxide) spheres and one 5 mm zirconia bead
- Maxwell RSC Blood DNA Kit (Promega, AS1400)
- MN Bead Tube Holder (MACHEREY-NAGEL, 740469): Rubber-foam adapter for processing bead tubes with Vortex-Genie instrument

Instrument

2
- Maxwell RSC (Promega, AS4500): Automated nucleic acid purification platform
- Micro Smash (TOMY, MS-100): Beads cell disrupter
- Vortex-Genie Mixer
- Heating block
- Microcentrifuge

Preparation of fecal samples

3 Place 50-100 mg of fecal sample into tube.

4 Add 1 ml of PBS per 100 mg of feces.

5 Mix thoroughly by vortexing or pipetting.

6 Allow the sample to stand for 2 min to sediment large debris.

7 Transfer 300 µl of the suspension to 1.5 mL tube.

8 Centrifuge at 18,000 x g for 3 min.

9 Discard the supernatant.

10 Resuspend the pellet (~30 mg of feces) in 300 µl of PBS.

11 Incubate at 70°C for 10 min and cool to room temperature.

Mechanical cell disruption by beat beating

12 Transfer 300 µl of the suspension to EZ-beads tube.

13 Lyse cells either by using disruption device (13.1) or vortex mixer (13.2).

13.1 Place the EZ-beads tube in Micro Smash instrument and disrupt cells at 2,500 rpm for 2 min.

13.2 Place the EZ-beads tube on MN Bead Tube Holder attached to Vortex-Genie mixer and vortex for 5 min at maximum speed.

14 Briefly spin the tube.

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Automated DNA extraction using Maxwell RSC Blood DNA Kit

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

16 Mix thoroughly by vortexing for 10 sec.

17 Incubate at 56°C for 20 min.

18 Briefly spin the tube.

19 Transfer the supernatant (~600 µl) to 1.5 mL tube.

20 Centrifuge at 18,000 x g for 3 min.

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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