VERSION 2

AUG 03, 2021

DNA extraction from fecal samples V.2

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ABSTRACT

DNA extraction from fecal samples

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bs9inh4e

Protocol Citation: Yoshiyuki Matsuo 2021. DNA extraction from fecal samples.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.bs9inh4e> Version created by Yoshiyuki Matsuo

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Protocol status: Working
We use this protocol and it's working

Created: Mar 12, 2021

Last Modified: Aug 03, 2021

PROTOCOL integer ID:
48138

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.

Automated DNA extraction using Maxwell RSC Blood DNA K..

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.

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- 22** Add 50 µl of Elution Buffer to elution tube.
 - 23** Start the extraction run following the manufacturer's instructions.