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Improved methods for high yield genomic DNA of cat stools using DNeasy PowerSoil Pro Kit.

Hajar Fauzan Ahmad¹

¹Faculty of Industrial Sciences and Technology (FIST), Universiti Malaysia Pahang, Malaysia.

Hajar Fauzan Ahmad

Faculty of Industrial Sciences and Technology (FIST), Univer...

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Protocol status: Working
We use this protocol and it works for us for extraction of gut microbiota from cat stool samples.

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Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up

- 1 to 250 mg (ideally 200 to 250 mg) of cat stool and 800 µl of Solution CD1, together with 25 µL of Proteinase K. Vortex briefly to mix.
- 2 Vortex at maximum speed for 10 min. Upon completion, transfer to Eppendorf ThermoMixer to continue incubating, and mixing almost for 20 minutes at 65°C.
- 3 Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).
- 4 Add 200 µl of Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x g for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided).
- 5 Add 600 µl of Solution CD3 and vortex for 5 s.
- 6 Load 650 µl of lysate to an MB Spin Column. Centrifuge at 15,000 x g for 1 min. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.
- 7 Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
- 8 Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.
- 9 Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

- 10 Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided). Centrifuge at up to 16,000 x g for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
- 11 Add 100 µl of Solution C6 to the center of the white filter membrane, and let the C6 remain at filter membrane for 10 minutes.
- 12 Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Expected result