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Genomic DNA extraction from animal faecal tissues using DNeasy blood and tissue kit

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We use this protocol and it's working

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Abstract

Faecal tissues are difficult to lyse and contain lower amount of DNA compared to other animal tissues such as liver and duodenum. This protocol was optimised to extract maximum amount of DNA using 50 mg of faecal tissues following Qiagen DNeasy blood and tissue kit.

Materials

Reagents and Kits

- DNeasy Blood & Tissue Kits (Qiagen)
- RNase A (Qiagen) - 2.5 ml
- Qubit broad range assay kit
- SYBR Safe DNA Gel Stain
- Invitrogen 1 Kb plus DNA ladder
- Gel loading dye
- Glass beads

Equipments

- 1.5 mL DNA LoBind Eppendorf tube
- 0.5 mL DNA LoBind Eppendorf tube
- Heat block at 56°C
- Microcentrifuge
- Bead beater
- Petri dish
- Scalpel
- Pipettes and pipette tips
- Gel tank
- Qubit
- Nanodrop
- Weighing machine to weigh tissues

Before start

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Autoclave 2 ml tubes containing 0.15 mm silica beads before starting
- Preheat the heat block to 56°C
- Fill glass beads upto 1.5 mm in a 2 ml tube

Tissue subculturing

1 Thaw the samples in ice and bring it to room temperature

2 Using a scapel, place the tissue in a petri dish and cut 50 mg of tissue. Check the tissue weight to confirm. Place it in a 2 ml eppendorf tube filled with 0.5 cm of purified silica beads.

DNA extraction

3 Add 324 µl of buffer ATL and 36 µl of proteinase K into each of the bead tubes containing sample.

For reagent only control, take empty bead tube and add only buffer ATL and proteinase K.

4 Place the bead tubes into the bead beater for 2 × 20 sec and spin it down.

5 Transfer the supernatant (as much as possible) into a new 1.5 ml eppendorf tube and discard the pellet.

Note: The supernatant may contain tiny tissue fragments which can be lysed in the next step

6 Incubate the samples at 56°C in heat block for 10 min to 30 min. Actual time depends on tissue lysis

7 Add 4 µl of RNase A (100 mg/ml) to the lysed samples, vortex and incubate for 5 min at room temperature.

8 Vortex and transfer 200 µl of the sample solution into a new 1.5 ml eppendorf tube.

9 Add 200 µl of buffer AL and vortex.

10 Add 200 µl of ethanol pipetting up and down. Vortex.

Note: Step 10 must be done immediately after step 9

11 Tranfer the entire content of the eppendorf tube into a spin column provided with the kit.

12 Centrifuge at 6,000 rcf for 1 min. Discard the flow through and collection tube.

13 Place a new collection tube under the spin column and add 500 µl of buffer AW1

14 Centrifuge at 6,000 rcf for 1 min. Discard the flow through and collection tube.

15 Place a new collection tube under the spin column and add 500 µl of buffer AW2.

16 Centrifuge at maximum speed (13,000 rcf) for 3 mins

17 Discard the flow through and place the same collection tube back into the column. Perform centrifuge for 1 min at maximum speed

18 Discard collection tube and place a 1.5 ml eppendorf tube under the column and add 100 µl of buffer AE. Let it stand for 1 min.

19 Centrifuge at 6000 rcf for 1 min.

20 Pipette the eluted DNA from the eppenforf tube and add it into the column again for re-elution

21 Let it stand for one min and centrifuge at 6000 rcf for 1 min.

22 Discard the filter column. Store 50 µl of eluted DNA at 4°C for quantitative analysis and store the rest at -20°C to prevent repeated freeze-thaw cycle.

Gel electrophoresis

23 Prepare 0.8% agarose gel and run at 100 V for 40-45 min to check the quality of DNA extracted

Qubit analysis

24 Make sure the qubit is standardised prior to starting

25 Use qubit broad range DNA assay kit and perform analysis using 1 µl of extracted DNA. Note down the concentration value (ng/µl)

Nanodrop

26 Use 2 µl of distilled water as blank and 2 µl of buffer AE as standard. Clean the surface between each read.

27 Add 2 µl of sample DNA into the surface of the nanodrop and read. Clean the surface before next use. Note down the A260/280, A260/230 and concentration (ng/µl)