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Foreskin Tissue DNA Extraction

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We use this protocol and it's working

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Abstract

Extracts total DNA from foreskin tissue, as used in Maust et al. 2022

Materials

Supplies

2 ml screw top microcentrifuge tubes
3mm steel beads (1 per sample)
scalpels
plate sealing film
proteinase K (20 mg/ml)
Absolute ethanol
PowerSoil Pro plates (Cat no 19311)
DNeasy 96 Powersoil Pro QIAcube HT kit (Cat no 47021)

Equipment

scale
heat block
Qiagen TissueLyser II
Qiagen QIAcube HT



Preparation

- 1 Add steel bead to 1.5 ml tube
- 2 Add 800 μ L CD1 to tube
- 3 Weigh tube (with bead and solution)

Tissue

- 4 Dissect approximately 25 mg (3 mm³) piece of tissue
Fragment tissue with scalpel
Add tissue to tube (with bead and solution)
- 5 Weigh tube and calculate net tissue weight.

Pre-robot processing

- 6 Process tubes on TissueLyzer II, 2 min at 30 Hz
Rotate block 180° and repeat
- 7 Centrifuge tubes briefly to collapse foam
- 8 Spin PowerBead Pro plate to ensure beads are settled at the bottom
- 9 Add contents of each specimen tube (except steel bead) to a PowerBead plate well
- 10 Add 5 μ L of 20 mg/ml proteinase K to each well
- 11 Seal plate with film
Vortex briefly to mix



- 12 Incubate plate at 65° C for 60 min or until tissue is mostly digested
- 13 Process plate on TissueLyser II, 5 min at 25 Hz
Rotate plate 180° and repeat
- 14 Centrifuge plate at 3000 x *g* for 7.5 min
- 15 Transfer approximately 350 µL supernatant from each well to fresh S-block using well-vator
- 16 add 300 µL of solution CD2 to each well and mix thoroughly by pipetting
- 17 Seal the plate with film
Centrifuge at 3000 x *g* for 7.5 min at room temperature
- 18 Avoiding the pellet, transfer 500 µL of supernatant to fresh S-block using well-vator

QIAcube

- 19 Follow DNeasy 96 PowerSoil Pro Protocol for QIAcube HT (page 17 onward)
Elute in maximum volume (120 µL)
Include vacuum performance check
- 20 Seal plate and freeze at -20°C