Foreskin Tissue DNA Extraction

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ABSTRACT

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

**integer ID:**

72091

**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 µL 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.
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 µL 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 180º 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)
    Incude vacuum performance check

20  Seal plate and freeze at -20ºC