Nov 01, 2022

Foreskin Tissue DNA Extraction

DOI
dx.doi.org/10.17504/protocols.io.4r3l2774jg1y/v1

Brandon Maust[1,2]

[1]Center for Global Infectious Disease Research, Seattle Children's Research Institute;
[2]Division of Infections Disease, Department of Pediatrics, University of Washington School of Medicine

Brandon Maust

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Protocol Citation: Brandon Maust 2022. Foreskin Tissue DNA Extraction. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.4r3l2774jg1y/v1

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

Created: October 31, 2022

Last Modified: November 01, 2022

Protocol Integer ID: 72091

Keywords: foreskin tissue dna extraction, total dna from foreskin tissue, foreskin tissue, dna extraction, total dna, dna, extraction

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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

Troubleshooting

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 mm^3) 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 180º 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 65º 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