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Cell isolation from dorsal mouse skin for single-cell RNAseq

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ABSTRACT

We identified heterogeneity of the skin cell populations upon Enterococcus faecalis infection.





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

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PROTOCOL integer ID:

68336

Funders Acknowledgement:

NMRC

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MATERIALS

Consumables

- Hank's Balanced Salt Solution Ca²⁺/Mg²⁺-free
- Dulbecco's Phosphate Buffered Saline Ca2+/Mg2+-free
- Dispase
- Collagenase type I
- Liberase TM Research Grade
- 0.25% Trypsin/EDTA
- Bovine Serum Albumin
- Trypan Blue
- Ice

Instruments

- CO₂ supplemented 37°C incubator
- Chromium Instrument/X, 10X Genomics
- Countess 3 Automated Cell Counter, Invitrogen
- 15ml and 50ml tubes-compatible centrifuge
- 1.5ml tube-compatible centrifuge

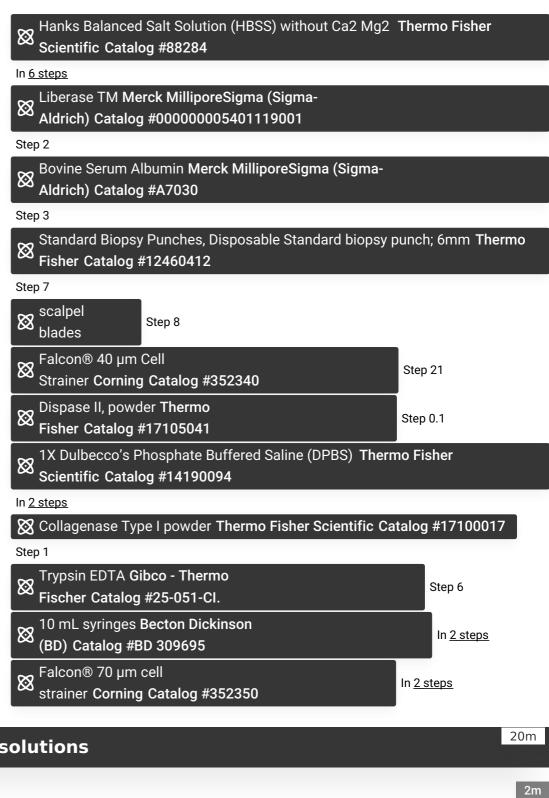
Surgical tools

- 6-mm punch
- Scissors
- Forceps
- Bistouri
- Blades G23
- 10mL syringes
- 21G needles

Other labware

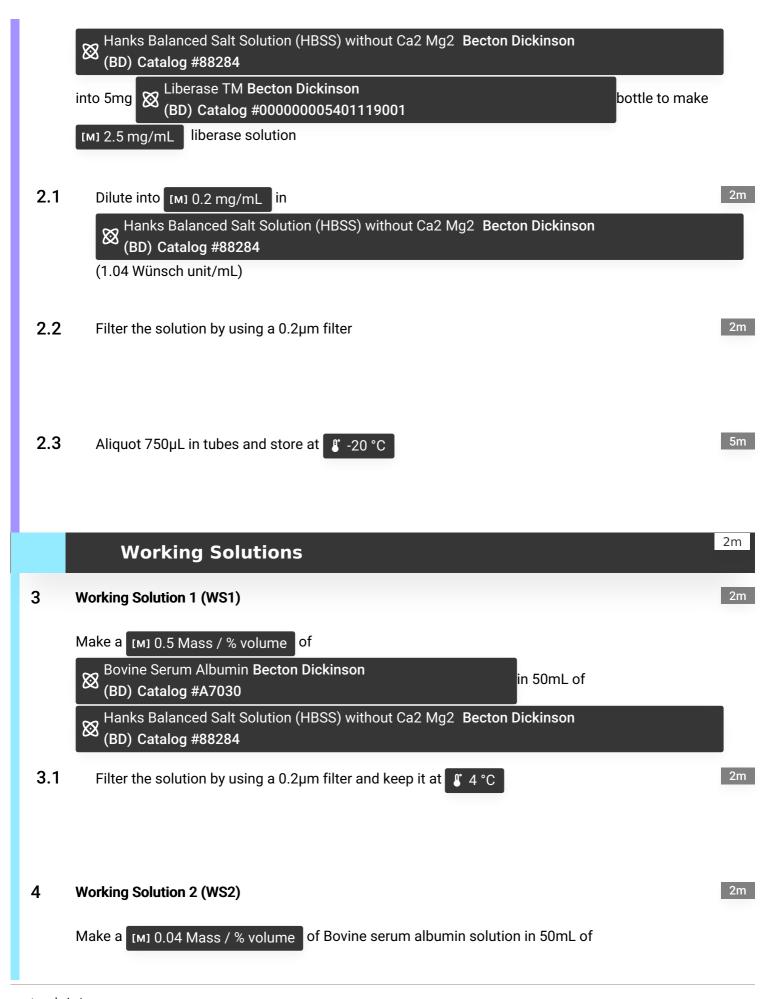
- 50ml centrifuge tubes
- 15ml centrifuge tubes
- 1.5ml Protein LoBind tubes
- Disposable haemacytometer (4 per sample)
- 10mm dishes
- 6-well plates
- Pasteur pipettes

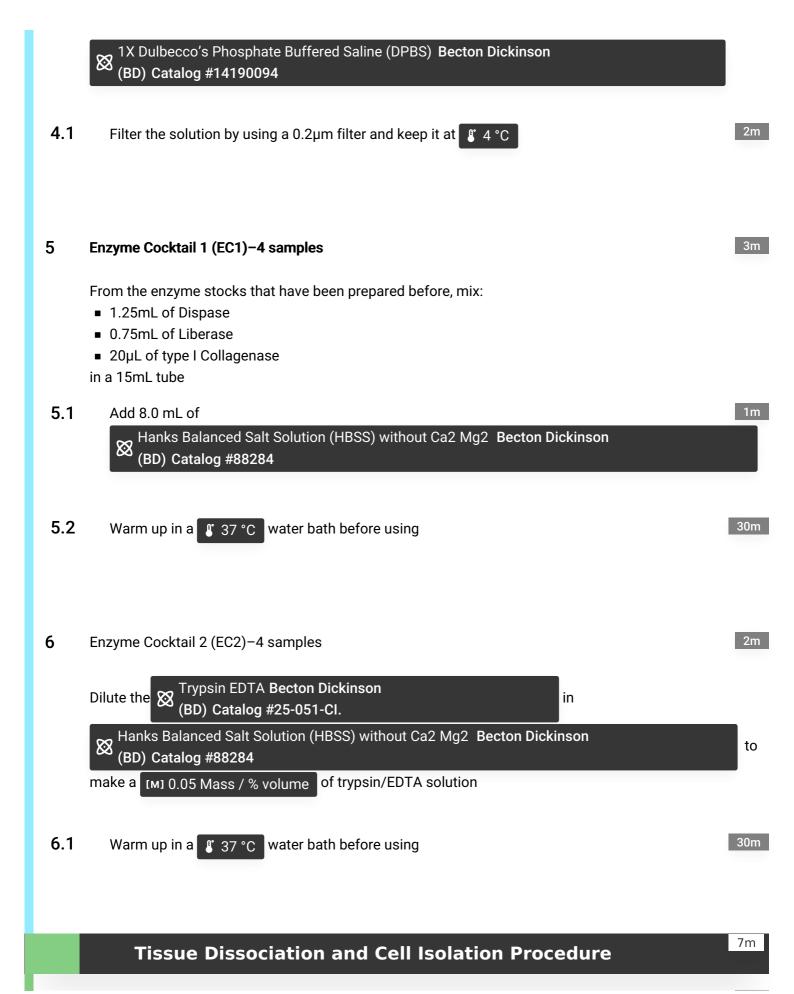
PROTOCOL MATERIALS

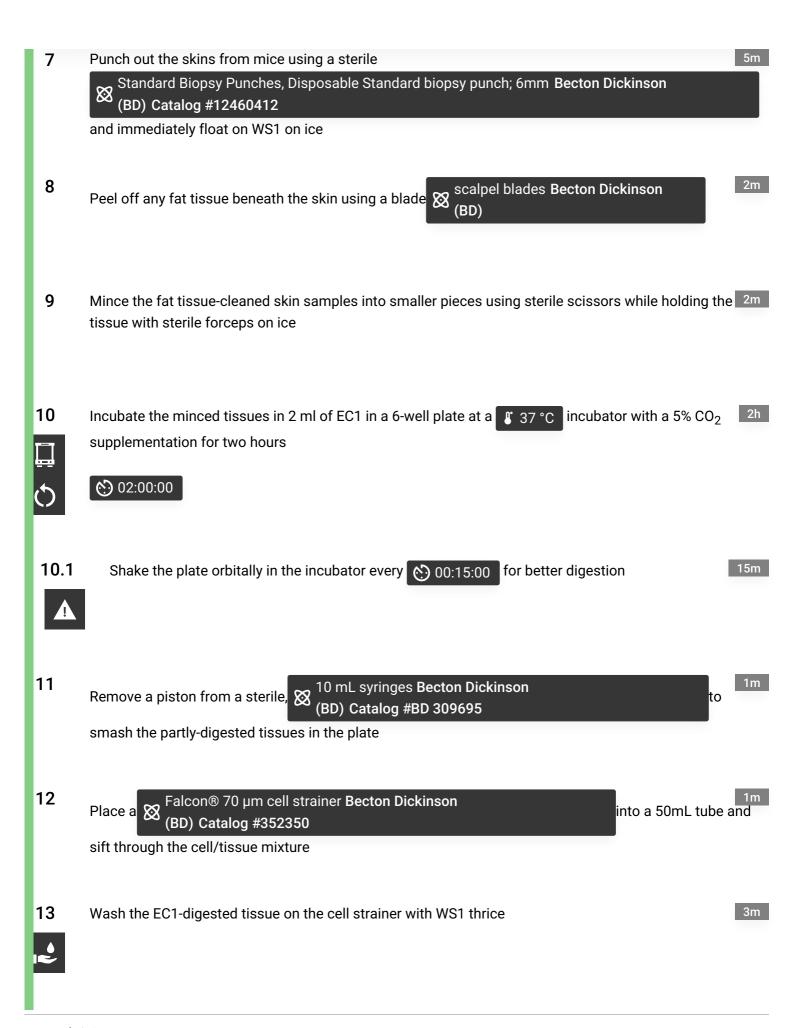


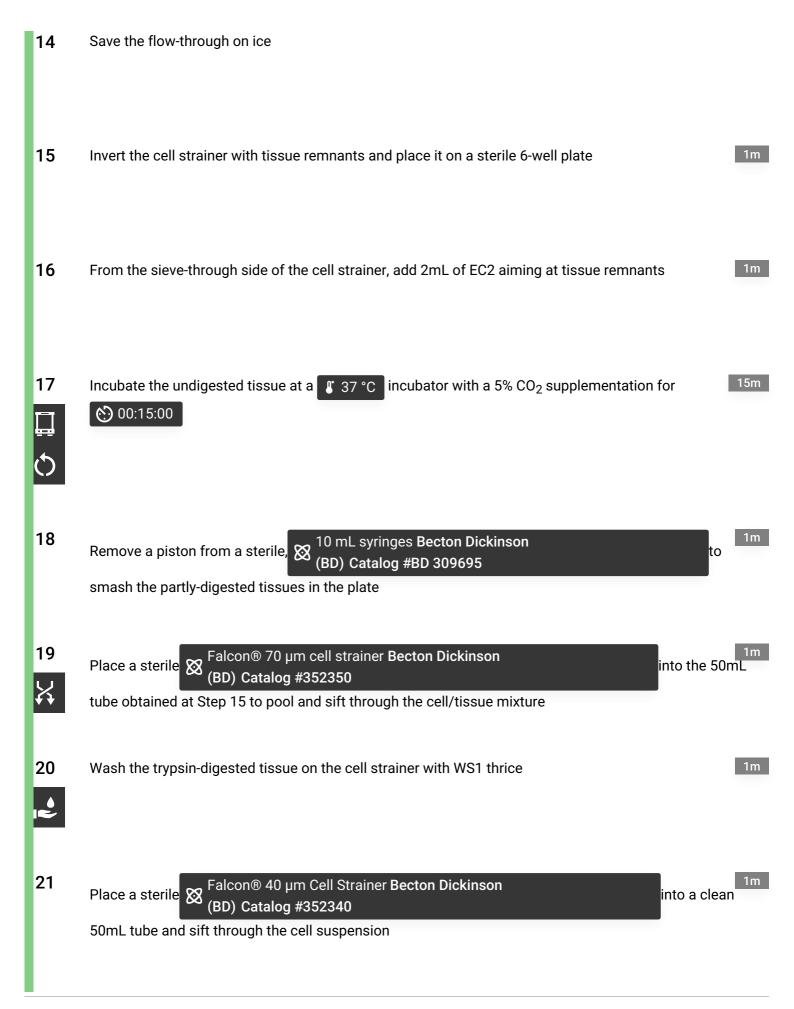




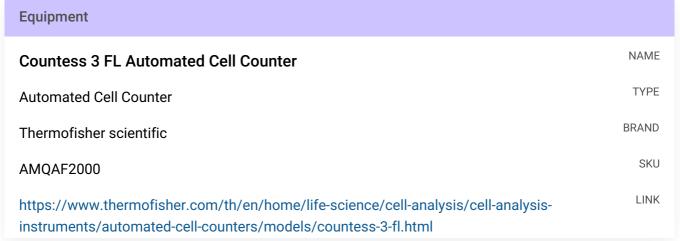








22 Centrifuge the pooled cell suspensions at 300 x g, 20°C, 00:10:00 10m 23 2m Carefully aspirate the supernatant and resuspend the pellet in 1mL of WS2 24 Centrifuge the pooled cell suspensions again at 300 x g, 20°C, 00:05:00 25 Carefully aspirate the supernatant and resuspend the pellet in 500µL of WS2 26 Determine the cell viability by mixing 10µl of the cell suspensions with 10µl of trypan blue 2m Equipment NAME Countess 3 FL Automated Cell Counter TYPE **Automated Cell Counter BRAND**



26.1 Take at least four counts per sample



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26.2 Remember to tick "Trypan Blue correction" on the Countess cell counter





27 Average cell numbers for each sample and dilute each cell suspension with WS2 to adjust the cell number to 700-1200 cells/µL as indicated in 10X Genomics Chromium Next GEM Single Cell 3' Reagent Kits v3.1 Protocol (CG000315)

27.1 Aim for cell viability above 70% for each sample



Expected result

Cell viability: 70% per count

27.2 If the cell viability is below 70%, you may choose to remove dead cells using a dead cell removal kit



28 Immediately proceed with Chromium Next GEM Single Cell 3' Reagent Kits v3.1 Protocol (CG000315)