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Cell isolation from dorsal mouse skin for single-cell RNA-seq

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

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Abstract

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

Materials

Consumables

- Hank's Balanced Salt Solution Ca^{2+}/Mg^{2+}-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

- CO_2 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

- Dispase II, powder **Thermo Fisher** **Catalog #**17105041
- 1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific** **Catalog #**14190094
- Collagenase Type I powder **Thermo Fisher Scientific** **Catalog #**17100017
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- Liberase TM **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**000000005401119001
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A7030
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- 1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific** **Catalog #**14190094
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- Trypsin EDTA **Gibco - Thermo Fisher Scientific** **Catalog #**25-051-CI.
- Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific** **Catalog #**88284
- Standard Biopsy Punches, Disposable Standard biopsy punch; 6mm **Thermo Fisher** **Catalog #**12460412
- scalpel blades
- 10 mL syringes **Becton Dickinson (BD)** **Catalog #**BD 309695
- Falcon® 70 µm cell strainer **Corning** **Catalog #**352350
- 10 mL syringes **Becton Dickinson (BD)** **Catalog #**BD 309695
- Falcon® 70 µm cell strainer **Corning** **Catalog #**352350
- Falcon® 40 µm Cell Strainer **Corning** **Catalog #**352340

Troubleshooting

Enzyme solutions

20m

1 **Dispase Solution** 2m

Dissolve 0.5g of Dispase II, powder **Thermo Fisher Catalog #**17105041 in a 50ml of 1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #**14190094 to make [M] 10 mg/mL of Dispase II solution

0.2 Filter the solution by using a 0.2µm filter 2m

0.3 Aliquot 1.25mL in tubes and store at -20 °C 5m

1 **Collagenase type I Solution** 2m

Dissolve 1g of Collagenase Type I powder **Thermo Fisher Scientific Catalog #**17100017 in 4mL of Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284 to make a [M] 250 mg/mL collagenase type I solution

1.1 Make a stock solution 2m

i.e. 67500U/mL x 4mL = 50U/µL x V_{max}
V_{max} = 5.4mL
5.4 - 4.0 = 1.4mL to add the stock to make the stock solution

1.2 Filter the solution by using a 0.2µm filter 2m

1.3 Aliquot 20µL in tubes and store at -20 °C 5m

2 **Liberase TM Solution** 2m

Punch through the cap with a syringe to add 2mL of

Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284

into 5mg

Liberase TM **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**000000005401119001

bottle to make [M] 2.5 mg/mL liberase solution

2.1 Dilute into [M] 0.2 mg/mL in 2m

Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284

(1.04 Wünsch unit/mL)

2.2 Filter the solution by using a 0.2µm filter 2m

2.3 Aliquot 750µL in tubes and store at -20 °C 5m

Working Solutions 2m

3 **Working Solution 1 (WS1)** 2m

Make a [M] 0.5 Mass / % volume of

Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7030 in 50mL of

Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284

3.1 Filter the solution by using a 0.2µm filter and keep it at 4 °C 2m

4 **Working Solution 2 (WS2)** 2m

Make a [M] 0.04 Mass / % volume of Bovine serum albumin solution in 50mL of

1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #**14190094

4.1 Filter the solution by using a 0.2µm filter and keep it at 4 °C 2m

5 **Enzyme Cocktail 1 (EC1)–4 samples** 3m

From the enzyme stocks that have been prepared before, mix:
- 1.25mL of Dispase
- 0.75mL of Liberase
- 20µL of type I Collagenase

in a 15mL tube

5.1 Add 8.0 mL of 1m
Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284

5.2 Warm up in a 37 °C water bath before using 30m

6 Enzyme Cocktail 2 (EC2)–4 samples 2m

Dilute the Trypsin EDTA **Gibco - Thermo Fisher Scientific Catalog #**25-051-CI. in
Hanks Balanced Salt Solution (HBSS) without Ca2 Mg2 **Thermo Fisher Scientific Catalog #**88284
to make a [M] 0.05 Mass / % volume of trypsin/EDTA solution

6.1 Warm up in a 37 °C water bath before using 30m

Tissue Dissociation and Cell Isolation Procedure 7m

7 Punch out the skins from mice using a sterile 5m
Standard Biopsy Punches, Disposable Standard biopsy punch; 6mm **Thermo Fisher Catalog #**12460412
and immediately float on WS1 on ice

8 Peel off any fat tissue beneath the skin using a blade scalpel blades 2m

9 Mince the fat tissue-cleaned skin samples into smaller pieces using sterile scissors while holding the tissue with sterile forceps on ice 2m

10 Incubate the minced tissues in 2 ml of EC1 in a 6-well plate at a 37 °C incubator with a 5% CO_2 supplementation for two hours 2h

02:00:00

10.1 Shake the plate orbitally in the incubator every 00:15:00 for better digestion 15m

11 Remove a piston from a sterile, 10 mL syringes **Becton Dickinson (BD) Catalog #**BD 309695 to smash the partly-digested tissues in the plate 1m

12 Place a Falcon® 70 µm cell strainer **Corning Catalog #**352350 into a 50mL tube and sift through the cell/tissue mixture 1m

13 Wash the EC1-digested tissue on the cell strainer with WS1 thrice 3m

14 Save the flow-through on ice

15 Invert the cell strainer with tissue remnants and place it on a sterile 6-well plate 1m

16 From the sieve-through side of the cell strainer, add 2mL of EC2 aiming at tissue remnants 1m

17 Incubate the undigested tissue at a 37 °C incubator with a 5% CO_2 supplementation for 00:15:00 15m

18 Remove a piston from a sterile, 10 mL syringes **Becton Dickinson (BD) Catalog #**BD 309695 to smash the partly-digested tissues in the plate 1m

19 Place a sterile Falcon® 70 µm cell strainer **Corning Catalog #**352350 into the 50mL tube obtained at Step 15 to pool and sift through the cell/tissue mixture 1m

20 Wash the trypsin-digested tissue on the cell strainer with WS1 thrice 1m

21 Place a sterile Falcon® 40 µm Cell Strainer **Corning Catalog #**352340 into a clean 50mL tube and sift through the cell suspension 1m

22 Centrifuge the pooled cell suspensions at 300 x g, 20°C, 00:10:00 10m

23 Carefully aspirate the supernatant and resuspend the pellet in 1mL of WS2 2m

24 Centrifuge the pooled cell suspensions again at 300 x g, 20°C, 00:05:00 5m

25 Carefully aspirate the supernatant and resuspend the pellet in 500µL of WS2 1m

26 Determine the cell viability by mixing 10µl of the cell suspensions with 10µl of trypan blue 2m

Equipment

Countess 3 FL Automated Cell Counter	NAME
Automated Cell Counter	TYPE
Thermofisher scientific	BRAND
AMQAF2000	SKU
https://www.thermofisher.com/th/en/home/life-science/cell-analysis/cell-analysis-instruments/automated-cell-counters/models/countess-3-fl.html	LINK

26.1 Take at least four counts per sample 8m

26.2 Remember to tick "Trypan Blue correction" on the Countess cell counter 1m

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)** 5m

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)**