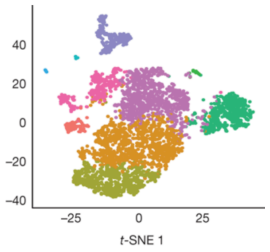
Apr 05, 2019

Colonic epithelial cell isolation for Single Cell RNA-sequencing



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Human Cell Atlas Metho...

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

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Abstract

Protocol for isolation of single colonic epithelial cells from human endoscopic biopsies.
Reference - https://doi.org/10.1038/s41586-019-0992-y

Before start

- Transport medium (500ml) – DMEM (high glucose) 500mL, Penicillin/Streptomycin 5ml, HEPES 5ml (1M)
- HPGA (500ml) - HBSS 500mL, Penicillin/Streptomycin 5ml, HEPES 5ml (1M)
- Wash medium (50ml) – HPGA + 1mM EDTA + 1mM DTT
- Chelation medium (50ml) – HPGA + 1mM EDTA
- PBS + 0.04% BSA (50ml)

Before Start: Place chelation medium in water bath (37 degrees) and wash medium / transport medium on ice

Starting material: >2 pairs of colonic biopsies collected during endoscopy

1 Biopsies transported on ice in 10ml of transport medium

2 Wash with 10ml of cold (4degree) wash medium, shake, remove media, repeat a total of 3 times

3 Transfer biopsies to 5mL warm (water bath at 37 degree) chelation medium

4 Incubate at 37C for 20mins – shake after 10mins and 20mins

5 Remove supernatant, Transfer biopsies to fresh 5ml warm chelation medium

6 Incubate at 37C for 10mins

7 Vortex 2 × 5s (or 5-10 hard shakes)

8 Allow biopsies to settle. Remove supernatant with FCS-coated pasteur, keep supernatant and replace 5ml warm chelation medium on biopsies. Return to 37C water bath.

9 Examine supernatant. If it contains crypts, spin down at 300G 4mins 4C

10 Resuspend crypt pellet in 2ml cold transport medium, place in 15ml Falcon on ice.

11 Repeat steps 5-10 and pool crypt containing fractions (3 or max 4 times)

12 Spin down pooled crypt suspension (300g, 4mins)

13 Resuspend in 3ml warm TrypLE Express with 50ug/ml DNAse at 37C

14 Incubate 45mins at 37C in incubator with agitation or rotation (e.g. MACSmix.)

15 Quench with 3ml of Transport medium supplemented with 5% FCS

16 Filter with 70um cell strainer (prepped with FCS) into 50ml Falcon, wash through with 5 ml of transport medium +5% FCS

17 Remove filter, wash with 15ml Transport Medium supplemented with 5% FCS

18 Spin filtrate 300G 4mins

19 Remove supernatant. Wash pellet with 10ml Wash medium

20 Spin 300G 5mins.

21 Very carefully remove supernatant until est. 1ml remains. Do not disturb pellet, resuspend pellet in this.

22 Run through 30um cell strainer (prepped with FCS) and wash through with 5ml of PBS + 0.04% BSA

23 Spin 300G 4 mins, aspirate until <1ml. re-suspend in <1ml of remaining PBS +0.04% BSA, transfer to Eppendorf and add additional volume of PBS +0.04% BSA to make 1ml total.

24 Take 10uL for count (Trypan blue, Invitrogen Countess or haemocytometer). For 10x count each sample x3 to obtain average count and viability. Ensure high viability.

25 Centrifuge (300G, 5 mins) and re-suspend using live count to concentration of 1000 cells/ul (1×10^6 / ml).

26 Process on 10x using manufacturers protocol