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Working

## Colonic epithelial cell isolation for Single Cell RNA-sequencing

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Human Cell Atlas Method Development Community

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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>

### THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

<https://doi.org/10.1038/s41586-019-0992-y>

### PROTOCOL STATUS

#### Working

We use this protocol in our group and it is working

### SAFETY WARNINGS

#### BEFORE STARTING

- 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 x 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 ( $1 \times 10^6$  / ml).
- 26 Process on 10x using manufacturers protocol



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