Gut_All Cells_Human

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Abstract

This protocol has been tested by me on human adult colon samples and given good results for scRNAseq. I suggest that you do an enrichment step afterwards, because the dissociation can be quite variable results in terms of the fraction of immune vs non-immune cells that are retreived.

Citation: Kylie James Gut_All Cells_Human. **protocols.io**
dx.doi.org/10.17504/protocols.io.q3xdypn
Published: 18 Jun 2018

Materials

D-PBS (Without Ca++ and Mg++) 500 mL 37350 by Stemcell Technologies

Liberase DH 5401054001 by Roche

Hyaluronidase 385931-25KU by Merck Millipore

HBSS Media 41122100-007 by Sigma

DNAse II D8764-30KU by Sigma Aldrich

Protocol

Step 1.

Receive samples. Take about 4mm^2 sample for OCT

Step 2.

Wash samples twice with HBSS medium. In petri dish, mince sample into 0.5cm pieces.

Step 3.

Incubate the samples in 2ml HBSS medium containing 1.07 Wunsch units/ml (2.14wu) Liberase DH (Roche Applied Science, Germany) and 70 U/ml hyaluronidase (Calbiochem/Merck, Germany) for 45 min at 37C on a shaking platform at 600 rpm.

Step 4.

Homogenise the sample every 15 min the solution by pipetting up and down with a 1ml pipette for 1 min.

Step 5.
Pass cells through a 40mM sieve. Spin them down at 300 g at 4C for 10 min.

Step 6.
Resuspended in 300ml PBS buffer containing 100 IU/ml DNAse II (Sigma Aldrich, Missouri, USA).

Step 7.
Proceed with bead enrichment as per manufacturers protocols. Typically this will be a Miltenyi CD45+ enrichment.

Step 8.
Count cells and dilute to the desired concentration with PBS.

Step 9.
Load cells (aim for 5000/run) on to the 10X.

Step 10.