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Human Liver Tissue Dissociation for 10x Single Cell RNA-seq

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Ankur Sharma[1], Ramanuj dasgupta[2]

[1]Genome Institute of Singapore; [2]GIS

Human Cell Atlas Metho...

Ankur Sharma
Genome Institute of Singapore

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

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Abstract

Liver Tissue dissociation of single-cell RNA-seq (10x Single-cell RNA-seq)

Buffers

1 **Before tumor arrives**

Note

Prepare all buffers fresh on the day of experiment

- Prepare dissociation buffer (2x) Collagenase IV (Gibco 17104-109)
- 4.3mg for 10ml of RPMI
Add 50µl of DNAseI
**Sterile filterand keep it at 37 °C **
** 5 ml of (2x) dissociation buffer + 5 ml of RPMI (without serum) per tumor **

- Prepare Liver Dissociation Buffer
-15 g BSA
1 ml EDTA (0.5M)
500ml DPBS
**Sterile filterand keep it at 4 °C **

- Prepare Liver Dissociation Buffer-2
0.1 g BSA
100ml DPBS
**Sterile filterand keep it at 4 °C **

-Prepare 70µ & 40µ filters, 15 & 50 ml falcons and surgical blades, RBC lysis buffer

Dissociation

2 -Check if tumor arrived in MACS Tissue Storage Solution (130-100-008)

Note

Please provide 15ml falcons with 5ml MACS Tissue Storage Solution to your clinical collaborators to be kept at 4o C

-Transfer into 10cm dish with warm 5ml of RPMI media

-Cut into small pieces using scalpels

-Transfer the chopped tumor (5ml in RMPI) into 50ml falcon with 5ml of 2x dissociation buffer

-Incubate at 37 °C for 40:00:00

-Re-suspend with 10ml pipette followed by 16z needle, re-suspend gently

-Add 25ml of Buffer-1cold pour the content through a 70µ strainer into a new 50ml falcon tube

-Rinse the strainer with 10ml of Buffer-1

-Discard strainer, Centrifuge @ 800g, 06:00:00 4 °C

-Remove supernatant, add 5ml of 1x RBC lysis buffer (BioLegend420301) to cells, incubate for 5mins in ice

-Add 10ml of Buffer-1, Centrifuge @ 800g, 06:00:00 4 °C

-Discard supernatant, add 5ml of Buffer-1, pour the content through a 40µ strainer into a new 15ml falcon tube

-Rinse the strainer with 5ml of Buffer-1

-Centrifuge @ 800g, 06:00:00 4 °C

Proceed for dead cell removal

Dead Cell Removal

3 Miltenyi Kit: 130-090-101

-Prepare 1x binding buffer by dilution of 20x binding buffer solution

-Collect cells centrifuge at 300g and remove sup

-Re-suspend cells in dead cell removal microbeads

-Mix well and incubate for 15:00:00 at 25 °C

-Place LD column in a MACS separator, prepare by rinsing with 2ml of 1x binding buffer

Note

In our experience large columns gives much better viable cell recovery compare to medium and smaller columns

-Apply cell suspension onto the column. Rinse with 2ml of 1x binding buffer

-Collect effluent as live cell fraction

-Centrifuge @ 800g, 06:00:00 4 °C

-dissolve the cell pellet in Buffer-2

MACS depletion of CD45 positive cells

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- Prepare MACS buffer PBS (pH 7.2) 0.5% BSA 2 mM EDTA **Sterile filterand keep it at 4 °C

Note

In our experience this procedure works only when you have more than 0.5x10^6total cells

- Resuspend upto 10^7 cells in 80 µlof MACS buffer and add 20 µl CD45 microbeads (130-045-801)

- Mix well by pipetting 10 times and incubate for 15:00:00 at 4 °C

- Add 2ml of MACS buffer and Centrifuge @ 800g, 06:00:00 4 °C

-Resuspend the cell pellet in 1ml of MACS buffer

-Place LD column in a MACS separator, prepare by rinsing with 2ml of MACS buffer(In our experience large columns gives much better viable cell recovery compare to medium and smaller columns)

-Apply cell suspension onto the column. Rinse with twice with 2ml of MACS buffer

-Collect 4ml of effluent as CD45 negativecell fraction

-Plunge the column with 2ml of MACS buffer and collect this effluent as CD45 positivecell fraction

-Count the number of CD45+ and CD45- cell fractions (at least thrice and on two different cell counters)

Enrichment of Hepatocytes

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Note

In our experience we have observed unproportionate number of immune cells in 10x data (after cell ranger and filtering for good quality of cells i.e. cells with more than 200 genes). Therefore, we adopted this strategy to deplete CD45+ which increase the number of hepatocytes in 10x experiments.

- Add equal number of CD45+ and CD45- cells to achieve the final concentration of 1000 cells/µl in total of 100µl solution

- Count cells (at least twice on two different cell counters)

-Proceed for 10x experiment