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Dissociation of Jejunum cells for clumps sorting

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ABSTRACT

Single-cell RNA sequencing combined with spatial information on landmark genes enables the reconstruction of spatially-resolved tissue cell atlases. However, such approaches are challenging for rare cell types since their mRNA contents are diluted in the spatial transcriptomics bulk measurements used for landmark gene detection. To overcome the limitations in reconstructing spatial expression profiles of rare cells, we present ClumpSeq, an approach for sequencing small clumps of attached tissue cells. Sequencing clumps increase the capture rate of rare cell types without the need for antibody enrichment and utilizes the spatial information of the major tissue cell type. We use this approach to reconstruct spatial maps of all intestinal secretory epithelial cell types along the crypt-villus axis, revealing zonated immune-modulatory programs and heterogeneous migration patterns. ClumpSeq can be applied for reconstructing spatial atlases of rare cell types in other tissues and tumors.

ATTACHMENTS

dsmrbbmpx.pdf

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EXTERNAL LINK

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MATERIALS TEXT

Material:

- DPBS (Ca/Mg-free)
- EDTA ([M]**0.5 Molarity (M)**)
- DMEM high glucose
- FBS (10%)
- FACS buffer
- Hepes ([M]**1 Molarity (M)**)
- Hoechst
- Zombie Green Fixable Viability Kit
- Reserpine ([M]**200 Milimolar (mM)** in acid acetic)
- DNase I
- **100 µm** mesh

Dissociation of Jejunum cells for clumps sorting 1h 5m

1 Prepare buffers:

Buffer1 (25ml):

A	B
DPBS (-/-)	24.5 ml
EDTA 0.5M (10mM)	500 µl

Buffer2 (50ml): For Hoechst

A	B
DMEM high glucose	44.5 ml
10% FBS	5 ml
Hepes 1M (10mM)	500 µl

2 

Set a centrifuge to **4 °C** .

3 Warm at **37 °C** **15 mL** of buffer1 and **5 mL** of buffer2.

4 Prepare a tube with cold DPBS and put it **On ice** .

5 Thaw the FBS.

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IMPORTANT: The whole procedure needs to be done on ice!!!.

6 Collect the tissue: proximal Jejunum - take **8 cm** of tissue, starting **8 cm** distal of the stomach.

7 

Wash the tissue: use a syringe filled with DPBS to flush the inside.

8 Lateralize the tissue to flat it.

9 Remove adjacent fat.

10  20m

Incubate the tissue for **00:20:00** in buffer1 **On ice** .

11 Move the tissue in the pre-warmed buffer1 and cut it into small pieces (~ **1 cm**).

12 Add **1 U** of DNase I in the tube.

13  5m

Incubate for **00:05:00** at **37 °C** - shake every minute.

14 Filter the solution through a **100 µm** mesh.

15  5m

Spin down in the cold centrifuge (**4 °C**) for **00:05:00** at **300 g** .

16 Discard the supernatant.

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17 Resuspend the pellet in **8 mL** cold DPBS + **2 mL** FBS and use the pipette to break the pellet gently.

18 Filter the solution through **100 µm** mesh.

19 

5m

Spin down in the cold centrifuge (**4 °C**) for **00:05:00** at **300 g** .

20 Discard the supernatant.

21 Resuspend the pellet in **5 mL** of pre-warmed buffer2 with Hoechst (1:500) + Reserpine (1:4000) for **00:05:00** a **Room temperature** (keep it in the dark).

5m

22 

5m

Spin down in a cold centrifuge (**4 °C**) for **00:05:00** at **300 g** .

23 Discard supernatant.

24 Resuspend in DPBS and add 1:500 Zombie for **00:15:00** **Room temperature** (Zombie works only in DPBS!!!).

15m

25 

5m

Wash 2x (**00:05:00** **300 g** **4 °C**).

26 Discard the supernatant.

27 Resuspend the pellet in FACS buffer (accordingly to the pellet).

The sample is ready for the FACS sorting!!!

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