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# Dissociation of Jejunum cells for clumps sorting

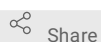
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1 Works for me



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[dx.doi.org/10.17504/protocols.io.bvq3n5yn](https://dx.doi.org/10.17504/protocols.io.bvq3n5yn)

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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

[dsmrbmpx.pdf](#)

## DOI

[dx.doi.org/10.17504/protocols.io.bvq3n5yn](https://dx.doi.org/10.17504/protocols.io.bvq3n5yn)

## EXTERNAL LINK

<https://www.nature.com/articles/s41467-021-23245-2>

## PROTOCOL CITATION

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<https://dx.doi.org/10.17504/protocols.io.bvq3n5yn>

## KEYWORDS

Jejunum cells, Clumps sorting, ClumpSeq

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## CREATED

Jun 11, 2021

## LAST MODIFIED


Jun 17, 2021

## OWNERSHIP HISTORY

Jun 11, 2021 Urmilas

Jun 16, 2021 Rita Manco

**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.

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.





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** <sup>5m</sup> a  
 **Room temperature** (keep it in the dark).

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!!!). <sup>15m</sup>

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!!!

