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JAX-Sen: Mouse kidney dissociation for single-cell RNA sequencing

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Cellular Senescence Net…

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We use this protocol and it's working

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Abstract

These samples are part of the JAX-Sen project in the SENNET Consortium. We aim to study and characterize senescence in the C57Bl/6 mouse kidney. We compare young (6 months old) and old (24 months old) mouse kidneys using scRNAsequencing. This protocol describes the single-cell dissociation of the kidneys before library preparation and sequencing.

Reagents and Materials

1

- 5 ml Protein lobind tubes
- 1.5 or 2 ml Protein lobind tubes
- Micro scissors/ scalpel
- Ice-cold PBS
- Wide-bore pipette tips
- GentleMACS dissociator
- GentleMACS C tubes
- DMEM + 10% FBS
- ACK lysis buffer – 2 ml/sample
- SS_buffer– 5 ml/sample
- 40um cell strainer
- 70uM strainer

SS_Buffer:

	A	B	C	D	E	F	G	H	I
		Stock conc.	Working conc.	50 ml	100 ml	250 ml	500 ml	20 ml	
	EDTA	0.5 M	2 mM	0.2	0.4	1	2	0.08	ml
	BSA	10%	2%	10	20	50	100	4	ml
	FBS	100%	15%	7.5	15	37.5	75	3	ml
	DPBS	1X	make up	32.2	64.4	161	322	12.88	ml

Procedure

2 Collect shipment from the warehouse.

2.1 Fill up ice buckets and prep them with reagents:
Bucket 1(small): Enzyme mix, PBS, SS_buffer
Bucket 2 (large): For mincing tissues and sample tubes

2.2 Confirm if the sample is cold. Take pictures. Transfer sample to 5 ml tubes.

2.3 Prepare enzyme mix by adding 2.35 mL of serum-free RPMI 1640 or DMEM, 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A of the Multi Tissue Dissociation Kit 1 into a

gentleMACS C Tube for up to 0.5 g of tissue.

2.4 Mince mouse kidneys (2-4 mm^3) with sterile micro scissors.

2.5 After cutting, transfer the sample into labeled 5 ml tubes.

2.6 Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

2.7 Run the gentleMACS Program Multi_B. If using the heating function of the gentleMACS Octo Dissociator with Heaters run program 37C_Multi_E.

2.8 After termination of the program, detach C Tube from the gentleMACS Dissociator.

2.9 Resuspend sample and apply the cell suspension to a MACS® SmartStrainer (70 µm) placed on a 5 mL tube.

2.10 Wash MACS SmartStrainer (70 µm) with **4 mL of ice-cold PBS.**

2.11 Centrifuge at **300 x g, 4°C, 7 min** to get rid of the supernatant. Aspirate supernatant.

2.12 Resuspend the cell pellet in 2 mL **ACK Lysing buffer**, and incubate on **ice for 3 min** to exclude RBCs.

2.13 Add 5 mL **SS_buffer** to stop ACK Lysing buffer reaction, then collect cells by centrifugation **500 x g, 4°C, 5 min**.

2.14 Resuspend the pellet in **1ml** of **ice cold PBS.**

2.15 Filter the cell suspension through **Flowmi 40um cell strainers** tube to remove debris and non-dissociated tissue fragments.

2.16 Collect cells by centrifugation **500 x g, 4°C, 5 min** and proceed to Flex protocol.