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JAX-Sen: Mouse heart 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 heart. We compare young (6 months old) and old (24 months old) mouse hearts using scRNAsequencing. This protocol describes the single-cell dissociation of the hearts 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
- 0.25% Trypsin/EDTA (Gibco, 25200056). - 2 ml/sample
- 20 mg/mL collagenase A and B (Sigma, 10103578001, 11088807001) – 2 ml/sample
- DMEM + 10% FBS
- 100 µm cell strainer (Corning, 431752)
- HBSS (Ca^{2+}/Mg^{2+} free) (Gibco, 14170120) – 2 ml/sample
- ACK lysis buffer – 2 ml/sample
- SS_buffer – 5 ml/sample
- 40 um cell strainer
- 70 uM 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): PBS, HBSS, DMEM+FBS, SS_buffer

Bucket 2 (large): For mincing tissue

2.2 Confirm if the sample is cold. Take pictures.

2.3 Place tubes on ice and mince the tissue (<1mm) with sterile micro scissors.

2.4 After mincing, transfer the sample into labeled 5 ml tubes.

2.5 Centrifuge at **500 x g, 4°C, 5 min** to get rid of the supernatant.

2.6 Resuspend the pellet in **ice-cold PBS (Ca+ and Mg+ free)** and pipette up and down gently with wide-bore pipette tip.

2.7 Centrifuge at **500 x g, 4°C, 5 min** to get rid of the supernatant.

2.8 Resuspend the pellet in **ice-cold PBS (Ca+ and Mg+ free)** and pipette up and down gently with wide-bore pipette tip.

2.9 Centrifuge at **500 x g, 4°C, 5 min** to get rid of the supernatant.

2.10 Add 2 ml of **0.25% Trypsin/EDTA** to the pellet and enzymatically digest at **37°C for 10 min** with slow rocking. ***Check at 5 mins for dissociation, under the microscope.***

2.11 Stop the digestion by adding an equal volume (1ml) of **DMEM/10% FBS**.

2.12 Centrifuge at **500 x g, 4°C, 5 min** to get rid of the supernatant.

2.13 Resuspend the pellet 2 ml of **20 mg/mL collagenase A and B** mixture and incubate the samples at 37 °C until the tissues were into single cells.

2.14 Vigorously pipette samples with 1 ml wide-bore pipette tips or if needed, regular tips.

2.15 Filter cells through **MACS SmartStrainers (70um)** and wash 1 ml **HBSS (Ca^{2+}/Mg^{2+} free)** through the strainer.

2.16 Centrifuge at **500 x g, 4°C, 5 min** to get rid of the supernatant.

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

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

2.19 Resuspend the pellet in **1ml** of **HBSS (Ca^{2+}/Mg^{2+} free)** (Gibco, 14170120)**.**

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

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