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# JAX-Sen: Mouse placenta single-cell dissociation for single-cell RNA sequencing

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Cellular Senescence Net...

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

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**Keywords:** cell dissociation of the placenta tissue, cell rna, placenta tissue, cell dissociation, senescence, sequencing, cell, single cell rna

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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. This protocol describes the single-cell dissociation of the placenta tissue before library preparation and sequencing.

## Reagents and Materials:

1 1. 6 cm Petri dishes
2. Ice-cold MACS buffer
3. 5 ml Protein lobind tubes
4. Micro scissors/ scalpel
5. Ice-cold PBS
6. Wide-bore pipette tips
7. TrypLE express (10 volumes)
8. Dispase (0.4 g/ml)
9. Collagenase
10. DMEM + 10% FBS
11. ACK lysis buffer
12. CS_buffer
13. 40 um cell strainer (Flowmi)
14. 70 uM strainer

## Procedure:

2 Add sample labels and collect shipment from the warehouse.

## Procedure:

3 Fill up ice buckets and prep them with reagents:
Bucket 1: PBS, MACS buffer, DMEM, DMEM+FBS, SS_buffer
Bucket 2: For mincing tissue, either in 6 cm dishes or in tubes – protocol A
Bucket 3: For mincing tissue, either in 6 cm dishes or in tubes – protocol B
Bucket 4: extra ice

4 Confirm if the sample is cold.
Notes:
*Optional: Place the sample on a dish with **MACS buffer** and take pictures.*

5 Place tubes on ice and mince the tissue (<1mm) with sterile micro scissors (or) mince using a scalpel on 6 cm dish w/MACS buffer on ice.

6 After mincing, transfer the sample into labeled 5 ml/ 10 ml tubes.

7 Centrifuge at 800 x g, 4°C, **5 min** to get rid of the supernatant.

8 Resuspend the pellet in **ice-cold PBS** and pipette up and down gently with wide bore pipette tip.

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11 Centrifuge at 800 x g, 4°C, **5 min** to get rid of the supernatant.

12 Add 10 volumes (~500 uL) of **[1937.5 uL TrypLE + 50 uL Dispase + 12.5 uL RNAse inhibitor + 250 uL DNAseI]** to the pellet and enzymatically digest at **4°C for 35 min** with slow rocking.
***Check every 5-10 mins minutes for dissociation, under the microscope.***

13 Stop the digestion by adding an equal volume (~500 uL) of **DMEM/10% FBS**.

14 Filter cells through MACS SmartStrainers (70um)

15 Wash 1.5 ml DMEM through the strainer. ----------à this is the filtrate cell suspension
Filtrate: Leave on ice.
Residue:
1. Collect the residue with wide bore pipette tips and transfer them to a new 2 ml conical tube.
2. Add collagenase Type IV (0.5 mg/mL) and DNase I (0.2 mg/mL) to the residue and enzymatically digest for 10 min at 37 °C.
[500 ul DNaseI + 1.25 mg collagenase + 12.5 uL RNase inhibitor + 1862.5 uL DPBS (make up to 2.5 ml)]
3. Filter cells through MACS SmartStrainers (70um)
4. Wash 1.5 ml DMEM through the strainer. ⟶ this is the residue cell suspension

16 Pool the filtrate and residue cell suspensions and proceed to step 16

17 Centrifuge at 800 x g, 4°C, **5 min** to get rid of the supernatant.

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

19 Add **5** mL **CS_b**uffer to stop ACK Lysing buffer reaction, then collect cells by centrifugation 800 x g, 4°C, **5 min**.

20 Resuspend the pellet in **1**ml of **CS_buffer.**

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

22 Collect cells by centrifugation 800 x g, 4°C, **5 min** and proceed to 10X Flex protocol/ cell sorting.

23 CS_buffer (Cell suspension 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 | 2mM | 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 |