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JAX-Sen: Mouse pancreas 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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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 pancreas. We compare young (6 months old) and old (24 months old) mouse pancreas using scRNAsequencing. This protocol describes the single-cell dissociation of the pancreas before library preparation and sequencing.

Reagents and Materials

1

- 15 ml conical tubes – 4 per sample
- 1.5 or 2 ml Protein lobind tubes
- Micro scissors/ scalpel
- Ice-cold PBS
- Histopaque-1077 – Sigma H8889
- Wide-bore pipette tips
- Collagenase solution
- Wash buffer
- 100 µm cell strainer (Corning, 431752)
- ACK lysis buffer – 2 ml/sample
- SS_buffer – 5 ml/sample
- 40 um cell strainer
- 70 uM strainer
- 10 ml syringe and 18G needle if available

2 ***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

2.1

- ***Collagenase solution*:**
  For each mouse, dissolve 4mg collagenase powder in 10mL serum-free Medium 199 (or MEM). Prepare fresh daily. Fill 5mL syringe with 3mL collagenase P (Roche Applied Science 11 249 002 001) and 15mL tube with 5mL collagenase; label tubes and store on ice until ready to use. Note: each lot of collagenase must be tested experimentally to determine optimal working concentration. Use 0.4mg/mL as starting point.

- ***Wash buffer***:
  DMEM containing 20% FBS.

- ***Stopping media:*** DMEM + 10% FBS + 1:100 dilution Glutamate CMRL media

Procedure

2.2 Collect shipment from the warehouse.

2.3 Fill up ice buckets and prep them with reagents:
Bucket 1(small): PBS, HBSS, DMEM+FBS, SS_buffer
Bucket 2 (large): For mincing tissue

2.4 Confirm if the sample is cold. Take pictures.

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

2.6 After mincing, transfer the sample into labeled 15 ml tubes containing **5 ml of 0.4mg/ml collagenase solution.** Incubate at 37°C for 10 mins.

2.7 Remove and shake tubes vigorously until pancreatic tissue is completely dissociated with no large chunks visible, 10-20 seconds. Alternatively, triturate using 10 mL pipet until well-dispersed.

2.8 **Immediately** dilute digest with cold wash buffer to 15mL total volume. Invert several times to mix. **Keep all the tubes cold**.

2.9 Centrifuge at **300 x g, 4°C, 1 min** to pellet pancreatic tissue.

2.10 Decant supernatant into waste beaker. Add **12mL wash buffer** and resuspend pellet by shaking or with 10mL pipette.

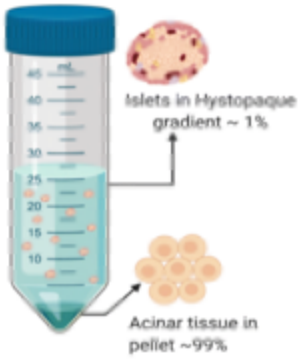
2.11 Repeat wash (steps #2.9-2.10) ***twice***. After the final spin, decant supernatant, then invert open tubes onto a paper towel and tap gently to remove all residual buffer.

2.12 Add **5mL Histopaque-1077** and gently resuspend pellet using 10mL pipette.

2.13 Hold tube at 45° angle and *slowly* layer **5mL wash buffer** on top of gradient layer (**10mL syringe** is helpful for slowly dispensing buffer). Do not allow buffer to mix with Histopaque! Very carefully transfer tubes to centrifuge.

2.14 **Turn off centrifuge brake**. Centrifuge at 300xg/4°C for 25 minutes.

2.15 *Islets should be visible floating at interface between Histopaque and wash buffer, or slightly below*. Remove islets from interface using sterile transfer pipette and transfer to new 15mL conical tube. Centrifuge at 300xg/4°C for 25 minutes. *Other cells (mostly acinar): in the pellet.*



Split the 2 layers and treat them as separate samples going forward.

Proceed to islets protocol and acinar+ducts protocols that we use for human pancreatic cells.

Acinars and Ducts:

2.16 Wash pellets from histopaq gradient **3x** with 10 ml cold PBS

2.17 Add 3 ml TrypLE E to the pellet and incubate at 37C (waterbath), mixing with pipette approximately every 5 mins. Check at 10 mins and every 5 mins following for single cells. (Ducts should take 10-15 mins, and acinar should take slightly longer ~20 mins. But we have a mixture here, so check under the microscope and stop reaction accordingly)

2.18 Add **3X stopping media (9 ml).**

2.19 Centrifuge at 1300 rpm/4°C for 2 minutes. Discard supernatant.

2.20 Wash again with **3 ml stopping media** and pellet cells. Resuspend in 1 ml of stop media.

2.21 Filter through 40 uM Flowmi filter.

2.22 Count cells and proceed to Flex protocol.

Islets:

2.23 Centrifuge the islets suspension at 130g/RT for 5 minutes.

2.24 Add 1.5 ml accutase, mix with pipette and incubate at 37C. Mix every 2 mins, checking at 6 mins.

2.25 Add CMRL media to approximately 15 ml total volume.

2.26 Centrifuge at 500xg/4°C for 5 mins.

2.27 Resuspend in 1 ml of CMRI media.

2.28 Filter through 40 uM filter.

2.29 Count cells and proceed to Flex protocol.