Adult mouse ear dissociation (on ice)

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Working
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Human Cell Atlas Method Development Community

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ABSTRACT

This protocol is used to dissociate adult (8-10 wk) mouse ear "on ice" (in order to reduce gene expression artifacts). The cell yield is 9000 cells/mg (213000 cells total from 23 mg tissue), with 98% viable. The protocol involves a 2-layered dissociation, incubating on ice with *bacillus licheniformis* enzyme. The dissociation is augmented by the use of a dounce homogenizer to help break up the tissue.

Ears adult mouse dissociation protocol.pdf

PROTOCOL STATUS

Working
We use this protocol in our group and it is working

GUIDELINES

***Bacillus Licheniformis* Enzyme Mix (1 mL per 23 mg tissue):**
100 µL b. lich 100 mg/mL (10 mg/mL final conc. - Sigma, P5380)
1 µL 0.5 M EDTA (0.5 mM final conc. - Sigma, A8806)
899 µL DPBS (no Ca, Mg) ThermoFisher (cat. #14190)

Preparing enzymes:
The enzyme is made up in DPBS (#14190). It is aliquoted and stored at -80 °C at 100 mg/mL in 100 µL aliquots.

Reagents
Bovine Serum Albumin - Sigma (A8806)
Hypothermosol FRS – Sigma (H4416)
Red Blood Cell Lysing Buffer – Sigma (R7757)

Required supplies:
2 mL dounce homogenizer – Bellco (1984-10002)
Centrifuge for 1.5 mL, 15 mL conicals
Pipettes and pipet tips
15 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters - Miltenyi (130-098-458)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Isolating tissue

1 Dissect out ear tissue and place in ice-cold hypothermosol on ice.

2 Using forceps, transfer ear tissue to petri dish on ice. **Mince ear tissue thoroughly on ice for 3-4 min on ice into 1-mm3 pieces** using razor blade while manipulating tissue with forceps – you will need to use grinding motion and vigorously break up tissue.

00:04:00 **mince tissue**

1st layer

3 Place **23 mg minced tissue** into 1 mL B. Lich enzyme cocktail. Incubate on ice. **23 mg tissue**

4 Shake every min; triturate every 2 min with p1000 w/tip cut (start triturating at 2 min) for 20 minutes.

00:01:00 **shake vigorously** 00:02:00 **triturate 10x** 00:20:00 **digest on ice**

5 After 20 mins of triturating on ice, use pipet to **transfer digest mix to 2 mL dounce homogenizer**. Use 10 strokes of Pestle A every 2 min (4 series total, 8 min). Digest mix should become turbid. 00:02:00 **dounce homogenize**

6 At **28 min total digest time**, let tissue chunks settle in dounce homogenizer for 2 min. Transfer **90% of supernatant (containing released cells)** using 1 mL serological pipet onto a 30 µM filter placed on a 15 mL conical.

00:02:00 **let tissue settle**

7 Rinse filter w/5 mL ice-cold PBS/BSA 0.04%. **Save flow through containing released cells on ice and keep filter on tube for 2nd layer.**

2nd layer

8 Add additional 1 mL b. lich enzyme mix to residual tissue chunks in dounce homogenizer. Continue incubating on ice.

9 **Use 10 strokes of pestle A every 2 min. for 15 additional minutes** (45 min. total time).

00:02:00 **dounce** 00:15:00 **additional digest time**

10 **Transfer entire volume to same 30 µM filter on 15 mL conical**. Rinse with additional 5 mL ice-cold PBS/BA 0.04%.

Preparing cells for single cell sequencing

11 Centrifuge cells for 300 g for 5 min at 4 °C.

12 Remove supernatant & re-suspend in 100 µL ice-cold PBS/BSA 0.04% (leave in 15 mL conical).

13 Add 1 mL RBC lysis buffer to cell mix. Triturate 10X. Let sit on ice for 2 min.

14 Add 12 mL ice-cold PBS/BSA 0.04% to dilute RBC lysis buffer.

15 Spin 300 g for five min. Remove supernatant. Re-suspend in 100-200 µL PBS/BSA 0.04%.

16 Examine using hemocytometer with trypan blue.

17 Adjust concentration to 1,000 cells/µL for Chromium or 100 cells/µL for DropSeq.