Skin adult mouse dissociation protocol **9/14/18**

***Bacillus Licheniformis* Enzyme Mix (1 mL per 23 mg tissue):**
100 µL b. lich 100 mg/mL (10 mg/mL final) (Sigma, P5380)
1 µL 0.5 M EDTA (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
Enzymes, trypsin inhibitor, BSA and DNAse are made up in DPBS (no Ca, no Mg) from Thermo Fisher (14190).
Bovine Serum Albumin - Sigma (A8806).
Hypothermosol FRS

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)

Cell yield:
Skin: 3000 cells/mg (69,750 cells total from 23 mg tissue)

Protocol:

1. After euthanizing mouse, remove hair using Nair: dab with Nair, wait 30 secs, wipe with wet paper towel.
2. Isolate tissue and place in ice-cold hypothermosol.
3. Scrape off underlying layer of fatty / connective tissue using scalpel before proceeding.
4. Mince skin tissue thoroughly on petri dish on ice for 3-4 min on ice into 1-mm^3 pieces using razor blade while manipulating tissue with forceps – you will need to use grinding motion and vigorously break up tissue.
5. Place 23 mg minced tissue into 1 mL B. Lich enzyme cocktail. Incubate on ice.
6. Shake every min; triturate every 2 min with p1000 w/tip cut (start triturating at 2 min).

7. 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.
8. Transfer back to 1.5 mL tube using 1 mL serological pipet. Mix thoroughly and allow to settle on ice 2 min.
9. Save 70% (700 µL) of supernatant, leaving chunks at the bottom of the tube; apply to 30 µM filter on 15 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%. Save flow through on ice and keep filter on tube for 2nd layer.
10. Add additional 1 mL b. Lich enzyme mix to residual tissue chunks.
11. Triturate every 2 min, shake every min while incubating on ice for 20 additional mins. (50 min. total digest time).
12. Transfer entire volume to same 30 µM filter on 15 mL conical. Rinse with additional 5 mL ice-cold PBS/BA 0.04%.
13. Centrifuge at 300 g for 5 min at 4 °C. Remove supernatant & re-suspend in 100 µL PBS/BSA 0.04%. Examine using hemocytometer with trypan blue.
14. Adjust concentration to 1,000 cells/µL for Chromium or 100 cells/µL for DropSeq.