HUMAN ISLET SORTING FOR ALPHA, BETA, AND ACINAR CELLS

- Based on protocol from Markus Grompe's lab

- Recommended starting material – 30,000 to 40,000 IEQs

I. BEFORE STARTING

- Make sure water bath is on and to temp (37C).
- Make sure centrifuge is available (50ml conicals)
- For Qiagen DNA/RNA AllPrep kit, Prep RLT Plus Buffer

II. SET-UP

1. Thaw trypsin in water bath (0.05%, at least 6ml)
2. Thaw FBS
3. Label sorting tubes for samples
    - Negative control
    - Islet cells
4. Label TWO sets of collection tubes (sorting tubes) and put 500ul 1XPBS in each tube
    - Alpha
    - Beta
    - Acinar
5. Prepare 2% FBS (50ml 1XPBS + 1ml FBS), keep on ice

NOTE: keep 2% FBS, 1XPBS, trypsin and FBS on ice

III. PROCEDURE

1. Incubate islets.

2. Combine all islets into TWO 50ml conical tubes.

3. Centrifuge 4min, 1200rpm, RT.

4. Take off supernatant and use supernatant to rinse residual islets out of flask. Spin again, 4min, 1200rpm, RT.

5. Add 3ml 0.05% trypsin to each 50ml tube, pipette up and down (NOTE: re-suspend in 1ml trypsin using P1000 and then add additional 2ml)

6. Incubate at 37C (water bath) for 9 min. Pipette up and down every 3 min. (t = 7min, 4min, 2min, 0min)

7. Remove tubes from water bath. Passage contents of BOTH tubes through ONE strainer into ONE 50ml conical in the following order. (NOTE: use p1000, tip is on strainer, pressure, swirl. 40uM nylon strainer, ref = 352340).
    - Add 1 ml 100% FBS to each tube.

- Passage contents of both tubes through one strainer.
- Swirl tube, and if clumps visible vortex quickly (2 sec).

8. Going forward, cells remain in ONE conical tube.

9. Centrifuge: 4min, 1200rpm. Take off supernatant. Re-suspend in 25ml 1XPBS. Vortex quickly, then centrifuge 4min, 1200rpm. Take off supernatant.

10. Re-suspend in 1-2ml 2% FBS.

11. Count cells (10 microliters cells, 10 microliters trypan blue, 10 microliters into either side of a Countess chamber).

12. Adjust volume so cells are at a concentration of $5x10^6$ in 2% FBS.

13. Remove 100,000 cells (20ul) and put in FACS tube labeled "Negative Control". Add 480ul 2%FBS for final volume of 500ul. Store on ice until sort.

14. Add ALL THREE primary antibodies (HIC1-2B4, HIC3-2D12, HIC1-1C10) to cells at 1:50 (see below for detailed information; different batches of antibody may require optimization).

15. Incubate for 30 min on ice. Swirl tube every 10 min.

16. Centrifuge: 4 min, 1200rpm. Remove supernatant.

17. Wash by re-suspending in 25ml PBS, centrifuge 4 min, 1200rpm. Re-suspend in 2% FBS to bring concentration back to $5x10^6$ cells/ml.

18. Add BOTH secondary antibodies (APC-conjugated anti-mouse IgM and PE-conjugated anti-mouse IgG) to cells at 1:200.

19. Incubate for 30 min at 4C, with tubes wrapped in foil. Mix (swirl) every 10 minutes.

20. Centrifuge: 4 min, 1200rpm. Take off supernatant. Wash: re-suspend in 25ml PBS, centrifuge 4 min, 1200rpm. Re-suspend in 2% FBS to bring concentration back to $10x10^6$ cells/ml. Transfer to FACS tube. Add DAPI at 1X for both labeled cells and negative controls cells (stock is 10,000X). Sort.

21. After sort:
    I. 50,000 alpha, beta, and acinar cells aliquoted for ATACseq
    II. 5,000 alpha and beta cells plated for single-cell calcium imaging
    III. 5,000 alpha and beta cells plated for patch clamping
    IV. Remainder of cells: Lyse cells for use in Qiagen DNA/RNA AllPrep kit: for >500,000 cells, use DNA/RNA Universal AllPrep kit; for <500,000 cells, use Qiagen DNA/RNA ALLPrep Micro kit.
        - Centrifuge cells, then carefully remove all supernatant by aspiration.
        - Loosen pellet by flicking and add RLT Plus buffer (prepared with Beta-mercapto ethanol)
            - $<5 \times 10^6$ cells, 350ul

- $5 \times 10^6 – 1 \times 10^7$ cells, 600ul

V. Pipet the lysate directly into a QIAshredder spin column and centrifuge for 2 min at maximum speed.
VI. Continue with AllPrep protocol, or snap freeze and store at -80 for future use.

IV. Ab Information

Three primary antibodies, obtained from Grompe lab at OHSU

- **HIC1-2B4** (HPi1) is a mouse IgG1 that labels all human islet cells (to slightly varying degrees; beta cells are a little bit brighter than the others).
- **HIC3-2D12** (HPa3) is a mouse IgM that differentially labels endocrine subtypes. Alpha, Gamma, and Epsilon cells are brightly labeled, Delta cells are moderately labeled, and Beta cells are dim-to-negative. This antibody also dimly labels duct cells, but these can be easily distinguished by their HIC1-2B4 negativity.
- **HIC1-1C10** (HPx2) is a mouse IgM that labels acinar cells.

Two Secondary antibodies, purchased from Jackson ImmunoResearch:

- R-Phycoerythrin-AffiniPure F(ab')2 Fragment Goat Anti-Mouse IgM, μ Chain (Cat # 115-116-075)
- Allophycocyanin-AffiniPure Goat Anti-Mouse IgG (subclasses 1+2a+2b+3), Fcγ (Cat # 115-135-164)