Epithelial single cell isolation protocol (CiCi and Di modified)

i) Mouse intestinal Crypt isolation

1. Cut out intestine and place into ice cold HBSS w/o Ca^{++}/Mg^{++}
2. Open intestine longitudinally. Wash with ice cold HBSS w/o $Ca^{2+}Mg^{2+}$ five times.
3. Cut the tissue into approximately 1cm pieces.
4. Place tissue into a 40ml conical tube with final EDTA concentration of 30mM:

   2.4ml of 0.5M EDTA
   37.6ml ice cold HBSS w/o $Ca^{2+}Mg^{2+}$
5. Incubate on ice for 20-30min.
6. Wash tissue with ice cold HBSS w/o $Ca^{2+}Mg^{2+}$ Five times.
7. Keep in ice cold HBSS w/o $Ca^{2+}Mg^{2+}$ for 20mins.
8. Shake vigorously by hand 3-5' (~180 shakes/minute) for 4min.
9. Take supernatant into a new tube.
10. Repeat 3 times shaking.
11. Use 70um cell strainer to filter supernatant
12. Spin at approximately 225-250xg for 5min at 4 ºC.

ii) Epithelial Single cell dissociation

13. Remove supernatant and reconstitute the crypts isolated from 1 mouse small intestine with 2-3ml TryPLE Express contain 500uM NAC, 10uM Y-27632

    10ml TryPLE Express:
    10ul 500mM N-Acetyl-L-cysteine
    100 1mM Y-27632 dihydrochloride ROCK inhibitor.
14. Incubate for 6-8 min in a 37^{O}C water bath while gently shaking by hand or stirring by a P1000 pipette occasionally.
15. Return the dissociated crypts to ice bath for another 1min.
16. Transfer dissociated samples to 40ml ice cold wash media:

    50ml of Advanced DMEM/F12 media

500ul L-GlutaMax, 1:100
500ul of HEPES, 1:100
500ul of Penicillin (200U/mL)/Streptomycin, 100X
50ul of N-acetyl cysteine, 100X
50ul of Y-27632, 10X

17. Shake the 50ml tube for about 30sec to promote the dissociation and filter the suspension into another 50 ml falcon tube.
18. Spin at 1500rpm (500g) for 5min at 4^{O}C.
19. Re suspend pellet in 3ml of ice-cold staining media:

50ml of advanced DMEM/F12
500ul L-GlutaMax, 1:100
500ul of HEPES, 1:100
500ul of Penicillin (200U/mL)/Streptomycin, 100X
50ul of N-acetyl cysteine, 100X
500ul of Y-27632, 100X

20. Use 20µm syringe filter to separate any remaining mass
21. Spin at 1500rpm (500Xg) for 5min at 4^{O}C
22. Use the dead cell removal kit to remove dead cells. (Protocol provided with the kit).
23. Count cells for culturing.

Dead cell Removal Protocol

1. Prepare 1x Binding buffer (0.25 ml of 20x Binding buffer stock + 4.75ml of double distilled water) in 15ml tube. *Handle under sterile condition
2. Collect cells, centrifuge at 300xg for 8 min, and remove supernatant completely.
3. Resuspend cells with 100 µl of Dead Cell Removal Microbeads. (if two mice, use 200 µl, or if fewer cells, just use 100 µl)
4. Mix well and incubate for 15 minutes at R.T.
5. Place column in MACS Separator, and rinse with 500 µl of 1x Binding buffer.
6. Apply cell suspension onto the column.
7. Let the negative cells pass through; rinse 500 µl of 1x binding buffer for 4 times.
8. Collect effluent as live cell fraction.

(References)

Wang, F., Scoville, D., He, X.C., Mahe, M.M., Box, A., Perry, J.M., Smith, N.R., Lei, N.Y., Davies, P.S., Fuller, M.K.*, et al.* (2013). Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. Gastroenterology *145*, 383-395 e381-321.