**OL Extraction** (Popko lab)

All amounts geared for one extraction.

1. **Day 0**
   1. Two 150mm plates, one labelled Ran-2 and one labelled GC
      1. 20mL 50mM Tris-HCl pH 9.5 + 60uL goat anti-mouse IgG+IgM
   2. One 100mm plate labelled O4
      1. 10mL 50mM Tris-HCl pH 9.5 + 30uL goat anti-mouse IgM, Mu chain specific
   3. Incubate O/N at 4C
   4. One set of panning plates is sufficient for 1-2 rat brains or 2-3 mouse brains
2. **Prepare buffers**
   1. 10mL EBSS, equilibrate with at 10% CO2  at 37
   2. 10mL papain, 60mm 5% CO2/95% O2 on wet paper towels at 34
      1. Once the color turns slightly orange, add 200 units papain and 2mg L-cysteine
      2. Warm in water bath to help dissolve.
      3. Once clear, filter and then add 200uL DNase
   3. 500uL DPBS w/o Ca++/Mg++ in 60mm
3. **Add secondary antibodies to panning plates**
   1. PBS 3x
   2. RAN-2: 4mL Ab + 8mL DPBS/0.2% BSA
      1. *Mouse: 20uL Thy1.2 + 12mL DPBS/0.2% BSA*
   3. GC/O1: 4mL Ab + 8mL DPBS/0.2% BSA
   4. GC/O4: 1mL Ab + 8mL DPBS/0.2% BSA
4. **Dissection**
   1. Cut off head, skin, and skull
   2. Remove cortex and place in 500uL DPBS
   3. Dice cortex with scalpel
5. **Dissociation**
   1. Add papain buffer to diced brains and incubate with CO2/O2 for 45’, shaking every 15’
   2. Coat plates with pDL: Rinse coverslips 3x with ddH2O, then dry in large dish. Place 100uL 1x pDL on each. Incubate >20’ RT
   3. Make buffers
      1. 10mL Lo Ovo + 200uL DNase (9mL PBS + 1mL Lo Ovo)
      2. 6mL Hi Ovo (5mL DPBS + 1mL Hi Ovo)
      3. 15mL panning buffer (12.5 DPBS + 1.5mL 0.2% BSA)
      4. 10mL 30% Fetal Calf Serum in DPBS
6. **Tritruation**
   1. Transfer dissociation into 15mL, let settle
   2. Aspirate papain buffer and gently add 2mL Lo Ovo. Let settle, aspirate again.
   3. Add 2mL Lo Ovo and tritruate with 5mL pipette tip. Let settle, remove 1mL. Add 1mL fresh Lo Ovo and repeat.
   4. Tritruate with 1000uL pipette until there are no chunks.
   5. Spin 15’ at 1000rpm
   6. Aspirate and resuspend in 8mL panning buffer.
   7. Pre-wet nulon mesh filter with 2mL panning buffer
   8. Filter cells through and rinse filter with remaining 5mL panning buffer

1. **Panning**
   1. Before adding cells, rinse each panning plate 3x PBS
   2. Add cells to Ran-2 (astrocytes) panning plate for 30’ at RT
   3. Shake a bit, transfer supernatant to GC/O1 (oligodendrocyte) panning plate for 30’ at RT
   4. Shake thoroughly before dumping the supernatant.
   5. Trypsinize cells briefly (leave on for no more than 1’) then treat with FBS. Pipet vigorously to remove all cells from bottom. Spin down to change media.
   6. Transfer to O4 plate for 45’ at RT.
   7. Shake thoroughly before dumping the supernatant.
   8. Trypsinize for no more than 1’ then treat with FBS. Pipet vigorously to remove all cells from bottom.
   9. Spin down cells and resuspend in 8mL. Count before plating. Incubate at 10% CO2.