NOTE: This method is adapted from the Schwarz video article published in Jove in May 201521.

1. **Preparation for Tissue Collection (**Continuing from **2.5 Day of Experiment**)
   1. Fire polish 3 glass Pasteur pipettes in decreasing diameters
      1. 1 = 6.5 mm diameter
      2. 2 = 5.5 mm diameter
   2. Turn on water bath to 37 °C
2. **Perform 3.0 Perfusion – 4.7 Tissue Dissection**
3. **Prepare Enzyme Mix 1** 
   1. Prepare Enzyme Mix 1
      1. Mix 50 μl of Enzyme P with 1,900 μl of Buffer Z for each sample
   2. Place Enzyme Mix 1 in the water bath at 37 °C
4. **Manual Dissociation** 
   1. Use a 1 mL pipette tip to smash the tissue into as small of pieces as possible.
   2. Using a microcentrifuge, spin the samples at 300 x *g* for 2 min at room temperature. Aspirate and discard supernatant.
   3. Add 1,900 μl of warm Enzyme Mix 1 to each sample.
   4. Incubate the samples for 15 min in the 37 °C water bath, inverting the tubes several times every 5 min.
   5. Prepare Enzyme Mix 2 in a 200 μl tube.
      1. Mix 20 μl of Buffer Y with 10 μl of thawed Enzyme A
   6. Add 30 μl of Enzyme Mix 2 to each sample. Invert gently. Do not vortex.
   7. With a fire polished 6.5 mm diameter Pasteur pipette, dissociate the sample by triturating up and down 30 times. Avoid bubbles.
   8. Incubate for 15 min in the 37 °C water bath, invert tubes several times every 5 min.
   9. With a fire polished 5.5 mm Pasteur pipette, dissociate the sample by triturating up and down 30 times. Avoid bubbles.
   10. Again, using a fire polished 5.5 mm Pasteur pipette, dissociate the sample by triturating up and down 30 times. Avoid bubbles.
   11. Incubate the samples for 10 min in the 37 °C water bath, invert tubes every 5 min
   12. Apply single-cell suspension to a 70-μm cell strain placed in a 50 mL conical tube.
   13. Apply 10 ml of D-PBS to the filter. Discard filter.
   14. Centrifuge the sample at 300 x *g* for 10 min at room temperature. Aspirate and discard supernatant.
   15. Proceed to **7.0 Debris Removal**

Schwarz, J. M. Using fluorescence activated cell sorting to examine cell-type-specific gene expression in rat brain tissue. *J Vis Exp*, e52537, doi:10.3791/52537 (2015).