Hypothalamus Cell Dissociation

Introduction

For dissociating single cells into suspension for 10x sequencing. Protocol adapted from Worthington Papain Dissociation System, with adaptions from B. Raj.

Materials

- 4% Agarose solution (in H20)
- Petri Dishes (plastic and glass)
- Forceps
- Dissection needles
- Super-glue
- Razor blades
- Leica Vibratome
- Paintbrush
- Microscope slides
- DPBS (w/out CaCl and MgCl) (+ BSA)
- Neurobasal media (+ N2 or B-27 supplement)
- Papain Dissociation System (Worthington)
  - Papain + DNAse (equilibriated with CO2) (resuspended in neurobasal media w/out supplement)
  - Inhibitor solution (in EBSS, with DNAse) (equilibriated with CO2)
- 35 micron snap-cap filters
- 20 micron filters (for 15ml or 1.5ml tubes)

Procedure

Prepare 10X Solutions

1. Equilibrate to room temperature. Vortex and centrifuge briefly before use.

   A. Single Cell 3′ Gel Beads (from -80C)
   B. RT Reagent Mix (-20C)
   C. RT Primer (-20C)
   D. Additive A (-20C)

2. Place on ice

A. RT Enzyme Mix (-20C)

3. Obtain

A. Oil
B. Single Cell Chip(s)

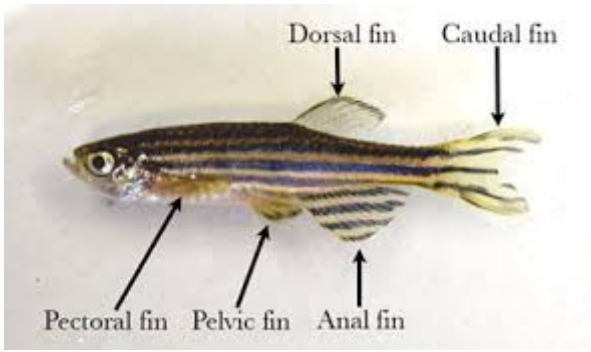
Dissection + Sectioning

4. Turn on water baths (for agarose, and cell dissociation).

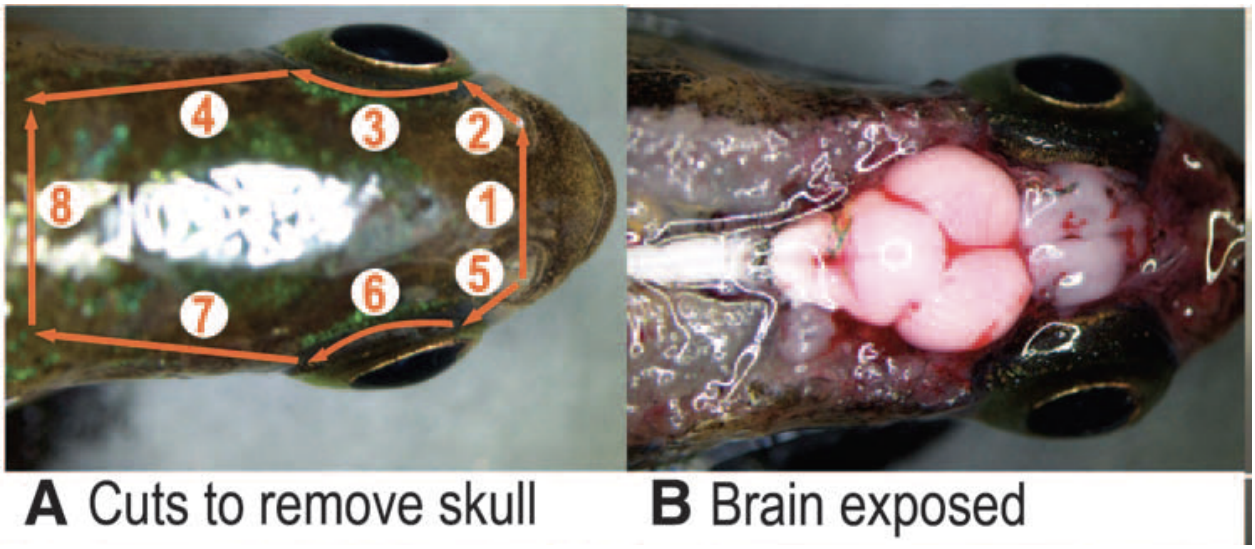
5. Liquify 4% agarose solution in ddH2O at 70 Deg C (to prevent evaporation).

6. Mix 50:50 with pre-warmed Neurobasal media (+ supplement), and incubate at 42 Deg.

7. Place fish into anasethesia and wait until they stop moving. Place spoon on ice, wait until sufficiently cooled. Collect fish using an embryo strainer, and place on cold spoon to euthanize fish. Decapitate fish at a level posterior to the pectoral fin and transfer to petri dish for further dissection. Dissection is preformed in Neurobasal media + B27 (NM+).

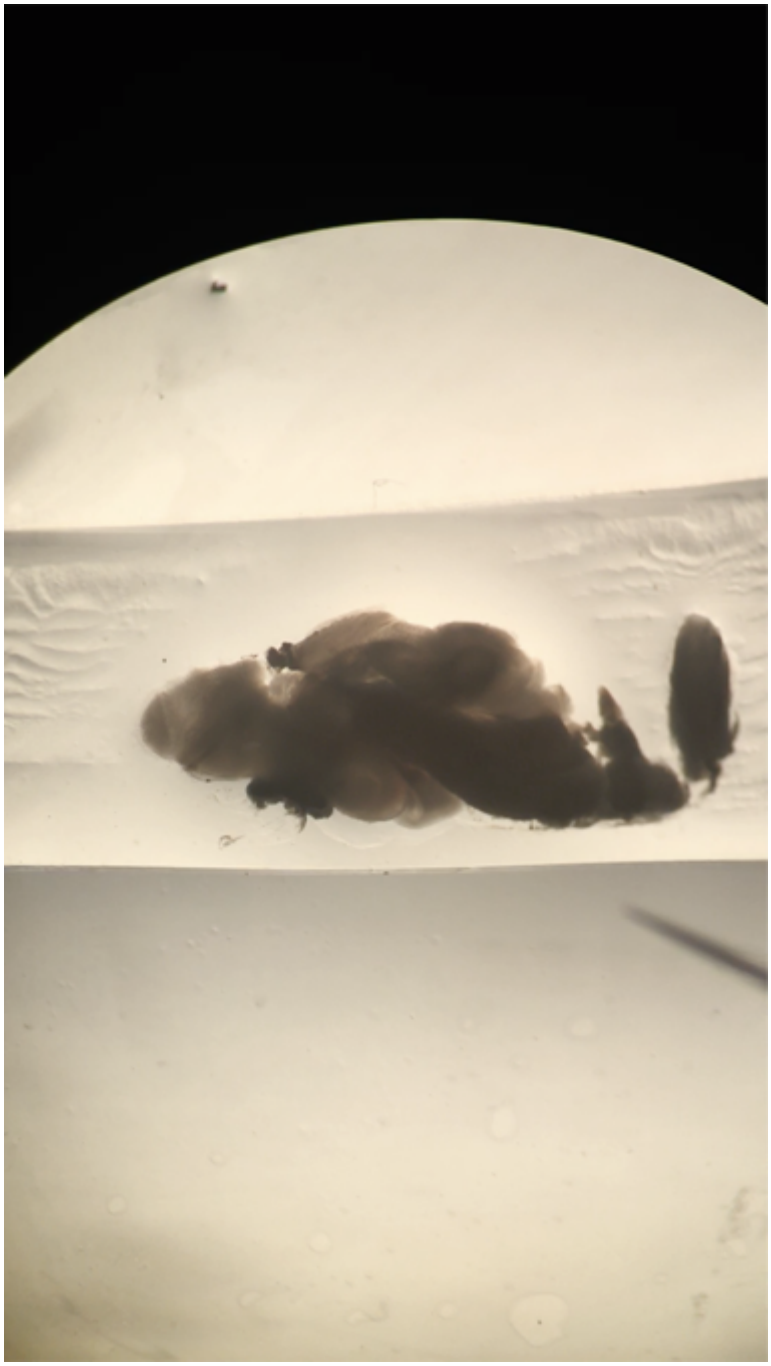


8. Pin head to dish by placing one pin in each eye socket and a third pin behind the brain so that the head is upright, with the skull on top. Remove the skull (crack it open from the eye socket) and open spinal cord by inserting forceps and opening them. Remove the brain by tearing beneath the brain with forceps / pull on optic nerves from underneath. Collect multiple brains in NM+ (up to 3 or 4).



9. Add brains to a small plastic petri dish and fill with pre-warmed agarose solution (step 1). Place petri on ice-cold heatblock (normally stored in -20 Deg Freezer), to allow agarose to solidify from the bottom up, while maintaing brains suspended in solution using either forceps or dissecting needles.

10. Trim agarose blocks so that brains are incapsulated in a square/rectangle of agarose with a thin layer surrounding on all sides. Glue agarose blocks to vibratome blocks and section brains to 500um thickness in 50:50 PBS:NM+. Maintain uncut blocks in NM+ (place several drops onto it using a transfer pipette). Using a paintbrush, manipulate sections onto a slide, place in petri dish and cover in NM+. Using dissection needles, remove the hypothalamus + pre-optic area from each section individually and resuspend in NM+.



Dissociation and Counting

11. Wash tissue in 1ml NM (without B27) by pelleing at 300g for 30sec - 2min. Resuspend in 500-1000ul papain solution and transfer to a 15ml falcon tube (papain resuspended in Neurobasal media with added DNAse). Papain solution should be pre-innoculated with CO2, and topped up during incubation. Incubate in waterbath at 37 Deg for 15mins.

12. Titrate tissue by micropipette until large chunks dissociate (1ml tip, ~20x times). Pellet (300g) then resuspend in 1ml inhibitor solution (with CO2).

13. Wash with 1ml NM+ and filter through a 20um eppendorf tube filter. Pre-wet filter with 1ml, then add cell-suspension, pellet, then wash through filter with another 1ml NM+. Resuspend final cell-suspesion in NM+ for cell viability counting. 30-60 ul per brain dissection.

14. Count cells using heamocytometer (for consistency). Dilute to 1000 cells/ul and load 17.4ul into each lane of a 10X chip.