



JAN 16, 2024

OPEN  ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.261ged79dv47/v1

Protocol Citation: Mel Feany 2024. Single cell sequencing preparation in adult *Drosophila* Brain.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.261ged79dv47/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

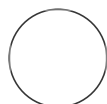
Created: Jan 16, 2024

Single cell sequencing preparation in adult *Drosophila* Brain

Mel Feany^{1,2}

¹Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815



Beatrice Weykopf

ABSTRACT

This protocol covers the preparation of adult *Drosophila* Brains for single cell RNAseq

Last Modified: Jan 16, 2024

PROTOCOL integer ID:
93630

Keywords: ASAPCRN

Funders



Acknowledgement:

ASAP

Grant ID: ASAP-000301

Fly brain dissection

15m

- 1 Dissect 20 male and 20 female brains from 10-day-old flies on ice-cold Schneider's medium with FBS (Gibco, filtered 10% FBS)
- 2 After a quick spin down, remove the supernatant and wash the brains with ice-cold PBS
- 3 Incubate the brain at  25 °C with 300 µl of 0.05% trypsin-EDTA (Fisher Scientific) with continuous pipetting every 5 minutes
- 4 Pass solution containing brain chunks through a 25-gauge needle (25G 5/8), without introducing air bubbles, roughly 50 times
- 5 After dissociation, the solution was through a 10 µm pluri-select cell strainer (Fisher Scientific), and 400 µl of ice-cold Schneider's medium containing FBS was added to inactivate trypsin
- 6 Centrifuge for  00:15:00 at 600xg and remove the supernatant without disturbing the pellet

15m

- 7 Suspend pellet in sterile PBS containing 0.04% BSA

Single-cell quantification

15m

- 8 Quantify the number of cells and measure viability using an AO-PI reagent (Logos Biosystems)

Prepare sequence libraries

- 9 Encapsulate cells, prepare and sequence libraries using the manufacturer's protocol (10X genomics & Illumina Inc.)