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

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Single cell sequencing preparation in adult Drosophila Brain

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ABSTRACT

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

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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.)