JAN 29, 2024

OPEN ACCESS

DNA damage assessment in the adult Drosophila brain via comet assay

Mel Feany[1,2]

[1]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

ASAP Collaborative Research Network

Beatrice Weykopf

ABSTRACT

This protocol describes how to determine DNA damage in the adult drosophila brain using the comet assay

DOI:
dx.doi.org/10.17504/protocols.io.eq2lyjrrplx9/v1

Protocol Citation: Mel Feany 2024. DNA damage assessment in the adult Drosophila brain via comet assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyjrrplx9/v1

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

Created: Jan 29, 2024

Last Modified: Jan 29, 2024

PROTOCOL integer ID: 94347

Keywords: ASAPCRN

1 Prepare a Lysis solution for one slide by mixing 20 mL of lysis buffer (Trevigen Comet Assay Reagent Kit) and 2 mL of DMSO

2 Melt LMAgarose in a boiling water bath, aliquot to 1.5ml tubes, and place at 37 °C until use

3 Place comet slide at 37 °C until use

4 Dissect 2 adult fly brains per the desired genotype in ice-cold PBS

4.1 Homogenize brains with a blue pestle

5 Combine head homogenate with 100 µL 37 °C agarose and immediately pipette 75 µL onto Comet Slide

6 Place slides flat at 4 °C for 20 minutes

7 Immerse slides in prechilled Lysis Solution On ice for 00:45:00 45m

8 Immerse in a freshly prepared alkaline solution (prepare 25 mL l by mixing 0.3 g NaOH and 125 µL of 200mM EDTA in dH2O). pH>13 for 00:30:00 at Room temperature in the dark 30m

9 Drain excess buffer and wash in 1X TAE twice for 00:05:00 each 5m

10 Run for 00:10:00 at 23V/6mA in TAE_one volt per cm electrode to the electrode 10m

11 Drain excess buffer and rinse in dH_2O

12 Immerse slides in 70% Ethanol for 00:05:00 and then air-dry slides Overnight 10m

13 The next day, prepare SYBR Green (Thermofisher) dilutant by mixing 1 µL in 10 mL of TE buffer (10 mM Tris-HCL pH 7.5, 1 mM EDTA in dH_2O) and place 50 µL on each circle of the comet assay slide for 00:05:00 in the dark 5m

14 Drain excess buffer and mount slides without DAPI in permount (Fisher Scientific)

15 After mounting and drying the slides, take images with 20X objective of the epifluorescence microscope and analyze the comet tails using the Image J Comet assay plugin