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DNA damage assessment in the adult *Drosophila* brain via comet assay

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We use this protocol and it's working

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Abstract

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



- 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 μ L  37 °C agarose and immediately pipette  75 μ L 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 I by mixing  0.3 g NaOH and  125 μ L 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 dH₂O



- 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 dH₂O) 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