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Genetic interaction analysis using ERGs in D. melanogaster

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

This protocols describes the recording of electroretinograms (ERGs) and calculation of genetic interactions of fly mutants for a transheterozygous screen.

Electroretinograms (ERGs)

1 For every measurement male flies are collected (including the control) 4±1 d after eclosion from the transheterozugous crosses reared in a temperature and light-controlled incubator at 25 °C and 12-hour light-dark cycle

2 Flies are sedated with CO2 and immobilized on glass microscope slides using double-sided tape

3 Filamented glass micropipettes are used to generate electrodes using the Laser-pipette-puller P2000.

4 Glass electrodes (borosilicate, 1.5 mm outer diameter) filled with 3 M NaCl are placed in the thorax as a reference and on the fly eye for recordings

5 The flies are exposed to darkness for 3 seconds, followed by 1 second of LED light illumination inside a Faraday Cage.

6 This is repeated 4 times for each fly (5 times in total).

7 Light-evoked signals are amplified by a DC amplifier and the amplified signal is processed by a data acquisition device (Clampex) and Axosope 10.7, connected to a PC running Clampfit 10.7 software (Molecular Devices).

8 First control flies are recorded to to assess whether the recording is stable and conditions are comparable to previous recordings

9 ERG traces are analyzed for depolarisation amplitude and On/Off peaks in IGOR Pro 6.37 (WaveMetrics) using a custom-made macro.

Analysis for genetic interaction using non-interacting model

10 For all ERGs the depolarization amplitude is determined and normalized to the mean depolarization amplitude of the control of the individual experiment.

11 The expected depolarization amplitude for every gene pair is calculated by multiplying the normalized mean depolarization amplitude of the single heterozygous mutants.

12 The genetic interaction strength is calculated by subtracting the modelled expected depolarization (step 9) from the individual observed depolarization amplitude of double heterozygous gene pairs