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Locomotor Assay in *Drosophila melanogaster*

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[Abstract] This protocol describes a simple locomotor assay in *Drosophila melanogaster*. In brief, the locomotor of each single fly in the culture dish is recorded by a web camera. The moving time, walking length, speed and the locomotor trails of the single fly could be quantitatively analyzed.
Keywords: Locomotor, *Drosophila*, Video analysis, Motion detection, Motion trail, Behavior

[Background] This protocol was implemented in the previously published study (Liu *et al.*, 2016). In that study, this assay was combined with the optogenetic system by simply providing the proper excitation light.

Materials and Reagents

1. 3.5 cm culture dishes with black paper inside
2. *Drosophila* strains to be analyzed
3. Ice for anesthesia

Equipment

1. Empty vial for cold anesthesia
2. Fluorescent lamp (Bannet T5 8 W, China)
3. Infrared LEDs (TUOENS, model: TS-6036A)
4. Web camera (Omiky, model: CEL USB 2.0 50.0M PC Camera, catalog number: CEL9002255) with the IR filter removed; Replacing the IR filter with the floppy disk (the black floppy disk contained in the hard shell) to filter the visible light
5. Behavior room with the temperature and humidity controlled

Software

1. MATLAB (MathWorks, R2013a)

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Procedure

1. Set the environmental illuminance (provided by the fluorescent lamp) to be 1,000-1,300 Lux, the temperature to be 24 ± 1 °C and the humidity to be 40-60%.
2. Transfer the flies into the empty vial and anesthetize them on ice for 10 min.
3. Transfer one fly to each culture dish, and let the flies recover from the anesthesia for at least 10 min.
4. Record the locomotion of the flies for 2 min with the web camera equipped with a visible light filter (Figures 1A and 1D; Video1). The fly is light up and the background is dark (Figure 1B). The angle of the infrared LEDs should be adjusted before recording to reduce the reflection on the dish.

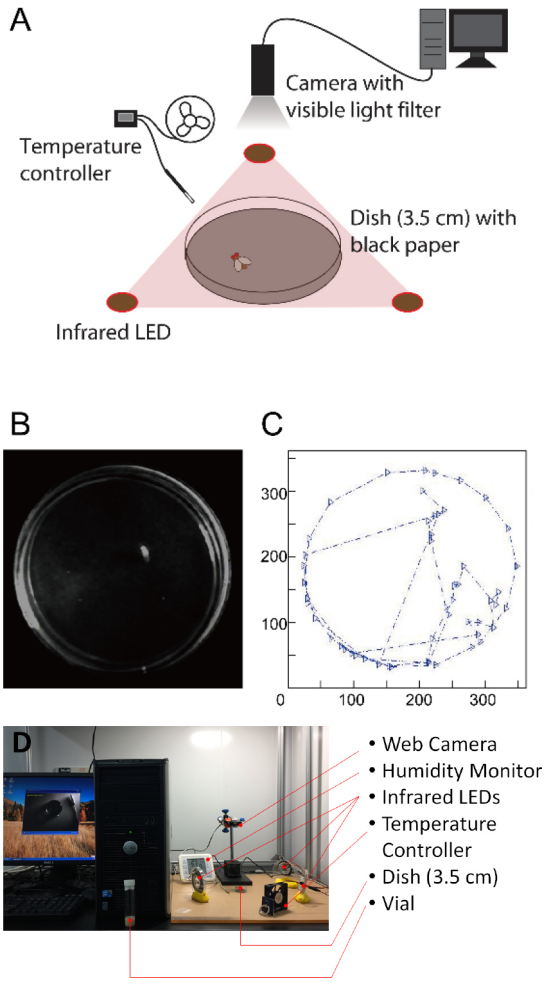

Figure 1. Locomotor assay in *Drosophila melanogaster*. A. The schematic diagram of the locomotor assay; B. A photo of the dish with a fly inside; C. The motion trial of a recorded fly; D. A photo of the setup.

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Video 1. Recording of the locomotor behavior of the flies

Data analysis

1. Analyze the photos with MATLAB (MathWorks, R2013a; For MATLAB code for video analysis, see Supplemental file 1) (Figure 1C). Figure out the coordinate of the fly on each photo, and then calculate the moving time, walking length and speed of the fly, and analyze the locomotor trail of the fly.
2. Individuals with the walk length less than half of the fly body length during recording should be excluded.
3. The Wilcoxon signed rank test should be applied to evaluate differences between matched samples.

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