ROOM1

**# Title: Type in the title/name**

> Locally Activatible BioLuminescence (LABL) Oscillations of Distinct Clock Measured in Young and Aged fruit flies (*Drosophila melanogaster*)

**## Date: 2022-10-03**

**## Purpose (Research Hypothesis)**

> Measure the transcriptional oscillation of distinct neuronal and peripheral clocks in live Drosophila.

> To characterize the expression pattern of LABL in distinct neurons and tissues using different Gal4 drivers.

**## Abstract**

This dataset contains measurements of luminescence oscillations in both young and aged *Drosophila melanogaster*, aiming to investigate the functioning of different neuronal clocks. The data provided reflects the activity under controlled experimental conditions.The main results show that despite loss of PDF signaling, circadian oscillation continued in peripheral clocks in non-neuronal tissues. LABL detection also facilitated observation of circadian differences dependent on anatomical location, mutation and age. Thus, confirming distinct differences between clocks.

**## Contributors**

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**## Biosample**

> Description of biological entity that was monitored / measured/ experimented on - *Drosophila*

Sample type: Brain and whole fly

In vivo

Ex vivo

Age of fly group (young): 1-3 days old

Age of fly group (old): 39-41 days old

> Genetic info / Strains (what to include here):The following fly strains were used in the study: tim-UAS-Gal4 (A3), Pdf-Gal4, DvPdf-Gal4, R18H11-Gal4, Clk4.1-Gal4,, *perS* and *perL*, elav-Gal4; *Pdfr5304* (*han5304*); Mai179-Gal4; Clk9M-Gal4; 3xUAS-FLP2::pest; PDF receptor mutant *han5304*; esg-Gal4; NP3084-Gal4.

G-TRACE flies (32251); mef2-Gal4 (27390); C564-Gal4 (6982); LSP2-Gal4 (6357).

LABL was constructed into an attB cloning vector for Drosophila embryo injection.

**## Husbandry**

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Example entry

Environment/feeding Laboratory setting with controlled temperature, humidity, and light-dark cycles: Flies were reared on standard cornmeal/agar/yeast/molasses medium at room temperature (22 °C) or 18 °C in ambient laboratory light.

Population density: Maintained to prevent overcrowding.

**## Entrainment**

> Light-Dark Cycles: 12h Light / 12h Dark to synchronize circadian rhythms before the experiment.

Temperature: Consistent temperature (25 °C) maintained throughout the entertainment and experimental period.

**## Experiment design**

> What is crucial for circadian study with flies

**## Protocols**

>Luminescence Recording: Procedure for measuring and recording luminescence signals.

Data Normalization: Techniques used to normalize luminescence values.

Statistical Analysis: Methods applied to calculate SEM and fit S-curves.

LABL was constructed into an attB cloning vector for Drosophila embryo injection and PhiC31-mediated genome integration. Luminescence of flies was measured using the LumiCycle 32 Color.

Actimetrics analysis software was used to normalize the exponential decay of luminescence signal over days using a polynomial curve fit, with no smoothing. Custom python code was used to organize luminescence data into 30 min bins and quantify peaks.

Circadian behaviour was determined by measuring the locomotor activity in the analysis of time in constant darkness using the Drosophila Activity Monitor System 5 (Trikinetics).

Fluorescence microscopy and immunohistochemistry were implemented to measure loss of mCherry signal from LABL plasmid in S2 cells. Protein samples were quantified using a Lowry Assay (Bio-Rad Cat. 5000112) and transferred toPVDF membrane using standard methods.

Matlab was used to simulate N=100 sinusoidal oscillators at increments of 0.5 hr each, each with a 24-hr period for 14 days.Finally, Bioluminescence images were acquired with a UPLSAPO 40 x by exposing the brains for 5 min with an EM gain of 400. For each brain, a Z-stack of 13 slices was taken, each slice containing a bioluminescence, a fluorescence (200ms), and a bright field image. All images were processed in FIJI (Schindelin et al., 2012) and combined with bright field images in GIMP.

**## Measurements**

**### Measured data**

* Bioluminescence
* Fluorescence signals
* Immunoblotting: Lowry Assay

**### Measurement technique**

* Luciferin-luciferase
* Fluorescence Imaging

**## Licence**

Not applicable