Unwinding the genetic clock

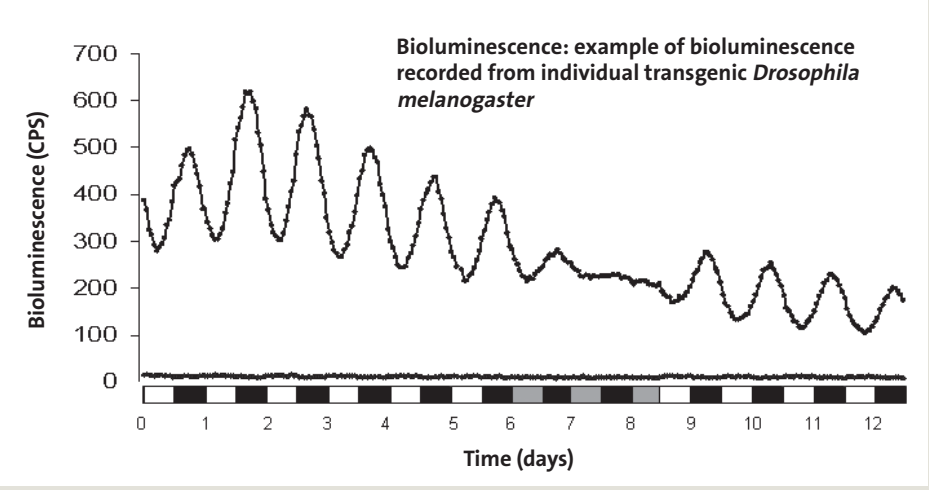
Researchers at the Institute of Entomology (ENTU) in the Czech Republic are studying insect circadian clocks and have used Tecan's Infinite® M1000 plate reader to automate hourly luminescence measurement of transgenic *Drosophila*. Automation enables large-scale screens of mutant *Drosophila* strains to be performed, involving hundreds of measurements over a period of up to two weeks.

Scientists at the Laboratory of Molecular Chronobiology in the Department of Molecular Biology and Genetics, Institute of Entomology, České Budějovice, are studying the circadian clock in *Drosophila melanogaster*, the fruit fly, to better understand the cellular and molecular mechanisms underlying internal rhythms. Dr David Doležel, a researcher in the Laboratory of Molecular Chronobiology, explained: "Insects are very convenient for genetic research, and studying *Drosophila* is valuable for mammalian research. Like humans, insects have an internal clock so that they know whether it is morning or evening, and the circadian genes – which are essential for this clock – are quite conserved between insects and humans. To study the expression of these genes, which are cyclically expressed over a 24-hour period, we have created transgenic *Drosophila* containing luciferase reporter genes controlled by the promoter of the circadian gene under study. Individual flies are placed into 96-well microplates, one fly per well, and surrounded by agar containing food, water and luciferin. We then measure the luminescence caused by activation of their reporter genes at hourly intervals for up to ten days."

"To reliably perform this assay on a large scale, we needed a microplate reader that would enable us to automate plate transfer; this is essential for our research as measurements are performed at regular intervals throughout the day and night. We also wanted an instrument with a luminescence module that was equipped with filters, as this would allow us to discriminate between red and green luciferases, enabling us to follow multiple genes per well. Tecan's Infinite M1000 plate reader satisfied all our requirements; it can be used with a variety of microplate formats, it is available with luminescence color filters and it is equipped with a reliable stacker system which automatically transports the microplates to and from the reader. Its compact size also means that it can be fitted into our laboratory incubator, allowing us to precisely control light and temperature and investigate the effect of environmental variables on the regulation of the circadian gene being studied."

David continued: "The Infinite M1000's microplate format enables us to load hundreds of flies into the plate reader at the same time. The bioluminescence emitted by the living flies is then measured at hourly intervals for up to two weeks, providing us with a fully automated system to screen for mutations that affect circadian gene activity and to test new variations that we have created in house. In a typical experiment, we measure bioluminescence from around 500-600 flies in eight plates, usually four to six individuals per mutant strain. To ensure light can access the flies, we alternate empty transparent plates with those containing flies, and in one month we can screen for between 200 and 400 different mutant strains. Of those strains, 99 % will be discarded after the two week screen, only retaining the mutants of interest to create new mutations for further study."

"We also use *Drosophila* tissue cultures or isolated organs to study circadian gene expression through luminescence, and these too are in 96-well format for the luciferase assay. This format is useful for developing new assays, and we have already successfully been able to independently measure the luminescence of red and green luciferases in *Drosophila Schneider 2* cells, thanks to the Infinite M1000's luminescence filter system. This strategy allows us to follow multiple



In this experiment, a promoter of the circadian gene *timeless* drives luciferase expression in a temporal pattern identical to *timeless* mRNA, enabling scientists to trace periodic expression in individual flies for two weeks. The black and white bars under the panel indicate when lights were off (12 hr) and on (12 hr), respectively. Gray bars indicate subjective daytime in darkness (12 hr). (Dolezelova and Doležel, unpublished)

D. melanogaster (left), classical model of genetics compared to the housefly, *Musca domestica*

target genes in a single assay, which we can hopefully extend to living flies in the future. The Infinite M1000 can also be used with other formats – such as 24-well microplates – which, while not used in our current research, may be useful for other assays in the future; it is good to know that this option is available to us."

"We have been very impressed by both the performance and the flexibility of the Infinite M1000, and Tecan was very happy to allow us to visit its premises in Austria to test the system with our own samples before we committed ourselves to purchasing the instrument. We spent an entire day testing the reader for our assay, and then Tecan's own engineers further developed the instrument's stacker system to meet our requirements. We were delighted to have this opportunity to trial the plate reader to see how it would handle our *Drosophila* cultures. Genetic research on insects requires the use of huge numbers of these organisms, and the Infinite M1000 reader makes this large-scale screening possible," David concluded.

To find out more about Tecan's Infinite M1000 plate reader, visit **www.tecan.com/infinitem1000**

For more information on the Institute of Entomology, visit **www.entu.cas.cz/en/**

Recent technologies allow genetic modification of new model organisms, in this example the housefly, *Musca domestica*. The Green Fluorescent Protein (GFP) expressed in the eyes was used as a marker to trace the presence of a transgene delivered by piggyBac transposon. Three transgenic and one control fly were photographed under white light (left) and GFP fluorescence after blue light excitation (right). (Doležel, Hediger & Bopp, unpublished)