APPLICATION NOTE

MONITORING CIRCADIAN GENE EXPRESSION IN MICRO-ALGAE

Luminescent imaging of luciferase reporters expressed from a circadian promoter in the algal species *Ostreococcus tauri*

Abstract

Expression of firefly luciferase from a circadian-regulated promoter is the most convenient and reliable way to image circadian gene expression in vivo over long-time series. For photosynthetic organisms, constant light conditions are required in between luminescence reads. We report how the Berthold TriStar² multimode plate reader equipped with luminescence module, in combination with an external LED illumination adaptor, can be used to image circadian rhythms in the micro algae *Ostreococcus tauri*.

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Introduction

Most living organisms possess an endogenous timekeeper to aid efficient negotiation of daily changes to the environment. This timekeeper, the circadian clock, regulates many aspects of the metabolism and physiology of an organism to anticipate predictable changes in the external environment. In plants, this anticipation aids the responses to temporally predictable biotic and abiotic stress [1]. Conversely, disruption of circadian timekeeping severely compromises plant health and reduces yields [2-5]. It is therefore imperative that we understand the

intricate regulation of circadian rhythms in plants, including the factors that affect motion of the transcriptional clockwork itself. The extensive influence of circadian rhythms arises to a large extent from the rhythmic regulation of up to a third of the total transcriptome in the model plant Arabidopsis [6]. These rhythmic gene products are involved in a broad range of cellular processes including metabolic pathways, hormone signalling, and stress responses.

Testing circadian defects in photosynthetic organisms relies on imaging the rhythmic expression of firefly luciferase from circadian clock gene promoters, to allow assessment of circadian phenotypes in transgenic lines (cf. [7, 8]) or upon treatments such as pharmacological inhibition (cf. [9, 10]). Circadian imaging under constant conditions allows the analysis of treatment effects on circadian period, phase, or amplitude.

Here, cells of the marine eukaryotic species *Ostreococcus tauri*, transgenically expressing the rhythmic luminescent reporter CCA1-LUC [11], are imaged under constant light conditions for 5 days in the presence of pharmacological inhibitor X or mock vehicle. In a second experiment, the effects of overexpressing transgene Y are analysed compared to the parent CCA1-LUC line. Luminescent traces directly reveal how either treatment affects circadian gene expression in these cells.

Experimental procedures

Cells of *Ostreococcus tauri* transgenically expressing CCA1-LUC [11] were grown under 12h/12h light/dark cycles in a LEEC PL3 growth cabinet. Medium consists of (24 g/l NaCl, 4 g/l Na_2SO_4, 0.68 g/l KCl, 200mg/l $NaHCO_3$, 100 mg/l KBr, 25 mg/l H_3BO_3, 3 mg/l NaF, plus hydrous salts: 50 mM $MgCl_2*6H_2O$, 10mM $CaCl_2*2H_2O$, 0.1 µM $SrCl*6H_20$), supplemented with Guillard's F/2 marine enrichment solution (Sigma) and 10 nM H_2SeO_3. Full medium was adjusted to a salinity of 30 ppt. For imaging, 40 µl of one-week old cultures were transferred to wells of 384-well microplates (Greiner BioOne) and 50 µl of fresh media supplemented with 2 µM luciferin (BioSynth AG) was added. For drugging experiments, 10 µl of 10x inhibitor concentration or vehicle controls in media was added to the wells. Transgenic lines overexpressing transgene Y were generated as described previously [11, 12]. Plates were sealed with TopSeal-Aplus seals (Perkin-Elmer) before being transferred to the Tristar² Multimode Microplate Reader. The external light array was fitted with a single layer of Moonlight Blue filter (Lee lighting). Graphs were made using GraphPad Prism.

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Material

- Berthold TriStar² Multimode Microplate Reader
- Luminescence module
- RGB LED illumination array for microplates

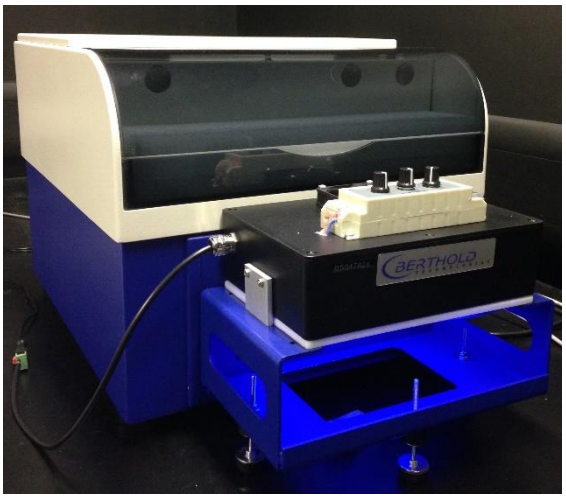

Figure 1: The TriStar² multimode microplate reader with external RGB LED array.

Instrument settings

The external light was set to 2 µmol/sec/m² as measured by a hand-held PAR radiometer (Irradian Ltd). The TriStar² was operated by the ICE software (Berthold Ltd). Microplates with 384 wells were used in this experiment, reading every well. Endpoint luminescence readings were taken for 1.5 seconds per well. An unload command was used with a delay time of 50 minutes (3000 seconds), leading to data with a time resolution of approximately 1 hour. The software was set to read 150 repeats. Screenshots of the instrument settings (left panel) and the results window (right panel) are displayed in Figure 2.

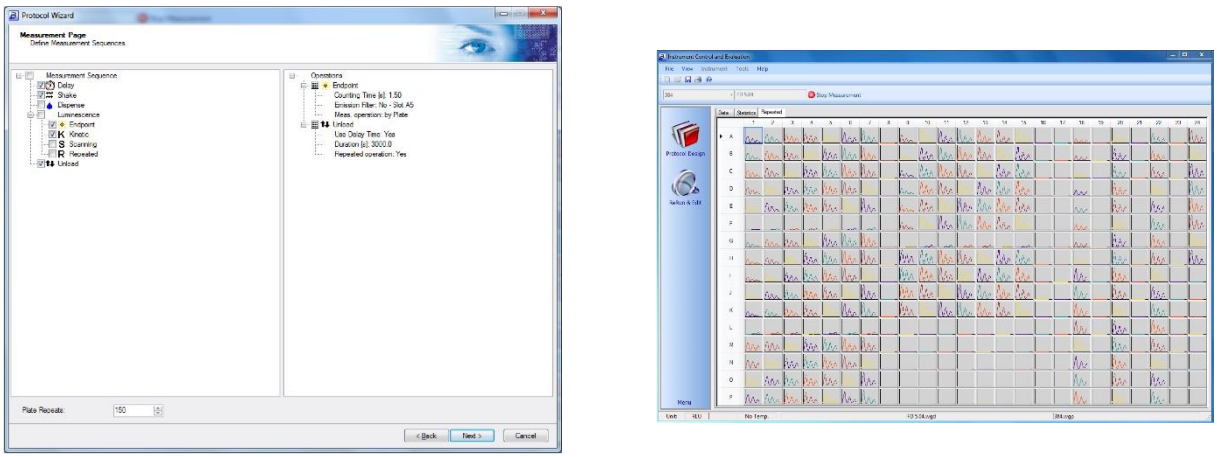

Figure 2: Screenshots of the ICE software. *Left*: Screenshot of instrument settings. *Right*: Screenshot of results window.

Results

We have imaged CCA1-LUC circadian rhythms in constant light for 5 days in the presence of a range of concentrations of pharmacological inhibitor X or mock treatment. Stable and persistent circadian expression rhythms are visualised using the methods described here (Fig. 3, black traces). In the presence of increasing concentrations of the pharmacological inhibitor X, circadian rhythms are increasingly fast (blue and green traces) and of decreased amplitude. Circadian gene expression was also analysed upon overexpression of a transgene, Y, in the CCA1-LUC parent background. As can be seen in Figure 4, overexpression of gene Y (blue traces) leads to clearly slower circadian rhythms compared to the parent line (black traces).

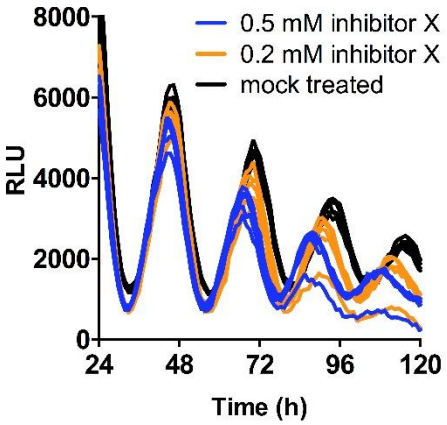

Figure 3. Circadian rhythms in CCA1-LUC luminescence upon pharmacological inhibition. Black lines are mock treated controls, orange and blue lines are treated with 0.2 or 0.5 mM of pharmacological inhibitor X (5 replicate wells are plotted per treatment).

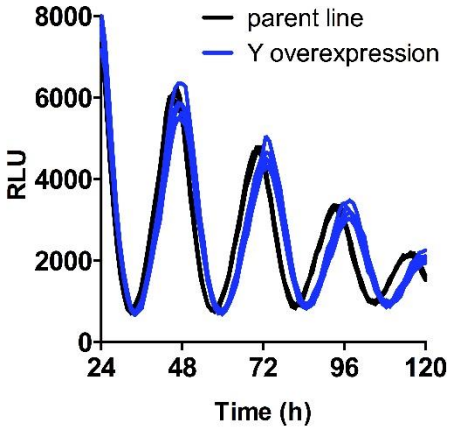

Figure 4. Circadian rhythms in parent CCA1-LUC line versus lines overexpressing transgene Y. Black lines are CCA1-LUC parent line controls, blue lines are transgenic lines overexpressing transgene Y in addition to the CCA1-LUC transgene (5 replicate wells are plotted per line).

Conclusions

The TriStar² multimode plate reader offers a reliable and versatile platform to image luminescent rhythms from circadian reporters. The system is compatible with a range of plate formats (plates from 6 wells to 384 wells), which allows great experimental flexibility. The external light array adds further flexibility as the red, green, and blue LEDs are independently adjustable and can be equipped with an external socket timer switch. The ICE software is intuitive and user friendly, whilst offering all the necessary flexibility to match your experimental needs. Throughout a long circadian experiment, the software interface provides a great overview of all data collected during the run. The customer service provided by Berthold has always been second to none.

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