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Gene expression

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WellReader: a MATLAB program for the analysis of fluorescence and luminescence reporter gene data

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ABSTRACT

Motivation: Fluorescent and luminescent reporter gene systems in combination with automated microplate readers allow real-time monitoring of gene expression on the population level at high precision and sampling density. This generates large amounts of data for the analysis of which computer tools are missing to date.

Results: We have developed WellReader, a MATLAB program for the analysis of fluorescent and luminescent reporter gene data. WellReader allows the user to load the output files of microplate readers, remove outliers, correct for background effects and smooth and fit the data. Moreover, it computes biologically relevant quantities from the measured signals, notably promoter activities and protein concentrations, and compares the resulting expression profiles of different genes under different conditions.

Availability: WellReader is available under a LGPL licence at http://prabi1.inrialpes.fr/trac/wellreader.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 BACKGROUND

Experimental techniques based on fluorescent and luminescent reporter gene systems have become powerful tools for the real-time monitoring of gene expression in living cells. The reporter systems are obtained by fusing the promoter region (and possibly part of the coding region of a gene of interest) to a reporter gene. The expression of the reporter gene, which generates a light signal (fluorescence or luminescence) that is easy to measure, thus reflects the expression of the gene of interest [e.g. Zaslaver *et al.* (2006)].

Thermostated and agitated automated microplate readers allow emitted fluorescence and luminescence signals to be measured on the level of populations of microorganisms. They are typically designed to read a 96-well microplate, where each well contains a cell culture expressing a reporter gene or a control (cells without reporter constructs or growth medium only). All wells are successively read to measure in a single experiment the optical density of the cell culture, which is approximately proportional to the number of cells in the population, and the luminescence and fluorescence intensity. This allows *in vivo* monitoring of the expression of several dozens of genes in parallel. Several applications in systems biology and biotechnology have confirmed the interest of monitoring gene expression by means of this approach [Kobiler *et al.* (2005); Lu *et al.* (2002); Onnis-Hayden *et al.* (2009); Ronen *et al.* (2002)].

In order to correctly interpret the reporter gene measurements, the primary data produced by the microplate reader must be translated into biologically relevant quantities, such as promoter activities and (relative) protein concentrations. This requires background corrections, data smoothing and fitting procedures, and mathematical transformations of the measured signals. Computer tools are essential for this, since automated microplate readers generate huge amounts of data, typically several thousands of data points per experiment. To our knowledge, no user-friendly computer tools for analyzing population-level fluorescence and luminescence reporter gene data exist in the public domain. The program WellReader aims at filling up this gap, thus facilitating the exploitation of the technology for the monitoring of gene expression in microorganisms.

2 DESCRIPTION OF FUNCTIONS

WellReader is equipped with a graphical user interface, structured around a visual representation of the microplate. This allows the user to access and control the analysis functions of the program (Fig. 1): an outlier detection module, a data smoothing and fitting module, a background correction module, modules for computing promoter activities and protein concentrations and a module for comparing expression profiles. In each step of the analysis, intermediate results can be stored for later use and exported to MATLAB for further analysis.

Outliers occasionally occur due to instrument or experimental errors, and they are removed either manually or by a simple outlier detection algorithm that can be parametrized by the user. After elimination of outliers, the data are interpolated by means of cubic smoothing splines with a user-defined smoothing parameter. Background levels of absorbance, fluorescence and luminescence systematic may cause systematic errors in the measurements. WellReader therefore allows the user to define background wells, and subtract the background levels from the uncorrected signals after appropriate normalizations. Relative measures of protein concentrations and promoter activities are then computed from the corrected absorbance, florescence and luminescence data.

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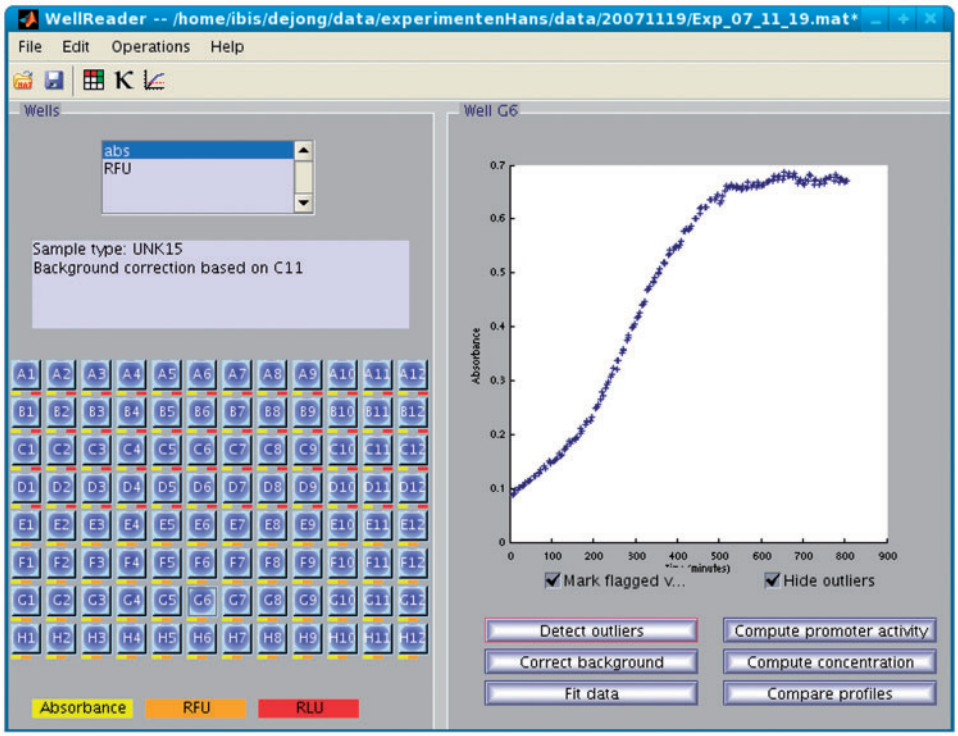

Fig. 1. Screenshot of WellReader main window. It shows the microplate and the absorbance data for one of the wells. The analysis functions listed at the lower right-hand side of the screen can be applied to the selected well.

This involves kinetic models of the temporal expression of reporter genes, as well as user-defined values for the half-lives of reporter proteins (e.g. GFP and luciferase) and host proteins. A detailed description of the data analysis procedures can be found in the Supplementary Material of this article.

All functions operate on the same underlying data structure, thus allowing the user to switch back and forth between the stages of the analysis process, and quickly investigate the consequences of, for example, different choices for smoothing parameters or background corrections.

3 IMPLEMENTATION AND DOCUMENTATION

WellReader has been implemented in MATLAB using the Spline toolbox (MathWorks). The program has been tested under Windows and Linux with different versions of MATLAB (R2007a, R2009a). In addition to the source code, a stand-alone executable is available to run WellReader outside the MATLAB environment. WellReader imports data in the plain text format generated by the Fusion Alpha microplate reader of Perkin Elmer as well as an XML format. This allows the user to load data from other plate readers, after a suitable transformation of the output files of the latter.

An online user manual is available at the WellReader web site together with a tutorial and an example dataset, concerning key global regulators involved in different stress responses of *Escherichia coli*. The results of the analysis of the dataset will be reported elsewhere.

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Conflicts of Interest: none declared.

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