Getting Started with WellInverter in Eight Simple Steps

Hidde de Jong, Michel Page, and Valentin Zulkower

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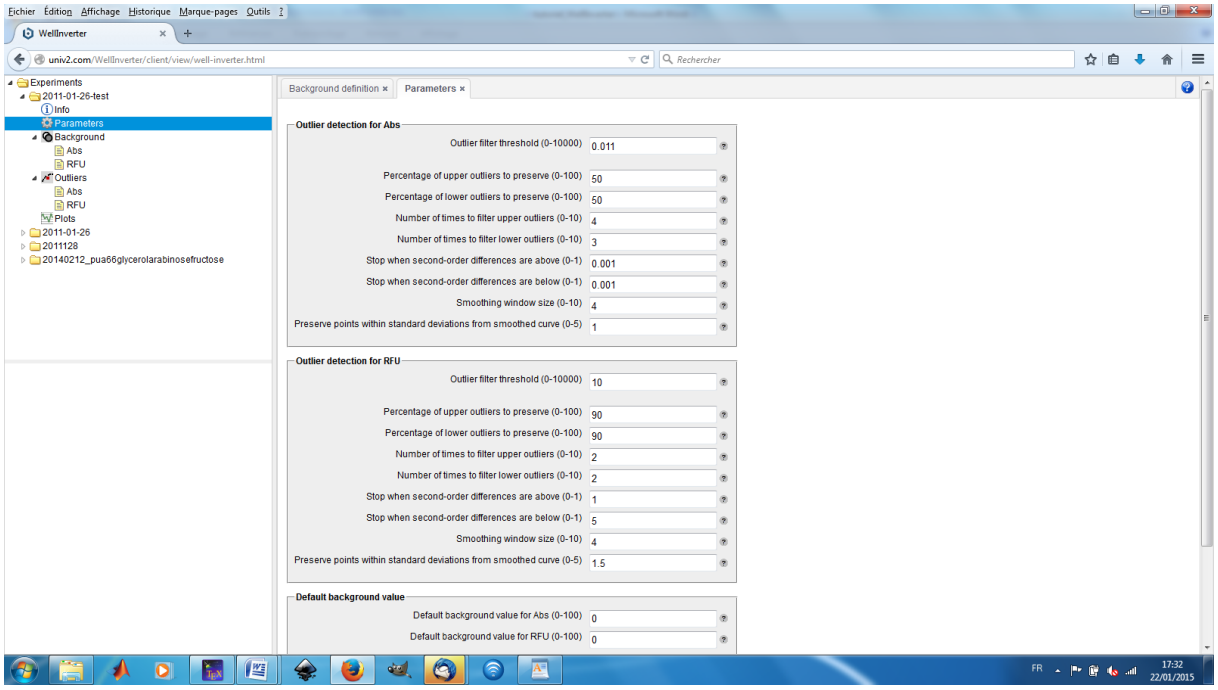
WellInverter is a web application for the analysis of reporter gene data, based on powerful linear inversion methods. In this tutorial we describe how to get started with WellInverter, in eight easy steps, from uploading your reporter gene data to performing the analysis and downloading the results. For more details on the linear inversion methods, see the accompanying paper (Zulkower et al., submitted for publication). For more details on the different options in each step of the analysis, you may also wish to consult the online help pages of WellInverter, accessible from within the application by following the "?" symbols.

Step 1: Upload your data file

Right-click on *Experiments* to open the upload window. Select your Tecan CSV data file and upload it to the WellInverter server.

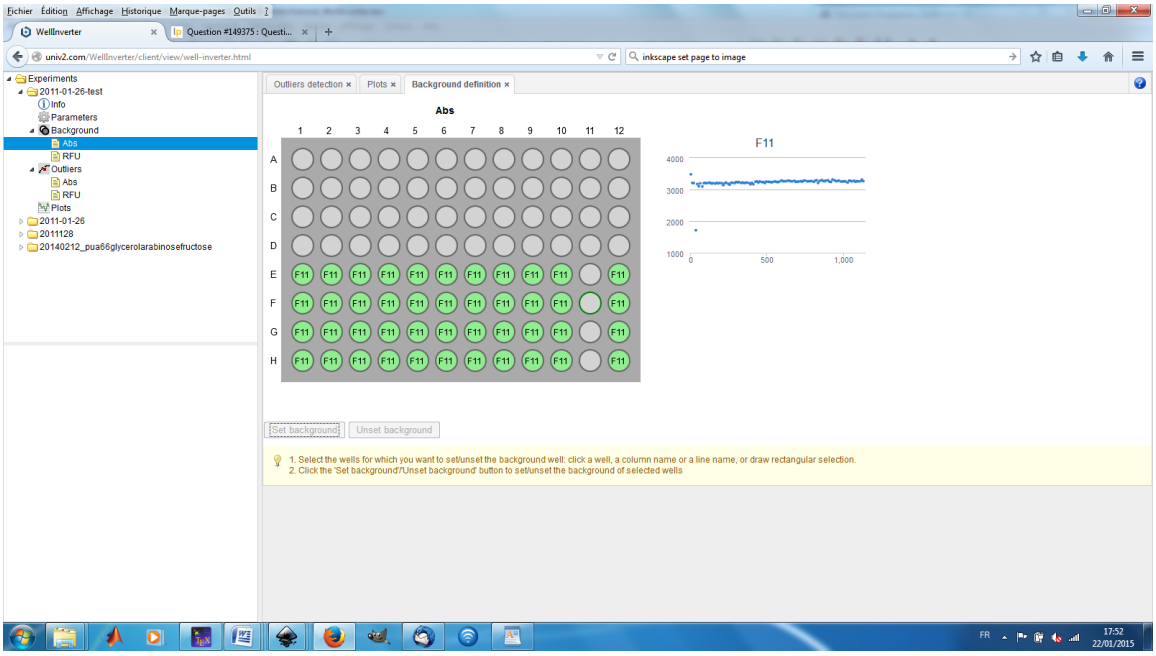
Step 2: Define the parameters for analyzing the data

Click on *Parameters* to open the window for defining the parameters of the analysis. You can set a number of parameters for outlier detection, background subtraction, and well synchronization. If there are little or no outliers, you do not have to change the default values for outlier detection. If your microplate contains wells with background controls, you do not need to define a default background level. If you do not want to synchronize the data in the wells on the microplate, you can set the maximum shift to 0. After changing parameter values, do not forget to save them by clicking on the *Save* button.



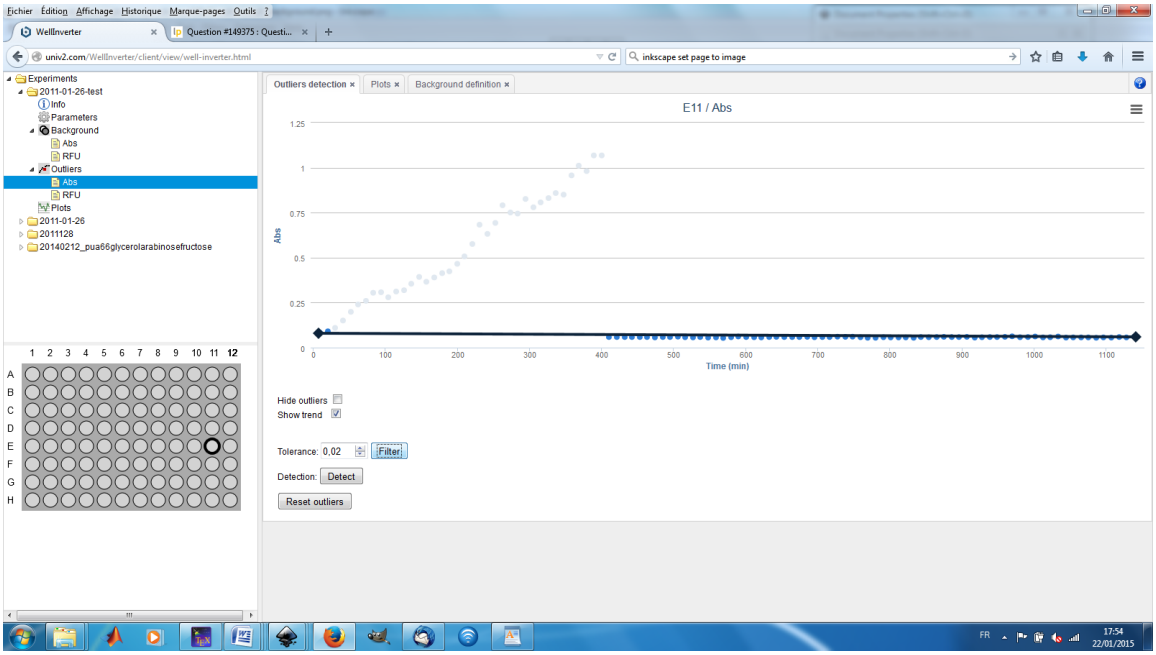
Step 3: Define the background for your measurements

Click on *Background* to open the window for defining the background levels to be subtracted from the primary data. First, select the wells for which you want to define a background, by clicking on individual wells, by clicking on column/row identifiers, or by drawing a rectangle with the mouse. Second, indicate that you want to set the background well for these selected wells, by clicking on the corresponding button. Third, select the well that contains the background control. Backgrounds can be defined for all different types of measurements in your data file, typically absorbance and fluorescence measurements.



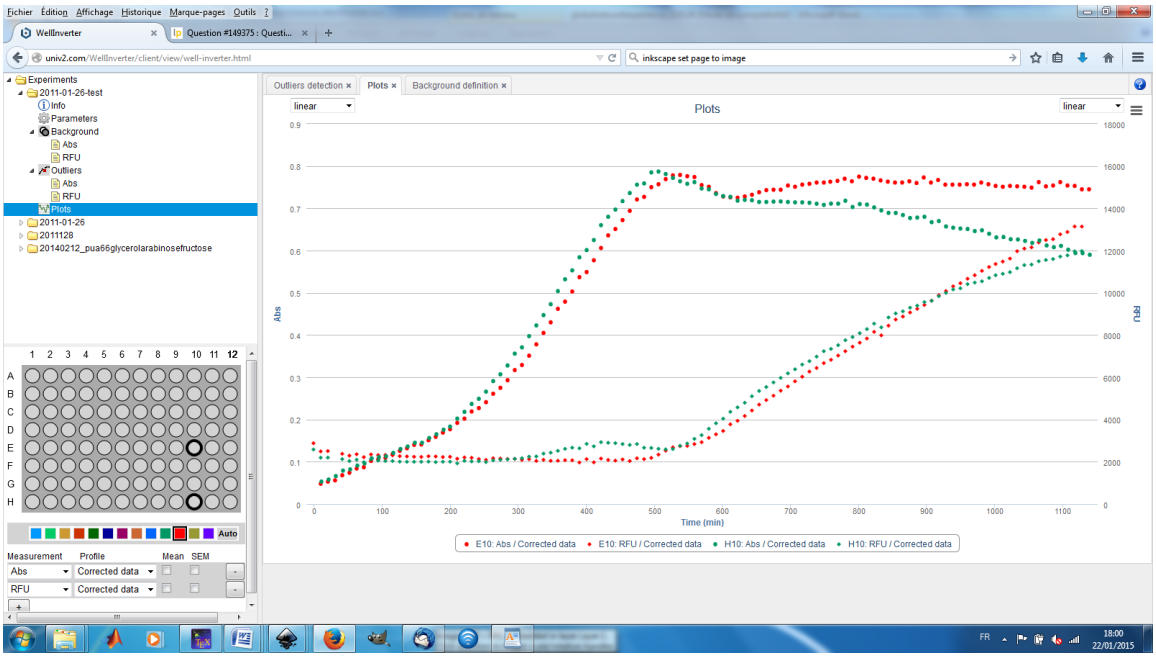
Step 4: Detect potential outliers in your data

The data from growth experiments in the microplate often contain outliers due to instrument failure or to specific experimental conditions (*e.g.*, the use of glass beads to improve aeration of a growing bacterial culture). WellInverter provides several alternatives for detecting and removing outliers. Click on *Outliers* to open the corresponding window. The first and simplest option to label data points as outliers is to click on the data point, which removes it from the data set (indicated by a change of color of the data points, from deep blue to grey-blue). The second alternative is to draw manually a trend in the data, by checking the option *Show trend* and by selecting consecutive points of a curve representing the trend in the data. All data points diverging by more than the indicated value in the *Tolerance* field are classified as outliers and removed from the data set when clicking on the *Filter* button. A third alternative is to press the *Detect* button, which launches the automated outlier detection algorithm. The latter option is particularly useful when you want to treat one or several microplates automatically. In that case, the outlier detection algorithm first needs to be calibrated and tested on a few typical wells on the microplate, by choosing appropriate parameters in Step 2.



Step 5: Plot the data after background correction and outlier detection

You can plot the treated data (background subtraction, outlier removal, synchronization) and compare the effect of these transformations with the primary data by clicking on *Plots*, which opens the plotting window. You can visualize several wells, by selecting them on the microplate representation, and different types of measurements per well. The colors can be automatically selected (by clicking on the *Auto* button) or manually chosen, by clicking on a color in the color bar. The latter option is useful, for example, when you want to display the contents of replicate wells in the same color. The *Measure* field allows you to select the different types of measurements (typically absorbance and fluorescence), the *Data* field the different types of data (primary data, outlier-corrected data, synchronized data, and background-corrected data). When selecting *Mean*, the mean of the replicates is displayed as a separate curve, while *SEM* shows the standard error of the mean as a shaded region.



Step 6: Compute growth rates, promoter activities, and protein concentrations

The growth rate, promoter activity, and protein concentration can be selected in the *Data* field of the plotting window. These quantities are automatically computed using the linear inversion methods. Like for the data from which they are computed, you can display the mean and the standard error of the mean for the selected wells.

Step 7: Download the data and analysis results

The data and analysis results are stored on the WellInverter server, but you are advised to regularly save the WellInverter files locally on your computer. You can do this by right-clicking the name of the experiment and selecting the option *Download WellInverter (JSON) experiment file*. The data are stored in the JavaScript Object Notation (JSON) format.

Step 8: Export your analyzed data

Sometimes it is convenient to export the (partial) results of the analysis in order to treat them in more depth by means of other programs. You can export the data and the analysis results by right-clicking the name of your experiment and then selecting the option *Export data and analysis results*. All data and analysis results plotted during the session will be exported in text fields that can be read into your favorite data analysis program.