[iGEM 2017] Plate Reader

Introduction

This is a basic overview of the Synergy H1 microplate reader. The plate reader is fairly intuitive and easy to use, and has an extensive searchable manual which can be accessed using the help button.

Materials

- Synergy H1 microplate reader
- Griener Bio-one plates
  - *Currently our plate reader's software is configured to use* Griener Bio-one plates*, and this protocol is written with the assumption that you are using this plate. While there are other types of plates which are compatible with the plate reader, it is necessary to prime the software with its specifications prior to its usage. Different plates and lid sizes can be enabled by going to the systems toolbar and selecting plate types. Please read the appropriate Manual and Documentation for instructions on how to do this.*
- (Optional) Plate Lid

Procedure

Turning on the Plate Reader

1. There is a power switch on the bottom left of the front of the machine. Turn it on. The plate reader will take a few minutes to start up, and it will involve a lot of repetitive long-lasting laser sounds. The plate-loading tray will open outward automatically at the end of the procedure.

2. If you are not yet ready to load in your plate, press the stick-like button at the bottom right of the front of the plate reader to close the plate-loading tray.

Preparing Samples in a 96-well plate

3. Load samples into the plate. Each well holds a maximum of 200 uL volume. We recommend that you do not exceed 150 uL if you are performing an assay which involves shaking/rotating the plate inside the plate reader.

Configuring the Software to measure your samples: Establishing a Protocol

4. Sign into the laptop next to the plate reader with **the following credentials**:

   user: .\BioMaker
   password: bsmsISC3033&&

5. Open Gen5 software (looks like a plate)

6. Create a New Protocol

   Once a relevant protocol has been created for your experiment (for example to measure GFP fluorescence and OD600 simultaneously), you can proceed to the next section.

7. Navigate to procedure (under protocol tab).

   Here you will specify your actual measurement protocol, which are the instructions that the plate reader will follow in collecting your data. On the left window you have Actions which can be inserted into the protocol. Once inserted, you can click 'Options' on a selected Action to configure its properties. Relevant Actions include:
   - READ : Perform an optical measurement. Can be configured to measure absorbance, fluorescence, luminescence and Time resolved fluorescence. Wavelengths can be manually specified (for sfGFP use 485 excitation and 520 emission), as can the gain.
   - START/END KINETIC : This is analogous to a WHILE loop. Actions inside the Kinetic Loop will take place repeatedly for the duration of the loop. The absolute duration of the Loop, as well as the frequency with which the Actions within will be performed, can all be configured. *Note that it is possible for your manually-specified frequency to contradict your manually-specified total duration. This is a common source of error in creating a Protocol.*
   - SHAKE : Will shake or rotate the plate. For long time-course assays, you should place a Shake Action inside your Kinetic Loop to keep your solution mixed.
   - SET TEMPERATURE : Set the temperature inside the plate reader. *Note: Set Temperature should be placed at the beginning of the Protocol, and it will maintain this temperature throughout the course of the experiment.*

   CRITICAL Also, at the top of this window there should be specification of the Plate Type you are using, as well as a checkbox for 'Plate Lid'. Set these options appropriately! **Failure to do so can damage the interior of the plate reader!**

8. Save your protocol.

Configuring the Software to measure your samples: Setting up the Experiment

9. Now, create a 'New Experiment'.

10. If you have configured the procedure to select wells at runtime then you can click and drag with your mouse the relevant wells on the plate, and the plate reader will only take measurements at those wells.

    Although there is a way to specify names for your samples at this menu, it is very cumbersome and unintuitive. Since your final data output will include the well coordinates associated with your readings anyway, we recommend that you store a key matching well coordinate to sample ID externally.

11. Click the green 'Play' button to begin your read. This will automatically cause the plate loading tray to open.

Loading the Plate into the Plate Reader

12. Insert your plate so that A1 aligns with the 'A1' marked on the plate-loading tray.

13. (if applicable) place the lid on your plate.

14. Press the stick-like button on the bottom right of the front of the plate reader to close the plate-loading tray. (This can also be done inside the software).

15. Your run will begin automatically!

Finishing the Measurement and Exporting your Data

16. Upon conclusion of the run, the plate-loading tray will open automatically. Remove your plate from the machine and close the tray by pressing the stick-like button at the bottom right of the front of the plate reader.

17. You can then measure additional plates. If you are finished, you can export your data by going to the plate tab and clicking the export button. Choose your desired export format, and then save in a folder somewhere. The computer is not internet enabled, so a flash drive must be used to transfer data.

18. If you are finished with all your measurements, turn off the plate reader using the switch at the bottom left of the front of the machine.