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iGEM TU/e 2015
Biomedical Engineering

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Measuring Fluorescence

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Measuring (bio)luminescense and fluorescence

Where innovation starts

1   Preparation samples

Estimated bench time: 20 minutes
Estimated total time: 20 minutes
Purpose: Prepare samples for fluorescence assay

1.1   Materials

- 384 Black wells plate
- dH_2O
- Eppendorf tubes
- Protein samples
- PCR tube

1.2   Setup & Protocol

- Make the desired protein concentrations and mixtures in a PCR tube.
- Load the samples in the 384 wells plate with a maximum of 90 µl of each sample. (The maximum loading volume may depend on the brand that is used, so search for the well-plate specifications beforehand.)

2 Measuring fluorescence

Estimated bench time: 30 minutes
Estimated total time: 30 minutes
Purpose: Analyses of the bioluminescence of the expressed proteins.

2.1 Materials

- Prepared 384 Black wells plate with loaded samples
- Tecan Safire2 plate-reader

2.2 Setup & Protocol

- For the measurement of fluorescence, open the program (Excel-file) of the Tecan Safire2 plate-reader and edit the measurement parameters.
    - Select the fluorescence measurement.
    - Plate: select the plate that is used and the wells that contain the samples.
    - Excitation wavelength: select the excitation wavelength to one suitable for the fluorophore that needs to be measured.
    - Emission wavelength: it's possible to scan over a broad wavelength range and determine the emission of the fluorophore. If you want to only measure the excitation at a certain wavelength, you need to select emission wavelength instead of emission scan.
    - Nr. of reads: select 10 (default)
    - Select endpoints measurement if you only want to perform one measurement.
- Insert the plate into the Tecan Safire2 plate-reader and start the measurement.