Horse Feces Plate Reader Fluorescence

Purpose:

To focus on quantification, a similar experiment was undertaken using a plate reader. The expansion of the experiment via different sample preparation methods was to see if there was a cheap method (filtering), that would show the presence of the reporter system more easily and quantitatively. From the initial experiment that was previously described, it was decided that amilGFP would be the best candidate for this follow up experiment. With this in mind, a liquid culture of OD600 = 2.13 expressing amilGFP was used in all sample preparations below.

To find the ultimate sample preparation for fluorescence measurements, two different methods are used:

- Samples with only feces and *E.coli*+GFP

- Samples with feces and *E.coli* which has been filtered with LB-media

Method:

For the measurements a multifunctional microplate reader was used, called Tecan infinite© 200.

Sample preparation

Three different kinds of samples were prepared, one with feces mixed with overnight culture with bacteria that express GFP, and the second and third samples consisted of feces mixed with diluted mixtures of overnight culture that expressed GFP and were filtered using coffee filters.

-The first sample preparation consisted of horse feces (a mixed batch was prepared with 10 g of feces from 5 different horses) mixed with a liquid culture of amilGFP at different concentrations (1, 3, 8, 15, 25, 50 and 75 %) respectively, with the total weight of mixed samples totaling at 1 g.

-In the second and the third sample preparation, 1 g of horse feces was mixed with LB-media and liquid culture of amilGFP at different concentrations (5, 10, 15, 25, 50, 75 and 100%).

Fluorescent measurements

Three different excitation and emission wavelength were used for this experiment, as followed:

Configuration	Excitation	Emission
1	396	509
2	488	510
3	503	512

However the last configuration was only measured on samples that were more than 1 day old. Therefore, the reliability of the results should be considered carefully.

Samples were put into the machine and read, and then graphs were made to determine the concentrations of GFP expressing bacteria needed in order to be detected in horse manure.