Reporter System, Weekly Notes

Week 1 (11/6-17/6)
Troubleshooting of fluorescence cell lines + Laboratory Planning, making LB and LB+c plates, regular LB and LB+Agar)

Week 2 (18/6-24/6)
Chromoprotein lines obtained from another lab were expressed and put into equine feces in various forms (liquid from cultures and solid from LB plates) “Horse Poop Analysis (initial)” protocol was used

Learned that Chromoproteins are not observable with the naked eye and UV light. However solid cultures mixed into equine feces were visible

Week 3 (25/6-1/7)
Attempted using activated charcoal before performing the “Horse Poop Analysis (initial)” protocol. Theory behind this: Charcoal can absorb impurities and stay in a solid form so it can be later extracted. There is also evidence that activated charcoal absorbs bilirubin-like structures (that are present in feces).

The results were slightly better when using activated charcoal, but not significantly.

Tried using solid cultures combined with horse manure. Works better with more water mixed.

Also “calibrated” the spectrophotometer for cell counts, so we would know which spectrophotometer was the most accurate for *e. Coli* growth curves.

Week 4 (2/7-8/7)
Preparation for measuring fluorescence with GFP with a plate reader. Different methods of sample preparation were done.

Week 5 (9/7-15/7)
Preparation of measuring fluorescence with GFP. Overnight cultures were made. Tested out some reads with the multiplate reader with feces and amilGFP. Some blanks showed more fluorescence than samples with amilGFP. New configurations on wavelengths were discussed and the whole measuring fluorescence of amilGFP was done.

The samples from the earlier day was measured again, with the two old settings and one new setting.

Week 6 (16/7-22/7)
Analysis and compilation of data was done

Made competent cells and flash froze them using “competent cell protocol” Tested them using “Transformation” protocol from the iGEM website using competent cell test kits

Week 7 (23/7-29/7)
Repeated competent cell/transformation protocol.

Ordered UnaG sequence for potential new reporter system that utilizes bilirubin. Expected to come August 14th.

Attempted transformations again, very low efficiency

Week 8 (30/7-5/8)
The transformation was successful for some of the plates.
Parts of lab group helped other subgroups with stuff.

Did new competent cells and did the transformation before freezing the cells. New protocol was followed, where cells were spinned down and took out 800 µL of the supernatant and then use the concentrated solution with the pellet for plating. The new protocol was used on Mueller-plates (the “wrong” plates).

The plates with 10 pg/µL didn’t show any transformation, 100 pg/µL worked for all plates (even the “wrong” plates).

Week 9 (6/8-12/8)
Subgroup work

Week 10 (13/8-19/8)
UnaG sequences was received in a low copy plasmid. Tried to ligate the UnaG sequences into a high-copy plasmid, with no success.
Proceeded with making same day competent cells and transformation of the plasmid with UnaG.

Week 11 (20/8-26/8)
Extraction of expressed UnaG from both the original sequence and from the modified sequence. Affinity chromatography was done with GE healthcare HisGravi trap.

Week 12 (27/8-33/8)
SDS-PAGE of the UnaG-proteins from the affinity chromatography. The modified UnaG-sequence was successfully ligated into backbone pSB1C3 by 2A-assembly and ready for submission into biobrick registry.