Week of May 7th:

- Received glycerol stocks from Dr. Lois Murray of last year's biobrick plus endoglucanase and primers

Week of May 14th:

- Using primers designed last year for canonical endoglucanase and beta-glucosidase coding genes, a PCR using Taq polymerase was performed on environmental cDNA from the porcupine microbiome. cDNA was created last year.
- 150 mg of fecal sample from the porcupine was mixed with 3 mL of water to create a slurry. This was streaked onto MacConkey plates.

Week of May 21st:

- Ran PCR products through 0.8% agarose gel. PCRs all failed.
- 3 colony morphologies were identified on MacConkey-Porcupine fecal slurry plates. Labelled "Bulls-eye", "Light Red", and "Dark Red". These 3 colonies were streak-plated onto cellulose-degradation isolation plates. These plates were made the same as last year except the cellulose source was changed from Whatman paper to Carboxymethylcellulose. Plates were left overnight at 37 degrees.

Week of May 28th:

- A new colony was identified on the porcupine fecal slurry plates which was designated "Black".
- Single colonies from the Bulls-eye, Light Red, and Dark Red streak plates were grown up overnight in LB broth at 37 degrees, shaking. A single colony of Black was grown up the same way. Cells were spun down and kept at -20 degrees until next week.
- Endoglucanase+biobrick glycerol stocks from last year were streaked onto LB + Carbenicillin plates and grown overnight at 37 degrees. Single colonies were picked from the plates and grown in LB broth. Cells were spun down and kept at -20 degrees until next week.

Week of June 4th:

- Received pET26b containing bacteria on an LB plate from Jamie. Selected single colonies and grew up in LB culture overnight in the presence of Kanamycin as an antibiotic marker. pET26b was then miniprepped using Qiaprep Miniprep Kit from Qiagen.
- Miniprepped endoglucanase+biobrick using Qiaprep Miniprep Kit from Qiagen and diagnostically digested it with either no enzyme, PstI alone or PstI and NotI for 1 hr. These diagnostic digests were run through a 0.8% agarose gel and were successful.
- Performed colony PCR on the 4 colony morphologies following last year's protocol for doing so.

Week of June 15th:

- Ran all 4 colony PCRs through a 0.8% agarose gel. Gel extracted the light-red and black morphologies using QiaQuick Gel Extraction Kit. Re-PCR'd the dark-red and bulls-eye colonies. Sent light-red and black morphologies for 16S sequencing at GeneWiz but sequencing returned with no priming.

Week of June 25th:

- Amplified last year's Endoglucanase part out of the biobrick using PCR.

Week of July 2nd:

- Gel extracted endoglucanase PCR via QiaQuick Gel Extraction. Digested with BamHI and SacI for insertion into pET26b plasmid. Ligation attempted but transformation into Stbl3 E. coli revealed a failed ligation.
- 1 ug of pET26b miniprep digested with BamHI and SacI. Digested backbone then ran through a 0.8% agarose gel to confirm size and backbone was gel extracted via QiaQuick Gel Extraction.

Week of July 9th:

- Ordered identified beta-xylanase to be synthesized by IDT DNA.

Week of July 16th:

- Received synthesized beta-xylanase.
- PCR amplified endoglucanase from previous biobrick and beta-xylanase from IDT gene block.

Week of July 23rd:

- Gel extracted last week's PCRs using QiaQuick Gel Extraction.
- Digested both extractions for insertion into pET26b. BamHI/SacI for endoglucanase, BamHI/EagI for beta-xylanase.
- Digested 1 ug of pET26b with BamHI/EagI for beta-xylanase insertion. Then gel extracted as before.
- Purified both insertion digestions and ligated them overnight to the corresponding pET26b digestions.

Week of July 30th:

- Transformed endoglucanase and beta-xylanase ligations into Stbl3 E. coli and plated on LB plates containing Kanamycin. Incubated overnight at 37°
- Beta-xylanase transformation failed but endoglucanase transformation had some enrichment. Selected 3 colonies and grew up overnight in LB broth with Kanamycin at 37°, shaking.
- PCR amplified beta-xylanase from IDT gene block again. Changed Tm from 56° to 51°.
- Miniprepped endoglucanase batch cultures using QiaPrep Miniprep Kit and sent portions of the minipreps for sequencing via GeneWiz.

Week of August 6th:

- Gel extracted beta-xylanase PCR successfully. Digested as before and ligated as before. Transformed into STBL3s as before on kanamycin-LB plates.
- Endoglucanase+pET26b confirmed successful via sequencing!
- Selected 3 beta-xylanase colonies and grew up overnight as before in LB broth.
- Miniprep of these cultures failed due to user error. Transformed beta-xylanase/pET26b ligation into STBL3s again. Transformation failed (no enrichment).

Week of August 13th:

- Received alpha-L-A, beta-glucosidase, and endoxylanase gene blocks from IDT!
- PCR amplified all 3 and gel extracted all 3 via QiaQuick Gel Extraction Kit.
- Digested endoglucanase+pET26b and beta-glucosidase gel extraction with SacI and HindIII for operon construction.
- Purified beta-glucosidase and ligated to endoglucanase+pET26b.

Week of August 20th:

- PCR amplified beta-xylanase once again, gel extracted, digested with BamHI and SacI and ligated to pET26b. Transformed into STBL3s as before. Great enrichment, selected 4 colonies and grew up overnight in LB broth as before. Miniprepped all 4 selected colonies as in QiaPrep Miniprep Kit.
- Re-digested and gel extracted pET26b+endoglucanase for 3 hours with SacI and HindIII as Jacob extracted the wrong band. Beta-glucosidase re-ligated to new digestion. Transformed into STBL3s as before. Transformation failed as there was no enrichment.

Week of August 27th:

- Last week of summer means vacations all around! Nothing to report for this week.

Week of September 3rd:

- Digested beta-glucosidase, endoxylanase, and alpha-L-A gel extractions from a few weeks ago with either SacI/HindIII (beta-glucosidase and endoxylanase) or HindIII/XhoI (for alpha-L-A) for insertion into pET26b. Also digested pET26b the same (1 sample SacI/HindIII, 1 sample HindIII/XhoI)
- Gel extracted both backbones and purified inserts (beta-glucosidase, endoxylanase, and alpha-L-A). Ligated inserts to appropriate pET26b extractions.
- Transformed all ligations into STBL3s as before.
- Beta-xylanase minipreps from two weeks ago sent for sequencing at GeneWiz. Sample 1 confirmed as working!

Week of Septermber 10th:

- All transformations from last week turned out as lawns. Re-digested both versions of pET26b (SacI/HindIII and HindIII/XhoI) for 4 hours and gel extracted as before.
- PCR amplified beta-glucosidase, endoxylanase, and alpha-L-A for insertion into biobricks. Ran through a 0.8% agarose gel to confirm amplification. Only beta-

glucosidase and alpha-L-A amplified correctly. These two were gel extracted as before and digested with NotI.

- Mackenzie Midiprepped RFP-containing biobrick for use as backbone for all of our biobrick parts. 1 ug of RFP-biobrick midiprep digested with NotI and gel extracted as before.
- Beta-Glucosidase ligated to NotI-digested biobrick.
- Endoxylanase PCR amplified again for insertion into biobrick with less primer concentration and a longer extension time at 72^{o}.

Week of September 17th:

- Ran out endoxylanase PCR for insertion into biobrick from last week, did not work again. Re-PCR amplified using even less primer concentration and a longer extension time. Finally worked after running through a 0.8% agarose gel. Gel extracted as before.
- Beta-glucosidase+biobrick ligation transformed into STBL3s. Plated on LB+carbenicillin plates. 4X enrichment the following day and 4 colonies were selected and grown up in LB broth with carbenicillin (1:1000). Cells were pelleted and miniprepped as before. All 4 samples sent for sequencing at GeneWiz.
- Made 3 types of solid media for enzyme assessment: carboxymethylcellulose and glucose + basic M9 media, cellobiose + basic M9 media, and glucose + basic M9 media.
- Beta-glucosidase+pET26b, endoxylanase+pET26b, alpha-L-A+pET26b, and alpha-L-A+BioBrick transformed into STBL3s and plated on appropriate media (Kanamycin+LB for pET26b, LB+carbenicillin for BioBrick).

Week of September 24th:

- No transformations from last week had any enrichment.
- New PCR amplifications: alpha-L-A for BioBrick, endoxylanase for pET26b and BioBrick, and beta-glucosidase for pET26b (4 PCRs total). Gel extracted all PCRs as they all worked. Both biobrick amplifications digested with NotI, both pET26b amplifications digested with SacI/HindIII. Purified and ligated to appropriate backbones.
- BioBrick ligations transformed into STBL3s and plated on appropriate media. ~3X enrichment for both. Selected 4 colonies from both and grew up overnight in LB broth + carbenicillin as before.
- Genewiz sequencing of beta-glucosidase+BioBrick showed a truncated version of the insert.
- PCR amplified beta-xylanase for insertion into pET26b.
- Transformed endoglucanase+pET26b miniprep into BL21 DE3s for enzyme assay. Serial diluted colonies (10^{-3} to 10^{-5} in Magnesium Chloride salt solution) and streaked onto carboxymethylcellulose (CMC) and glucose + basic M9 media plates for determination of colony growth on plates without expression of protein (ie. no IPTG).

Week of October 1st:

- Bacteria grew after 48 hours incubating at 37^{o} on the CMC and glucose + M9 media plates. Single colonies were visible on the 10^{-5} dilution plate while 10^{-3} and 10^{-4} were lawns of bacteria.
- Transformed empty pet26b into BL21 DE3s and plated as before.
- Overlaid 40 uL of 0.1 M IPTG onto 2 CMC and glucose + M9 plates and plated 50 uL of a 10^{-5} dilution (in dH_2O) of both endoglucanase+pET26b BL21s and pET26b BL21s. Incubated at 37^{o}C for 5 days (growth seen at 3 days). Repeated onto 2 new plates via streaking instead of plating dilutions. Incubated for the same time (growth seen at 3 days).
- Ordered a new beta-xylanase gene block from IDT DNA as the current gene block contained a NotI site and an EcoRI site.
- Endoxylanase+biobrick and alpha-L-A+biobrick cultures miniprepped and sent for sequencing.
- PCR amplified beta-glucosidase for insertion into biobrick. Gel extracted after running through 0.8% agarose gel and digested with EcoRI and PstI. 1 ug of Biobrick digested with EcoRI and PstI in preparation for ligation.
- Beta-glucosidase + pET26b ligation transformed again and plated onto appropriate solid media. No enrichment the next day.
- Sequencing from GeneWiz confirmed alpha-L-A is successfully cloned into the biobrick but endoxylanase is not. 8 colonies selected from previous transformation and grown overnight in batch culture as before.
- PCR amplified new beta-glucosidase for insertion into pET26b.

Week of October 8th:

- Assayed endoglucanase enzyme activity on single colony dilution plates via a Congo Red wash (0.5% Congo Red in H_2O). Possible haloing around 2 colonies on endoglucanase+pET26b plate. Second Congo Red wash on streak plates confirms this as a false positive as we saw haloing on both the endoglucanase+pet26b streak plate and the negative control.
- Gel extracted beta-glucosidase from last week as before. Ligated to pET26b and transformed into STBL3s as before. ~3X enrichment. 5 colonies selected and grown up overnight in batch culture as before. 5 cultures miniprepped as before.
- Beta-glucosidase ligated to biobrick and transformed as before. No enrichment.
- 8 endoxylanase cultures miniprepped as before and sent for sequencing. Sequencing confirmed that 1 out of 8 was successful! 2 biobricks completed.
- Made new endoglucanase assay plates following last year's recipe for cellulose media. Substituted whatman paper for carboxymethylcellulose and added 8 g of glucose.
- Grew up endoglucanase+pET26b BL21 DE3s and pET26b BL21 DE3s in batch culture for 17 hours overnight as before. At 17 hours, 5 uL of 0.1M IPTG was added to the cultures and cultures were returned to incubator for another hour. After that hour, cultures were streaked onto two of the newly made CMC+glucose+Congo Red plates and incubated at 37^{o} for 2 days. At 2 days of growth, halos had formed around the streaks on both the negative control and the sample of interest plate.
- New beta-xylanase gene block arrived from IDT.

Week of October 15th:

- New beta-xylanse successfully cloned into biobrick.
- Beta-glucosidase successfully cloned into biobrick.
- Beta-glucosdiase+pET26b sequencing returned negative. Beta-glucosidase re-PCR'd for insertion into pET26b and gel extracted. Decided to abandon beta-glucosidase+pET26b and focus on beta-xylanase in the interest of time.
- Transformed beta-xylanase+pET26b into BL21 DE3 E. coli for fluorophore and Coomassie blue experiments.
- Optimized endoglucanase+pET26b transformed into BL21 DE3 E. coli and Stbl3 E. coli

Week of October 21st:

- Optimized endoglucanase successfully cloned into biobrick.
- Beta-xylanase+pET26b and optimized endoglucanase+pET26b grown overnight in LB Broth+Kanamycin before induction with IPTG. Cell pellet collected and frozen for 1 hour at -20°. After 1 hour, boiled at 95° for 5 minutes in 200 µL Laemmli buffer before diluting 1:1 with Protein Sample Buffer. Both protein samples ran on SDS-PAGE. Optimized endoglucanase transferred to membrane and probed for His-tag. Successful! Beta-xylanase stained with Coomassie Blue for total protein expression. Failed.
- Beta-xylanase assayed for enzymatic activity via fluorophore protocol. Repeated 3X before results satisfactory.
- BL21 DE3s with beta-xylanase+pET26b assessed again for total protein expression compared to empty vector using IPTG. Successful! Band at ~50 kDa.