Ramya Prathuri's Notebook:

5/30 – 6/6:

Began brainstorming project ideas. Came up with several 2-component systems for biosensor applications in E. coli. Some of these sensors include ComX/ComP, AgrB and AgrD. Decided to use indigo as biosensor reporter. Began searching for ways to implement indigo pathway in E. coli. Began cloning several homologous glucosyltransferases to test in vivo for indican production.

6/7 – 6/14:

Identified particular 2 component systems such as pknB that utilize a serine/threonine kinase instead of a histidine kinase as the latter's mechanism would prove too difficult to engineer cross reactivity into. pknB has PASTA domains that detect beta-lactam rings such as those on antibiotics. Ordered primers to clone pknB into E. coli. Checked to make sure pknB would not result in inclusion body in E coli – search was inconclusive. Began cloning many of the promoter parts we would be using in E. coli. Co-transforming FMO expressing plasmids with various cloned GTs.

6/15 – 6/22:

Co-transforming did not work properly – probably because both plasmids on ColE1 origin. Began cloning FMO plasmids onto p15A origin of replication which has a lower copy number. Continued promoter assemblies for E. coli. Also began cloning FMO and GT onto multigene assemblies. Talked about potentially aiming to detect toxic intermediates using indigo sensor. Started researching potential toxic intermediates.

6/23 – 6/30:

Some toxic intermediates/ toxic chemicals we discovered included luteolin, and urushiol (toxic chemical in poison ivy). We also began designing plasmids to test if indigo production was toxic to E. coli as we work to optimize production. To do this, we were designing mutated FMO and mutated p450 enzymes to act as controls for the actual indigo producing parts. Used HPLC to see if any indican was generated in vivo with multigene assemblies with FMO and GTs. No positive results on that yet.

7/1 – 7/7

Wrote program to quickly analyze HPLC data. Did toxicity experiment. Received parts for glucosidase cloning, so we began to do that as well. Analyzed data for toxicity – we found that indigo production was not toxic as growth rate between control and indigo producing cells.

7/8 – 7/15

Homologous GT testing did not seem to produce good results. Next idea was to use cDNA libraries to find GTs from plants. To enrich for the type of B-glucosyltransferase, we would design degenerate primers that utilize a region of homology for all UDP-glucose utilizing GTs. Began designing degenerate primers. Also started cloning other enzymes that could do FMO function such as human p450 enzyme. Tested several of the glucosidases we found that potential work on indican. One of them, B. Circulans glucosidase, was the only one that worked.

7/16 – 7/23

Began doing mRNA extraction from 4 indigo producing plants. Ran into several technical difficulties during reverse transcription to get cDNA.

7/24 – 7/31

Because of tryptophan being the raw material for indigo synthesis, we tried to use minimal media to directly monitor the amount of tryptophan being added to the solution. We found that this works, except growth is exceptionally slow. Continued cDNA synthesis troubleshooting - finally achieved good concentrations of cDNA. Proceeded to try to use degenerate primers to try to enrich for GTs. Also being cloning for another GT thought to catalyze indican formation, NTGT2 from the tobacco plant.

8/1 – 8/8

Lots of cloning to try and troubleshoot cDNA library work. Began calibrating blue white screen that would be used to screen cDNA library.

8/14 – 8/21

Learned about oleD being a very promiscuous GT that acts on a variety of substrates that look and act like indoxyl. We borrowed the template for this enzyme from the Anderson Lab and purified it from pBad expression system. Continued to do some cloning for GT expression. Quantified amount of oleD protein in our purification.

8/22 – 8/29

Set up reactions with oleD protein and indoxyl substrate as well as several controls such as kaempferfol, a substrate for which oleD apparently glucosylates to 100% conversion. The positive controls worked in turning kaempferol into kaempferol glucoside, however no indican production was observed on LC-MS analysis.

8/30 – 9/6

Attempted to prevent indoxyl from oxidizing to indigo in solution by adding sodium sulfite an oxygen scavenging agent. Reset up the reactions same as last week and ran the samples through LC-MS. Again, no indican was observed. Found that a mutation for oleD called the 'ASP' mutation could lead to increased promiscuity. Decided to try to clone that version from current oleD. Also cloned two extended versions of oleD. Began cloning for registry.

9/7 – 9/14

Planned for proof of concept with glucosidase, indican, and cloth. Helped Roy work on indigo titer work. Continued cloning for oleD and started some assays with already purified oleD (same assay as before).

9/15 – present

Worked on presentation materials.