June 4

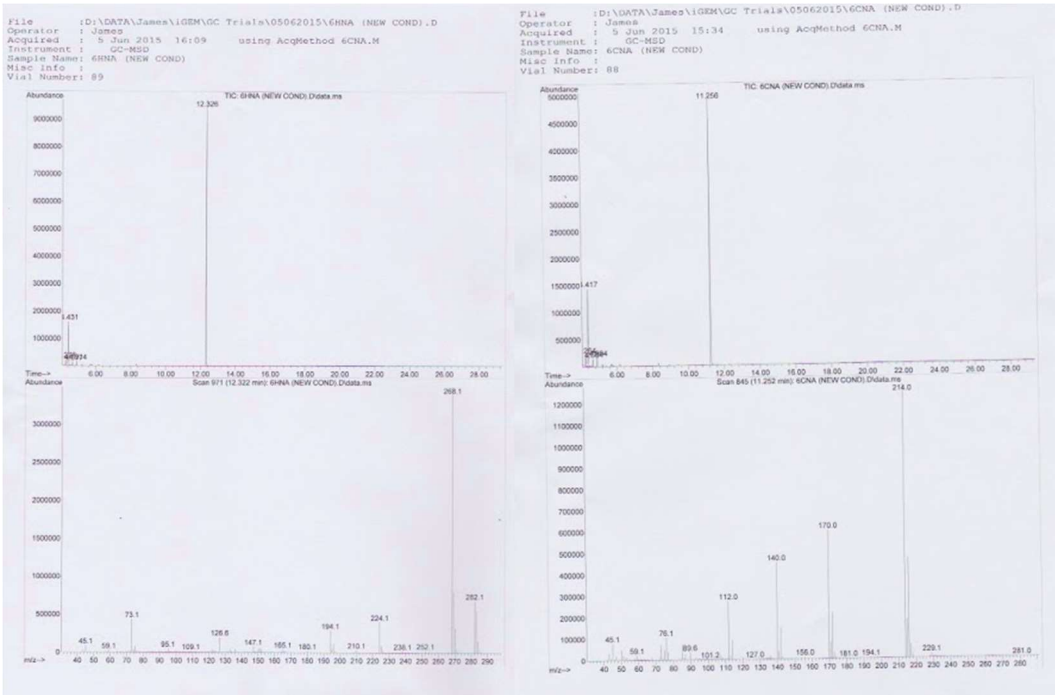
Goals: Prepare 6-CNA and 6-HNA samples to run on the gcms and test what they may dissolve in best.

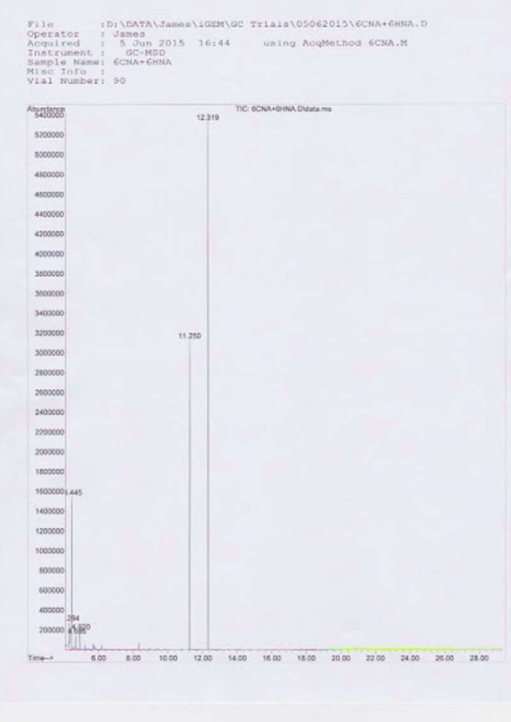
Methods: Prepared 2mg/ml 6-CNA in ethyl acetate and 2mg/ml 6-HNA in ethyl acetate stock solutions. 6-CNA dissolved readily in solvent, 6-HNA did not. Diluted 6-CNA sample to 0.5mg/ml and ran in GC/MS, which provided no clear signal. Then tried 0.25mg/ml concentration, ran it and got a distinct peak at 26 ish minutes.

June 5

Goals: Prepare 6-CNA and 6-HNA samples to run on the gcms and test what they may dissolve in best.

Methods: Prepared 2mg/ml 6-CNA in acetonitrile and 2mg/ml 6-HNA in acetonitrile stock solutions. 6-CNA dissolved readily in solvent, 6-HNA did not. Discarded acetonitrile as solvent. Prepared 1mg/ml 6-CNA in methanol and 1mg/ml 6-HNA in methanol stock solutions. After vortex and placing on heat block (100C then 50C), 6-HNA dissolved completely. From each stock solution we took 20 microliters, removed solvent, added 180 microliters of pyridine and derivatizing agent. We ran them through GC/MS, 6-CNA peaks at 26.9 min. At initial part of analysis pyridine appeared. Adjusted program to decrease time of run (quicker temperature increase) and adjusted initial temperature (80C), providing a cleaner spectrum (no pyridine at beginning) for both 6-CNA and 6-HNA. 6-CNA: 11ish minutes. 6-HNA: 12ish minutes. Runs now take approx 30 minutes. Ran both of them together, achieved good separation.





June 8

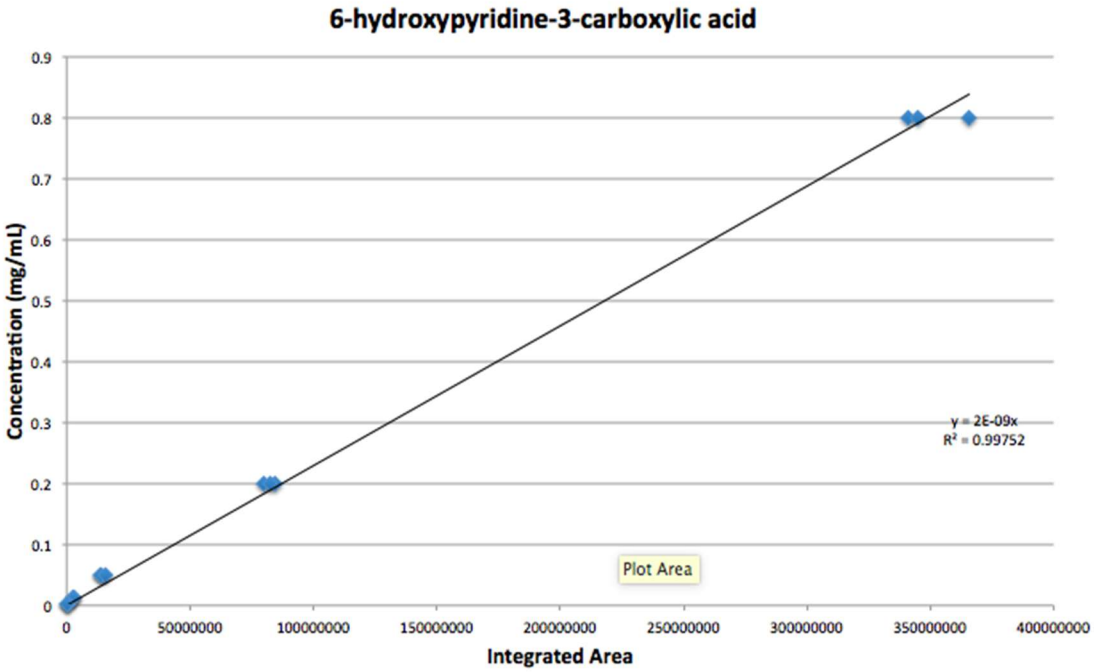
Goals: To run a sample with both 6-CNA and 6-HNA on gcms to get a standard.

Methods: Added 1ml of M9 salts, 9ml dH2O aseptically. Then added 0.1mM of 6HNA and 6CNA (139 microliters and 157 microliters respectively). Added approx 10ml EthylAcetate, vortex 30 secs, short centrifuge and then extracted the ethyl acetate (0.9 g/ml). Extraction was repeated 2 times more. Then solvent was evaporated via nitrogen drying and all 3 aliquots in the test tubes were combined into 1 fraction.

June 9

Goals: Prepare 6-CNA and 6-HNA samples to run on the gcms to make a standard curve for 6-HNA.

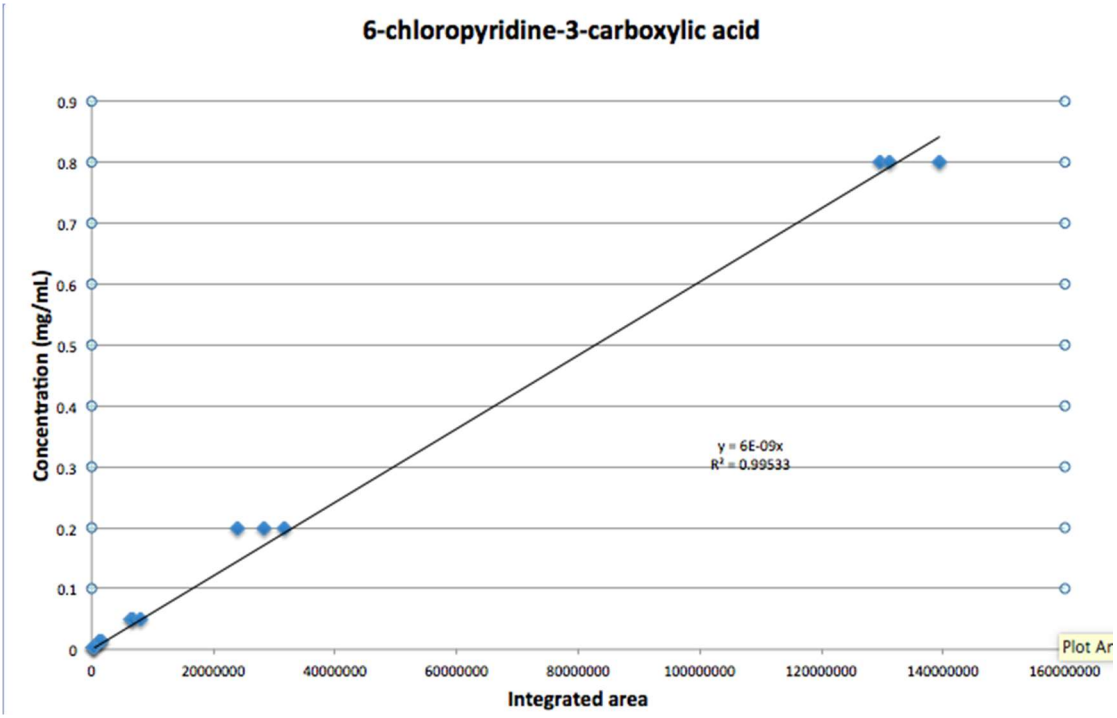
Methods: Created more stock solutions for 6-CNA and 6-HNA, both 0.8mg/ml. This time 6-HNA required no heating. The combined aliquots from June 8th's extraction were dried via Nitrogen gas. aliquots were made of 6-HNA(0.8mg/ml stock), where the concentration decreased by a factor of four, totaling 5 aliquots (0.8mg/ml, 0.2mg/ml, 0.05mg/ml, 0.0125mg/ml, 0.003125mg/ml). This was achieved by adding 50 microlitres of 0.8mg/ml 6-HNA and diluting it with 150 microlitres of methanol, this was subsequently repeated until five vials were full. This was repeated two more times totaling 15 aliquots. These were then dried by nitrogen gas and resuspended in 100 microlitres of pyridine and 100 microlitres of ethyl acetate. They were then vortexed and allowed to sit for thirty minutes. A blank was also made with 100 microlitres of pyridine and 100 microlitres of ethyl acetate. After thirty minutes the samples were ran on gcms.



June 10

Goals: Prepare the 6-CNA standard curve.

Methods: Same as June 9 but done with 6-CNA.



July 7

Goals: Do a restriction enzyme digest with CCH2 and LacI to get LacI upstream of CCH2.
Methods: Nano drop to test concentration of Nic C,D,E,F, CCH2, two human CYP and a reductase along with concentrations of plasmid with laci repressor and TAC promoter
Then restriction digest done with: (total 20 microL)

Cch2:	Laci repressor:
2 microL buffer.	Same
2 microL enzyme	Same
15 microL plasmid.	4.4 microL plasmid
1 microL water	11.6 microL water

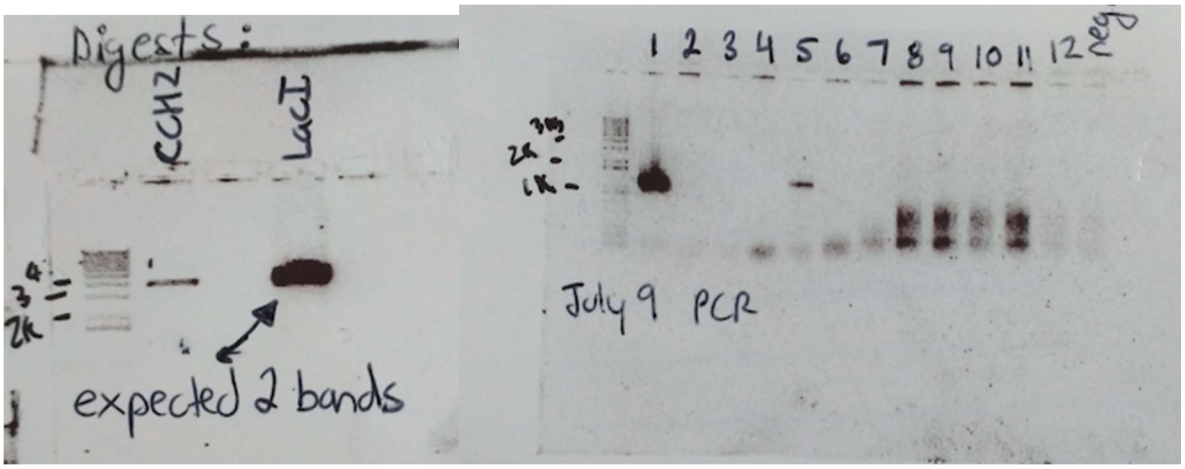
Then inoculate overnight to allow digestion.

July 8

Goals: Ligate and transform the digest products from July 7.
Methods: Heat inactivate the digestion enzymes at 80C for 20min. The negative control was the vector with the total insert volume substituted with water. Add DNA ligase and DNA ligase reaction buffer and flick and spin pcr tube. Incubate both microtubes with CCH2+LacI and just CCH2 at room temp. Then add DNA and heat shock at 65C in dh5alpha (50 microL with 250 microL LB) then rest. Inoculate one hour on the shaker to allow for expression. Finally plate aseptically on chloramphenicol plates (25 microg/mL). Then inoculate overnight.

July 9

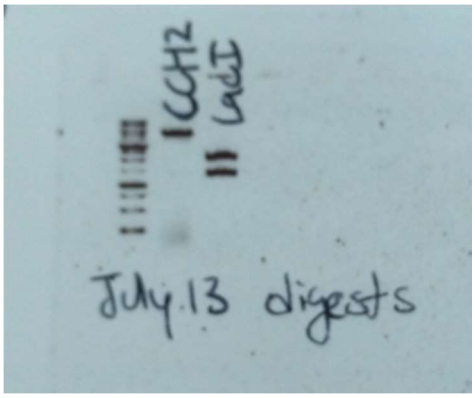
Goals: Due to the plates of the negative control being similar to experiential in number of colonies there potentially was going to be a lower chance of having a successful colony so a gel would be ran to see if the digest worked.
Methods: Ran CCH2 and lacI on gel to confirm correct length after restriction digest. Run 12 colonies for pcr with one neg control colony. Extension ran for 5min and final extension ran for 10min. All other settings for pcr same as protocol. Never used PCR product as the digest had failed.



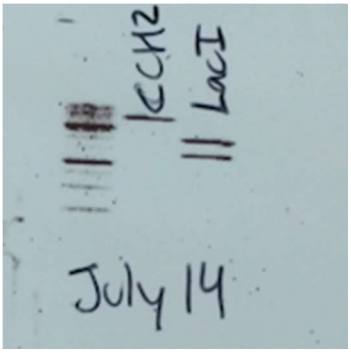
July 10

Goals: Make more colonies of CCH2 as there is none to redo a restriction enzyme digest to retry the failed digest from July 7.
Methods: Dilute of CCH2 made (2 microL CCH2/198micoL water) and transformed into e.coli to inoculate over the weekend.

July 13
Goals: Mini-prep overnight from July 10 of CCH2 and redo digest to get LacI upstream of CCH2
Methods: Mini prep the prepared CCH2 times 2. Nano dropped CCH2 with the elution buffer from the mini prep kit as a blank. Out of the two CCH2 vials, cch2 # 2 was used with a concentration of 272.2 ng/microL. The digestion was carried out with the same volumes for the lacI as on July 7. Digestion for CCH2 had amount added altered as concentration from July 7 was different (3.67microL). Used fast enzyme and fast cut buffer for digestion. Running the digestion products on gel the CCH2 had a single band approximately around 3kb and laci had two bands approximately at 1kb and 2kb. Then the ligation was done with the laci insert at three times. Did transformation and left on shaker to inoculate for 2 hours. Plated 100 and 200 microL of CCH2+LacI and the same for neg control.

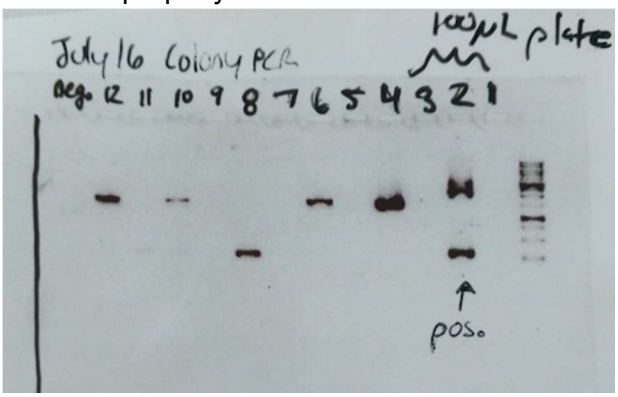


July 14
Goals: Use digested product from July 13 as the plates had a no growth on them.
Methods: Ran a gel to confirm July 13 digest still had the proper cuts. Then redid the ligation and transformation. Plating done at different concentrations to prevent a lawn from forming.



Jul 16
Goals: Do colony PCR on the plates from July 14.

Methods: Ran colony pcr with 12 colonies and one for the neg control. Thereafter a gel was ran and there was a positive colony. One successful colony had LacI upstream of CCH2. The gel for the successful colony had two bands where the lower one might have been where the PCR did not work properly.



July 19

Goals: Get vectors ready of all genes to begin standard assembly.

Methods: Prepared a liquid culture of the successful colony with the lacI and CCH2 using Chloramphenicol (50 microL) in 50mL LB. Left to inoculate overnight at 37. Also made overnight cultures of Nic C, D, E, F for assembly of the genes and made the same at the CCH2+LacI.

July 20:

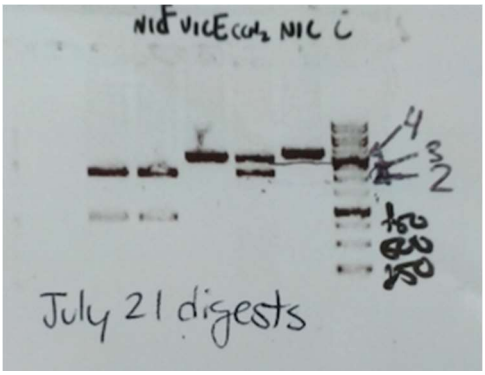
Goals: Miniprep overnights from July 19 to use for assembly.

Methods: Use mini-prep kit and store vectors at -20C.

July 21:

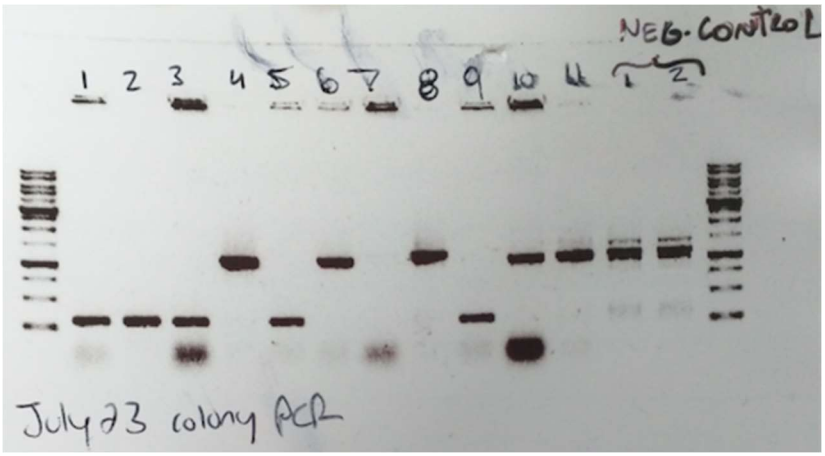
Goals: Assemble Nic F and E together and CCH2+LacI and Nic C together. Also prepare liquid culture of CCH2+LacI to run optimal protein expression experiment at different temperatures.

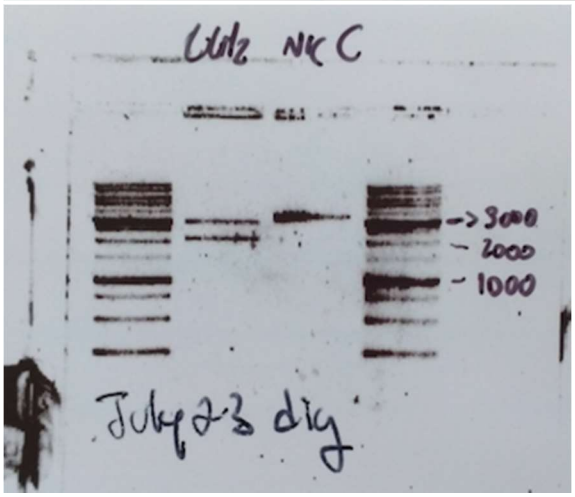
Methods: Digested Nic F, E, C and CCH2 and LacI (Nic C and Nic E linearized). Running on a gel the digests worked correctly. Next the a gel purification was performed of the digests. The gel purification yielded very low concentrations, that were not sufficient for using the ligation calculator hence the gel purified product was not used and the leftover digest product was used to ligate. Thereafter the ligation products were transformed and left to inoculate overnight. Also an overnight liquid culture of CCH2+Lac I was prepared.



July 22:
Goals: Continue preparing for CCH2+LacI protein expression experiments.
Methods: Transfer Jul 21, 10mL of liquid culture into 1L LB and add 1 mL chloramphenicol. Put on shaker in 37C room until optical density of 0.6-0.8 reached. OD=0.668. Cool on ice for 20min then add 1ml of IPTG. Put 50ml into five different erlenmeyer flasks and put in 16C, 20C, 25C, 30C, 37C shaker overnight.

July 23:
Goals: Continue preparing for CCH2+LacI protein expression experiments. Re-check the digests from July 21 and run colony PCR on the colonies of Nic F+E.
Methods: Take cultures of five temperature shakers and put into microcentrifuge tubes. Put 1.5mL of the culture and centrifuge 4min at >10000rpm. Get rid of supernatant and store cells in -20C. Put rest of the liquid cultures for the five different temperatures into 50ml falcon tube and centrifuge at 4000rpm for 20min at 4C. Then remove supernatant and store cell pellet in -80C. Colony PCR Nic F+E and double check the Nic C and CCH2+LacI digest from July 21.Colony PCR yielded nothing.





July 24:
Goal: Redo Nic F+E digest.

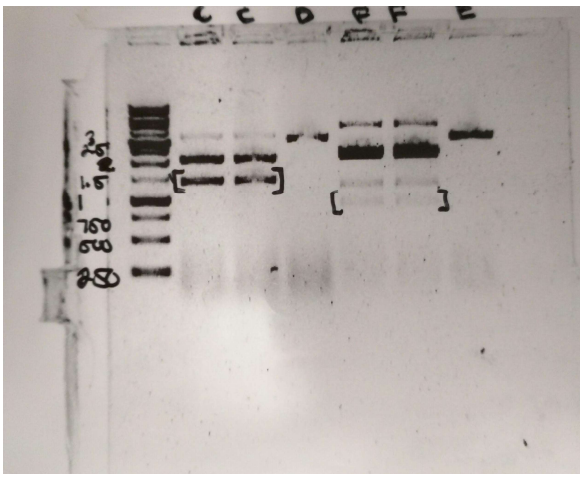
Method: Nic E and F is transformed for more of the vector as it is running out. Take Nic E and digest with EcorI and Xba1 and run on gel. Then the gel was purified for the digested product and ligated with Nic F and then transformed. (ligation=1 hr and expression=for 2 hrs).

July 27:
Goals: Make gels to run sds for the CCH2+LacI expression experiements prepared on July 21-23.
Methods: Make 2 40% SDS gels. First layer is separating, 1.5mL in a microtube with 4microL of TEMED. Thereafter is the rest of the separating with 8 microL of TEMED. Lastly is the Stacking layer with 6 microL of TEMED.

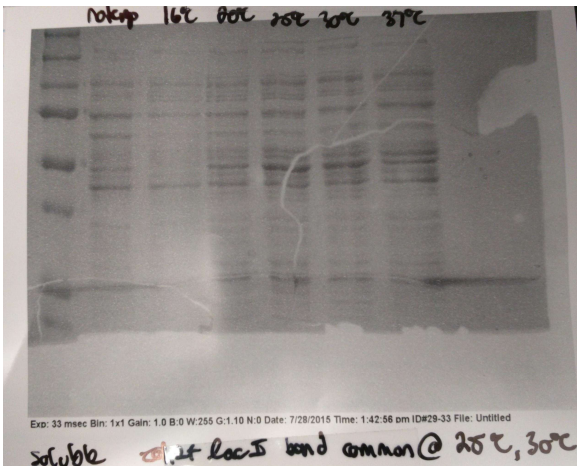
	Stacking	Seperating
Gel %	10	10
40% Polyacrylamide (ml)	0.5	2.5
1M TRIS (ml)	0.5	2.5
10 % Ammonium persulfate (mL)	0.04	0.1
10% SDS(ml)	0.04	0.1
TEMED (ml)	0.004	0.004
Water (ml)	2.92	4.8
Tot Volume (ml)	4	10

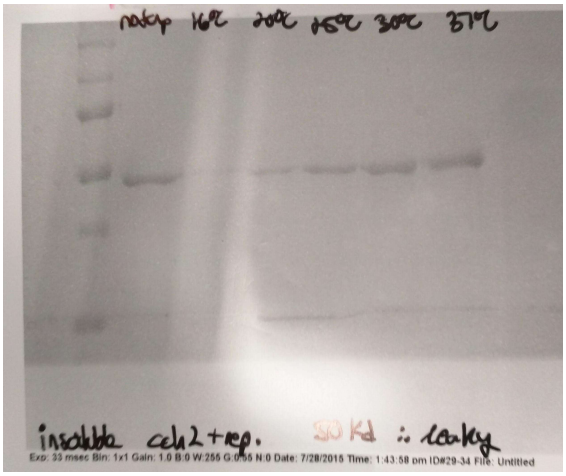
Store the gel in running buffer overnight in tin foil in 4C.
July 28
Goals: Digest Nic C, D, E, F and run sds of CCH2+LacI for optimal protein expression.
Methods: Mini Prepped an overnight liquid culture of CCH2+LacI, Nic C, Nic D, Nic E, Nic F, CCH2.
Lac I+CCH2: 358.1
CCH2: 75.3
Nic C: 103.3
Nic D: 68.2
Nic E: 101.6
Nic F: 256.9
Digested Nic C, Nic D, Nic F, Nic E. Assembly to be done out of order, compared to the order in the Nic cluster. Order= Nic F → Nic E and Nic C→Nic D. Ran a 40% SDS gel for the soluble and insoluble fractions of the CCH2+LacI. The samples were prepared by using the samples in the microtubes from July23. The cell pellet was taken and resuspended in a phosphate/salt buffer

and kept on ice. The resuspended samples were sonicated on the lowest settings for 10 seconds, four times in total. They were then spun down in a centrifuge for 20min at 4C, max rpm. This supernatant was kept and is the soluble fraction. Then the cell pellet was resuspended in a Urea buffer and spun down in a centrifuge for 20min at 4C, max rpm. This new supernatant was the insoluble fraction and the cell pellet was tossed. The expected protein fraction for the insoluble was leaky and displayed expression at all temperatures including the non induced fraction without IPTG. The soluble fraction had expression around 50 Kda and was most prevalent during 25C and 30C.



Nic F has an extra band hence there be contamination of Nic F as it is not the size of what we expected.





July 29
Goals: Purify the gel product from July 28 and ligate and transform.
Methods: Gel purified product from digest on July 28.
(ng/microL)
Nic C #1: 3.5
Nic C #2: 5.2
Nic D: 8.9
Nic F #1: 4.4
Nic F #2: 5.2
Nic E: 5.8
Ligated Nic E + Nic F and Nic C + Nic D and transformed. Also prepared no insert controls in the ligation. Left plates to inoculate overnight. (ligate 1hr and inoculate on shaker for 1hr for expression)

July 30
Goals: Prepare for resting cell assay to be done.
Methods: Plates from July 29 had no growth so digest ligation or transformation did not work. Prep work (LB in erlenmeyers for liquid cultures (50mL) and resting cell assay from Aug 3-7).

Aug 3
Goals: Prepare for resting cell assay to be done.
Methods: Prepare liquid cultures of a prepared PSB 1C3 with no insert (ie. no gene) from plate and of PSB 1C3 with CCH2 and a Lac I repressor. Let inoculate overnight on 37C shaker.

Aug 4
Goals: Start resting cell assay.
Methods: Transfer 10ml from the overnight liquid cell cultures from Aug 3, separately into 1L of LB. Add 1 ml of chloramphenicol to each and let sit on the 37C shaker until optical density of 0.6-0.8 achieved (no insert= 0.717 and CCH2+LacI= 0.628). Thereafter put on ice for 20min and put 750 microL of IPTG. Place the 1L cultures overnight on the 25C shaker.

Aug 5
Goals: Continue resting cell assay.
Methods: The 1L culture from the 25C from Aug 4 for the no insert and CCH2+LacI were spun down and the supernatant was discarded. Thereafter the cell pellets of each were washed once with m9 salts and again spun down. Thereafter the supernatant was discarded and the pellets resuspended in an m9 salt solution. 6-CNA was added to each, 250 microL of a 200mM solution

of 6-CNA dissolved in methanol. They were then incubated at 30 C overnight for approximately 48 hrs.

Aug 6

Goals: Do the cell lysate experiment.

Methods: The 25C pellets of CCH2+LacI were used from the protein expression experiments done on July 23. The pellets were thawed and resuspended in approx. 4mL of MOPS. This was then aliquoted into four microtubes (800 microL into each) of which two were for clear lysate and the other for whole cell. All four samples were sonicated for 10 sec at the minimum. The clear lysate had one sample sonicated twice and the other four times, each were then spun down (30min, 15K rpm and 4C) and the supernatant was kept. The whole cell samples were sonicated for 10 sec at the minimum. The whole cell had one sample sonicated twice and the other four times, these were not spun down. All samples were kept on ice. Each had 380 microL of MOPS added and 20 microL of 200mM 6-CNA solution in methanol. These were then incubated overnight at 30 C.

Aug 7

Goals: Continue resting cell assay and cell lysate experiment.

Methods: The overnights from Aug 5 were spun down and the supernatant kept. The supernatant was acidified with hydrochloric acid until the the supernatant of the no insert and CCH2+LacI had reached a pH=2-3. Then the supernatants were subsequently extracted via a liquid-liquid extraction with ethyl acetate. The top layer or the ethyl acetate was kept during each round of the extraction. The extraction was done two times with the leftover supernatant in the separatory funnel and ethyl acetate. The samples were then put into an RBF and roto-vapped. The solid residue leftover was resuspended in pyridine and approx. 100 microL was used in a 1:1 ratio with derivatizing agent. These samples were then ran on the gcms. The cell lysate fractions were acidified with hydrochloric acid until the the fractions of the whole cell and clear lysate reached a pH=2-3. Then the supernatants were subsequently extracted via a liquid-liquid extraction with ethyl acetate. The top layer or the ethyl acetate was kept during each round of the extraction. The extraction was done two times with the leftover supernatant in 2 mL microtubes. The ethyl acetate each time was transferred into a glass test tube which were then gassed down with nitrogen gas. After drying down the residue was resuspended with 600 microL of pyridine of which 100 microL was used. Derivatizing agent was used in a 1:1 ratio to pyridine and ran on the gcms.

Aug 9

Goals: Prepare overnight cultures for expression at different temperature of Nic genes (with LacI).

Results: Prepare the overnight cultures via picking up a colony on a pipet tip into 50mL of LB with 50microL of chloramphenicol. The cell lysate experiment from Aug 8 had degradation of 6-CNA to for 6-HNA, however 6-CNA was the most prevalent peak. The samples with sonification 4 times had less degradation than the samples sonicated 2 times. The resting cell assay had degradation of the 6-CNA as the 6-CNA peak of the CCH2+LacI was smaller than the one for

the no insert control, however the 6-HNA peak did not appear. The 6-CNA also was present as a fairly large peak.

Aug 10

Goals: Continue preparatory work for the different temperature expression of the Nic genes. Also overnight culture of CCH2+LacI and no insert for calculating rate of degradation.

Results: Nic C, D, E, F, X and a no insert had 10mL transferred into different 1L LB in 2L erlenmeyers, one for each construct. Then 1mL of chloramphenicol were added to each 1L culture and put on the shaker at 37C. After an optical density was reached of 0.6-0.8 the samples were removed and placed on ice for 20 min, where after IPTG was added and the cultures were placed into five different erlenmeyers at about 50mL of volume at five different temperatures (16C, 20C, 25C, 30C, 37C) overnight. Samples were collected also of the starter culture and the 1L culture before inducing to use as controls.

	optical density
Nic C	0.649
Nic D	0.656
Nic E	0.654
Nic F	0.600
Nic X	0.634
no ins.	0.720

Also overnight culture of CCH2+LacI and no insert for calculating rate of degradation.

Aug 11

Goals: Finish preparatory work for the different temperature expression of the Nic genes. Also continue preparatory work for CCH2+LacI rate experiment.

Methods: All the Nic samples for all the genes and the different temperatures had 1.5mL transferred into a microtube, thereafter the rest of the cultures were transferred to 50mL falcon tubes and the supernatant was disposed of. The CCH2+LacI and no insert overnight culture had 10mL transferred into 1L of LB with 1 mL of chloramphenicol. They were then placed on the 37C shaker to reach an optical density of 0.6-0.8. They were then chilled on ice for 20 min and 750 microL was added to each sample to induce expression. They were then placed at 25C overnight on a shaker. **NOTE:** each was done in doubles so there were two 1L cultures of CCH2+LacI and two of the no insert.

	Optical Density
no ins. #1	0.684
no ins #2	0.672
CCH2 #1	0.684
CCH2 #2	0.672

Aug 12

Goals: Collect samples from the CCH2 rate experiment.

Methods: The four 1L overnight cultures were spun down in the centrifuge and the supernatant discarded. They were then washed once in a m9 salt solutions, spun down and the supernatant discarded. The cultures were then suspended in 25 mL of m9. The CCH2 from both 1L cultures

was combined and the same for the no insert. Then 22.5 of m9 salt solution were put into 40 250ml erlenmeyer flasks. Thereafter 2.5 mL of the cell cultures, resuspended in m9 were put into the 40 erlenmeyers (so total of 20 erlenmeyer for CCH2 and 20 for no insert, with each one done in doublets). Lastly 20 microL of 200mM 6-CNA was added to each so that no ins #1, #2 and CCH2 #1, #2 were done at once at put on the shaker at 30C. The samples were collected at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48 hrs. The samples would be taken off the shaker, transferred into 50 mL falcon tubes, spun down at 4000 rpm, 20min, 4C and the supernatant transferred into another falcon tube. The cell pellet and supernatants were then transferred into the -80C and only the supernatant after freezing were transferred into the -20C freezer.

Aug 13

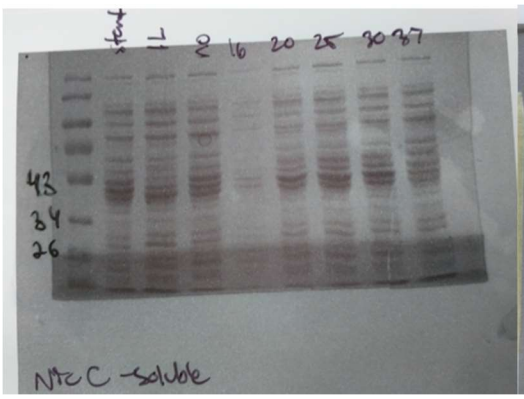
Goals: Prepare samples of Nic C and Nic D for SDS on Aug 14. Also continue collecting samples from the CCH2 rate experiment.

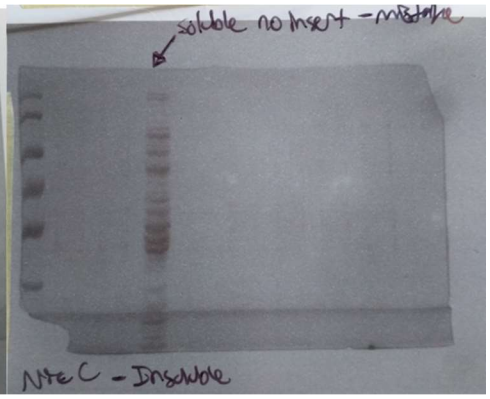
Methods: Sample collection done the same as Aug 12. The samples of the Nic C and D were taken out of the -20C were cell pellets from the starter culture, 1L before induction and the five different temperatures were collected. They were resuspended in 400 microL of MOPS and then sonicated 4 times and lowest setting. They were then spun down for 20 min, max at 4C. The supernatant was transferred into a microtube and labelled soluble fraction. The cell pellet was then resuspended. This time however it was resuspended in 400 microL of a Urea buffer. Then is was centrifuged at 4C, max for 20min. The supernatant was transferred into a microtube and labelled the insoluble. The cell pellet was then tossed. Part of the soluble and insoluble fraction were taken out and transferred into microtubes and stained with SDS dye.

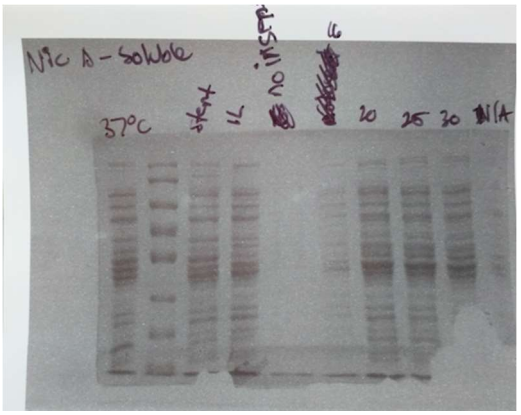
Aug 14

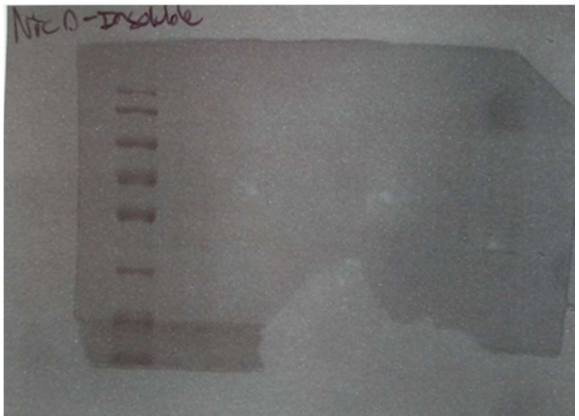
Goals: Prepare the samples to run on SDS and run SDS of Nic C and D.

Methods: Prep same as Aug 13 for the samples. The 40% polyacrylamide gel with a 10% percentage was ran. The protein expression was unclear and not visible on the gels. Nic C= 42.62 kDa, Nic D= 30 kDa.





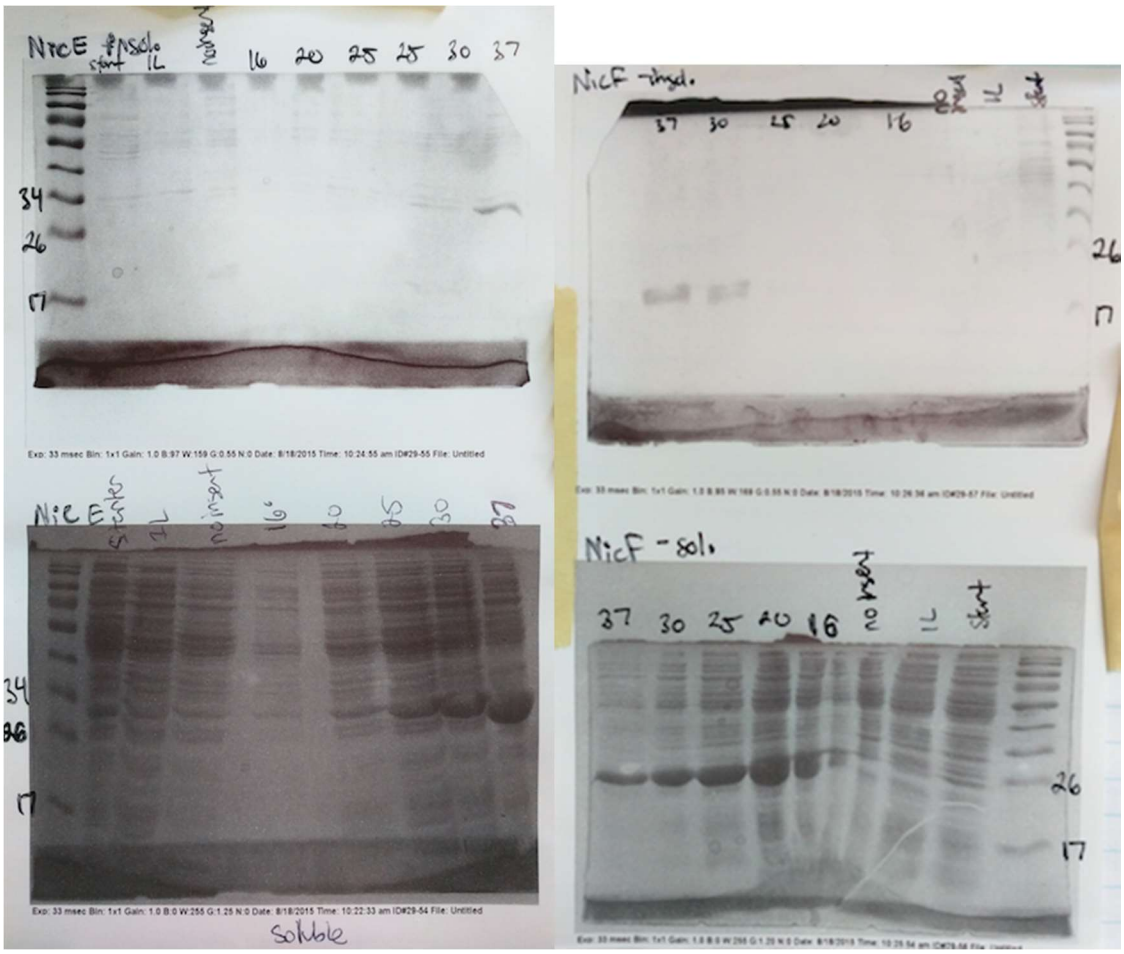




Aug 17

Goals: Prepare the samples to run on SDS and run SDS of Nic F and E.

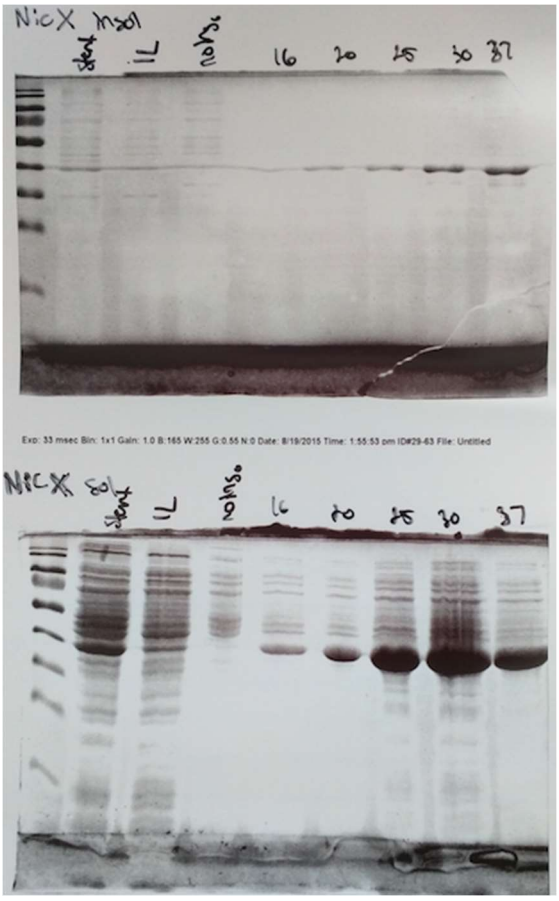
Methods: Prep same as Aug 13 for the samples. Nic F and E ran with 14% gel due to unclear results on Aug 14 and due to small protein size. Nic F= 23.9 kDa, Nic E= 27.97.

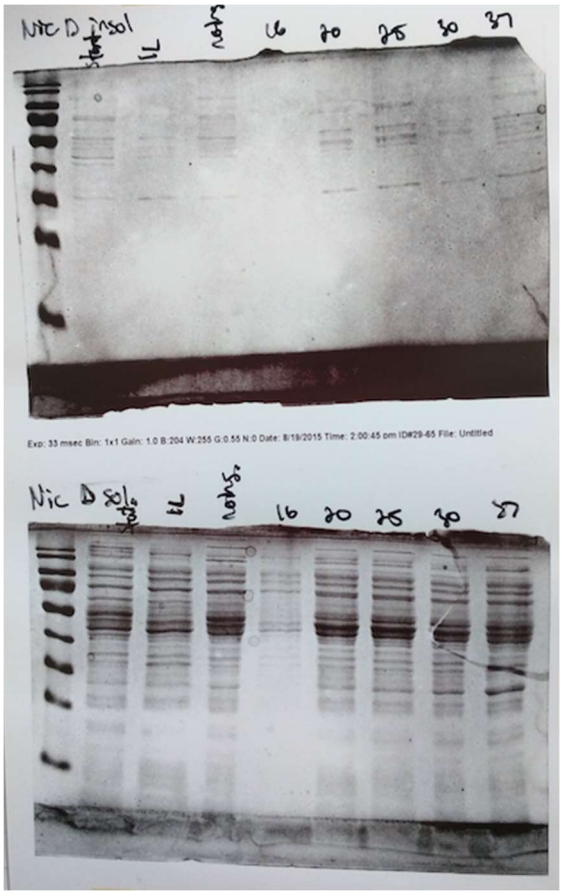


Aug 18

Goals: Prepare the samples to run on SDS and run SDS of Nic X and re run Nic D.

Methods: Prep same as Aug 13 for the samples. NIc X and Nic D ran with 14% gel. Nic X= 41.81, Nic D= 30 kDa.

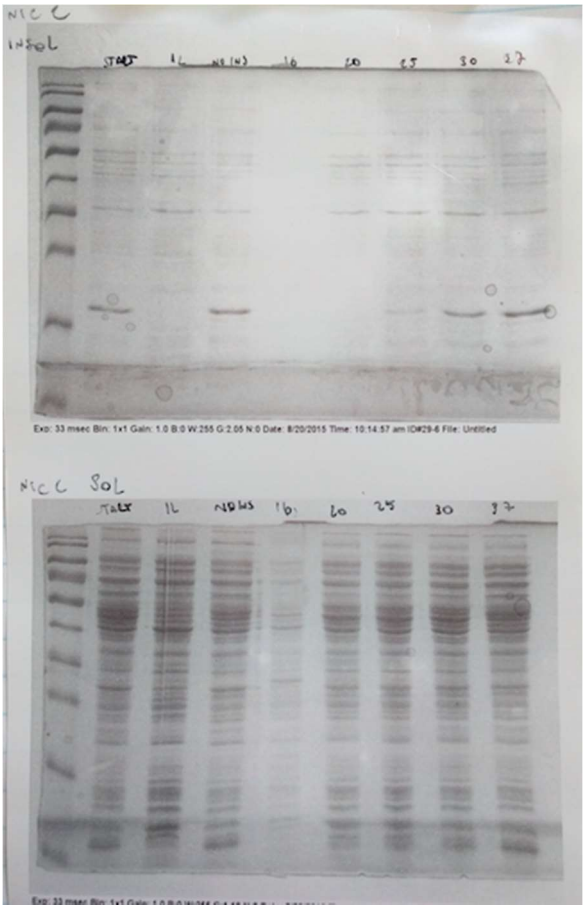




Aug 19

Goals: Re run Nic C on SDS.

Methods: Nic C ran on a 14% gel. Nic C= 42.62 kDa.



Aug 20

Goals: Prepare set one of the CCH2 data from the rate experiment to run gcms and run the gcms.

Methods: 1mL of the supernatants from the -20C from Aug 12-14 were taken and transferred into microtubes of CCH2 #1. The samples were acidified by 8 microL of hydrochloric acid, until pH=2-3. Then the samples were vortexed and spun down for 4 min at 15000 rpm and the supernatent kept and transferred into 2ml microtubes. 900 microL of ethyl acetate were added, vortexed and centrifuged at 15000 rpm for 3 min. Around 800 microL were removed from the top layer each time (ethyl acetate layer) and transferred into glass test tubes. After three extractions the sample in the glass test tubes was dried down with nitrogen gas. Thereafter the residue was resuspended in 100 microL of pyridine and transferred into vials for gcms and derivatizing agent was added in a 1:1 ratio.

Aug 21

Goals: Prepare set two of the CCH2 and no ins data from the rate experiment to run on gcms and make samples to re-run the 6-CNA and 6-HNA standard curve.

Methods: Same as the sample preparation done on Aug 20. The samples for gcms were dried down and resuspended in 100 microL of pyridine and stored in -20C overnight to run on the gcms another day.

Aug 24

Goals: Derivatize data set two for running on gcms and prepare for a new rate experiment and sole carbon testing on the final assembly.
Methods: Take the solutions out of -20C left on Aug 21 and add 100 microL of derivatizing agent, let sit one hour and run on the gcms. Prepare starter cultures of CCH2, No ins., CCH2+Nic cluster and Nic cluster for sole carbon and rate experiment.

Aug 25

Goals: Induce expression in overnight cultures from Aug 22.
Methods: The starters had 500 microL of starter transferred into 50mL LB and 50 microL of Chloramphenicol were added. The cultures were left at 37C on the shaker until an optical density of 0.6-0.8 was achieved. Then they were chilled on ice for 20 min. and had 50 microL of IPTG added and put on the 25C shaker overnight.

CCH2 #1	0.675
CCH2 #2	0.605
No ins #1	0.725
No ins #2	0.730
CCH2	0.624
no ins	0.743
Nic clusters	0.597
CCH2+ Nic's	0.637

Aug 26

Goals: Conduct the rate experiment for CCH2 with a shorter time point of 4 hours and Restart cultures for sole carbon experiment.
Methods: The cells were transferred into 50mL falcon tubes and spun down at 4000rpm for 20min at 4C. The supernatant was then discarded and the pellet resuspended in M9 to wash the cells. They were spun down again under the same conditions and the supernatant tossed. The cells were resuspended in 25mL M9. There were two fractions of the CCH2 and the no insert, so the fractions for the same gene were combined to normalize the cell. From this 25 mL were taken out and transferred into erlenmeyer. The two no inserts and the two CCH2 had 20 microL of a 200mM stock of 6-CNA in methanol added and left on the 30C shaker (where optimal protein expression is). 1mL samples were taken out of the flasks every 15 min for two hours and thereafter every 30 min for another two hours. These 1mL samples were spun down at 4C for 5min at max rpm's and the supernatant kept and the cell pellet tossed. The cells for the sole carbon experiment were tossed out. Restart cultures for sole carbon experiment of CCH2, NIC cluster, CCH2+NIC cluster and no insert

Aug 27

Goals: Induce cultures for sole carbon experiment and extract sample set two from Aug 26.
Methods: The extraction protocol is done the same as Aug 20. The protocol was carried out until the samples were dried down with nitrogen gas and resuspended in 100microL of pyridine. They were then stored at -20C to be run on Aug 28. Induction done the same as Aug 25.

Aug 28

Goals: Set-up the sole carbon experiment and extract samples for set one from Aug 26. Also run the gcms samples.
Methods: The extraction protocol is done the same as Aug 20. The protocol was carried out until the samples were dried down with nitrogen gas and resuspended in 100microL of pyridine. They were then stored at -20C to be run on Aug 31. 100 microL of derivatizing agent were added to the samples from Aug 27 and ran on the gcms. The induced cultures were spun down at 4C at max rpm's for 20 min, they were then resuspended in m9. The cultures were then spun down and resuspended in 50mL of m9. The cultures were then measured for the optical density and the amount being added from these cultures was adjusted so all cultures were at a 0.1 starting optical density. All genes were ran in duplicates (CCH2, No ins, NIC cluster, CCH2+NIC cluster). All genes had a glucose and nothing control, along with 6-CNA and 6-HNA addition. 6-CNA and 6-HNA were made dissolved in DMSO at 500mM and 200microL were added to get a final 2mM concentration the erlenmeyer flasks with a final total volume of 50mL.

	Of Culture added
No ins	3.68 mL
CCH2	3.13 mL
Full	3.93 mL
NIC's	3.60 mL

M9 was added to bring up the total volume to 50mL. 2.22M solution of glucose was made of which 45 microL was added.

Aug 29

Goals: Collect samples and OD values of the conditions set up on Aug 28.
Methods: 1 mL samples were collected from the erlenmeyers with different conditions setup and the optical density was measured and the 1mL samples were spun down at max rpm's for 5 min at 4C and the supernatant kept and the cell pellet in -80C.

Aug 30

Goals: Collect samples and OD values of the conditions set up on Aug 28.
Methods: Same as Aug 29. The cultures optical density values were decreasing, so the experiment will be done later with different conditions.

Aug 31

Goals: Run GC/MS samples from Aug 28.
Methods: 100 microL of derivatizing agent were added to the samples from Aug 29 and ran on the GC/MS.

Sept 1

Goals: Prepared overnight starter cultures for Cch2, a No Insert, Full construct and NICs in order to setting up a sole carbon source experiment

Methods: Prepare the overnight cultures via picking up a colony on a pipet tip into 50mL of LB with 50microL of chloramphenicol.

Sept 2

Goals: Induce expression in overnight cultures from Sept 1.

Methods: The starters had 500 microL of starter transferred into 50mL LB and 50 microL of Chloramphenicol were added. The cultures were left at 37C on the shaker until an optical density of 0.6-0.8 was achieved. Then they were chilled on ice for 20 min. and had 50 microL of IPTG added and put on the 25C shaker overnight.

CCH2	0.624
No ins	0.650
NICs	0.620
Full	0.609

Sept 3

Goals: Set up sole carbon source experiment using a plate reader

Methods: The induced cultures were spun down at 4C at max rpm's for 20 min, they were then resuspended in m9. The cultures were then spun down and resuspended in 30mL of m9. The cultures were then measured for the optical density and the amount being added from these cultures was adjusted so all cultures were at a 0.1 starting optical density. All genes were ran in triplicates (CCH2, No ins, NIC cluster, CCH2+NIC cluster). All genes had a glucose, DMSO and nothing control, along with 6-CNA and 6-HNA addition. Additionally, we added Chloramphenicol, Trace elements and IPTG to all samples at equal concentrations (20microliters per well).

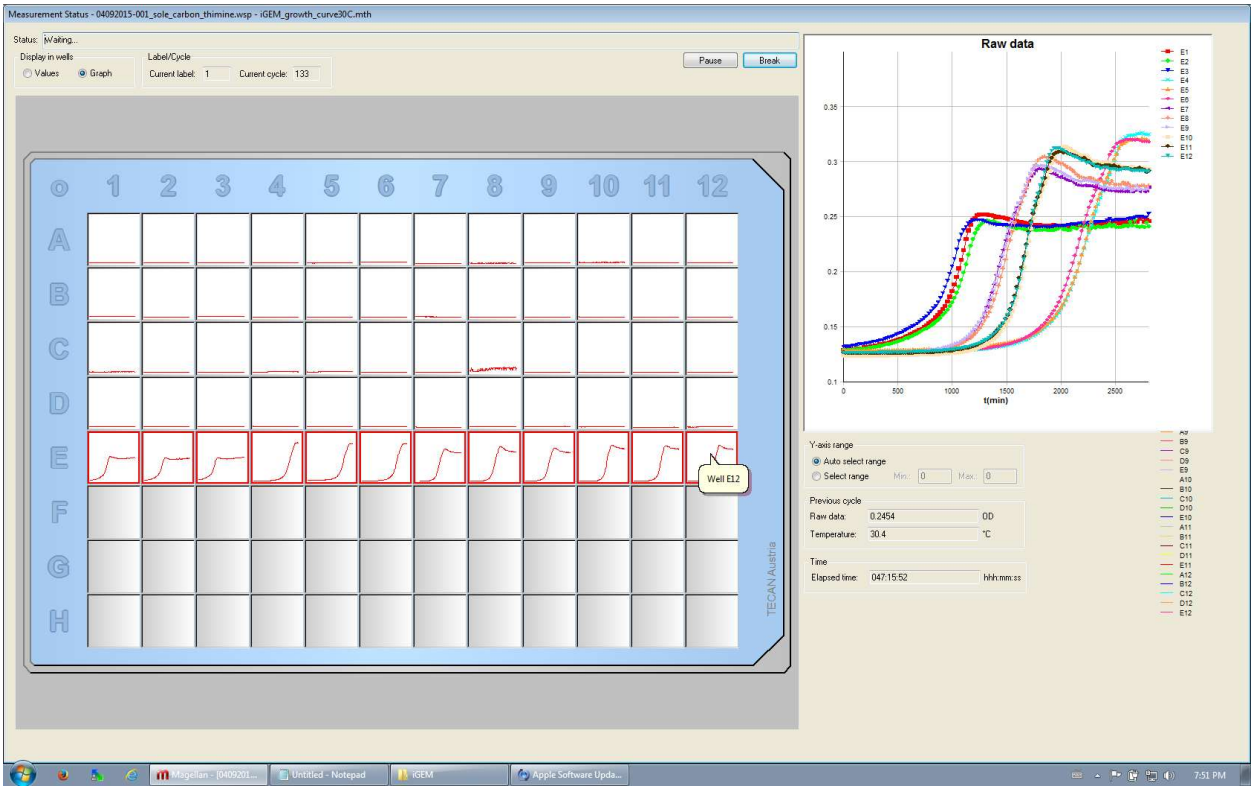
	No Ins	cch2	Full	Nics
M9+Chlor+IPTG				
M9+Chlor+IPTG +DMSO+ 6-CNA				
M9+Chlor+IPTG +DMSO				
M9+Chlor+IPTG +DMSO+6-HNA				
M9+Chlor+IPTG +glucose				

6-CNA and 6-HNA solutions were made using DMSO as a solvent at 500mM and the final concentration obtained was 2mM. Glucose final concentration was 20mM. Volume of solution added to each well was 200 microL, of which 1microL was cell culture and the remaining was the corresponding master mix.

Sept 4

Goals: Addition of Thiamine

Methods: After 24hs our plate showed no significant growth for any cultures, which suggested something was inhibiting their development. Thiamine was added at 3 uM. After 72hs, only the samples in glucose grew.



Sept 10

Goals: Prepared overnight starter cultures for Cch2, a No Insert, Full construct and NICs in order to setting up a sole carbon source experiment

Methods: Prepare the overnight cultures via picking up a colony on a pipet tip into 50mL of LB with 50microL of chloramphenicol.

Sept 11

Goals: Induce expression in overnight cultures from Sept 10.

Methods: The starters had 500 microL of starter transferred into 50mL LB and 50 microL of Chloramphenicol were added. The cultures were left at 37C on the shaker until an optical density of 0.6-0.8 was achieved. Then they were chilled on ice for 20 min. and had 50 microL of IPTG added and put on the 25C shaker overnight.

CCH2 #1 0.724
No ins #1 0.615
No ins #2 0.773
NICs 0.710
Cyp 6G1 and NICs 0.629
Cyp 6G1, cch2 and NICs 0.615
Cch2 and NICs 0.602

Sept 12

Goals: Set up 48 hs Imidacloprid degradation rate experiment sample and collected samples at 0 and 7hs

Methods: Set up of Imidacloprid degradation rate experiment was as follows. The induced cultures were spun down at 4C at max rpm's for 20 min, they were then resuspended in 15 ml of m9. The cultures were then spun down and resuspended in 20mL of m9.
The cultures included: full assembly (CYP 6G1+cch2+ nics), cch2 ,CYP 6G1 + Nics and a No Insert. The final volume of the solution was 25ml (15 ml of M9, 10 of culture, 25 microL of Chlor, Trace Elements and IPTG) placed in a 50ml Erlenmeyer Flask, which was covered completely in aluminum foil to prevent photodegradation.

Sample collection included removing 1mL and placing it in a 1.5mL Eppindorf tube, which was spun down at 4C at 15000rpm for 3 minutes. The supernatant was then transferred into dark 1.5mL Eppindorf tube and the samples were frozen at -80C.

Sept 13

Goals: Set up 6-CNA 24 hs degradation rate experiment, collected 24 h Imidacloprid degradation rate experiment sample

Methods: Collection of samples was as performed on Sept 12 and 24hs Imidacloprid sample was taken. Set up of 6-CNA degradation rate experiment was as follows. The induced cultures were spun down at 4C at max rpm's for 20 min, they were then resuspended in 15 ml of m9. The cultures were then spun down and resuspended in 20mL of m9.
The cultures included: full assembly (cch2+ nics), Nics and a No Insert. The final volume of the solution was 25ml (15 ml of M9, 10 of culture, 25 microL of Chlor, Trace Elements and IPTG) placed in a 50ml Erlenmeyer Flask. A sample was taken out at 0 and 5 hs.

Sept 14

Goals: Collect sample for 6-CNA degradation rate experiment at 24hs

Methods: Samples were collected as Sept 12.