May 25, 2015
Goal: To grow *G. apicola* and *S. alvi* on agar plates.
Methods: Growing *G. apicola* Protocol. *G. apicola* and *S. alvi* were streaked on LB and TSA plates and grown in 5% CO_2 balanced with nitrogen environment at 37°C for 48 hours. Four tubes of TSB flushed with 5% CO_2 balanced with nitrogen environment were inoculated with G. apicola and S. alvi. One of each bacterial culture was wrapped in tin foil to simulate a bee gut stomach. Bacterial Cultures were stored on a shaker at 37°C for 48 hours.
Results: Both the bacterial LB and TSA plates showed growth. The *S. alvi* plates had less growth than the *G. apicola* and were incubated for another 48 hours but displayed minimal additional growth. Attempting produce growth in liquid media yielded no growth in any four tubes.

May 27, 2015
Goal: To produce freezer stocks of *G. apicola* and *S. alvi*.
Methods: Two Cryotubes with 20% glycerol TSB media inoculated with our two bacteria and then set in 37°C whilst shaking for two hours and then placed in -80°C.
Results: Freezer stocks were not touched incase our bacteria were sensitive to freeze-thaw cycle. At least one *G. apicola* and *S. alvi* plate was kept in four degrees incase our freezer stocks yielded no viable cells.

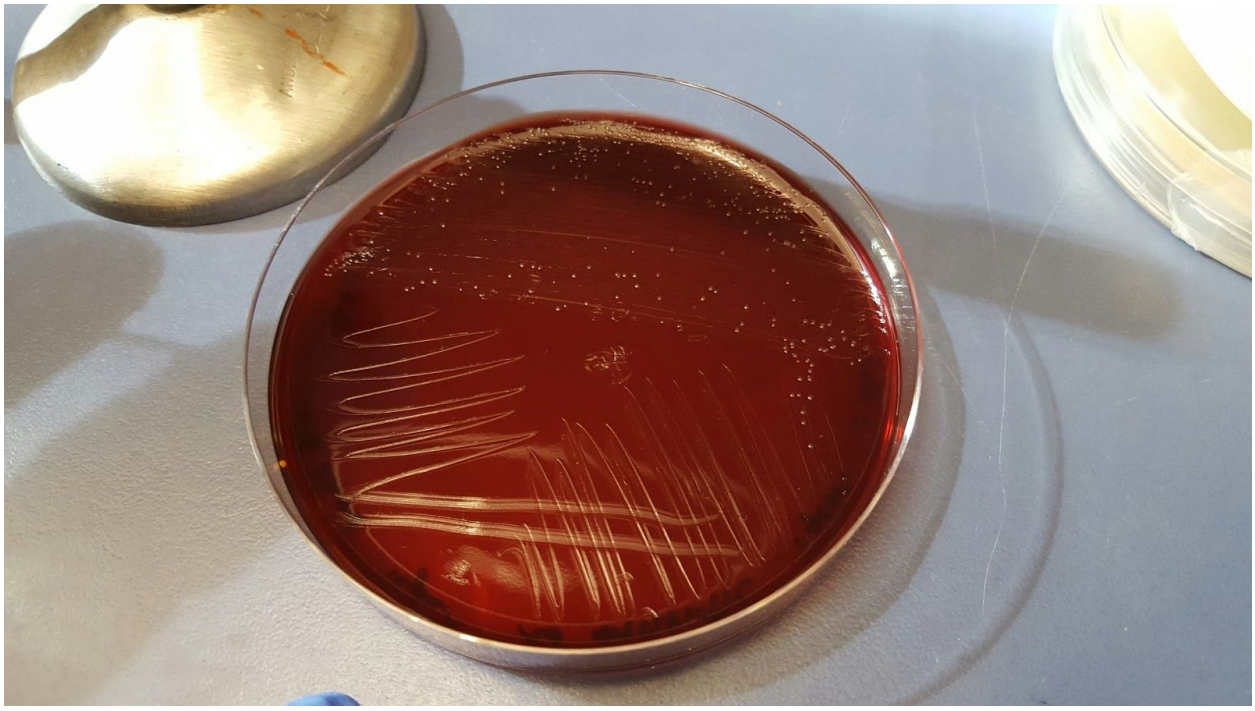
June 1 - 3, 2015
Goal: To prepare for electroporation.
Methods: Designed *G. apicola* and *S. alvi* specific primers. Plated *S. alvi* and *G. apicola* on ampicillin, chloramphenicol, and kanamycin plates to be certain of no unknown internal resistance plasmid or intrinsic resistance
Results: No colonies formed on the ampicillin, chloramphenicol, and kanamycin plates ensuring that both *G. apicola* and *S. alvi* are susceptible to those antibiotics.

June 1 - 2 ,2015
Goal: To transform *G. apicola* and *S. alvi* with the pBBR1MCS-2 plasmid through electroporation.
Methods: Campy Competent Cell protocol. Electroporation Protocol. The cells were electroporated with pBBR1MCS-2 plasmid with kanamycin resistance and plated overnight to recover on TSA plates. Additionally, a negative control of cells without plasmid was used to test if the cells could survive the electroporation. The next day, the cells that were only electroporated grew small colonies, indicating they were able to survive the electroporation and washing process. The recovered cells were plated on kanamycin plates.
Results: All three plates streaked out on June 3rd from the electroporation plates grew, therefore our samples were contaminated on no discernable information can be obtained as to whether the cells were able to be transformed.

June 3, 2015
Goal: To find a way to get better *S. alvi* growth.

Method: Streaked out *S. alvi* on 5% sheep blood agar plates to see if it would lead to more biomass than on the TSA plates.
Results: The blood agar plates from June 3rd produced small colonies but with minimal difference from growing on TSA plates.

Figure 1: *Snodgrassella alvi* streaked out on blood agar (5% sheep blood)



June 5 - 15, 2015
Goal: To find out why the streak plates of *G. apicola* have a different morphology than previous plates.
Methods: Four different TSA plates were used to find the source of the contamination. *G. apicola* was streaked onto two plates and the other two were blank, with no bacteria. One of each type were grown under the normal atmosphere and the remainder in 5% CO_2 balanced with nitrogen. All were grown at 37°C. Using the bacteria grown from these plates, a colony PCR was run with the *G. apicola* specific primers. **Colony PCR Amplification Protocol.**
Results: There was minimal growth on the *G. apicola* plates and no growth on the two blank plates, suggesting that the contamination is not from the plates. It was noted the streak plates were more liquid-y than usual and it is hypothesised that due to the different growing conditions, the bacteria displayed a new morphology. The new morphology was displayed as *G. apicola* from colony PCR.

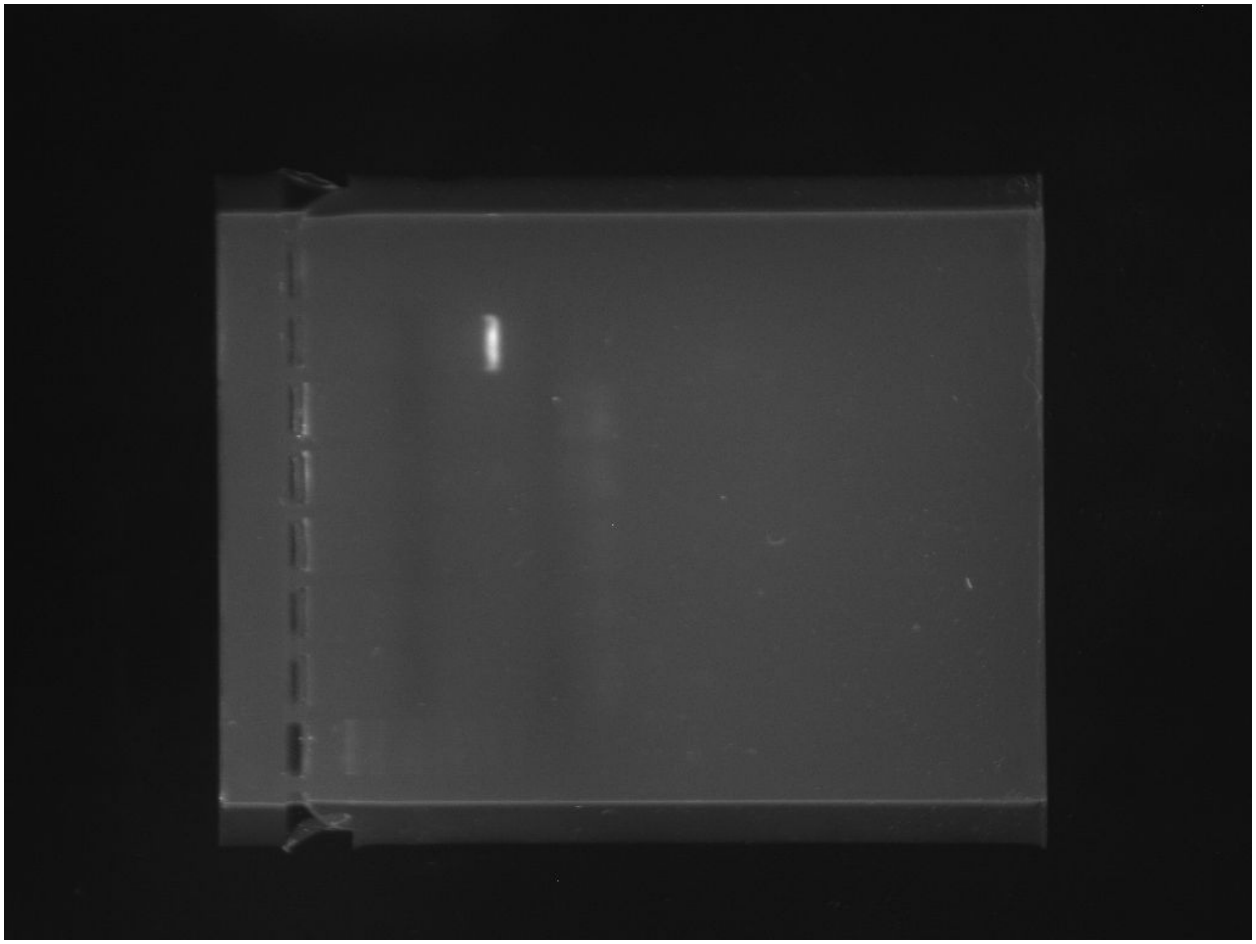
June 17 - 19, 2015
Goal: To transform *G. apicola* with the pSB1A3, pSB1C3, and pBBR1MCS-2 plasmids through electroporation.

Methods: Campy Competent Cell Protocol. Electroporation Protocol. The bacteria was electroporated with three different plasmids: pSB1A3 (ampicillin resistance), pBBR1MCS-2 (kanamycin resistance), pSB1C3 (chloramphenicol resistance). They were plated on TSA plates to recover overnight. **Colony PCR protocol** was performed on the cells grown on the resting plate with *G. apicola* specific primers.
Results: The colony PCR products showed that all cells on the resting plates were *G. apicola.* However, the pSB1A3 plasmid cells had to be discarded after spreading on plates that appeared to have pre-existing contamination on them. After one day, there was no growth on the kanamycin and chloramphenicol plates. After four days, there were no visible colonies on the chloramphenicol plate for the pSB1C3 plasmid. There was lots of biomass on the kanamycin plate for the pBBR1MCS-2 plasmid; however, the colonies were of a variety of sizes suggesting possible contamination, but all other morphological characteristics were the same. We did colony PCR on 3 colonies from the kan plate and streaked out 3 more on different Kan plates to see if colonies remain variable in size or shift to becoming more uniform. Ran gel electrophoresis on the PCR products from June 22 and found that two of the three colonies demonstrated amplification suggesting that *G. apicola* is our transformant. However, due to equipment error with the gel machine, our ladder did not appear, so we redid the gel on June 25 to ensure the correct amplicon length (and it was).

June 19 - 22, 2015
Goal: To determine whether *G. apicola* could survive the freeze-thaw process.
Methods: Campy competent cell protocol. The competent cells were frozen, then thawed and streaked onto TSA plates.
Results: The competent cells that were streaked onto the TSA plates produced colonies implying that *G. apicola* can survive the freeze-thaw process.

June 22 - 26, 2015
Goal: To transform *G. apicola* with the pBBR1MCS-2 and pSB1A3 plasmids through electroporation.
Methods: Campy Electroporation protocol.
Results: Notably, the cells that were electroporated with the pBBR1MCS-2 plasmid produced less biomass that was seen in the previous electroporation, possibly due to less competent cells surviving because of the stress of freeze-thaw process. Cells electroporated with the pSB1A3 plasmid did not want to dissolve in the TSA and refused to dry on the LB/Amp plate. The pSB1A3 plasmid produced no cell growth. The three Kan plates with *G. apicola* that were streaked from June 22 plate that had cells that were electroporated had produced no colonies so we streaked out the June 22 plate on Murphy lab LB kan plates to see if it's our plates or just the bacteria. The plate from June 23 that had the second trial of electroporated cells with pBBR1MCS-3 produced very large colonies (not characteristic of *G. apicola).* We ran a colony PCR for the plasmid and nothing amplified except for the plasmid suggesting the cells were growing on kanamycin without the plasmid.

Figure 2: DNA Agarose Gel Electrophoresis for pBBR1MCS-2 plasmid



From bottom to top: latter, Electroporated *G. apicola* 2, Electroporated *G. apicola* 3, Electroporated *G. apicola* 4, Electroporated *G. apicola* 5, purified pBBR1MCS-2 (positive), no template (negative)

June 29 - July 2, 2015

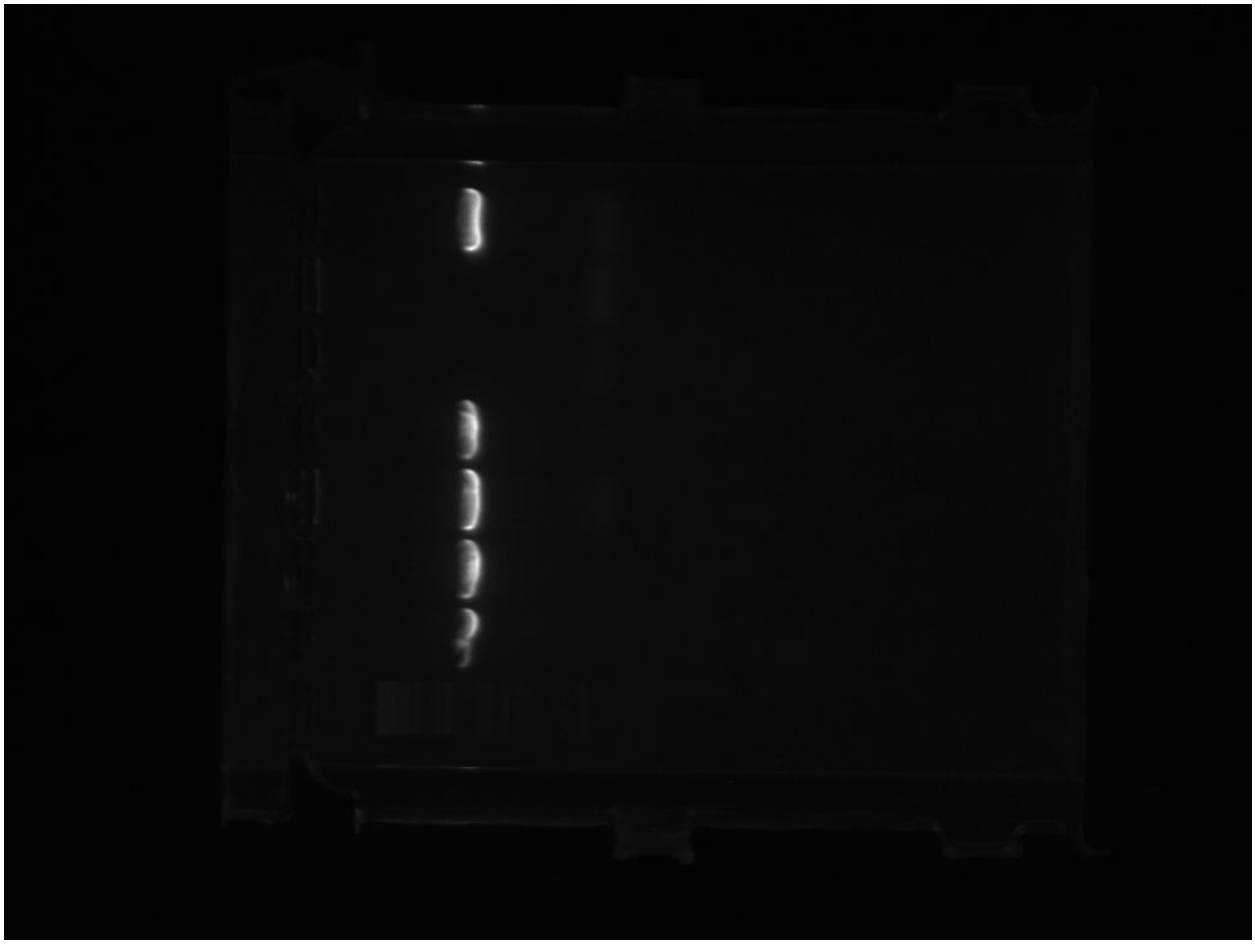
Goal: To test controls used for the electroporations of *G. apicola* with the pBBR1MCS-2 plasmid.

Methods: Electroporation protocol. A larger cell volume, 150 µL, was used for electroporation. Three separate electroporations occured: a negative control without plasmid, a regular electroporation with the pBBR1MCS-2 plasmid to be rested on TSA and then on kanamycin, and an adjusted electroporation with the pBBR1MCS-2 plasmid to be rested directly on kanamycin.

Results: After a day, there is no growth on the kanamycin plates but growth on the TSA plates. This indicates that the resting stage for the cells on TSA is required. Significant growth was observed three days after restreaking electroporated cells onto the selective antibiotic. A colony PCR using *G. apicola* specific primers and PCR using M13 primers specific to pBBR1MCS-2 was run on the electroporated cells grown on the kanamycin plates. The colony PCR showed all cells grown were *G. apicola* but did not contain the pBBR1MCS-2 plasmid.

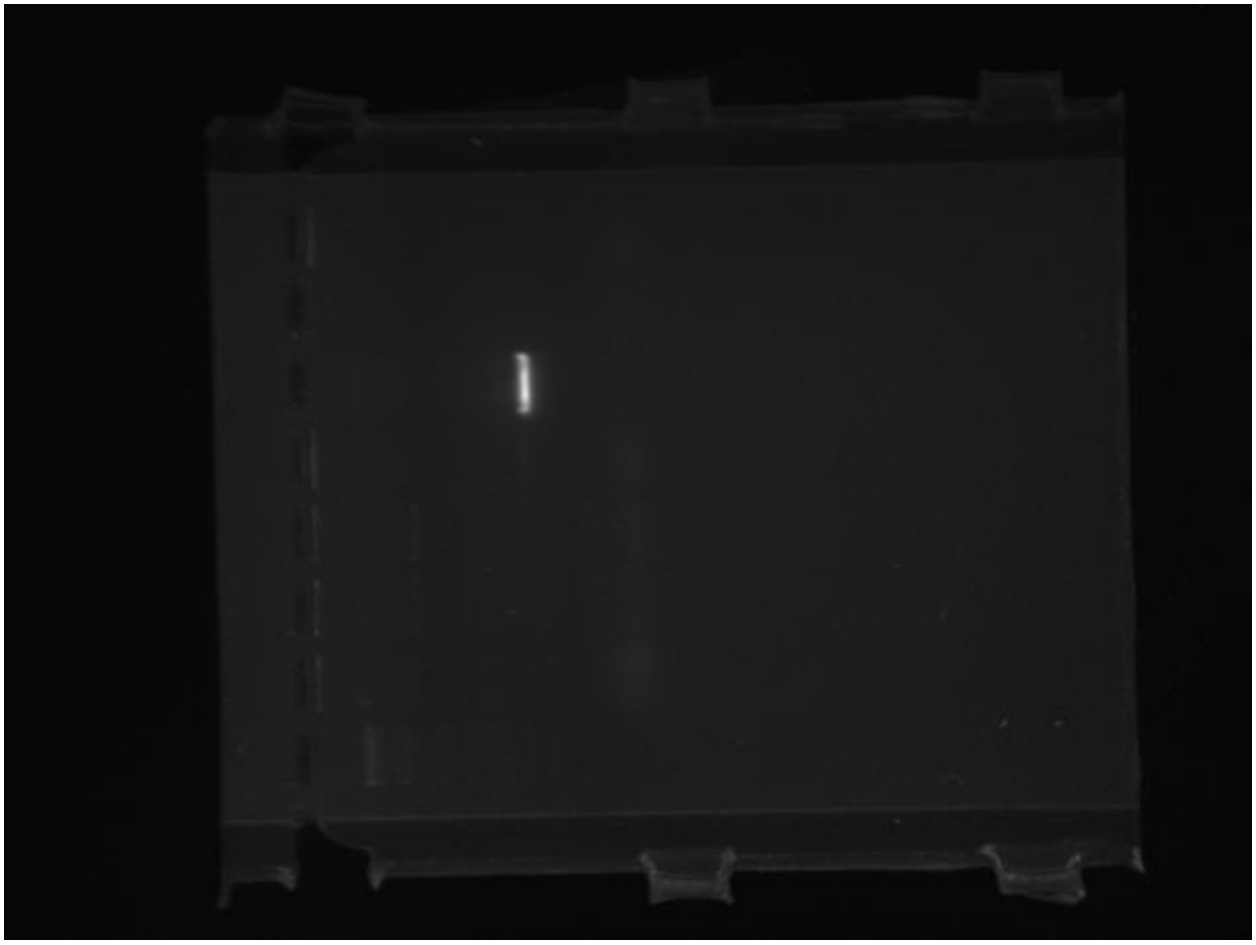
June 29 - July 2, 2015
Goal: To transform *G. apicola* with the pBBR1MCS-2 plasmid through heat shock.
Methods: Heat Shock Protocol with one plated directly onto kanamycin plates and another plated on to TSA to rest.
Results: After a day, there is no growth on the kanamycin plates but growth on the TSA plates. This indicates that the resting stage for the cells on TSA is required. Significant growth was observed three days after restreaking electroporated cells onto the selective antibiotic. A colony PCR using *G. apicola* specific primers and PCR using M13 primers specific to pBBR1MCS-2 was run on the electroporated cells grown on the kanamycin plates. The colony PCR showed all cells grown were *G. apicola* but did not contain the pBBR1MCS-2 plasmid.

Figure 3: DNA Agarose Gel Electrophoresis to see if cells on TSA Kanamycin plates were *G. apicola*



From bottom to top: Latter, Electroporated *G. apicola* 1, Electroporated *G. apicola* 2, Heatshock *G. apicola 1,* Heatshock *G. apicola 2, G. apicola* 1 (positive), *G. apicola* 2 (positive). Bands at 1300 bases confirm cells were *G. apicola.* Gel was used to confirm cells that were on Kanamycin plate were *G. apicola.*

Figure 4: DNA Agarose Gel Electrophoresis to see if cells growing on the TSA kanamycin plates had pBBR1MCS-2 plasmid



From bottom to top: Latter, Electroporated *G. apicola* 1, Electroporated *G. apicola* 2, Heatshock *G. apicola 1,* Heatshock *G. apicola 2,* Purified Plasmid (positive), Negative. Band at roughly 1kb indicate presence of plasmid in *G. apicola*

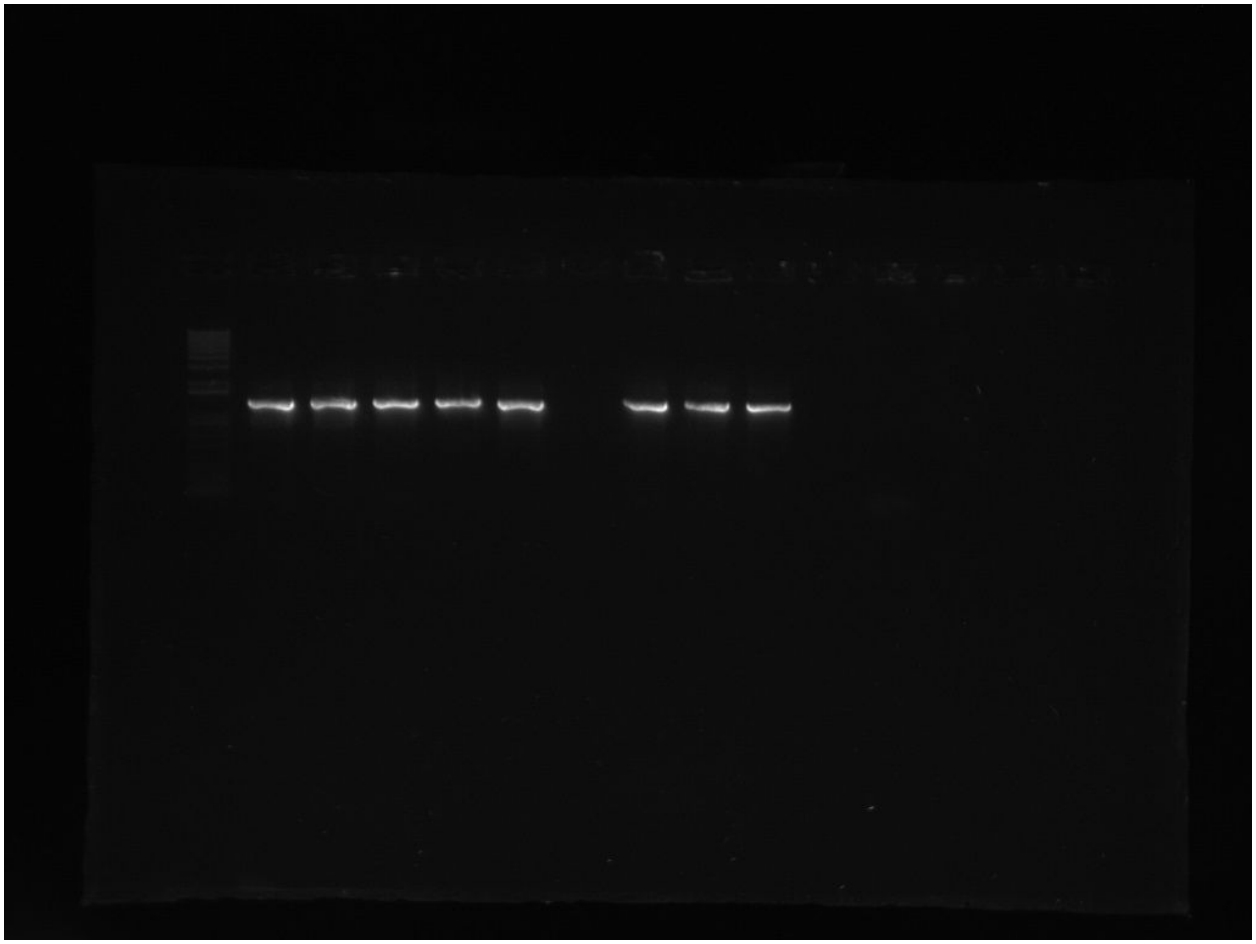
June 29, 2015
Goal: To see if colonies from June 22 pBBR1MCS-2 transformation continued to carry kanamycin resistance.
Methods: Streaked the transformed *G. apicola* from June 22 (that had a few colonies) onto a new kanamycin plate, and was incubated at microaerophilic conditions (5% CO_2 balanced with nitrogen, and 37°C).
Results: One colony grew very minimally and failed to grow upon streaking it out onto any other plates in attempts to produce more biomass.

June 30 - July 3, 2015:
Goal: To determine which media *S. alvi* grows best in.

Methods: *S. alvi* was inoculated into a 10 mL of MH Broth tube and 10 mL of brain-heart infusion tube flushed with 5% CO_2, and incubated at 37°C (while shaking).
Results: No growth was visible in any of the liquid cultures.

July 3 - 9, 2015
Goal: To determine whether the June 22nd electroporation products were *G. apicola*.
Methods: Campy Competent cell protocol. *E. coli* heat shock protocol. Colony PCR protocol. Transformed competent cells of G. apicola from campy protocol with pBBR1MCS-2 one set with heat shock and another with electroporation. Performed colony PCR using our *G. apicola* primers with eight unique colonies selected from both the electroporated and heat shocked transformations of *G. apicola* with the pBBR1MCS-2 plasmid (four from each plate) along with two negative controls (one with no plasmid and another with an *E. coli* colony to ensure the primers were selective). To ensure the product was *G. apicola*, the DNA was sequenced. DNA was isolated from the cleanup of a colony PCR gel.
Results: Blasted the sequences of our bacteria and found a 98.5% (sample H1) and 98.8% (sample 7) similarity to *G. apicola* indicating the transformed bacteria was indeed *G. apicola.*

Figure 4: DNA Agarose Gel Electrophoresis

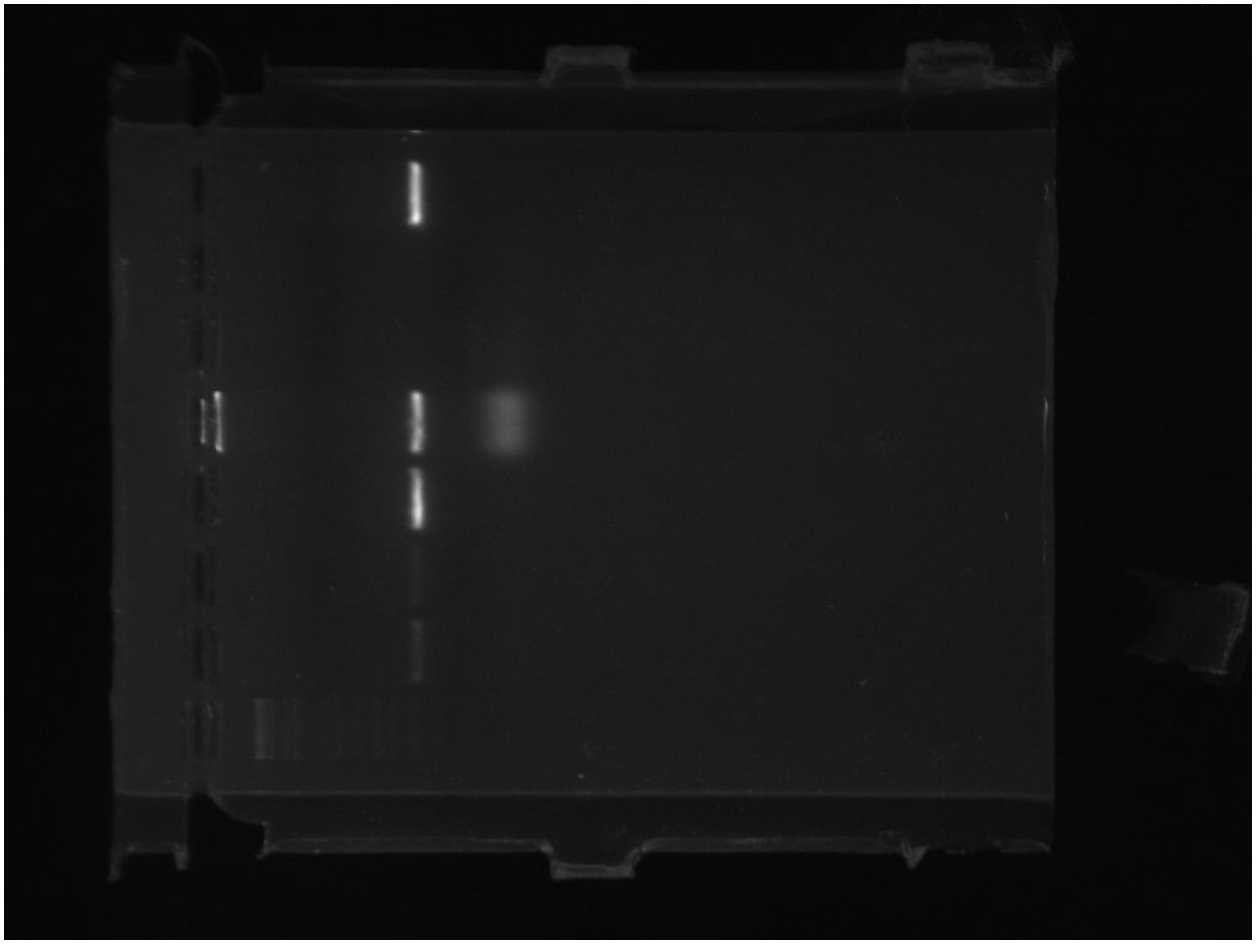


From Left to Right: Latter, Electroporated *G. apicola* 1, Electroporated *G. apicola* 2, Heat shock *G. apicola 1,* Heat shock *G. apicola 2,* Electroporated *G. apicola* 3, Electroporated *G. apicola* 4, Electroporated *G. apicola* 5, Electroporated *G. apicola* 6, Electroporated *G. apicola* 7, Negative, *E. coli*

July 5-8, 2015
Goal: To prepare for electroporation with *S. alvi*
Methods: Kanamycin was added to the blood agar plates for later use in the selection of the transformed *S. alvi* with pBBR1MCS-2. *S. alvi* was streaked out to produce large biomass for future transformations. PCR and a gel electrophoresis of the results were run on different colonies of *S. alvi* plates to ensure all plates contained *S. alvi* and were not contaminated.
Results: *S. alvi* grew small visible colonies and colony PCR confirmed they were indeed *S. alvi.*

July 6 - 9, 2015
Goal: To transform *G. apicola* with the RP1 plasmid through heat shock.
Methods: campy competent cell protocol and heat shock protocol.
Results: A bacterial smear/cloud formed on the initial transformation plate and was streaked out onto another TSA KAN plate since original KAN plate did not display individual colony growth (just cloudy growth). No visible growth appeared on the second streak plate and was suggested that too much biomass was present and enable initial growth.

July 7, 2015
Goal: To determine if the electroporated cells from June 22nd contained the pBBR1MCS-2 plasmid.
Methods: Miniprep was performed on the transformed *G. apicola* cells and PCR was performed on the obtained DNA with the M13 primers specific to the pBBR1MCS-2 plasmid. Miniprep was performed first because it was hypothesized that the primers could not reach the plasmid present in the cells.
Results: Refer to Gel
Figure 5: DNA Agarose Gel Electrophoresis



From bottom to top: Miniprepped pBBR1MCS-2 from “transformed” *G. apicola* cells 1, Miniprepped pBBR1MCS-2 from “transformed” *G. apicola* cells 2, *G. apicola* that grew on a second streak plate (kanamycin plate), *E. coli* Transformed with pBBR1MCS-2, *E. coli* no plasmid, no template (negative), purified pBBR1MCS-2 (positive)

July 7 - 24, 2015
Goal: To transform *S. alvi* with pBBR1MCS-2 plasmid through electroporation
Methods: campy competent cell protocol and electroporation. *S. alvi* was plated on blood agar plates to rest. Controls for this transformation were non-transformed *S. alvi* on two blood agar plates with one to be transferred onto another kanamycin blood agar plate.
Results: Only after seven days was there enough colony formation on the blood agar plates with the transformed *S. alvi* to be transferred onto a kanamycin blood agar plate. No growth was seen upon the transfer even after a week, all controls produced no colonies. It was concluded that *S. alvi* cannot be transformed with pBBR1MCS-2, as there was no growth.

July 8 - 14 , 2015
Goal: To determine whether *S. alvi* could survive the freeze-thaw process.
Methods: Competent *S. alvi* from the frozen stock were streaked on blood agar plates to check if they survived the washes and freeze/thaw.

Results: *S. alvi* colony formation was seen on the blood-agar plates indicating that the cells survived the washes to induce competence but did not get transformed.

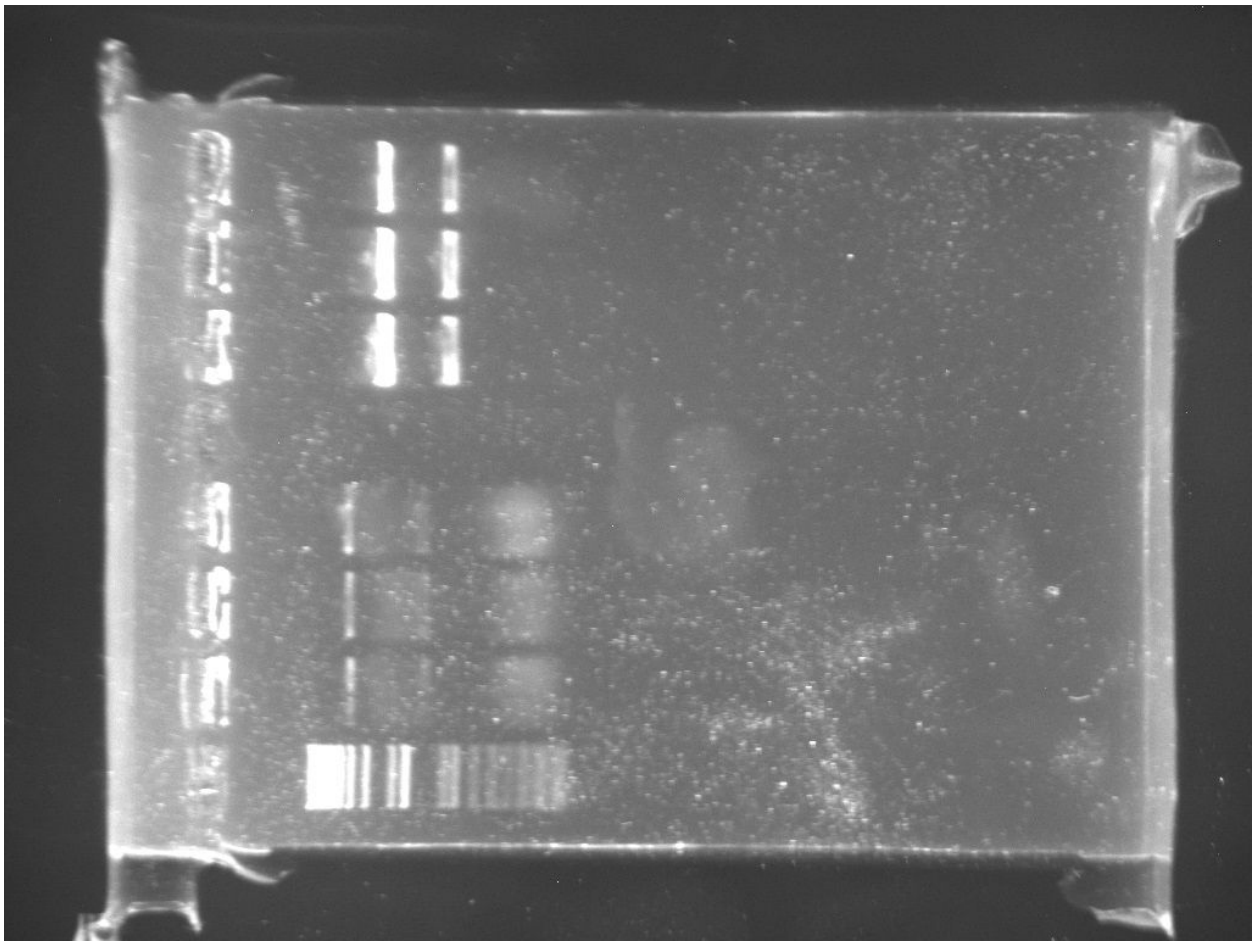
July 9 - 20, 2015

Goal: To insert the marker, a red fluorescent protein gene, into the plasmid pBBR1MCS-2, to help distinguish our “pro-bee-otic” and see if our cells really were transformed..

Methods: The insertion of the red fluorescent protein gene from the pSB1A3 plasmid into the pBBR1MCS-2 vector used SpeI and EcoRI restriction enzymes. The ligation product was then transformed into *E. coli* and plated onto TSA plates with kanamycin.

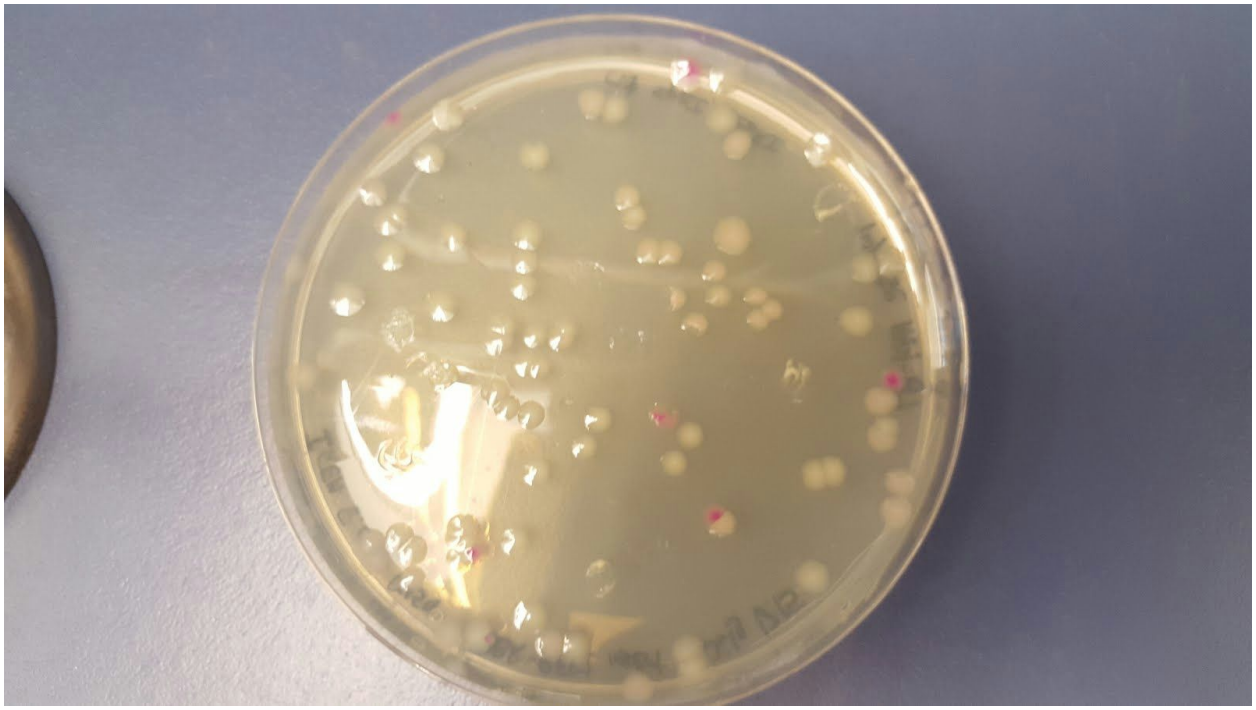
Results: Red colonies appeared on the kanamycin TSA plate, indicating the ligation was successful.

FIgure 6: DNA Agarose Gel Electrophoresis



From Bottom to top: Latter, Cut pBBR1MCS-2, Cut pBBR1MCS-2, Cut pBBR1MCS-2, Cut pSB1A3-RFP, Cut pSB1A3-RFP, Cut pSB1A3-RFP. Top band of the cut pBBR1MCS-2 is the the digested version of it. Lowerband of the cut pSB1A3-RFP is the RFP vector

Figure 7: *E. coli* Transformed with pBBR1MCS-2 with RFP insert



July 20 - 21, 2015
Goal: To see if *G. apicola* can survive in the sugar feed conditions for the honeybees.
Methods: A 40% sugar media was made 50% being water and 10% being TSB and *G. apicola* was transferred into the mixture and vortexed to produce a uniform mixture.
Results: *G. apicola* appeared to survive after two and twenty four hours in the sugar water mixture by streaking the solution onto TSA.

July 22 - 25,2015
Goal: To transform *G. apicola* with pBBR1MCS-2 and RFP-pBBR1MCS-2 through electroporation with adjustments to the original protocol
Methods: campy competent cells and electroporation protocol Transferred the transformed cells onto the respective kanamycin plates, using water instead of TSB incase cells survived due to the TSB used to transfer the cells.
Results: No growth of transformed cells. It is hypothesized that due to the low salt content of the water, the bacterial cells exploded and were unable to survive the transfer.

July 25 - 28 , 2015
Goal: To transform *G. apicola* with pBBR1MCS-2 and RP1 through electroporation with adjustments to the original protocol
Methods: campy competent cells and electroporation protocol An adjustment from the original protocol was using a PBS (Phosphate Buffered Saline) solution and used it instead of TSB to transfer the newly transformed *G. apicola* onto their respective antibiotic plates.

Results: No growth on any of the antibiotic selection plates.

July 27 - 29, 2015
Goal: To transform *G. apicola* with pBBR1MCS-2 and RFP-pBBR1MCS-2 through electroporation with adjustments to the original protocol
Methods: campy competent cells and electroporation protocol An adjustment for, the original protocol was using either TSB or PBS buffer to transfer the cells onto the selecting kanamycin plates.
Results: No growth on any of the antibiotic selection plates,

July 30 - August 6, 2015
Goal: To prepare for conjugations
Methods: Streaked out the *E. coli* on LB plates with the appropriate antibiotics and created freezer glycerol stocks with the cells. G. apicola, and the DH5alpha, S17, SM10 strains of *E. coli* were streaked onto varying concentrations of oxytetracycline: 5µg/mL, 10µg/mL, 30µg/mL, 75µg/mL, 100µg/mL. This was to determine the concentration which allowed G. apicola to grow but not *E. coli*. These plates were placed in 5% CO_2 balanced with nitrogen and left overnight to grow. Moreover, the S17 and SM10 strains of *E. coli* were streaked onto TSA, TSA kanamycin, and TSA streptomycin plates to see if the bacteria had resistance to the antibiotics.
Results: The TSA oxytetracyline streak plates of 5ug/ml, 10ug/ml, 30ug/ml displayed growth meaning the concentration was not sufficiently high enough to screen for G. apicola. Both the 75ug/ml and 100ug/ml plates killed off all the E. coli strains, but G. apicola produced colonies, therefore a sufficiently high enough concentration was determined to try transformation by mobilization. S17 *E. coli* was found to be internally resistant to streptomycin and SM10 *E. coli* became the designated strain for conjugations. It should be noted that the oxytetracycline was a strange color and did not dissolve fully in the water and it is hypothesised that the oxytetracycline used was bad.

July 29 - August 2, 2015
Goal: To transform *G. apicola* through conjugation with SM10 *E. coli* and the PKT210 and PRK293 plasmids
Methods: electroporation protocol. Obtained new plasmids from the Smit lab: pBBR3, pBBR4, PKT210, and PRK293. All four were given in *E. coli* liquid media with the appropriate antibiotics. Freezer stocks of all strains were produced and were mini-prepped to try transformation with. *G. apicola* was attempted to be transformed with the four new plasmids.
Results: All four plasmids attempted yielded no transformants on the antibiotic selection plates.

July 31 - August 3, 2015
Goal: To find a liquid culture for *G. apicola* to grow in.
Methods: A brain heart infusion broth with blood agar blocks added was created and inoculated with G. apicola flushed with CO_2 and left on a shaker at 37C. The LB liquid culture of G. apicola produced cells and was plated on TSA for colony PCR tomorrow. Another brain heart

infusion broth was inoculated with G. apicola however this time without blood blocks added, Culture was flushed with CO_2 and left on shaker at 37C.
Results: The brain heart infusion broth from July 31 appeared to have cells growing in it, 1ml was extracted and plated on TSA to ensure cell presence. No growth appeared after 48 hours in the microaerophilic jar at 37°C.

A**ugust 3 - 4, 2015**
Goal: To transform SM10 and S17 *E. coli* with the PIND4 and PTK210 plasmids.
Methods: *E. coli* SM10 and S17 were attempted to make competent following an Addgene protocol. Comp cells were subsequently transformed via heat shock with PIND4 and PTK210 and plated on appropriate antibiotics. *G. apicola* was inoculated into LB liquid media and flushed with CO_2 and placed on a shaker at 37°C.
Results: Transformed cells from August 3rd produced low colony number, with *E. coli* SM10 with the PRK-210 plasmid producing no colonies. Cells that did grow were inoculated into liquid culture to produce higher cell count. Comp cell protocol produced low transform efficiency and thus competent *E. coli* S17 and *E. coli* SM10 cells were recreated using a different protocol.

August 5 - 7, 2015
Goal: To transform *G. apicola* with pBBR1MCS-3 and pBBR1MCS-4 through electroporation
Methods: campy competent cell and electroporation protocol
Results: There were no visible colonies on the TSA antibiotic plates from the August 5th transformations.

August 5 - 10, 2015
Goal: To transform *G. apicola* through conjugation with the SM10 and S17 *E. coli* and the PIND4 plasmid
Methods: Began **conjugation protocol** with the PIND4 plasmid using both *E. coli* S17 and SM10 cells. Different proportions of the acceptor *G. apicola* and the transformed donor *E. coli* cells were used: 5:1, 2:1, 1:2, and 1:1. The conjugation protocol is listed in the protocols page.
Results: There was a significant amount of growth for the SM10 *E. coli* and *G. apicola* conjugations, so a colony PCR was performed to determine if the bacteria grown was indeed *G. apicola.* After PCR and DNA agarose gel electrophoresis, we concluded that the 1:1 ratio of *G. apicola* to SM10 *E. coli* possibly successful in conjugating the plasmid, since *G. apicola* grew on the kanamycin plate and was identified to be *G. apicola* through colony PCR. We found that PIND4 conjugation restreak grew on TSA kanamycin however colony formation looked strange after 2 days and it appeared to possibly be two colony types.

August 6 - 12, 2015
Goal: To transform *G. apicola* with the PRK293, PKT210, and PIND4 plasmids through electroporation.
Methods: electroporation and campy competent cell protocol

Results: The electroporation of PKT210 grew, but none of the PRK293 and PIND4 grew on the antibiotic plates. (aug11) The restreak from PKT210 looked promising, so we streaked it out again on two new TSA strep plates (one to put in aerobic conditions as a control) in preparation for a miniprep tomorrow. (aug12) The restreaked *G. apicola* and PKT210 grew in aerobic conditions, so we did not do a miniprep & digest to check for the plasmid, as it was contaminated.

August 6 - 12, 2015
Goal: To transform *G. apicola* through conjugations performed with both S17 and SM10 strains of *E. coli* and the PRK293 and PKT210 plasmids
Methods: In an attempt to get colonies forming near each other, we simply platted the the bacteria near each other and waited 24 hour to let them grow then transferred to the antibiotic selection plates..
Results: Both conjugations with PRK293 with SM10 and S17 *E. coli* grew small colonies on the kanamycin antibiotic plate. (aug12) Did a colony PCR on the conjugated *G. apicola* with PRK 293 and the contaminated restreak of PKT210 that grew aerobically - found that the conjugation was amplified, so we decided to repeat the conjugation in the upcoming week to confirm that *G. apicola* was transformed successfully. When retried, no growth was seen.

August 11, 2015: Received PBSPIISK(-) from Hancock labs, and inoculated 5mL LB with the transformed *E. coli* for miniprep tomorrow.

August 12-15, 2015
Goal: To transform *G. apicola* through electroporation with the PBSPIISK(-) plasmid
Methods: campy comp. cell and electroporation protocol. Freezer stocks of the strain was produced.
Results: No growth on any of the antibiotic selection plates.

August 13 - 14, 2015
Goal: To transform *G. apicola* through heat shock with the pBBR1MCS-2, pSB1A3, and PKT210 plasmids
Methods: campy competent cell protocol and heat shock protocol
Results: Aerobic control for heat shock with *G. apicola* competent cells grew, so we discarded all the transformed plates instead of transferring onto antibiotic.

August 13, 2015
Goal: To determine whether SM10 *E. coli* could survive freeze-thaw process.
Methods: Inoculated two 5mL LB test tubes with SM10 *E. coli* to ensure the cells are still alive after freezing them in -80C and prepare for another trial of conjunction next week.
Results: Bacterial biomass was present in multiple LB test tubes after 24 hours indicating that SM10 *E. coli* is capable of surviving the freeze thaw process.

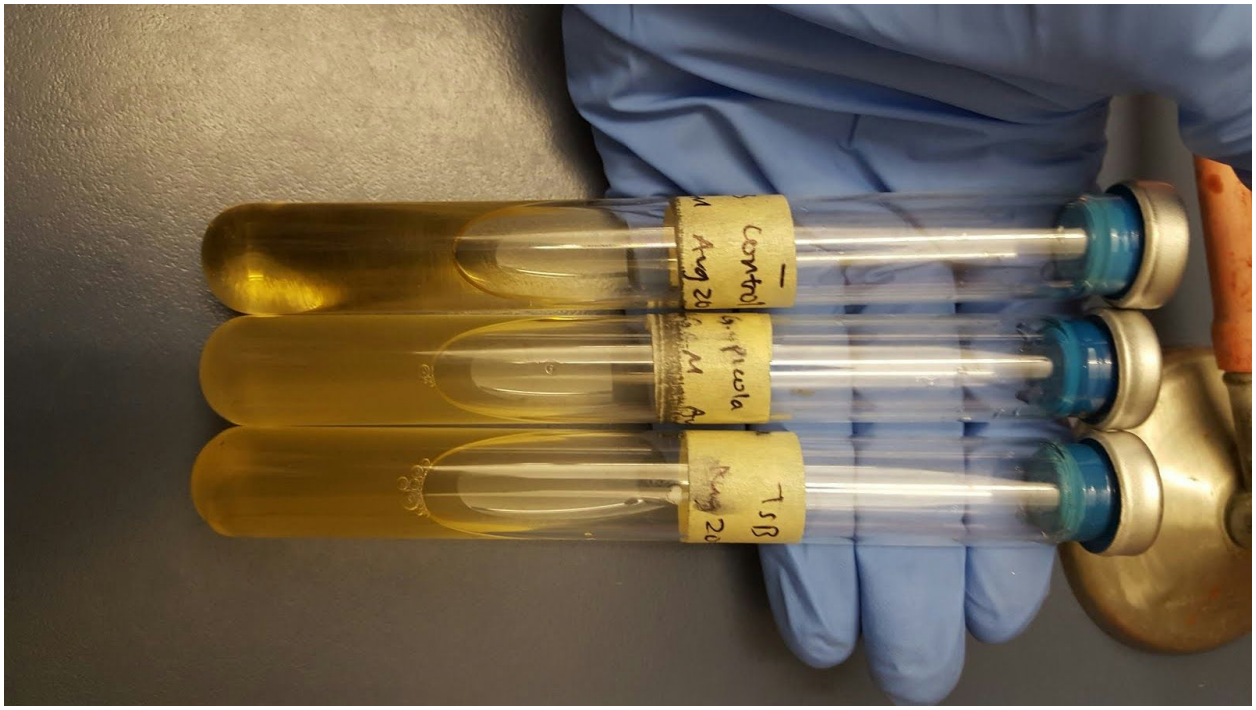
August 16 - 20, 2015
Goal: To transform *G. apicola* through conjugation with SM10 *E. coli* and with the PRK293, PKT210, RP1, PBSPIISK- plasmids.
Methods: Performed the **conjugation protocol** between E. coli SM10 with PIND4, PBSPIISK(-), PKT210, and a no plasmid control by plating both types of cells on a TSA plate. Each attempt had two replicates and were left overnight at 37°C with a 5% CO_2 balanced with nitrogen environment.
Results: Looked at the conjugation plates and they appeared to have a bacterial smear, likely meaning that the shear number of E. coli cells were too much for the oxytetracycline + other antibiotic plates. Bacterial lawns were present on the double antibiotic plates from the conjunction (appears to be E. coli), so we plan to retry them later this week (sunday). The hypothesis is that the oxytetracycline went bad.

August 17-20, 2015
Goal: To test if *G. apicola* could survive new competent cell protocols
Methods: Salmonella and Pseudomonus competent cell protocol After performing the protocols, the cells were streaked out both sets of competent cells onto two plates and left one under normal atmosphere at 37C to grow and the other in 37°C with a 5% CO_2 balanced with nitrogen environment. this was to ensure that the cells had survived the process and were not contaminated.
Results: no growth was seen on the plates left under the normal atmosphere whereas growth was seen on the plates that were left under the microaerophilic atmosphere. The colony morphology was the same as seen for *G. apicola* in the past and PCR would be done in the future.

August 18 - 20, 2015
Goal: To transform *G. apicola* through electroporation with pBBR1MCS-4
Methods: Transformed Competent G. apicola cells **(salmonella protocol)** with pBBR1MCS-4 (tet. res.) by **electroporation** plated on TSA to recover. were left overnight at 37°C with a 5% CO_2 balanced with nitrogen environment.
Results: The Electroporation of pBBR1MCS-4 yielded colonies on the negative control plate meaning that the tetracycline we used was bad.

August 20 -24, 2015
Goal: To grow *G. apicola* in liquid media.
Methods: Tried three more liquid cultures for G. apicola (TSB, two positive and one negative), cultures were flushed with 5% CO_2 balanced with nitrogen while hot, innoculated, and (environment is considered anaerobic for this trial) and left in 37C on shaker to grow.
Results: The anaerobic TSB appeared to have growth in the two positive tubes and no growth in negative. A PCR was run on the positive tubes and was confirmed by gel as gilliamella.

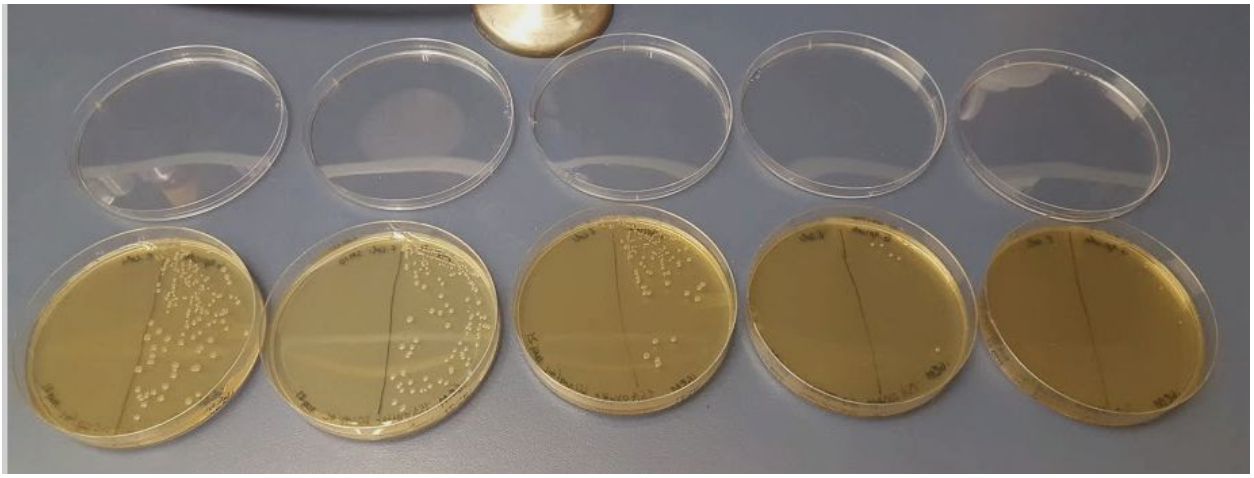
Figure 8: *G. apicola* in anaerobic TSB



From top to bottom: Control, *G. apicola* 1, *G. apicola* 2

August 21-23, 2015
Goal: To find the optimal antibiotic concentration for growth of *G. apicola* and inhibition of growth for *E. coli*
Methods: In preparation for conjugation and mobilization, a method of selecting for *G. apicola* was required. As *G. apicola* expresses a natural resistance to oxytetracycline, a spectrum of TSA plates with increasing oxytetracycline concentrations were used to determine what allowed growth of *G. apicola* whilst inhibiting the donor strain *E. coli* SM10. Colony PCR was performed on colonies to confirm selection for *G. apicola*. It was determined that 30 µg/ml of oxytetracycline was optimal, as it selected for growth of *G. apicola* and inhibited *E. coli* SM10.

Figure 9: Oxytetracycline concentration spectrum with *E. coli* SM10 on the left half of every plate and *G. apicola* on right



From left to right, plate oxytetracycline concentrations are 20, 40, 60, 80, and 100 µg/ml.

Results: It was determined that 30 µg/ml of oxytetracycline was optimal, as it selected for growth of *G. apicola* and inhibited *E. coli* SM10. 30µg/ml was chosen as both 20 and 40µg/ml oxytetracycline produced decent colony number but 40 appeared to have slightly lower colony number.

August 23 - 27, 2015
Goal: To transform *G. apicola* through conjugation with SM10 *E. coli* and with the PIND4,, PKT210, RP1, PBSPIISK- plasmids.
Methods: Performed the **conjugation protocol** between E. coli SM10 with PIND4, PBSPIISK(-), PKT210, RP1 and a no plasmid control by plating both types of cells on a TSA plate. Each attempt had two replicates and were left overnight at 37°C with a 5% CO_2 balanced with nitrogen environment.
Results: No cells grew upon the transfer plate containing oxytetracycline and selecting antibiotic for the plasmid. indicating that the conjugation was unsuccessful.

August 24, 2015
Goal: To see if growing *G. apicola* and *S. alvi* together could lead to faster growth.
Methods: Performed a cross streak of of G. apicola and S. alvi to see if grown together results in better growth as it had been previously described that they pass certain compounds between them.
Results: No enhanced visible growth was seen of *S. alvi* or *G. apicola.*

August 25 - 26, 2015
Goal: To test if *Salmonella* and *Campylobacter* protocols were inducing competence in cells by using them to transform SM10 *E. coli* with pSB1A3.
Methods:*Salmonella* and *Campylobacter* protocols

Results: Cells from the Campylobacter and Salmonella produced transformants on the antibiotic selection plates indicating that the protocols were able to induce competence in E. coli DH5alpha.

August 25 - 27, 2015
Goal: To transform *G. apicola* through electroporation with pBBR1MCS-4
Methods: Electroporated G. apicola competent cells **(from salmonella protocol)**
Results: Growth on the control plate of the tetracycline electroporation indicated that our selecting concentration was too low (our tetracycline is believed to be degraded).

August 25 - 27 - , 2015
Goal: To transform *G. apicola* through conjugation with SM10 *E. coli* and with the plasmids RP1, PBSPIISK(-), PIND4, and PKT210.
Methods: Rhodobacter conjunction protocol.
Results: Plates containing both *G. apicola* and *E. coli* for the conjunction were transferred to appropriate antibiotic plates selecting for the plasmid and oxytetracycline to select for *G. apicola*. No colony formation occurred on the plates and conjugation was deemed unsuccessful.

August 28 - 31, 2015
Goal: To transform *G. apicola* through electroporation with the pSB1A3, PKT210, and pBBR1MCS-2 plasmids
Methods: Liquid culture of *G. apicola* was grown to appropriate OD600 value and then washed with chemicals following the **salmonella electroporation** and **E. coli heat shock** protocols to induce competence. Cells were then attempted to be transformed using both protocols with pSB1A3, PKT210, and pBBR1MCS-2. Cells were allowed to rest for 1 hour in anaerobic TSB media at 37°C and then plated on antibiotic selection plates and grown in liquid media as well selecting for the plasmid.
Results: No growth on any of the antibiotic plates except for the one with pSB1A3. Liquid cultures yielded no viable cell growth. Ran a DNA agarose gel after pCR for the plasmid and *G. apicola* for the cells that grew on the restreak from the salmonella protocol electroporation with pSB1A3. Unfortunately, the cells were not G. apicola and did not have the plasmid.

August 30, 2015
Goal: To transform *G. apicola* through electroporation with the pBBR1MCS-4 plasmid
Methods: : G. apicola was **electroporated** with pBBR1MCS-4 with competent cells from the **salmonella protocol** and plated on tetracycline (100ug/ml) and oxytetracycline plates (60ug/ml). The tetracycline concentration is so high because it is believed that our tetracycline has either degraded or gone bad
Results: No growth on the antibiotic selecting media and no growth on the control plates indicating the concentration of tetracycline was high enough but the transformation was no successful.

September 2, 2015
Goal: To induce competence in *G. apicola*
Methods: One 100ml culture of cells grew and subsequently washed to induce competence **salmonella protocol**
Results: A PCR was done to ensure the original cells were G. apicola and that the competent cells were G. apicola (confirmed).

September 2, 2015
Goal: To induce competence in *G. apicola*
Methods: salmonella protocol
Results: Ran a gel to confirm that the cells that were washed yesterday were still G. apicola.

September 3, 2015
Goal: To transform *G. apicola* with the competent cells prepared on September 2nd with the pSB1A3, pSB1C3, pBBR1MCS-2, the PKT210 plasmids
Methods: electroporation
Results: No growth on any of the antibiotic selection plates and cell growth on oxytetracycline plates indicating that cells were still alive and that the transformation was unsuccessful.