Over these past eight weeks at Rutgers Cook College I worked with the biotechnology program performing three main experiments. The first was to produce Rifampicin resistant B. cepacia G4 bacteria. This was accomplished by placing the G4 bacteria in a very diluted solution of rifampicin and letting it grow in each test tube for 12 hours. After 12 hours, it was placed in the next highest dilution of a doubled concentration of rifampici, until finally it was able to grow in a concentration of .1 mg/ml. The production of G4 rifampicin resistant bacteria was important to our other experiments since we needed to be selective for what we grew on our LB plates.

The next project we worked on was mating of bacteria to produce mutants. We used three different types of bacteria to accomplish this: the Donor: OKM/JM109 a Helper: PRK co13/ HB101 and our two Recipients: B. Cepacia G4 and B. Cepacia DB01. A loop full of each was added to a 10% glycerol solution which was centrifuged for 5 minutes. The supernate was discarded and the cells were resuspended. We pipetted out 10 micro liters of the solution on a LB plate in circular blobs and allowed this to incubate over night. As result of this mating genes were mutated and now we had to see which genes had changed. First, we had to find an optimal concentration to plate out on a big plate of LB in order to use the colony picker. We found the optimal concentration by doing test matting using smaller plates and used 4 different concentrations which were a concentrated, $10^3$, $10^2$, and $10^4$. We found that $10^2$ and $10^4$ gave us the best growth for our weekly target of about 4000 colonies of bacteria. For the most part using the dilution of either $10^1$ or $10^2$ worked best for our mating.

Now we had to screen for the mutants that were produced during the mating on our six different carbon sources which were Benzoate, Phenylalmine, p-hydroxybenzoate, Phenyl-pyruvate, Tryptophan, and Tyrosine. Each week we would have to fill sixty 384 well plates using robots to save time. We used the robot again to inoculate the 384 well plates with the bacteria which were picked from the colony picking machine. We would let the plates grow over night and on the next day take absorbance readings from each well to see if anything was growing. If nothing was growing in one of the carbon sources it was considered to be a mutant and would go on to
for further testing for confirmation. We would then take all of our possible mutants and place them on 6 LB palates each one containing a carbon source. If something didn’t grow, and was consistent with our first reading, it would go on for a second test on LB to finally be a confirmed mutant. In any given week, we might start out with as many as 50 to 80 mutants and only end up with 6 to 16 confirmed mutants.

The next step in our project was to identify where the gene mutated was and where along the pathway of either synthesis or degradation for each mutant.

Working at the Rutgers lab was the first lab I have worked in. It was very different than working out of a lab book, which I was used to. This was also the first time I have worked with bacteria. I learned a lot about the basics of working with bacteria, such as, how to produce LB to grow it on, how to keep your sample contaminate free, how to streak out bacteria on to LB plates, and how to take it off. All these tasks should be very helpful in the future since I plan on getting into the field of pharmaceutical research and development. I could not have found a better summer job, and am very grateful to have been given such a good opportunity.