Elizabethkingia – To be or not to be…ta-lactamase

Group 22
1990-002

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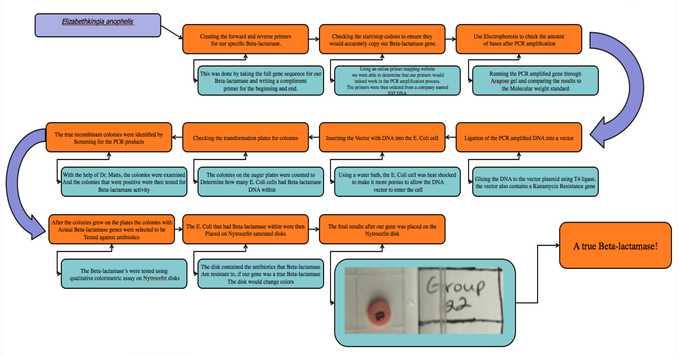
ABSTRACT

In this experimental process we attempted to determine if our designated strand of beta-lactamase enzyme was indeed a true Beta-Lactamase. In order to do this we indulged the gene code in PCR processes, electrophoresis process as well as the ligation process among others and as a final result concluded it to be or not to be indeed a true Beta-lactamase

INTRODUCTION

Elizabethkingia *anophelis* is a staph infection causing disease that transfers from mosquito host to bitten human, the disease is a completely penicillin resistant, meaning that it contains a specific gene in its DNA, *beta-lactamase*, that allows the infected cell to be immune to all penicillin based antibiotics. However, this disease is uncanny in a way that it contains more than one identified known *beta-lactamase* gene, whereas a cell only needs one gene to be resistant to penicillin based products. However, there have been over 20 identified genes hypothesized to be *beta-lactamase* genes and 3 identified true genes. Our experiment revolved around one gene of the many designated by our professor to other students experimental groups, and in the end our specific gene sequence was concluded to be a true *beta-lactamase* gene upping the amount of genes located in the disease to be true *beta-lactamase* to 4.

MATERIALS AND METHODS



RESULTS

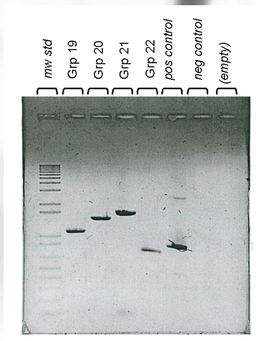
The first results we have found were the primers that were needed ordered to begin this experiment.

Forward Primer:
5' ATGAAGAATATAATGCGGGCACTG 3'

Compliment Primer:
5' TTAATTTGAAGCCTTTTGTTTTTG 3'

Later on when we got to the electrophoresis treatment we were happy to find that just as we had hypothesized, the base pairs amounts was in the ball park range of what we had concluded to on the gel treatment.

These are our augur plates with the colonies of E. Coli grown and ready to be screened for final testing.



Gel 5

Group	Sample
19	E.ano_bla267
20	E.ano_bla2038
21	E.ano_bla2045
22	E.ano_bla304
Pos	E.ano_strep2459
Neg	E.ano_strep2459 no primers

The final step is testing the Beta-lactamase gene that was ligated into the E. Coli cell. This is tested against a positive and negative controls on qualitative colorimetric assay on Nytrocefin disks. Our disk is below.

How ironic that in the beginning this disease is transferred via the blood taking of a mosquito and in the end our identification color, to see if our sequence was a true beta-lactamase, was red.

DISCUSSION

From an outsider's viewpoint, biochemistry is complicated science filled with variables that will never be understood. Yet, after only a semester of experimenting, our group has a better understanding of PCR reactions, primers, ligation reactions, vectors, along with the insight to the field of biochemistry. Our numerous amounts of discussions started with shared, confused expressions as we carried out the first of these reactions. Yet, with the explanation and help from Dr. Canaan and that of our TAs, the experiment process began to clear up. Nothing went wrong with any of our experiments, however, we did hit a small bump in the road with the reverse primers sequence which was quickly corrected by Dr. Canaan. Our PCR reactions went well, as we copied our strand of DNA millions of times through the PCR reactions. The results we obtained from the PCR reaction was used in a piece of agarose gel to determine if our PCR reaction was successful. From attracting our strand from one end of the gel to the other using a negative charge, we were able to determine the amount of base pairs to conclude that the PCR reaction was successful, for the amount of base pairs had to match with our estimated amount. After connecting our DNA strand to a vector, in the final home stretch of our experiment, we used a heat shock process to encode our DNA inside of an E. Coli cell. Within our vector however, we added kanamycin, which made the cell as a whole resistant to kanamycin, this in a way acted as a 'signal' – it showed us which ells contained the end products DNA and which didn't. From there we colonized the cells and identified which colonies were penicillin resistant and which died when exposed. All in all, it was a very interesting process as we discovered that the final result of our groups gene sequence proved to be a true beta-lactamase gene as our end product lit up red!

REFERENCES

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