ANALYZING LAKE ERIE BACTERIA USING PCR AND GEL ELECTROPHORESIS
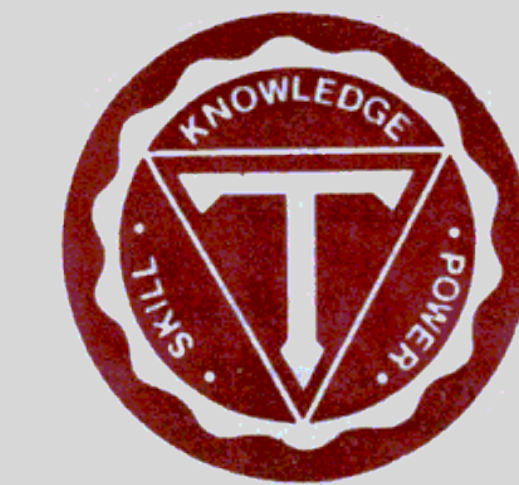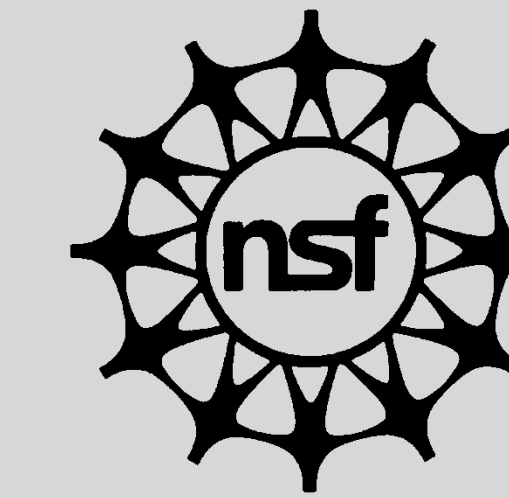## Identifying Both Genus and Species

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OBJECTIVES

1. Determine the presence of bacteria, in Lake Erie , using PCR amplification and analysis of Gel Electrophoresis
2. Determine the type of bacteria present in Lake Erie using molecular known genetic markers.

BACKGROUND

Fecal contamination of local waterways has long. been a problem for Buffalo and it's surrounding suburbs. Most primary sewer systems were built around the 1830's. Until this time, direct discharge of untreated sanitary and industrial waste into nearby bodies of water was the norm. Waterborne disease outbreaks were endemic. The completion of treatment plants in 1938 and updates to secondary plants in 1979 helped to reduce the problem.

Today human sewage contamination is a major health concern. High levels of fecal bacteria is a major contributor to degraded quality at local urban beaches, lakes and rivers. These fecal bacteria include Escherichia Coli and Enterococcus and have been used for water quality monitoring. These bacteria are found in both animal and human sources. The presence of the bacteria, bacteriodes, is a positive indicator for human fecal contamination . The presence of all three bacteria indicates sewage contamination is nearly ubiquitous in the urban environment.

In this investigation, the area of study was two local beaches, Woodlawn Beach and Beaver Island State Park where we collected, filtered and used PCR and gel analysis to identify genetic markers for each bacteria type. Our study indicated a positive result for all three bacteria types, E. Coli, Enterococcus, and Bacteroides. This study illustrates the molecular approaches designed to test for fecal pollution can be used to improve and prioritize remedial projects.


HYPOTHESIS

We think we will find E. Coli, Enterococci and Human Bacteroides in Lake Erie because these are the major fecal bacteria associated with the overflow of the sewer systems after a heavy rain.

MATERIALS AND METHODS

Materials: Thermocycler, automatic pipettes, pipette tips, ethidium bromide, gel electrophoresis apparatus , TBE buffer, Agarose, 100kp genetic ladder, dreamtaq, distilled water, known bacterial DNA, , unknown DNA , vacuum filtration, petri dishes, lake water, sample bottles, pcr tubes.,microcentrofuge and tubes. Primers (3' &5')

Methods:
1. Initially, we needed to develop accuracy while pipetting. We used various concentrations of dye and water. Then inserted tubes into spectrophotometer to produce a line graph. If line graph is not straight, repeat until it is.
2. Given a genetic ladder for comparison, use known samples of E. Coli, Enterococci , and bacteriodes to determine their KB length for Identification purposes
   a. This involved PC Ring each bacteria with a set of (4) controls:
      1. Known Sample
      2. 3' primer
      3. 5' primer
      4. no primers
   b. The purpose of the controls is to make sure no contamination occurred to samples.
3. Given unknown samples, follow same procedure to identify unknown samples as E.Coli , Enterococci and bacteriodes which will become our known genetic markers.
4. Repeat until gels run properly and can be used for comparison.
5. Collected samples from Olcott Beach and Beaver Island State Park.
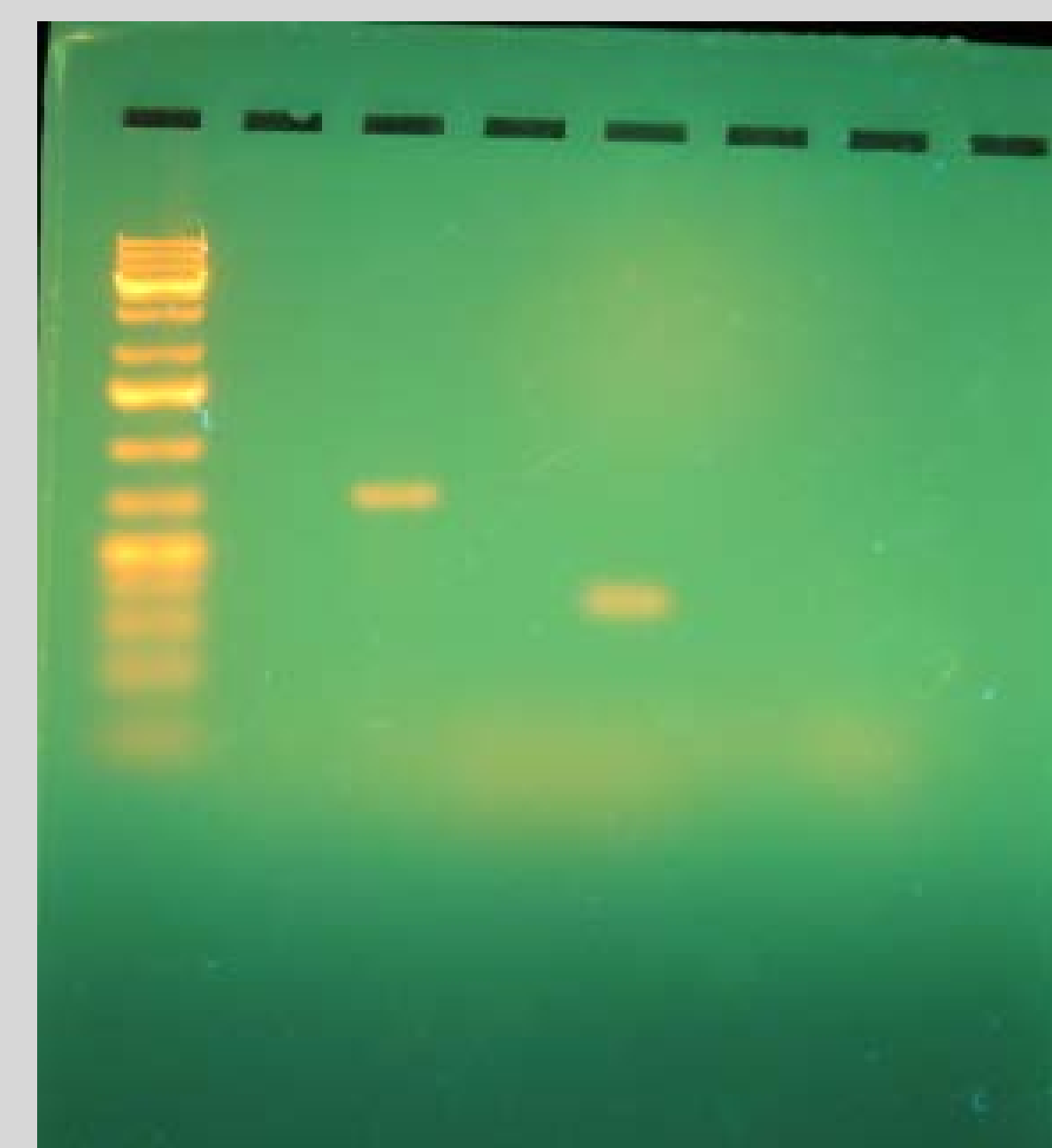
Positive results for Enterococcus facaelis

Vacuum filter set-up.

This involved using a sterile autoclaved bottle,. Held under water to fill bottle without contamination. Using a GPS, record the exact coordinates of samples taken.
6.Filter samples using a vacuum filter system and place filter on A pre-poured agar plate to grow. This agar contains specific nutrients for the growth of E. Coli. Other plates are specific to Enterococci and Bacteriodes.
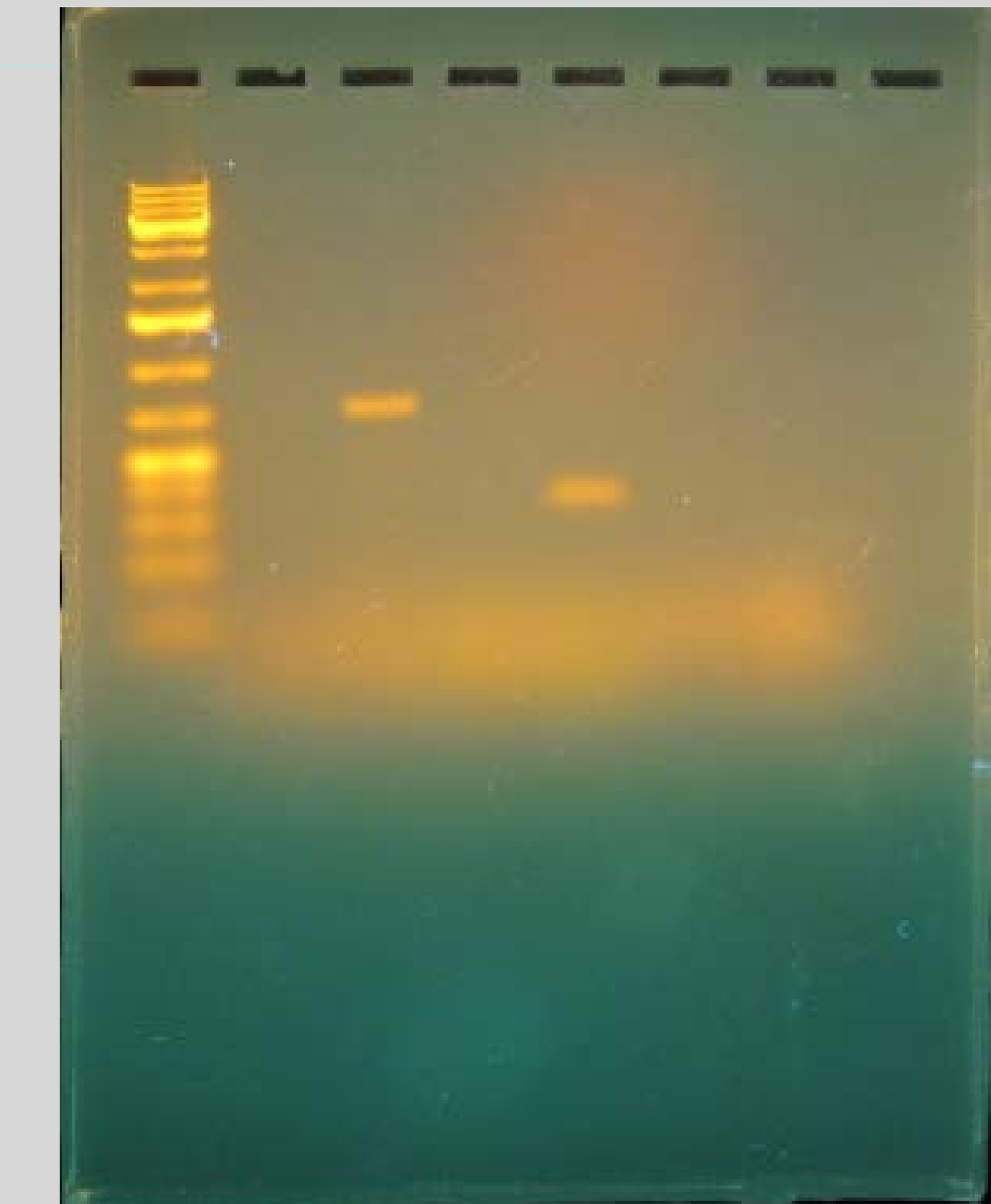

Example – Vacuum filtering.

7. Analyze samples using PCR and gel electrophoresis.
8. Positive result is indicated when matched to genetic ladder.

RESULTS
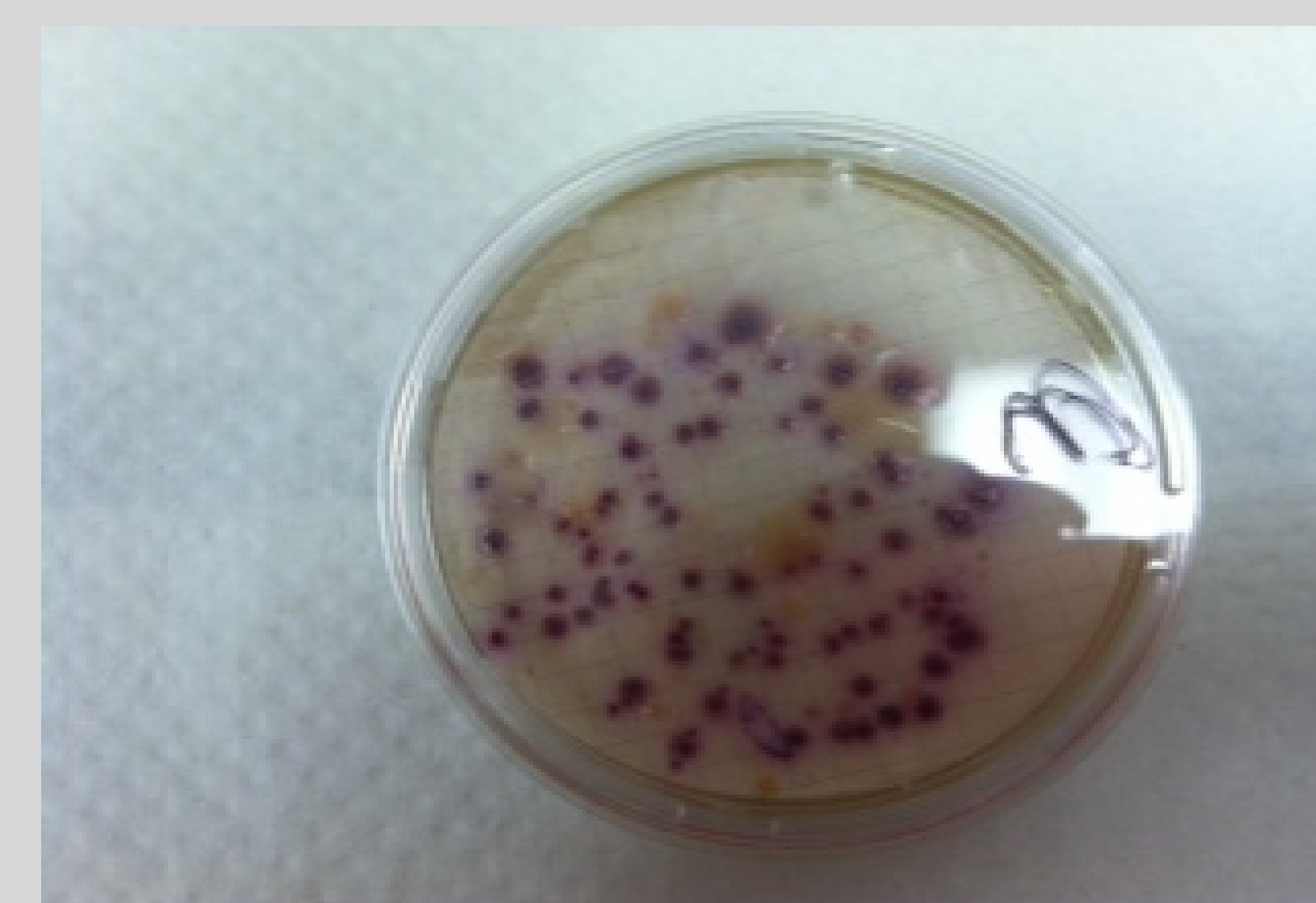
Positive results were obtained for the following types of bacteria.
+ - E. Coli grown on Agar plates.
EPA standard is 200 colonies per 100 ml water to close the beach. This amount below is insufficient.

Similar results were found for Enterococcus at Beaver Island.

CONCLUSIONS

Positive results were obtained from both Beaver Island and Olcott Beach for all three types of fecal bacteria. We were able to identify both genus and species for each.

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IMPLEMENTATION

Field trip planned for September to collect water samples while weather is good for collection.
Back at school water will be filtered and plated and bacteria will be stored for future use.
Practice Pipetting will occur during lab time in September.
Using PCR and gel electrophoresis will be used to ID the bacteria when conducting Genetics Unit.