



Interdisciplinary
Renewable & Environmental
Chemistry REU

ANALYSIS OF *E. coli* BACTERIA ON ATMOSPHERIC FILTERS

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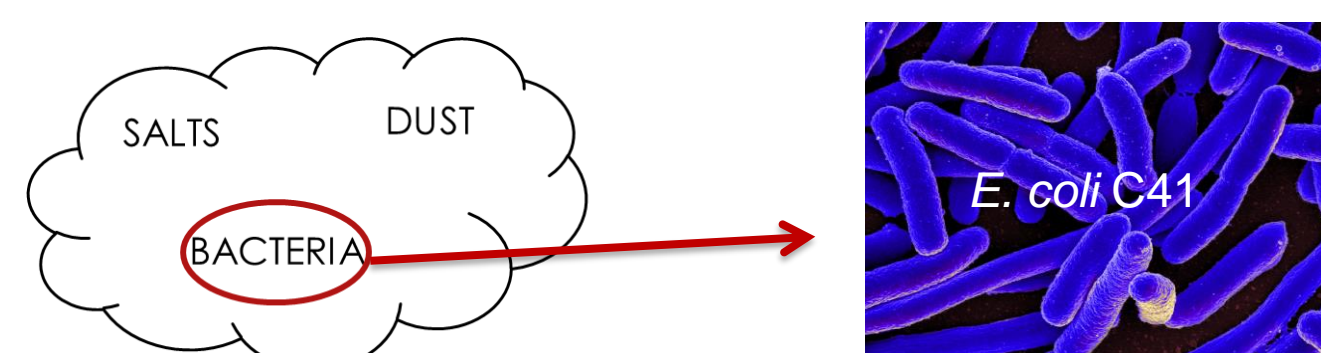
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INTRODUCTION

Bacteria cause ice to form at warmer temperatures, making them effective ice nuclei. Ice nuclei are responsible for causing precipitation. There are two ways to properly collect bacterial samples: 1. TSI atomizer and 2. Sparging Liquid Aerosol Generator (SLAG). We tested a known sample (*E. coli* C41) as a way to test the effectiveness of both methods, because it is a good model organism, safe, and commonly used.



The goal of the project is to demonstrate a proof of concept and establish the best way to collect atmospheric samples.

QUESTIONS

- Is DNA detected in the filters?
- How much DNA is in the filters?
- How does the amount of collection time impact how much DNA is collected?
- Did we find *E. coli*?
- What the best procedure to avoid bacterial contamination?

METHODS

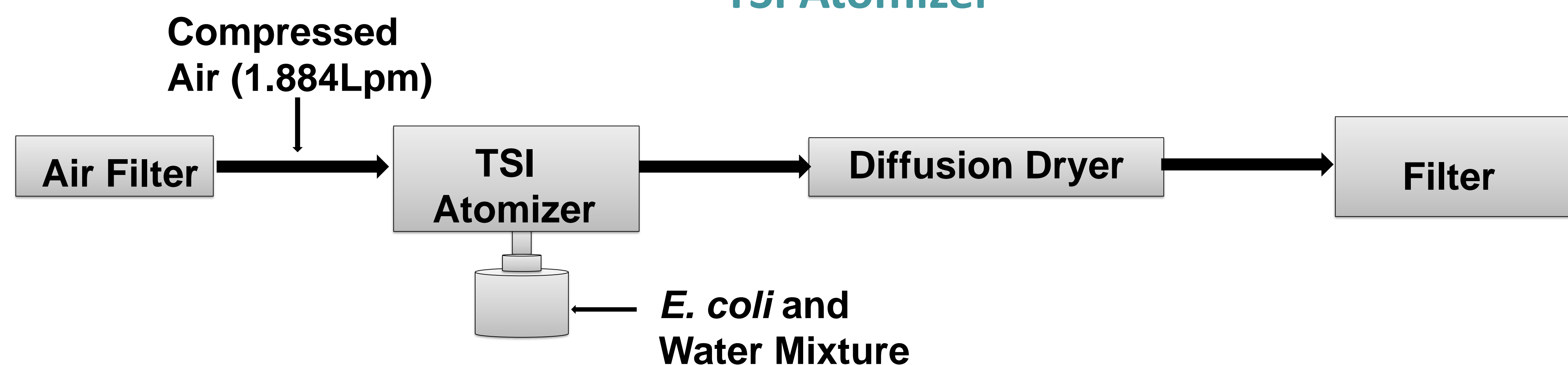
Atmospheric collections

- TSI Atomizer
- Sparging Liquid Aerosol Generator (SLAG)

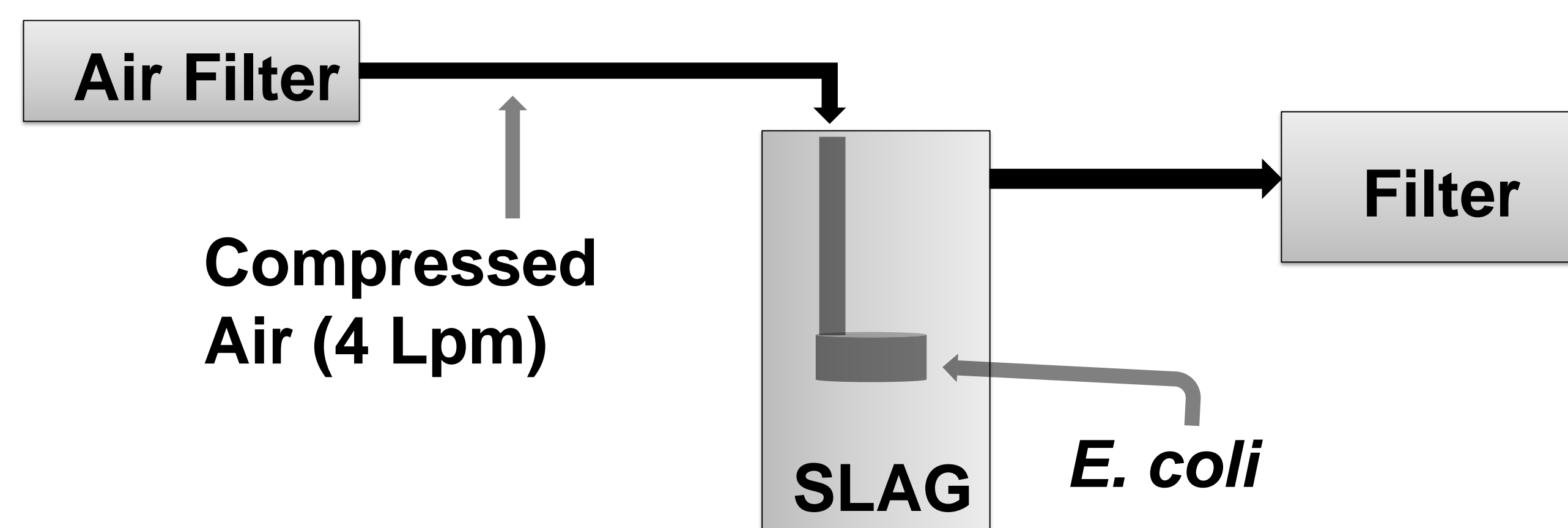
DNA identification

- Polymerase Chain Reaction (PCR)
- 16S rDNA (300-400bp)
- Dideoxy-sequencing
- Assembled forward and reverse sequences using the program Sequencher
- Compared resulting sequences with published sequences contained in National Center of Biotechnology Information (NCBI) using BLAST searches.

TSI Atomizer



SLAG



Sample	Time	DNA Concentration	Result	Identity (percentage)
TSI.Blank	Overnight	15.0 ng/μL	<i>Capriavidus sp.</i>	92%
TSI.A	Overnight	15.4 ng/μL	<i>Cupriavidus sp.</i>	95%
TSI.B	Weekend	48.8 ng/μL	<i>Propionivibrio limicola</i>	93%
SLAG.Blank	Hour	-0.3 ng/μL	No DNA	0%
SLAG.A	Hour	2.4 ng/μL	<i>Cupriavidus sp.</i>	97%
SLAG.B	Hour	3.1 ng/μL	<i>Zoogloeaceae bacterium</i>	91%

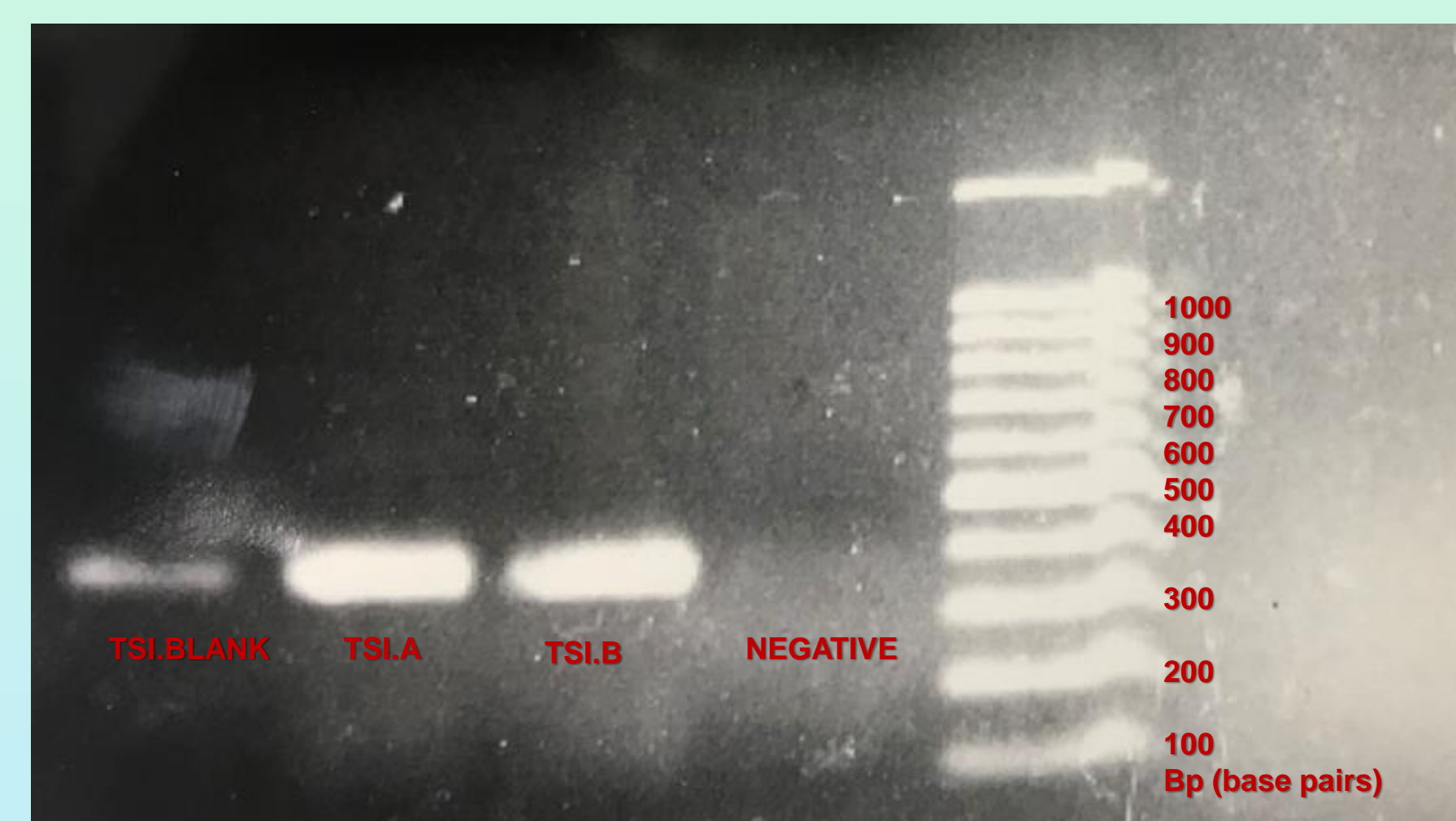


Figure 1. Results from one PCR amplification of TSI samples. This gel indicates that the TSI collecting device was contaminated. The ladder on the left is an indicator for amplicon size, indicating that the correct region is amplified.

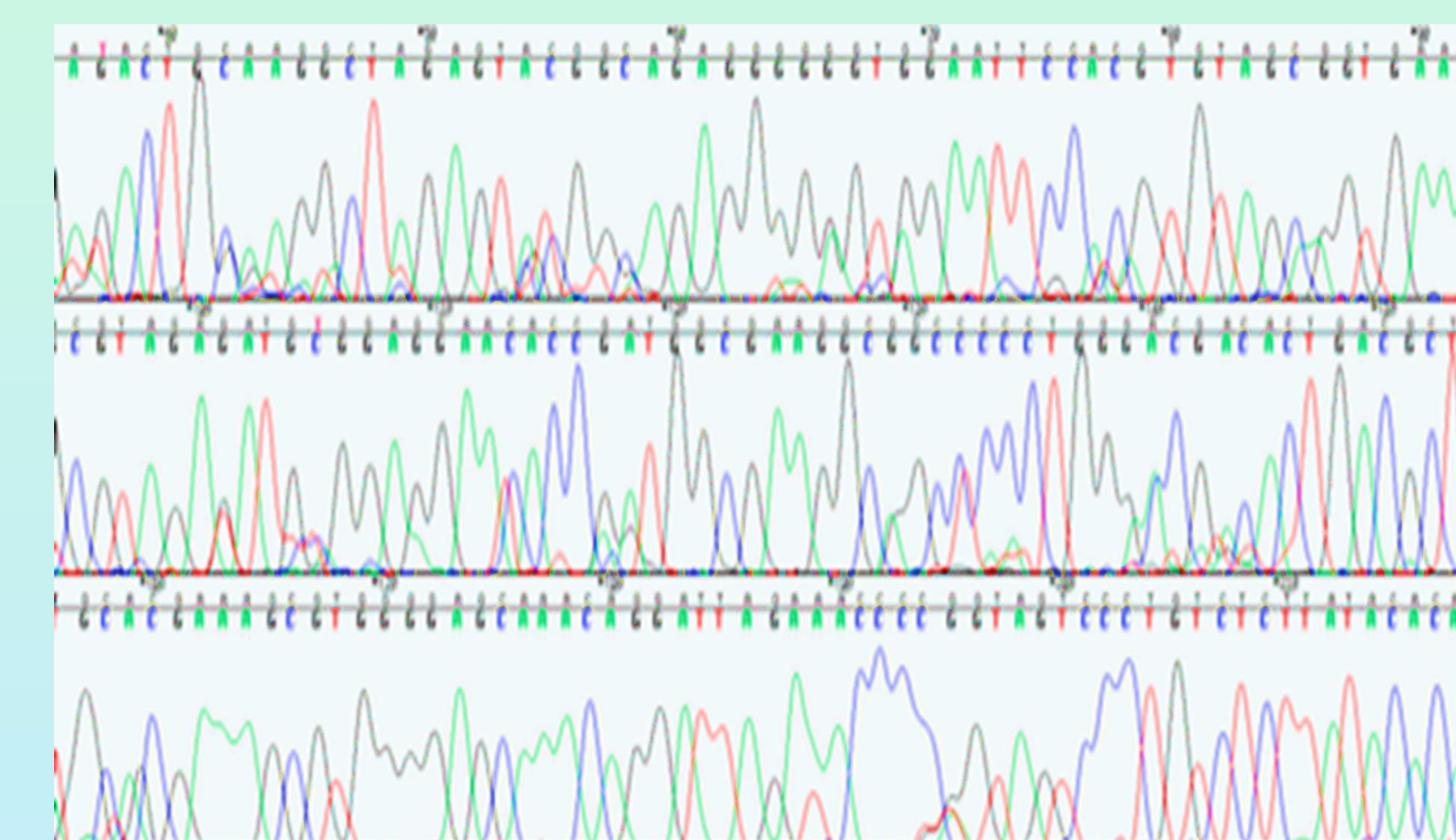


Figure 2. A representative DNA chromatogram that shows the DNA sequence for one bacterial fragment

CONCLUSIONS

- DNA was detected in the filters.
- Filters contain from -0.3ng/μL to 15ng/μL DNA.
- Longer collecting times result in higher concentrations of DNA.
- *E. coli* was not detected in samples. The collected bacteria appear to be contaminants from water.
- Bleaching and autoclaving equipment is effective for avoiding contamination.

FUTURE WORK

- Aerodynamic Particle Sizer (APS)
 - Measures the sizes of particles to verify the presence of bacteria
- Scanning Electron Microscope
 - Allows visualization of bacteria to see differences in TSI and SLAG collecting methods

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