

Identification of a Persistent Contaminant of an AKTA Protein Purification Instrument

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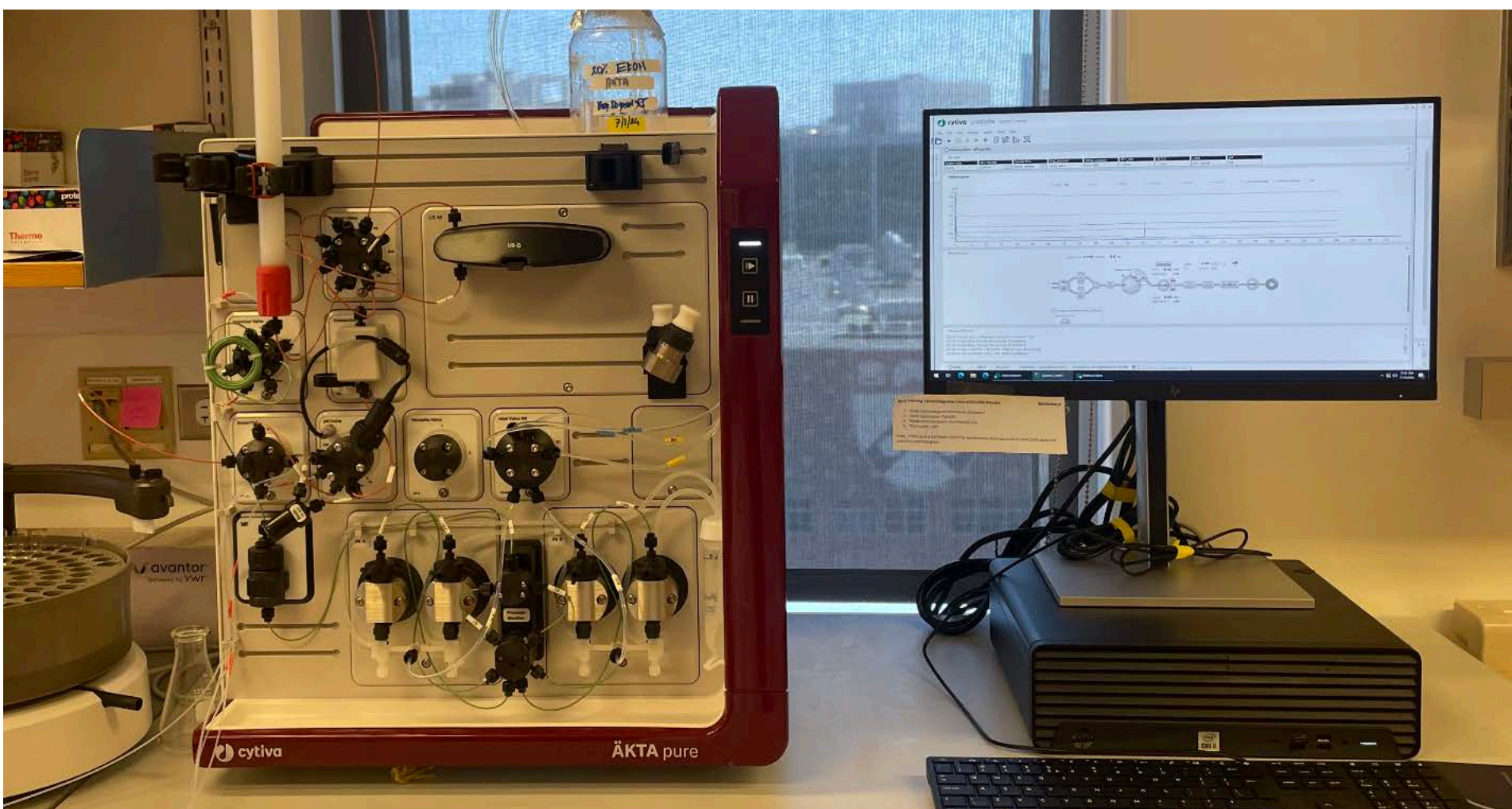
Objective

Contamination is common in healthcare and laboratory workspaces,, whether it be through patients, equipment, or machinery.

Our lab's AKTA Purifier is used to separate proteins. It was observed that there were bacteria contaminants within the instrument. Two bacteria were isolated and my project was to identify these bacteria.

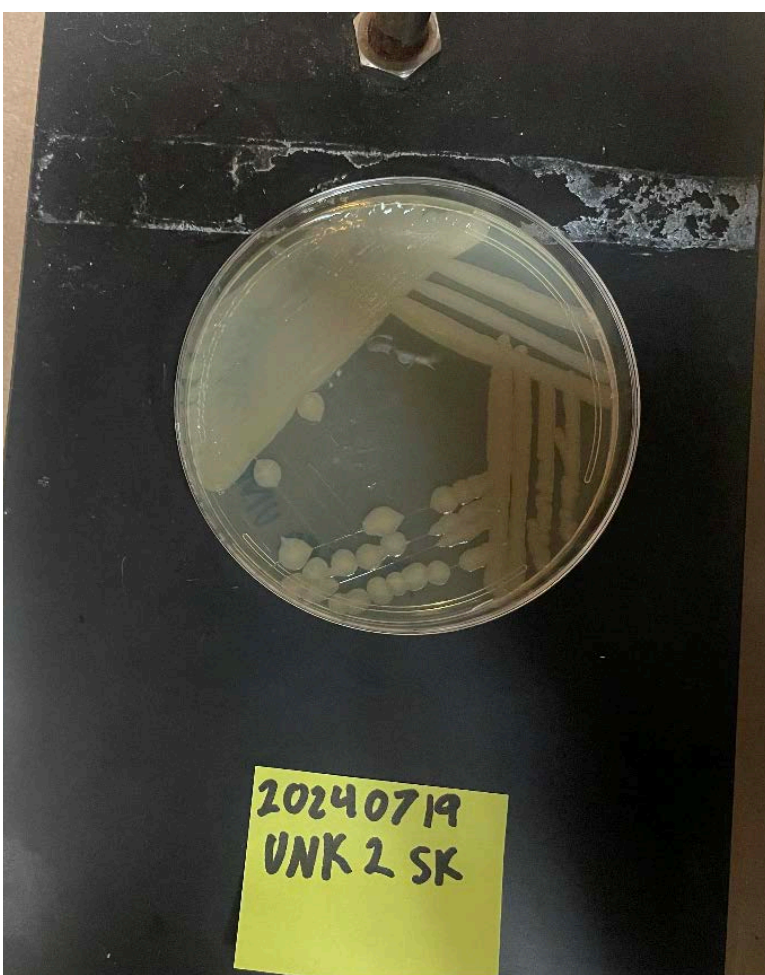
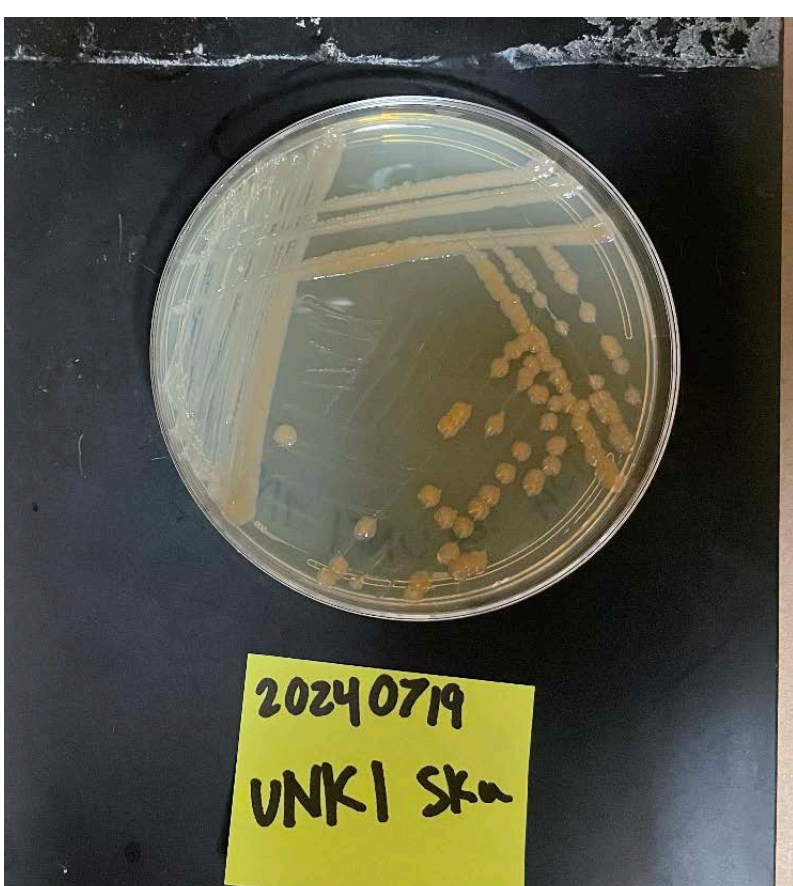
Methods

Source of Bacteria: AKTA Purifier



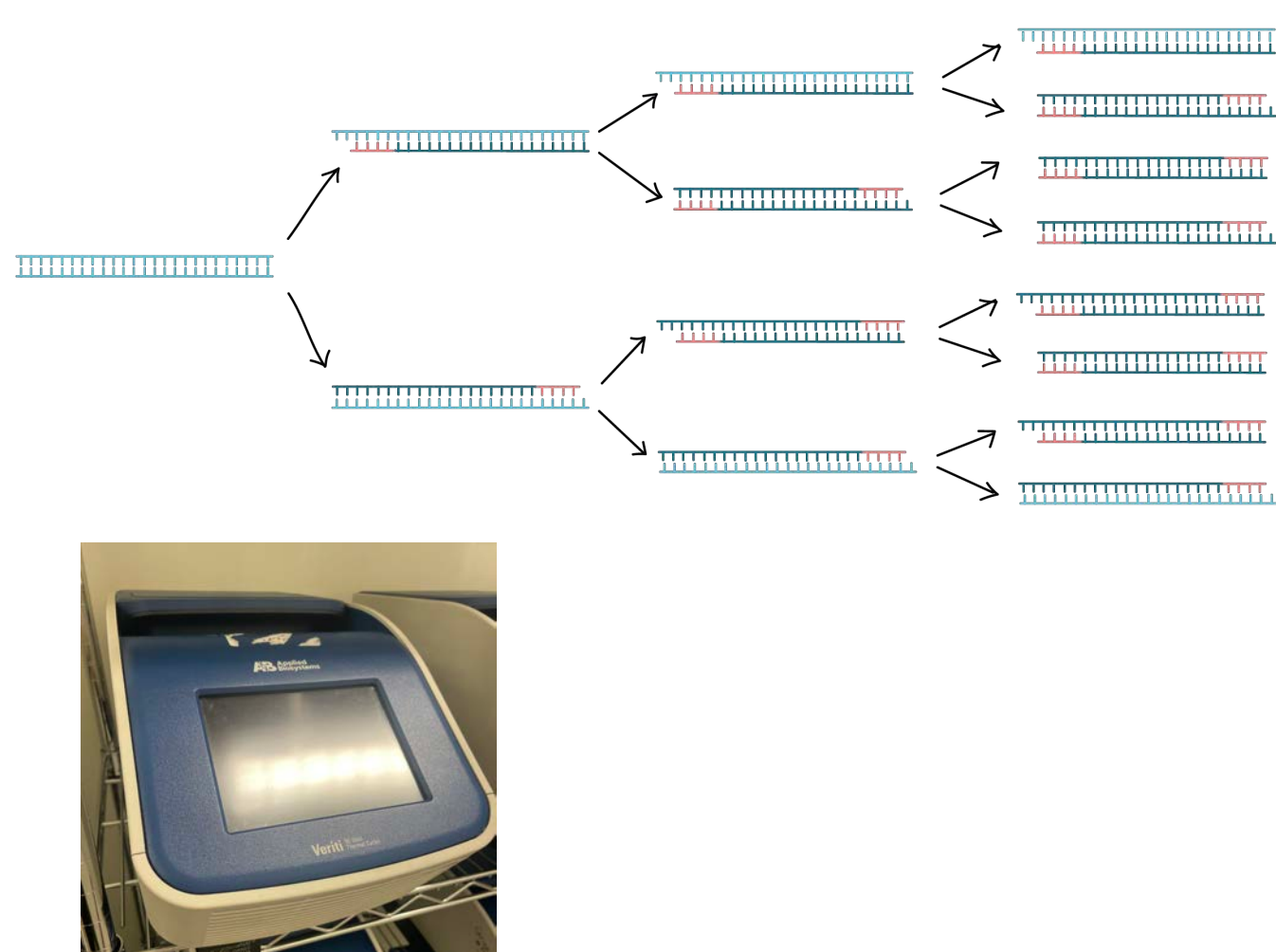
Streaking Plates for Isolation of Single Colonie

- o I streaked bacteria onto TSA plates, and placed them into a 37.0 ____ incubator.



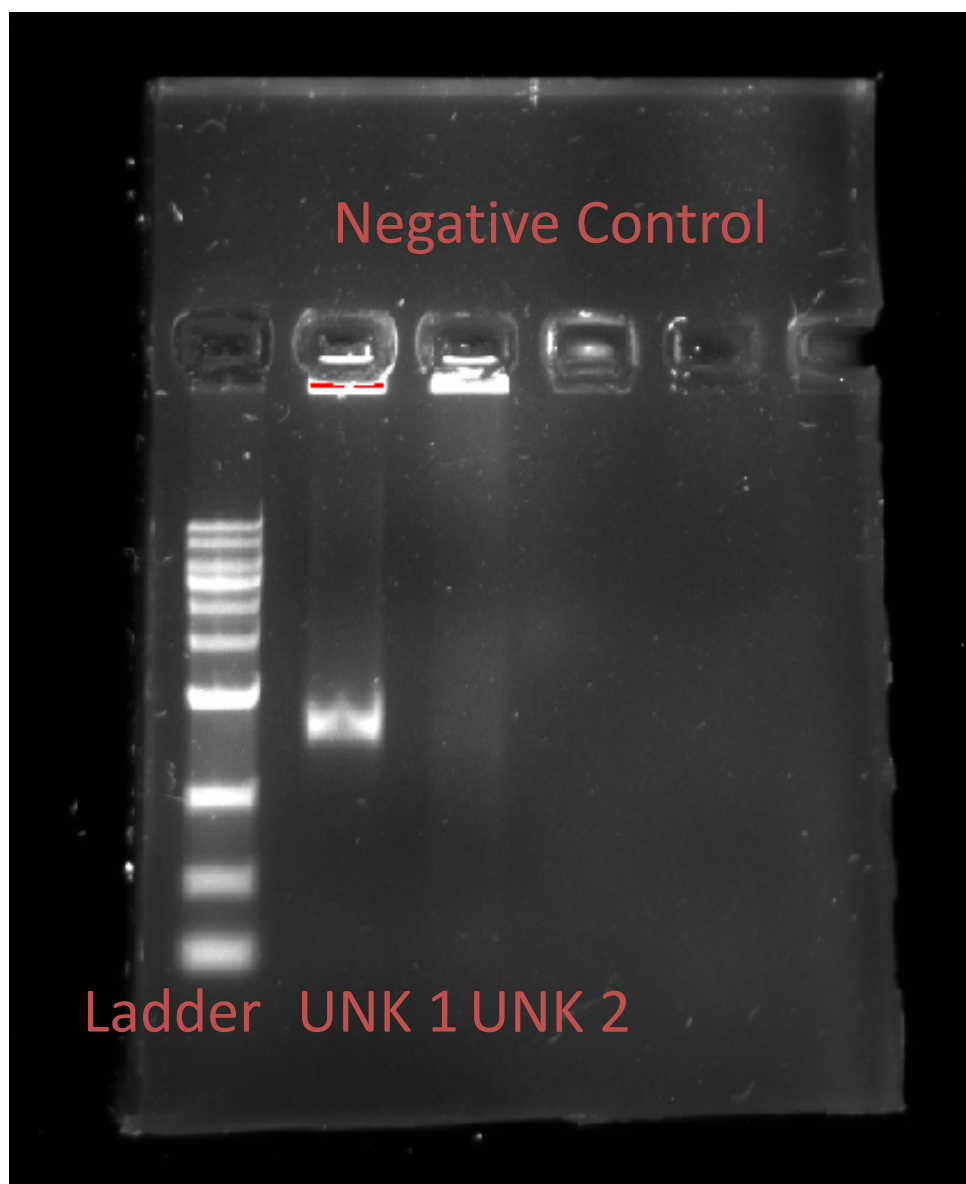
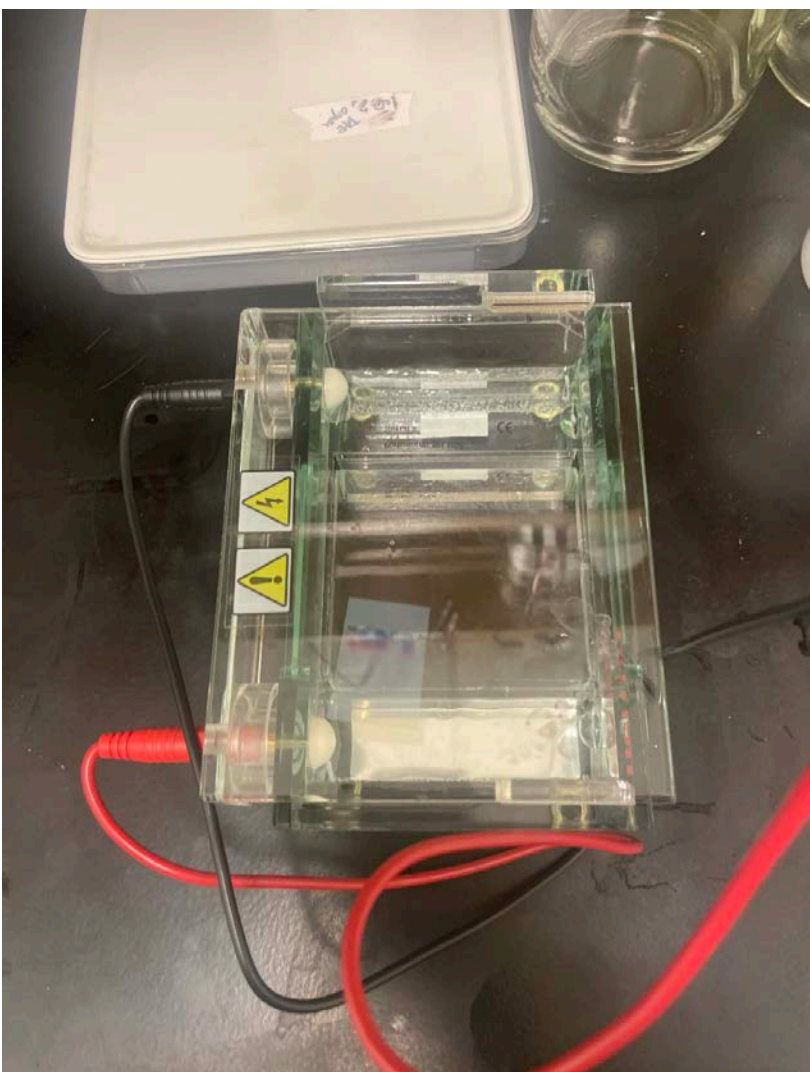
Colony PCR of 16s RNA Gene

- o Small piece of bacteria colony was placed in the mix in the PCR reaction mixture.
- o After mixing, it is put in a thermal cycler for 35 cycles.



Validating PCR Product with Agarose Gel

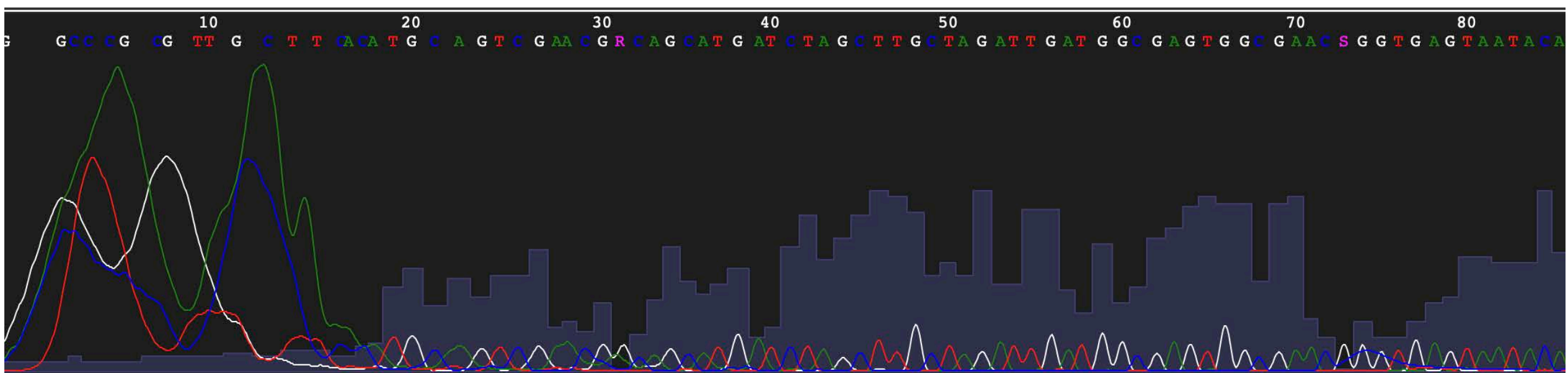
- o As confirmation for our PCR products for DNA Sequencing, we run a gel for UNK 1 and UNK 2 at 100 voltage.
- o I was able to obtain a PCR product from UNK 1 DNA, but not UNK 2.



DNA/Sanger Sequencing

- o The UNK 1 PCR product was sent for Sanger DNA Sequencing.
- o We place it into ApE editor to display the sequence.

Results



Blast

- o We used BLAST to search for similarity in base pairs of the PCR DNA against all bacterial sequences in the database
- o The sequence of UNK 1 was highly similar to *Ralstonia pickettii*.
- o The E-value for this result was 0.0, and the sequence was 99.4% identical.

Ralstonia pickettii strain_EEA-23.165_rpoN.html

Genomic RNA gene, partial sequence

Ralstonia pickettii

1757 1757 99% 0.0 99.38% 1444 MG822776.1

Sequence ID: MH151272.1 Length: 1441 Number of Matches: 1

View Match Previous Match

Range 1: 17 to 979

View Match Previous Match

Score	Expect	Identities	Gaps	Strand
1759 bits(952)	0.0	959/963(99%)	2/963(0%)	Plus/Plus
Query 15	CACATGCACTGGAACGACGATGATCTAGCTTCTAGATTGATGGCACTGGGCAACG	74		
Sbjct 17	CACATGCACTGGAACGACGATGATCTAGCTTCTAGATTGATGGCACTGGGCAACG	76		
Query 75	GTGAGTAACTACATCGAACCTGCTCTGATGGGGATACCTAGTCAAGATTAGCTAA	134		
Sbjct 77	GTGAGTAACTACATCGAACCTGCTCTGATGGGGATACCTAGTCAAGATTAGCTAA	136		
Query 135	TACCGCATACGACCTGAGGCTGAAGTGGGGGACCGCAAGGCTCATGCTATAGGAGCG	194		
Sbjct 137	TACCGCATACGACCTGAGGCTGAAGTGGGGGACCGCAAGGCTCATGCTATAGGAGCG	196		
Query 195	CCGATCTCTGATTAGCTAGTTGGTGAAGTAAAGGCTCACCAGGCGACATCAGTACCTG	254		
Sbjct 197	CCGATCTCTGATTAGCTAGTTGGTGAAGTAAAGGCTCACCAGGCGACATCAGTACCTG	256		
Query 255	GTCTGAGAGACGATCAGCCACACTGGGACTGAGACACGCGCCAGACTCTACGGAGGC	314		
Sbjct 257	GTCTGAGAGACGATCAGCCACACTGGGACTGAGACACGCGCCAGACTCTACGGAGGC	316		
Query 315	AGCACTGGGGAATTTTGACAAATGGGCAAGGCTGATCCAGCAATGCCGCTGTGTAA	374		
Sbjct 317	AGCACTGGGGAATTTTGACAAATGGGCAAGGCTGATCCAGCAATGCCGCTGTGTAA	376		
Query 375	GAAGGCTCTGGGTTTAAAGCACTTTTGTCCGGAAGAATGGCTCTGTATATACCTG	434		
Sbjct 377	GAAGGCTCTGGGTTTAAAGCACTTTTGTCCGGAAGAATGGCTCTGTATATACCTG	436		
Query 435	GGTCTGATATGCTATCTGGAACATAGCACTGGCTATCTATCTGCTAGCTGCTGCTA	494		

Conclusion

We identified the unknown bacteria as *Ralstonia pickettii*. *Ralstonia* is a common bacteria often found in hospital settings in sterile water, medications, blood culture bottles, and saline solutions. *Ralstonia pickettii* is also known to be soil-borne and it causes wilt disease to plants. *R. pickettii* is a very small bacteria that can pass through 0.2 mm membranes.

We concluded that in order to avoid future contamination, it is important that we filter our buffers through at 0.1 mm filter in future.

Acknowledgements

Throughout this internship, I have felt nothing but appreciation towards my team and colleagues. I would like to thank..

Dr. Grice for providing the materials to conduct this project.
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Lastly, for my colleagues and everyone else involved for making this experience memorable!