Development of quantitative PCR assays targeting varied-length sequences of the ND1 gene in human DNA

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Introduction

- Environmental DNA (eDNA) analysis is a rapidly growing research field with evolving applications. DNA is frequently used in criminal investigations. eDNA provides the potential for testing for targeted biological substances in bodies of water. This can aid in looking for missing persons, testing for biological substances, and other forensic applications. [1]
- Mitochondrial DNA has a higher abundance within a cell than nuclear DNA, and therefore often has a higher probability of being detected. [2]
- In turn, this study developed two assays targeting sequences of varying lengths from the ND1 coding region in the human mitochondrial genome based off of a previously published assay, with the purpose of testing how different DNA fragment lengths are distributed throughout the Chena River [3].

Research Objectives

- Develop quantitative PCR (qPCR) assays that target varying lengths of the human ND1 gene
- Test the ability of the developed assays to amplify target sequence
- Analyze extracted DNA from the three sampling sites to determine the ratio of longer:shorter human DNA relative to input sources

Methods

- Developed two assays using a previously published forward primer by designing reverse primers and probes
- eDNA sample collection (Figure 1)
  - Collected 3 field replicates and 1 field blank at each site using Smith-Root eDNA Citizen Scientist Sampler
  - Subsampled DNA filters
  - Performed DNA extractions on filtrates from Chena River

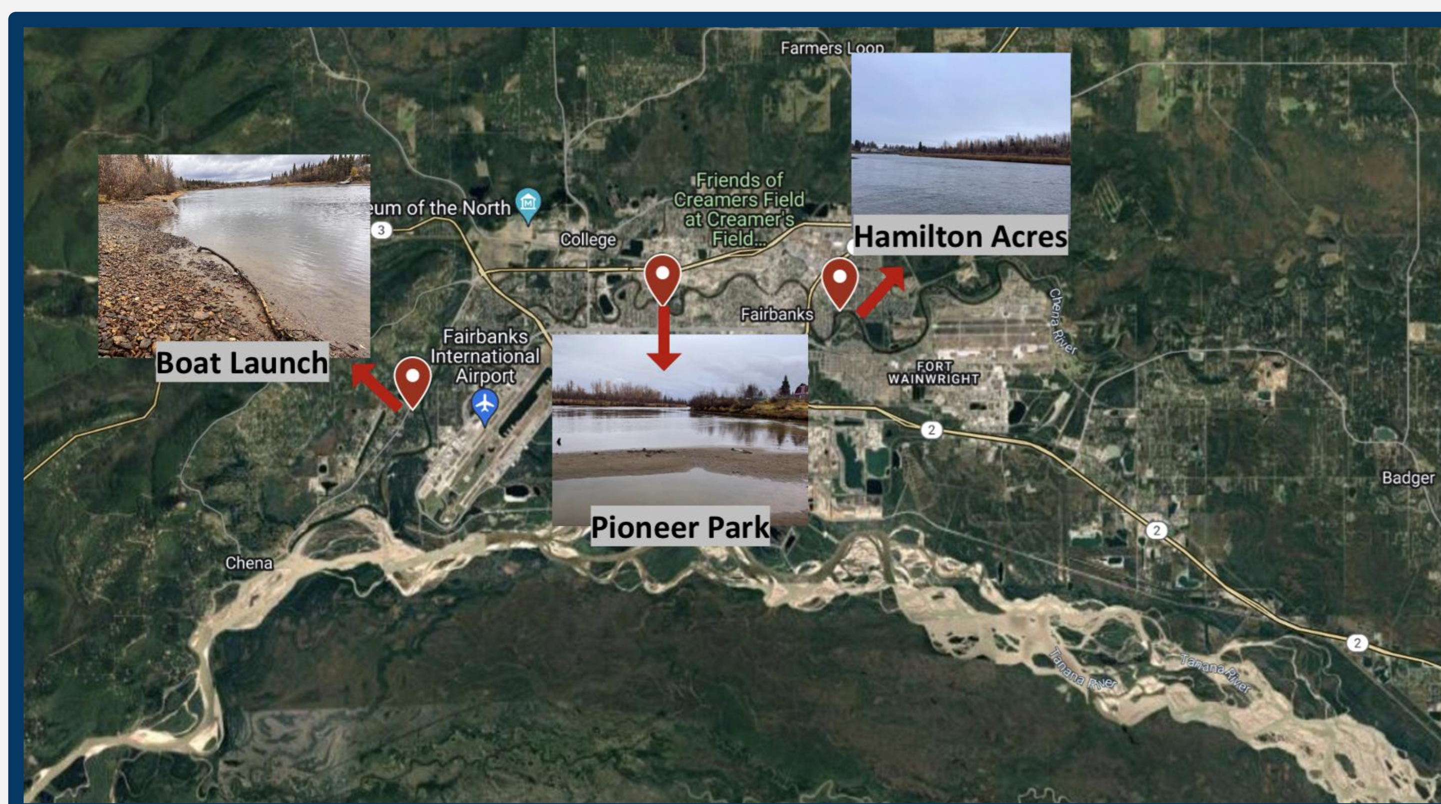

Figure 1. Upstream (Boat Launch), midstream (Pioneer Park), and downstream (Hamilton Acres) eDNA sampling sites on the Chena River. Red markers indicate sampling sites.

Assay Development

1. I isolated the desired ND1 human gene sequence from the GenBank database
2. In Primer3 I inputed the complete ND1 gene, desired forward primer sequence, and desired target sequence length
3. Primer3 generated options for reverse primers and probes
4. Of those generated by Primer3, I chose the best suited reverse primer and probe
5. I repeated steps 2-4 to produce assays that target longer sequence lengths

Preliminary Analysis

- Conducted gel electrophoresis to test PCR amplification of human DNA using mtND1_short assay
- Currently testing the sensitivity of the two developed assays using an ND1 oligonucleotide 10-fold dilution series
- DNA amplification of wells 1 and 4 with fragment lengths shorter than 100 base pairs suggests that the mtND1_short assay amplified the targeted DNA sequence (Figure 2)

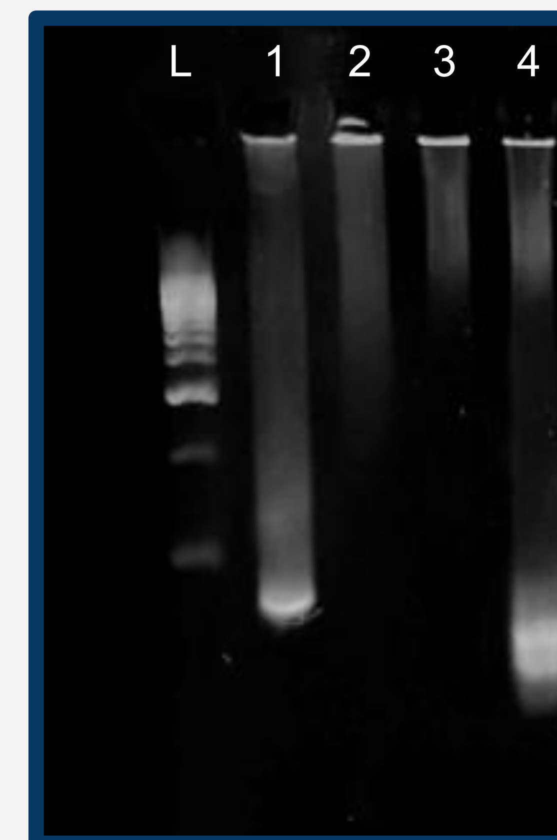

Figure 2. mtND1_short assay gel electrophoresis run on 2% agarose gel. L indicates well with 100 base pair ladder. Wells 1-4 were run with PCR products amplified using the mtND1_short assay with the following samples:

1. Positive control - ND1 fragment oligonucleotide
2. Negative control - H_2O
3. Coho salmon extracted DNA
4. Human spit

Table 1. Previously published assay and the designed probes and reverse primers. Blue tinted box indicate assay published by Timken et al. (2005). Orange tinted boxes indicate assays developed during this study.

Assay Name	Target gene	Forward Primer 5' to 3'	Reverse Primer 5' to 3'	Probe 5' to 3'	Target sequence length
mtND1_short	ND1	CCCTAAAACCC GCCACATCT	GAGCGATGGTG AGAGCTAAGGT	CCATCACCCTC TACATC	69
mtND1_med	ND1	CCCTAAAACCC GCCACATCT	TCGGTTGGTCTC TGCTAGTG	GTCAACCTCAA CCTAGGCCT	407
mtND1_long	ND1	CCCTAAAACCC GCCACATCT	TGAGTTCCTCGT AGCGGAAT	CACTAGCAGAG ACCAACCGA	678

Discussion

- Determining relationship between eDNA fragment lengths and qPCR amplification results could help determine where sources of the targeted DNA is originating
- Future directions
  - Test limit of detection of assays using synthetic ND1 oligonucleotide
  - Test assays for cross-amplification
  - Use assays for qPCR to quantify varying lengths of the human ND1 gene human DNA in the Chena River
- Hypotheses
  - Higher relative concentrations of DNA will be detected at the midstream and downstream sampling sites where there is more fresh human DNA input
  - The ratio of longer:shorter DNA fragments will be higher near sources of human DNA in the Chena River due to DNA transport characteristics in lotic systems

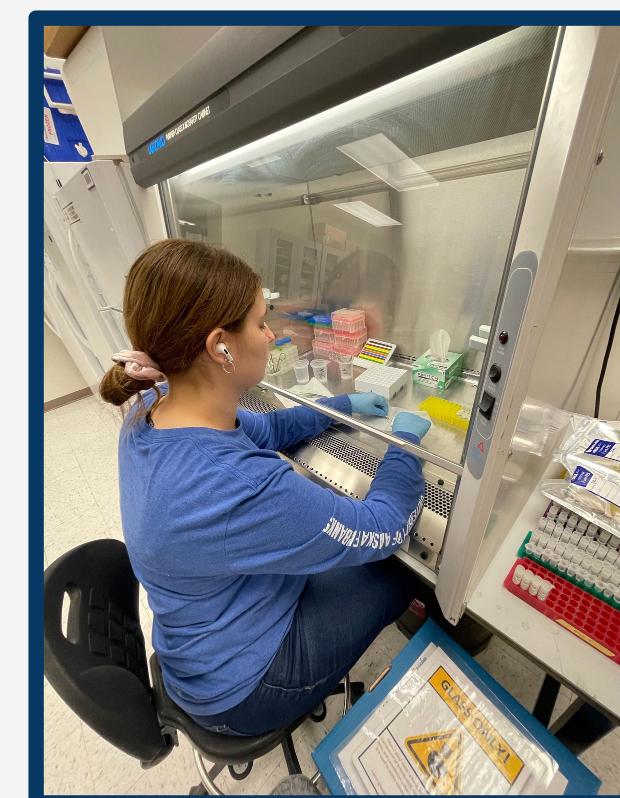

Figure 3. Katherine LeBlanc subsampling field samples.

Citations

[1] Antony Dass, M., Sherman, C. D., Nai, Y. H., Ellis, M. R., van Oorschot, R. A., & Durdle, A. (2022). Assessing the use of environmental DNA (eDNA) as a tool in the detection of human DNA in water. *Journal of Forensic Sciences*, *67*(6), 2299–2307. https://doi.org/10.1111/1556-4029.15124

[2] Mauvisseau, Q., Harper, L. R., Sander, M., Hanner, R. H., Kleyer, H., & Deiner, K. (2022). The multiple states of environmental DNA and what is known about their persistence in Aquatic Environments. *Environmental Science & Technology*, *56*(9), 5322–5333. https://doi.org/10.1021/acs.est.1c07638

[3] Timken, M. D., Swango, K. L., Orrego, C., & Buoncristiani, M. R. (2005). A duplex real-time QPCR assay for the quantification of human nuclear and mitochondrial DNA in forensic samples: Implications for quantifying DNA in degraded samples. *Journal of Forensic Sciences*, *50*(5), 1–17. https://doi.org/10.1520/jfs2004423

Acknowledgements

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