2020

The Impact of Submersion on the Quantification of Host and Bacterial DNA

Grace Sprouse

VIRGINIA COMMONWEALTH UNIVERSITY

The Impact of Submersion on the Quantification of Host and Bacterial DNA

Grace Sprouse, Baneshwar Singh
Department of Forensic Science and Virginia Commonwealth University

Abstract

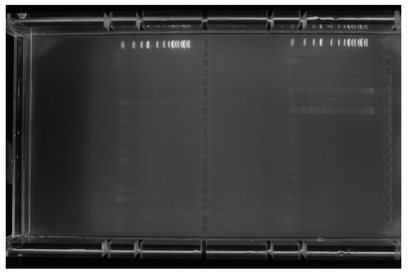
When remains are recovered from aquatic environments, as a result of water related accidents, a reliable method for identifying individuals and generating investigative leads includes, DNA analysis. Depending on the duration of submersion and stage of decomposition, tissue samples may not be intact enough to run DNA analysis. Given that bones have a specific physical and chemical make-up that provides protection from deterioration for longer periods of time, identifying individuals may rely on DNA obtained from skeletal remains. Microorganisms, which contribute to carrion decomposition, can be used be used as a clock to estimate postmortem interval (PMI) or minimum postmortem submersion interval ($PMSI_{min}$). While several studies (Crainic et al.[1], Mundorff et al.[2], and Finley et al.[3]) have focused on the changes in microbial communities for PMI model development, studies on changes in the quality and quantity of bacterial DNA over time, especially in aquatic environments, is not available in the literature. Because microorganisms contribute to the decomposition process, comparing bacterial DNA (16S rDNA) and nDNA may be able to aid in determining how long the remains have been submerged based on a bacteria:host DNA ratio, and informing researchers on the feasibility of using quantity of bacteria on remains over time for estimating the PMI or $PMSI_{min}$. The samples used in this study were obtained from long-term submerged skeletal elements (e.g., rib and scapula) using two different extraction methods (e.g., organic-phenol chloroform and solid-phase). Using 10” x 10” cages, each containing five ribs and scapulae, samples were submerged in a freshwater lake (e.g., Henley Lake) and river (e.g., James River). Approximately ca. 250 accumulated degree days (ADD), one cage was collected, totaling nineteen lake and twenty-four river collections.

Methodology

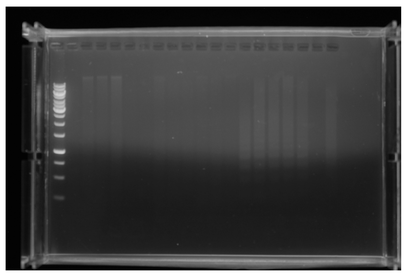
Three hundred and fifty-two DNA extracts were run on 1.0% agarose gel with purple 6X gel loading dye, and ethidium bromide to visualize the DNA. The bands that were visualized on the gel will be assessed using a 5-point scale to assess the quality of the DNA extracted from two different extraction methods. All samples will also be quantified using qPCR approach for both quantity and quality determination.

Results/Discussion

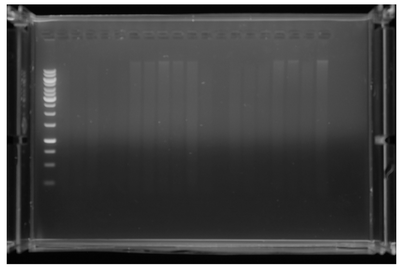
The gels pictured below are one of multiple 1% agarose gel completed in each run. These gel images show the smearing of the DNA extracts. A qPCR standard curve was developed but because of Covid-19 closing DNA quantification was not completed. The R^2 value of the practice standard curve was 0.998850.



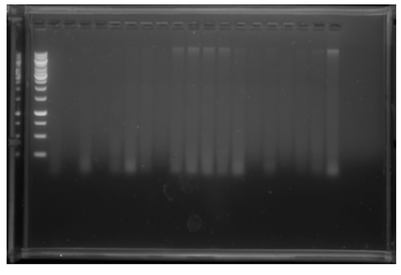
Run 1



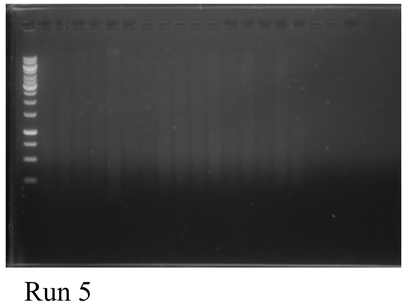
Run 2



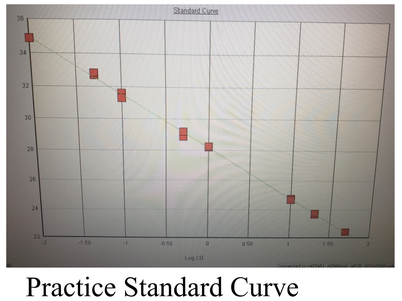
Run 3



Run 4



Run 5



Practice Standard Curve

Objectives

1. To determine extraction method (Organic or ChargeSwitch) that performs the best in recovery of nuclear DNA and bacterial DNA from waterlogged bones.

2. To determine extraction method (Organic or ChargeSwitch) that performs the best in recovery of good quality nuclear DNA from waterlogged bones.

Future Directions

The second half of the project will be completed when we return to campus after quarantine. The first task is to assess the bands for each extraction. A Chi-squared test will be preformed to compare the quality scores between the bone type, water location, and the extraction method. After statistical analysis of extraction quality, the quantity of each type of DNA will be assessed. Bacterial DNA will be quantified using primers for variable region v4 of 16S rDNA on a qPCR machine as described in Seashols-Williams et al.[4]. Nuclear qPCR will be performed using FH1733 porcine primer[4]. Both qPCR reactions will run on an ABI 7500 and will be analyzed by Chi Square test.

Works Cited

•1. Crainic, K., Paraire, F., Leterreux, M., Durigon, M. and de Mazancourt, P. Skeletal remains presumed submerged in water for three years identified using PCR-STR analysis. *Journal of Forensic Sciences*, **47(5),** 1025–1027. (2002).

•2. Mundorff, A.Z, Bartelink, E.J., and Mar-Cash, E. DNA Preservation in Skeletal Elements from the World Trade Center Disaster: Recommendations for Mass Fatality Management. *Journal of Forensic Science* (2009).

•3. Finley S.J., Benbow M.E., Javan G.T. Microbial communities associated with human decomposition and their potential use as postmortem clocks. *International Journal of Legal Medicine.* **129** (2015).

•4. Seashols-Williams S., Green R., Wohlfahrt D., Brand A., Limjuco T.T.A., Nogales F., Brooks J.P., Singh B. An accurate bacterial DNA quantification assay for HTS library preparation of human biological samples. *Electrophoresis,* 2824-2825 (2018).