

## B132 Massively Parallel Sequencing (MPS) of Diatom and Bacterial DNA: A New Approach for Freshwater Trace Evidence Analysis

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the value of bacterial and diatom DNA metabarcoding and MPS for the comparison and exclusion of freshwater trace evidence with questioned environments

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the rapid and discriminatory potential of molecular approaches for crime reconstruction in freshwater environments. This presentation will outline a novel MPS protocol, targeting *rbcL* and 16S rRNA markers, applied to bulk environmental and trace forensic samples to indicate the site of initial transfer.

Freshwater environments are frequently encountered in forensic cases as the location of death or an evidence deposition site. Although they are diverse and widespread, few forensic techniques are readily available to link persons, items, and scenes of forensic interest. Environmental bacterial and diatom communities transferred to a questioned sample may yield a powerful discriminatory tool.<sup>1</sup> Although MPS of bacterial communities has shown great promise in time of death estimations and in soil trace evidence comparisons, the potential for bacterial markers to infer a site of freshwater evidence transfer has not yet been explored.<sup>2,3</sup> Diatoms, an abundant and species-rich group of microscopic algae, are more frequently studied as a form of aquatic trace evidence and are useful indicators of drowning in forensic pathology.<sup>4</sup> While analysis typically uses microscopy to assess diatom morphology and surface structures, this approach is time consuming with few forensic experts available to support accurate species identification. To overcome these limitations, and to increase the potential for more diverse forensic applications, it is imperative to test and develop innovative technologies such as MPS for the analysis of freshwater evidence.

This research represents the first characterization of diatom DNA in forensic samples and investigates the reliability of diatom and bacterial sequencing to discriminate between freshwater environments and indicate the transfer site of immersed clothing items. An MPS protocol was developed for application to bulk environmental and trace forensic samples. Eighteen freshwater sites in and around Adelaide (South, Australia) were sampled in June 2019 and a 5L disturbed water sample collected from each. Four 100% cotton t-shirts were immersed in four of these environments and retained for analysis. DNA was extracted from a concentrated water sample and two different treatment methods were trialed to recover DNA from each clothing item. 16S rRNA (bacteria) and *rbcL* (diatom) gene regions were amplified and sequenced using Illumina® MiSeq®. Species were identified using the available reference databases and the relative abundance of each determined. To support reliable interpretations, statistical approaches including source-tracking, and likelihood ratio calculations were incorporated.

The results demonstrate the successful recovery and sequencing of both diatom and bacterial DNA from all environmental and forensic samples. The 18 freshwater sites were discriminated based on the overall species assemblage, and each clothing sample was successfully predicted to the correct control site regardless of the DNA recovery method used. Diatom DNA proved a more stable molecular marker on the questioned samples compared to bacterial DNA, although the inclusion of a second taxonomic marker offered an independent tool to enhance sample comparisons and exclusions. This presentation represents the first application of MPS and the first multi-organismal study of freshwater trace evidence samples for crime reconstructions. The technique offers a rapid and less subjective tool for forensic analyses of environmental markers, with future avenues for research highlighted.

### Reference(s):

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3. Young, J.M., Weyrich, L.S., and Cooper, A. (2014). Forensic soil DNA analysis using high-throughput sequencing: A comparison of four molecular markers. *Forensic Science International: Genetics*, 13, 176-184.
4. Scott, K.R., Morgan, R.M., Cameron, N.G., and Jones, V.J. (2019). Freshwater diatom transfer to clothing: Spatial and temporal influences on trace evidence in forensic reconstructions. *Science & Justice*, 59(3), 292-305.

### Massively Parallel Sequencing, Forensic Ecology, Trace Evidence