DETECTING ANCIENT DNA WITHIN BURIED ATACAMA SOILS. R. V. Nichols[1], P. H. Hoffman[1], M. B. Wilhelm[2], A. F. Davila[2], L. L. Jahnke[2], B. Shapiro[1], [1]Paleogenomics Lab, University of California Santa Cruz, Santa Cruz CA 95060 (ruthvnichols@gmail.com), [2]NASA Ames Research Center, MS 245-3, Moffett Field CA 94035.

Introduction: Understanding how biomolecules, such as DNA, can be preserved over geologic time is crucial for reconstructing the evolution of life and searching for signs of life on other planets. Well preserved functionalized lipid biomarkers have been isolated from buried soil horizons in the hyperarid core of the Atacama Desert that are between 40,000 and 2 million years old, and have been protected from water percolation by an overlaying halite unit that has acted as an aquiclude [1]. In addition to the extreme dryness, these soils have high amounts of clay and salts, and this combination is generally thought to preserve and protect DNA from degradation [2], making it is possible that DNA has also been preserved alongside the lipids. These conditions make it challenging to successfully extract DNA due to the adsorption of DNA to the clay surfaces. Here we present an optimized DNA extraction protocol for the Atacama soils and provide important lessons about the types of difficulties that may be encountered in searching for DNA in hyperarid terrestrial or martian soils of a similar age. This study also informs our understanding of the stability of DNA in relation to other biomolecules in hyperarid settings over these timescales.

Study Site: The hyperarid core of the Atacama Desert in northern Chile (Yungay region) has <2 mm of precipitation annually [3] and is one of the driest places on Earth. The extreme temperatures, aridity over decadal timescales, rare salts, low microbial and organic carbon content and UV radiation make it an excellent Mars soil analog and thus an ideal site to study biomolecule preservation.

Methods: Soil samples were collected at different depths with researchers wearing cleanroom suits, facemasks and gloves. All collection tubes were certified DNA and RNA free and all tools were autoclaved and sterilized prior to sampling.

To optimize the DNA extraction protocol we spiked into the soil known DNA, extracted it under multiple protocols and used qPCR to quantify and compare DNA yield. The base extraction protocol we used was the PowerLyzer kit from MoBio (Carlsbad, CA); it is one of the most widely used for soil DNA extractions and it is relatively robust for multiple soil types. Briefly, the kit uses glass bead beating to lyse cells, followed by inhibitor removal steps and the use of a spin filter to concentrate and elute the DNA.

After determining the best protocol, we extracted the DNA in a dedicated ancient DNA (aDNA) clean room where extreme care is taken to not introduce exogenous DNA into the samples. After extraction, the DNA was prepared into Illumina shotgun sequencing libraries [4] and sequenced on a MiSeq sequencing platform.

Results: Adding phosphate buffer and polyadenylic acid into the intitial lysis buffers of the PowerMax and PowerLyzer extraction kits greatly increased the yield of DNA compared to other protocols.

To find aDNA we processed nearly 500 g of soil across almost 150 different extractions. We found sequences for different microbes at extremely low numbers of reads (fewer than 10 per microbe). Most of the taxa identified in the soils are shared with those found in the extraction controls, indicating contamination during the library preparation process. Using the final extraction protocol we did find sequences (~10,000 reads) that have the potential to be aDNA because they were not found within the extraction control. However, they match no known reference sequences which makes it challenging to verify if the DNA is indeed ancient. It is possible that these sequences belong to something that has thus far never been sequenced. More work needs to be done to examine these samples and their sequences.

Discussion: Our results show that extracting DNA from soils high in clay and salt can be challenging and that an optimization step is critical before endogenous DNA can be successfully recovered. Our optimized protocol may be useful for informing DNA recovery from similar soils, such as those on Mars.

More work needs to be done to confirm aDNA within ancient Atacama soils. It may be that the unknown sequences will one day be matched to known reference sequences. Until then, we will continue to process more soil and attempt to match the sequences to known DNA databases. A major challenge in DNA identification in natural samples is discriminating between true positives (i.e. endogenous signals) and false positives (i.e. contaminants). This challenge is particularly relevant to the search for evidence of life beyond Earth.

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