**NANOPORE SEQUENCING IN THE ANTARCTIC DRY VALLEYS.** A. Bai[1], E. Zaikova[1], S. W. Tighe[2], D. S. Goerlitz[3], and S. S. Johnson[1]. [1]Department of Biology, Georgetown University; [2]University of Vermont; [3]Georgetown University Medical Center

**Introduction:** Nanopore sequencing is a method for sequencing DNA by measuring electrical current changes as nucleotides pass through small holes in a membrane across which a voltage has been applied [1]. The MinION, a handheld instrument developed by Oxford Nanopore Technologies, utilizes polymer membranes with protein nanopores for single-molecule, real-time, massively parallel DNA sequencing [2]. This approach allows direct sequencing of native DNA, thereby avoiding PCR bias and generating extremely long reads (>10kb) [3].

The MinION's small size and low power requirements make it a promising instrument for *in situ* life detection. Its capabilities have been demonstrated successfully aboard the International Space Station as mission scientists used the MinION to interrogate known sequences from *Mus musculus*, *Escherichia coli*, and lambda bacteriophage [4]. Our results demonstrate that the MinION can be used for *in situ* analyses of challenging environmental samples from remote and physically extreme planetary analog environments. The McMurdo Dry Valleys, located on the coast west of the McMurdo Sound in Victorialand, Antarctica, are characterized by extreme cold, aridity, and salinity, all of which present unique challenges to life. This hyper-arid polar desert environment results in a hydrologic cycle that mirrors aspects of Mars [5], and is of great astrobiological interest. The Dry Valleys and their paleolakes are dominated by microbial communities capable of surviving in extreme environments and contain extremely low biomass, presenting challenges to macromolecular analyses. In this study, we apply third generation sequencing technologies to metagenomic analyses of modern and paleomicrobial communities in the Dry Valleys.

**Methods:** The fragmented and fragile nature of ancient biomolecules can decrease the quality of sequencing data obtained from samples undergoing the standard preparation pipeline. We extracted genomic DNA from soil, modern and ancient microbial mats using a custom protocol developed in concert with Omega Bio-Tek to limit DNA fragmentation in the extraction process. Prior to extraction, all samples were incubated at 35°C for a minimum of 2 hours with a six-enzyme cocktail designed to achieve optimum cell lysis. Sequencing libraries were prepared in the field using the Oxford 1D library protocol. Custom software for offline use, was utilized to generate 1D reads in the field.

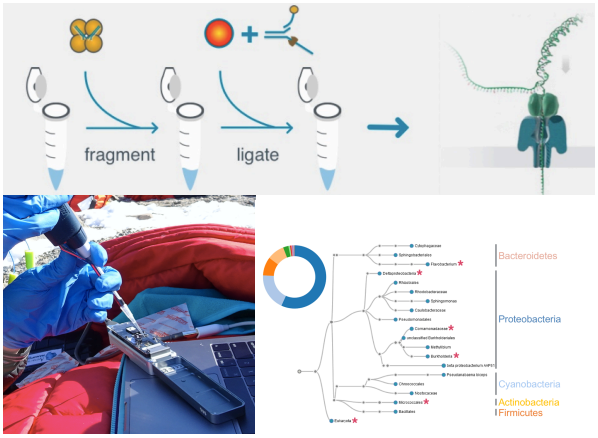


Figure 1: (A) Schematic of the Oxford 1D library preparation protocol for samples to be sequenced on MinION (B) *In situ* sequencing of paleomat samples demonstrating instrument capabilities. (C) One Codex-generated phylogeny of bacterial species detected in modern mat sample, with species detected in paleomat sample marked with red stars.

**Results:** The phylogenetic results from an ancient microbial mat sample with an adjusted radiocarbon age of 6kyr [6], analyzed with One Codex [7], are shown in Figure 1A. The longest ancient DNA fragment ever recovered from dead cells is two orders of magnitude shorter than our longest reads, suggesting the presence of intact cells in our samples [8, 9].

**Implications:** Sequencing tools have advanced rapidly in recent years. Our results help validate the field use of third generation sequencing technologies on microbial communities in a Mars analog environment. While further hardware development is still required, the demonstrated success of this novel technology helps to enable the possibility of returning remotely telemetered genomic data from space.

**References:** [1] Kasianowicz J. C. et al. (1996) *Proc Natl Acad Sci USA,* **93** (24): 13770–3. [2] Bayley H. (2006) *Current Opinion in Chemical Biology,* **10** (6): 628–637. [3] Laver T. et al. (2015) *Biomol Detection and Quantification,* **8** 1-8. [4] Castro-Wallace S. L. (2016) *bioRxiv* 077651. [5] Heldman J. L. (2013) *Planet Space Sci,* **85** (1): 51-58. [6] Hall B. L. and Henderson G. M. (2001) *Earth Planet Sci Lett,* **193** (3-4): 565-577. [7] Minot S, et al. (2015) *bioRxiv* 027607; [8] Johnson S. S. et al. (2007) *Proc Natl Acad Sci USA,* **104** (36): 14401-5. [9] Willerslev E. et al. (2003) *Science*, **300** (5620): 791-5.