

IN SITU LIFE AND BIOSIGNATURE DETECTION AT MARS ANALOG SITES USING THE OXFORD NANOPORE MINION SEQUENCER. K. F. Bywaters^{*1}, C. P. McKay¹, A. F. Davila² and R. C. Quinn³, ¹NASA Ames Research Center, Moffett Field, Mountain View, CA 94035 (*correspondence: kathryn.f.bywaters@nasa.gov, chris.mckay@nasa.gov), ²Carl Sagan Center, SETI Institute, Mountain View, CA 94043 (adavila@seti.org), ³Carl Sagan Center, SETI Institute/NASA Ames Research Center, Moffett Field, Mountain View, CA 94035 (richard.c.quinn@nasa.gov).

Introduction: Biomolecules are the most unambiguous and information-rich of all known biosignatures. The identification of biopolymers akin to DNA, RNA or proteins, would be difficult to refute as a successful life detection experiment. In the case of Mars, the search for biomolecules is inescapable, but few technological solutions exist for *in situ* identification.

The Oxford Nanopore MinION is a novel, miniature, off-the-shelf instrument capable of detecting biological polymers (DNA, RNA and proteins) without the need for amplification (i.e. the use of primers) and/or bulky sequencing equipment.

The MinION determines the structure of a biopolymer (such as the A, T, G, C sequence of DNA) by measuring changes in current across a membrane as the biopolymer passes through a protein nanopore. In contrast to traditional sequencing methods, prior knowledge of molecular structure or composition is not required.

Due to the versatility and portability of the MinION it has the potential to be used to conduct *in situ* field analysis of samples. These attributes make the MinION a prime candidate as an astrobiological life or biosignature detection instrument for planetary missions, such as Mars landers and icy world flybys.

The MinION Experiment: A proof-of-concept study is being performed to conduct *in situ* field analysis and to determine the detection limit of biopolymers, including DNA and RNA, contained in samples from the hyperarid Atacama Desert, the Mojave Desert and the Dry Valleys of Antarctica. These samples contain one of the lowest biomass levels on Earth and represent excellent Mars analogs for ground truthing instrument performance.

The results of this study will be used to establish measurement and instrument requirements needed for implementation of MinION technology on future NASA missions. By identifying the structure and possible survival strategies of the microbial communities in these extreme ecosystems, which may provide an analog of conditions on other worlds, a better understanding will be obtained about the conceivable adaptations and evolution of life beyond Earth. By testing of the MinION in analog settings this work will also assist in the development of tools for monitoring the adaptation of organisms in other planetary or space environments.

The MinION has been tested in the hyperarid core of the Atacama Desert at a field site located in the Yungay region. Preliminary results show that the MinION can detect DNA in halite samples from the hyper arid Atacama Desert. Endolithic communities containing cyanobacteria (*Halothece*) and associated heterotrophic bacteria in the halite deposits have already been characterized [1, 2]. The results obtained by processing the Atacama halite deposit will be discussed in the context of comparison to the previously described results obtained by traditional methods (PCR amplification of 16S rRNA genes) [2].



Figure 1. MinION in a halite field at the Yungay site in the Atacama Desert, Chile.

References: [1] Wierzbos J. et al. (2006) *Astrobiology*, 6, 415-422. [2] de los Rios et al. (2010) *Int. Microbiol.*, 12, 79-89.

Acknowledgements: Development for future NASA missions will be carried out in collaboration with David Deamer (an inventor on patents: US5795782A1, US6015714A1, and US7189503B2 used in MinION technologies) and his staff at UC Santa Cruz. K.B. acknowledges support from the NASA Postdoctoral Program. R.Q. and A.D. acknowledge support from the NASA Astrobiology Institute. The authors would like to thank Oxford Nanopore for granting access to the MinION Access Programme (MAP). K.B. thanks Brain Glass, PI of the NASA PSTAR funded Atacama Rover Astrobiology Drilling Studies (ARADS) project, and the ARADS team, for providing scientific, technical, and logistical support during the MinION field testing.