NUCLEIC ACID EXTRACTION AND SEQUENCING FROM LOW-BIOMASS SYNTHETIC MARS ANALOG SOILS

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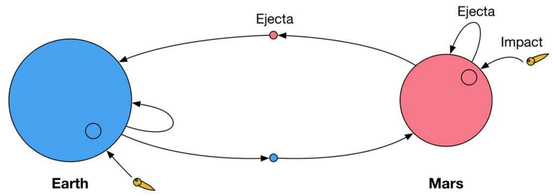
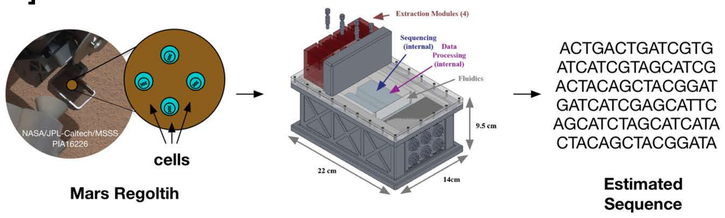
INTRODUCTION: Life on Mars, if it exists, may share a common ancestry with life on Earth due to the estimated billion tons of rock transferred between Mars and the Earth during the late heavy bombardment period [1]. Therefore, biological informational polymers (e.g., deoxyribonucleic acid - DNA, and ribonucleic acid - RNA) have the potential to provide unambiguous evidence of any ancestrally-related life on Mars and discern any forward contamination [2].



Lithological Exchange During the Late Heavy Bombardment (4.1 - 3.8 billion years ago)

We are developing an integrated nucleic acid extraction and sequencing instrument, the Search for Extra-Terrestrial Genomes (SETG), for life detection on Mars and Ocean Worlds [3-7].



Our goal for technology readiness level (TRL) 6 is to achieve a sensitivity of one part per billion: 10^6 bases detection from 10^4 *Bacillus subtilis* spores in 50 mg soil (10^6 spores/g). Given the current state-of-the-art in single molecule sequencing, including post-extraction losses, this goal requires at least a 5% extraction yield (~2 pg DNA) [3].

•Here we present DNA extraction results from Mars-relevant cell concentrations, e.g. similar to those observed in the Atacama Desert [8], in synthetic Mars analog soil [9].

•Futhermore, we characterize the sequencing efficiency of the current generation of Oxford Nanopore Technologies (ONT) MinION Mk-1b sequencers and R9.4 flowcells for low-input sequencing. We employ a "pore maintainer" (*Enterobacteria phage/Lambda* DNA) to preserve nanopore viability.

METHODS: EXTRACTION: Custom extraction cartridges were based on Claremont BioSolutions (CBIO) OmniLyse® mechanical cell disruption system. Coupled with binding and elution buffers, we conducted rapid (5 minutes) solid-phase nucleic acid extraction from tough-to-lyse organisms (*Bacillus subtilis* spores) [10-12].

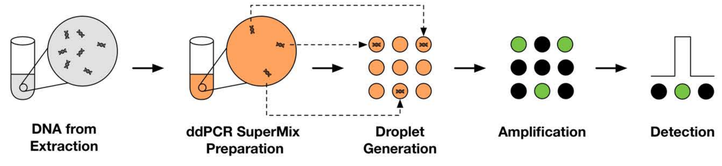
1. Approximately 1.6 x 10^4 spores and 50 mg of a "perchlorate" soil, an analog for the Phoenix lander site [9] was homogenized in 800 µL of 8x CBIO binding buffer (n=4).

2. Samples were then desalted in an Amicon® Ultra-0.5 column.

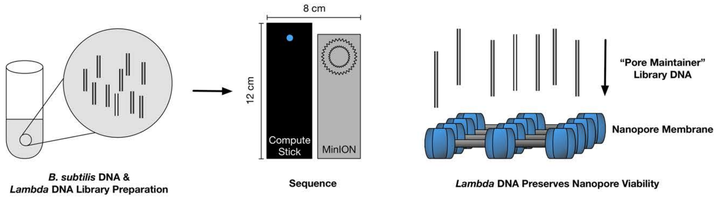
3. The soil/spore mixture was then re-suspended in 800 µL of 4x CBIO binding buffer and 4 µg of random hexamer primers, homogenized, and processed in our extraction cartridge resulting in 200 µL of CBIO elution buffer.

4. Lastly, a blank extraction (n=1) and ddPCR water control (n=3) were used to identify any sources of contamination.

EXTRACTION QUANTIFICATION: Extracted *B. subtilis* DNA was quantified using droplet digital polymerase chain reaction (ddPCR) with single copy primers (SpaC gene, forward: TGA GGA AGG ATGG GAC GAC A, reverse: AAC AGA TTG CTG CCA GTC CA). Extraction yield was calculated as extracted DNA / input DNA.



SEQUENCING: We prepared 0.2 ng of *B. subtilis* ATCC 6633 spore DNA within 250 ng of *Lambda* DNA using a transposase-based kit (SQK-RAD001), loaded half of the library (~125 ng), and sequenced on an Intel® Compute Stick (STK2MV64CC).



RESULTS & DISCUSSION: EXTRACTION: Extraction yields of *B. subtilis* spore DNA in water (Figure 1 & 2) were consistent with prior work on higher concentrations (~15% yield at 10^8 spores) [11]. All extractions from the perchlorate soil satisfied our 5% requirement except one sample for which a pump malfunctioned at the elution step (Figure 1 & 2).

Figure 1: Raw ddPCR Detections

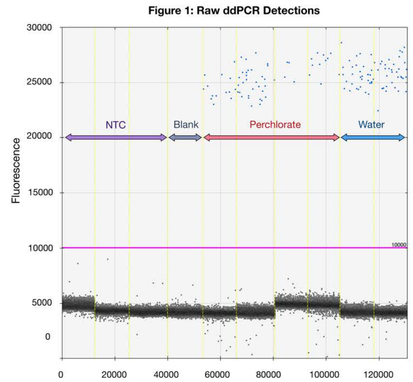


Figure 2: Extractions Results

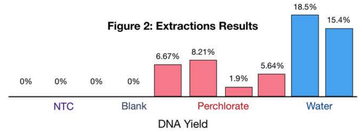


Table 1: Sequencing Metrics

Library to Sequening	Mass (ng)	Estimated Bases
Enterobacteria phage	125	2.47E+14
Bacillus subtilis ATCC 6633	0.10	1.97E+11
Sequencing Data	**Measured Reads**	**Measured Bases**
Total Raw Data	49,076	2.32E+08
High Quality Data	31,654	2.02E+08
Identified Species (mismatch)	**Mapped Reads**	**Bases**
Enterobacteria phage(12.7%)	31,624	2.02E+08
Bacillus subtilis subsp. Spizizenii str. W23 (15.1%)*	30	1.07E+05

SEQUENCING: Our sequencing metrics indicate that current nanopore sequencing is inefficient. From the theoretically available library (Table 1), only 8.7 x 10^{-5} % was sequenced. The causes of this detection remain to be explored; we speculate library may be lost due to adsorption to flow cell channels or other surfaces, the translocation-regulating motor protein may be knocked off of library molecules, or some library molecules floating in the headspace may simply never reach the nanopores.

CONCLUSION & SUMMARY: In this study, we have validated our extraction protocols to Mars-relevant cell concentrations and characterized sequencing efficiency. Our results suggest employing a pore-maintainer can improve low-input sequencing, however, future studies will focus on improving sequencing efficiency, required to achieve our future sensitivity goals. In addition, our sequencing results simulate the potential for a genomics-based instrument to detect low-levels of contamination, relevant to planetary protection.

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