

SETG: NUCLEIC ACID EXTRACTION AND SEQUENCING FOR IN SITU LIFE DETECTION ON MARS.

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Introduction: Theoretical and experimental evidence suggest that life on Mars, if it exists, may share a common ancestry with life on Earth due to substantial lithological exchange experienced during the late heavy bombardment period [1]. Moreover, widespread synthesis and delivery of amino acids and nucleobases, *the known building blocks of life*, from the early planetary nebula [2-4] may have biased life on Mars towards a familiar medium of hereditary transference via informational polymers (IPs) (e.g., deoxyribonucleic acid - DNA, and ribonucleic acid - RNA). Thus, biological IPs have the potential to provide *unambiguous* signatures of extraterrestrial life.

We are developing an integrated nucleic acid extraction and sequencing instrument: the Search for Extra-Terrestrial Genomes (SETG) [5,6] for *in situ* life detection on Mars (Fig. 1). SETG implements nucleic acid isolation [7] and leverages the sensitive technologies developed, such as nanopore sequencing, in the present genomics revolution.

Here we provide a brief overview of SETG, having recently achieved technology readiness level 4 (TRL-4) and outline the next steps in the instrument's development (TRL-5-6)

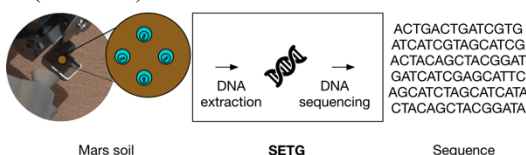


Figure 1: SETG overview, soil sample to genetic sequence.

Science Goals: Searching for IPs, including DNA and RNA, is critical to any comprehensive life detection strategy. Sequence data has revealed that all known life on Earth share ~400 “universal genes” (including the ribosomal RNA) [8,9]. These genes have remained “conserved” for the past 3-4 billion years (e.g., 16s rRNA) [10], thus comparing sequence data from Mars “life” and Earth life could discriminate forward contamination from true life detection and establish a possible common ancestry [11]. Our goals for SETG are to:

- identify related or unrelated nucleic acid-based life
- discern forward contamination among detections
- inform Mars Sample Return (MSR) of potential extant life

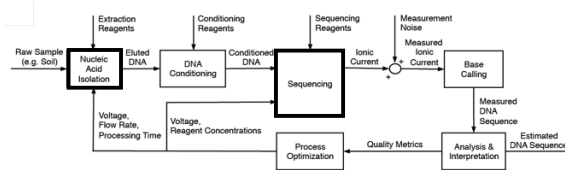


Figure 2: Provisional SETG functional block diagram; nucleic acid isolation and sequencing are discussed in this overview [5].

Instrument Overview: SETG consists of two primary subsystems: *nucleic acid extraction* and *sequencing* (Fig. 2).

Our *nucleic acid extraction* module is based on Claremont BioSolutions, LLC (CBIO) manual PureLyse® rapid (< 3 minutes) genomic DNA/RNA extraction kit [7]. PureLyse® facilitates solid-phase nucleic acid extraction and purification via mechanical bead-beating cell lysis in combination with binding and elution buffers. Furthermore, PureLyse® validations [12] have been conducted on a suite of synthetic Mars analog soils representing Gusev Crater (salt-rich), Meridiani Planum (acidic), Chrysis Planitia (alkaline), Vatistas Borealis (perchlorate-rich), and the global aeolian soil [13].

Sequencing is conducted using Oxford Nanopore Technologies (ONT) MinION Mk 1B sequencer with R9 flowcells (Fig. 3A). ONT performs strand sequencing using protein nanopores anchored in an electrically resistant membrane bilayer. When a voltage is applied to the bilayer, it creates an ionic current through the pores. Extracted IPs are conditioned to contain a motor protein that regulates the movement through the pores, and nucleobases are detected by monitoring the ionic current produced by the translocation of a single strand polymer.

TRL-4: In the context of SETG, TRL-4 implies *automated nucleic acid extraction* accompanied by *automated sequencing* (with intermediate manual handling).

Here we show the results of DNA extracted from 1.56×10^8 spores of *Bacillus subtilis* ATCC 6633 homogenized with 50 mg of synthetic perchlorate-rich Mars analog soil [13]. Spores of *B. subtilis* were selected as a worst case scenario of hardy, tough to lyse cells that can survive in extreme environments [13,14] while perchlorate-rich soil is a foreseeable sample candidate on Mars of astrobiological interest [15].

Automated nucleic acid extraction: Our automated extraction module is a custom Claremont BioSolutions SimplePrep X1 Automated Lysis and Nucleic Acid Extraction Platform (Fig. 3B). The X1 utilizes integrated PureLyse® cartridges to isolate DNA/RNA from tough to lyse microorganisms in soil samples. In addition, we implement sample conditioning protocols to mitigate soil-DNA interactions (e.g. DNA destruction and/or adsorption by soil) (Fig. 4) [12].

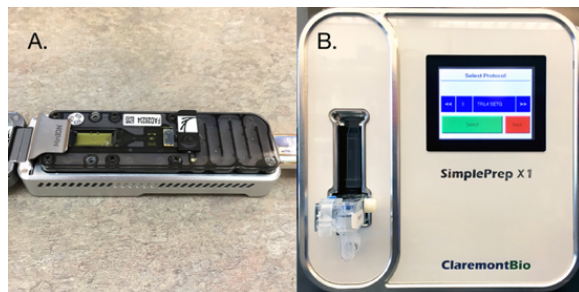


Figure 3: A). MinION Mk 1B with R9 flowcell B). SimplePrep X1 automated lysis and nucleic acid extraction platform.



Figure 4: TRL4 nucleic acid extraction workflow and sample conditioning protocol for synthetic Mars analog soils.

Automated sequencing: Two extractions (*B. subtilis* in perchlorate soil) with the X1 system were prepared using ONT's rapid sequencing kit (SQK-RAD001). This transposon-based method attaches platform specific adapters and motor proteins to DNA (IPs) in two five-minute steps. The conditioned DNA was then sequenced on a 48-hour cycle which yielded $\sim 4 \times 10^7$ sequences (Fig. 5) of positive *B. subtilis* ATCC 6633 detections (verified with Basic Local Alignment Search Tool, blast.ncbi.nlm.nih.gov/Blast.cgi).

TRL-5-6: The advancement of SETG from TRL4 towards TRL5 is focused on achieving fully-automated end-to-end sample loading to sequencing. We expect to achieve a less than 10 parts per billion (ppb) detection sensitivity from samples containing lower ranges of cell densities analogous to those present in the McMurdo Dry Valleys and the Atacama Desert (1×10^5 spores/g soil). TRL6 is focused on producing a fully-integrated SETG prototype compatible with Mars temperature and pressure, TRL6 will also target an ultimate detection sensitivity of less than 1 ppb [16,17]. Current work using CBIO manual PureLyse® kits and Droplet Digital Polymerase Chain Reaction (ddPCR) DNA detection techniques suggest 1 ppb sensitivity is attainable.

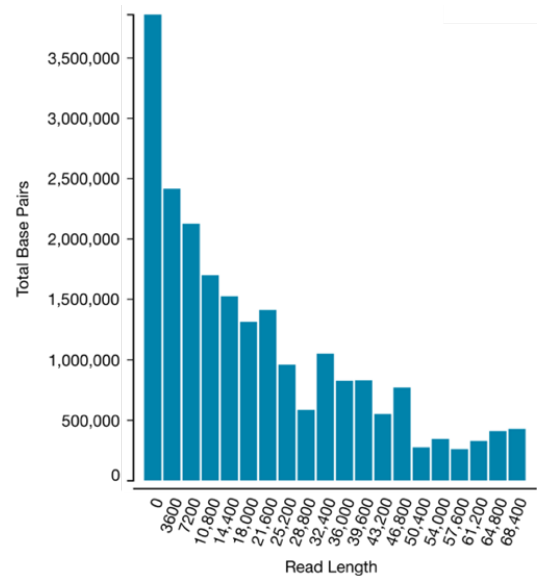


Figure 5: Total base pairs as a function of read lengths. Longer read lengths are a measure of quality sequencing as they aid in genome assembly, leading to more distinct discrimination between sequenced species.

Summary: Life on Mars, if it exists, may potentially be detected by SETG via *in situ* sequencing aboard a future rover or lander mission. Martian metagenomics could also provide unambiguous evidence of life on Mars and discern conserved genetic sequences, unveiling a Mars-Earth shared ancestry. SETG would also provide the first *in situ* characterization of forward contamination.

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Acknowledgments: This work was supported by NASA MatISSE award NNX15AF85G.