PROGRESS AND CHALLENGES FOR LIFE DETECTION VIA NUCLEIC ACID SEQUENCING. C. E. Carr[1-2] , A. Mojarro[1], J. Hachey[2], A. Pontefract[1-2], R. Doebler[3], M. Brown[3], G. Ruvkun[2], and M. T. Zuber[1*], [1]MIT Department of Earth, Atmospheric and Planetary Sciences, Cambridge, MA, [2]Massachusetts General Hospital, Department of Molecular Biology, Boston, MA. [3]Claremont BioSolutions LLC, Claremont, CA. [*]Full team: setg.mit.edu

Introduction: Life beyond Earth may be similar to life as we know it due to common building blocks [1-3], common physiochemical environments [4, 5], or common ancestry, e.g. Earth-Mars meteoritic exchange [6, 7]. Such life may utilize informational polymers (IPs) similar to life on Earth, e.g. deoxyribonucleic acid (DNA) or related IPs. Here we report progress developing the Search for Extra-Terrestrial Genomes (SETG) instrument [8-10], and characterize fundamental challenges for life detection via nucleic acid sequencing. We have elsewhere justified this approach and our nominal sample size of 50 mg [9, 10].

Assumptions. We target 1 ppb sensitivity (DNA mass/mass), in the range of low-moisture terrestrial analogs of Mars (**Fig. 1A**); energetic considerations may constrain maximum cell counts on Ocean Worlds to much lower densities. We consider *Bacillus subtilis* spores, which we use as a "worst-case" extraction challenge. With a 4 Mb genome, typical of bacteria, 1 ppb corresponds to $2{\cdot}10^5$ cells/g, or 10^4 cells/50 mg sample.

Overview: DNA is *extracted* (**Fig. 1B**) using a micromotor-based lysis and solid phase purification system (based on OmniLyse®, Claremont BioSolutions LLC). We then perform library preparation and nanopore-based strand *sequencing* (MinION, Oxford Nanopore Technologies), in which a motor protein produces controlled translocation of a DNA strand through a nanopore. The ionic current through each pore in an array is used to estimate the DNA sequence.

Target Requirements. We set an arbitrary target of "detection" as reading 1M bases, and set a sequencing target yield of 0.06%, reflecting extensive loss of nucleic acids during single molecule sequencing with a state of the art protocol [11]. This dictated a 5% extraction yield to achieve our 1 ppb goal (**Fig. 1C**).

Extraction Progress: We synthesized a variety of Mars analogs following Schuerger et al. [12] and used commercially-available OmniLyse® devices to develop strategies to mitigate the negative impacts of Mars-like soils on nucleic acid extraction [13]. In addition, we have recently demonstrated extraction using automated cartridges down to 10^4 spores/50 mg [14].

Sequencing Progress: Strand sequencing of DNA nominally uses 200 ng up to several µg to achieve up to 10 Gb/run. We have sequenced of as little as 0.1 ng DNA, with statistically significant detection of a known organism with <30 reads (~10^5 bases) [14]. In addition,

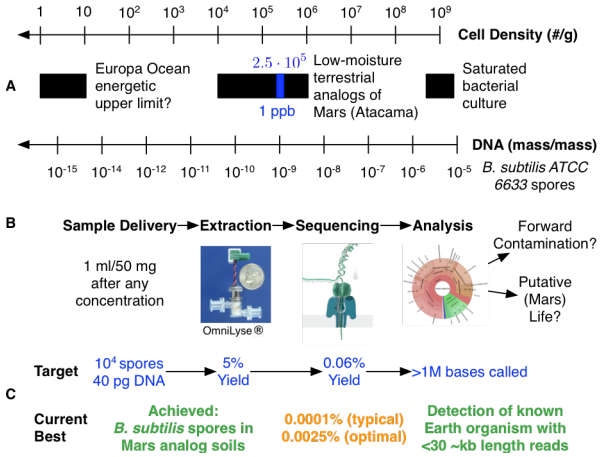

Figure 1. A) Cell density of representative environments compared to DNA mass fraction. **B)** SETG *in situ* sample processing steps. **C)** Comparison of project targets vs. selected current achievements.

despite low per-base accuracy (80-95% typical), we find that long nanopore reads can be classified taxonomically at higher rates than short (100-300 bp) next generation sequencing (NGS) reads [15].

Other accomplishments include detection of non-standard bases (inosine nucleosides) [16], and evaluating the failure modes of strand sequencing in the absence of DNA, revealing, with proper mitigation such as quality filtering, very low background noise [17].

Challenges. Spore extraction yields can be improved maximally 5-10X, in comparison to *Escherichia coli*, where yields already exceed 80-90%. Thus, to achieve sensitivities required for Ocean Worlds, sequencing yield must increase, through decreased losses (>10^2), amplification (~10^6 feasible), or both. Pre-concentration (~10^3) is also valuable. Compatibility with fragmented/damaged DNA and reagent stabilization also represent significant challenges. Advances in solid-state sequencing could reduce reliance on biological reagents, enhance the sequencing yield, and expand the range of target IPs.

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