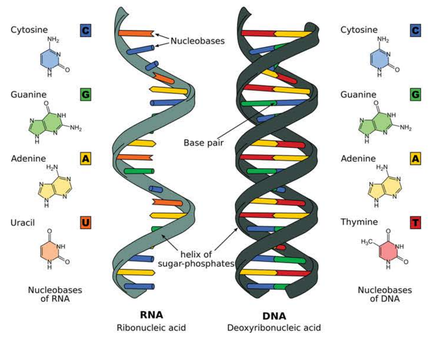
METHODS FOR EXTRACTING NUCLEIC ACIDS FROM MARS ANALOG REGOLITH

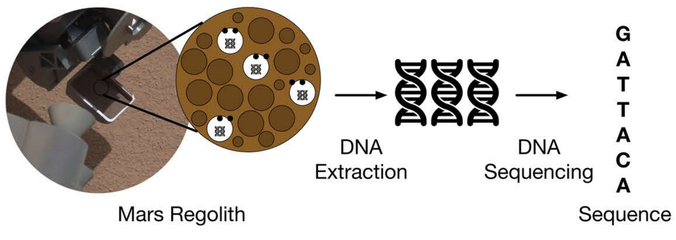
A. Mojarro[1,+], G. Ruvkun[2], M. T. Zuber[1], C. E. Carr[1,2,*]
[1]Department of Earth, Atmospheric and Planetary Science, Massachusetts Institute of Technology, Cambridge, MA, 02139
[2]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, 02114
[+]E-mail: mojarro@mit.edu *E-mail: chrisc@mit.edu

INTRODUCTION: Biological informational polymers such as nucleic acids (e.g. deoxyribonucleic acid, or DNA), have the potential to **provide unambigous evidence of life beyond Earth**. Our research group is developing a life-detection instrument that integrates automated nucleic acid extraction and nanopore sequencing, the **Search for Extraterrestrial Genomes (SETG) instrument [1]**.

•Our goal is to identify nucleic acids from extant or preserved life on Mars.



On Earth, metagenomic analysis of nucleic acids (DNA) **extracted from environmental samples has been a powerful tool for identifying new and unique "unculturable" microorganisms [2]**. However, complex matrices such as andisols (oxidized soils) have inhibitory side effects when extracting nucleic acids due to **competitive adsoption [3]** and the **production of destructive free radicals [4]** from Fe^{3+} and Fe^{2+} cycling in aqueous extractions.



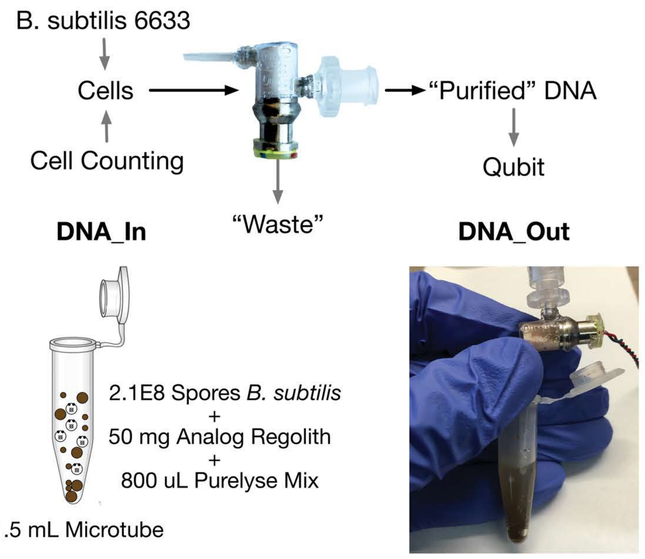
•In this study we utilize a miniature, two-step lysis and nucleic acid extraction PureLyse® module to extract nucleic acids from JSC Mars analog regolith.

•We report baseline DNA yields and outline the modifications required to attenuate soil-DNA interactions.

METHODS:

1. Spores of *Bacillus subtilis* ATCC 6633 were processed with and without 50 mg of JSC regolith following the standard PureLyse® protocol.

2. DNA concentration from two combined 200 ul elutions was quantified using a double-stranded, DNA-specific fluorometric assay.



RESULTS: Baseline processing of *B. subtilis* spores with PureLyse® in **water (control) produced a DNA yield of 15.6%** (Figure 1). We propose that resulting low yields from spores are the result of small acid-soluble proteins **(SASPs) that interfere with nucleic acid extraction [5]**.

B. subtilis spores processed with **JSC 1A lowered DNA yields to 12.9%** and **JSC Mars-1A lowered yields to 3.23%** (Figure 1).

Utilizing competitive binders is a common practice for improving yields with andisols [6]. We included random hexamer primers to JSC 1A, which increased DNA yields to 14.51% (Figure 1).

In JSC Mars-1A regolith, presumed DNA destruction by hydroxyl free-radicals was migitated by removing dissolved oxygen from the PureLyse® reagents through degassing in an anaerobic chamber. Yield increase from 3.23% to 6.79% in JSC Mars-1A using this methodology (Figure 1).

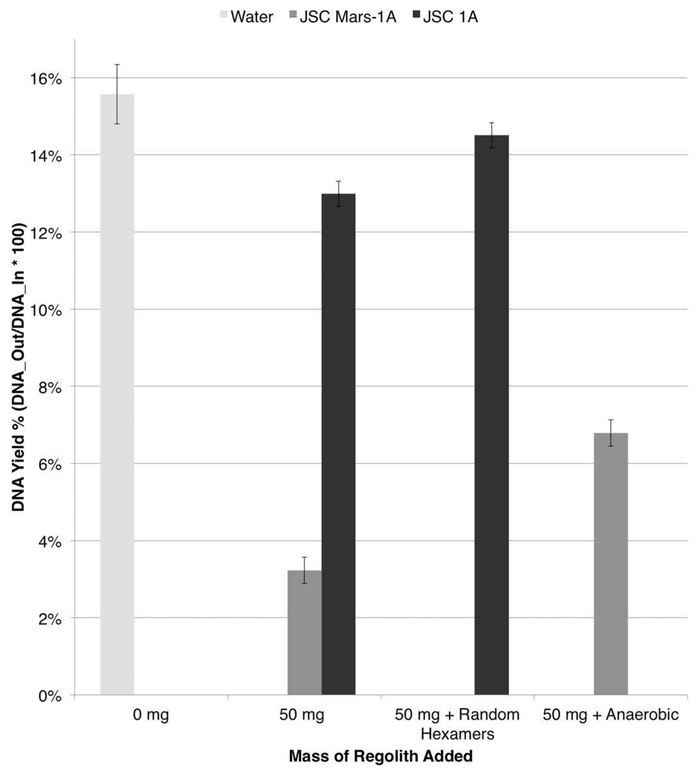


FIGURE 1: DNA extraction yields from a standard and modified PureLyse® protocol with *B. subtilis* ATCC 6633 in water, 50 mg JSC Mars-1A, and 50 mg JSC 1A (n=3) . DNA Yield (%) is defined as DNA Out / DNA In *100%.

CONCLUSIONS: In this study we demonstrate the feasibility of extracting DNA from Mars analog regolith using a simple extraction module and provide an analysis of yield through different protocols. Future studies will focus on extracting DNA from various types of Mars analog regolith and testing an automated nucleic extraction system in the field. In addition, further research will investigate the role of SASPs in nucleic acid extractions and the purity of isolated DNA for downstream genomic analysis.

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