DETECTING DNA IN SOIL, AEROSOLS AND ICE – A TECHNIQUE FOR APPLICATION TO FUTURE MARS, VENUS, AND EUROPA IN-SITU SAMPLE ANALYSES K. L. Craft[1], P. Thielen, Z. Chaudhry, K. Verratti, and C. Bradburne. Johns Hopkins University Applied Physics Laboratory (11100 Johns Hopkins Rd., Laurel, MD 20723, [1]Kate.Craft@jhuapl.edu)

Introduction: Detecting life, or evidence of past life, in a soil, atmosphere or ice sample will prove challenging due to the need for automated sample processing of complex samples. Previous work by [*1*] and [*2*] on Mars-analogue Atacama desert samples showed that, Synchronous Coefficient of Drag Analysis (SCODA) [3], coupled with sample desalination, enables concentration and purification of DNA from samples. In the past, salinity levels have been mitigated using centrifuges and dialysis [1; 2] which may not be amenable to easy automation on a spacecraft or rover. The paramagnetic nanoparticle DNA collection process tested here allows separation of DNA from salts and could be designed for *in-situ* sampling more easily.

Previous studies applying the SCODA technique obtained improved DNA purification and concentration over other extraction techniques used on samples from identical locations in the Martian-analogue Atacama desert [*1, 2*]. This is important considering that biomass at rover/probe-accessible locations in planetary samples may be extremely low abundance, if present at all. Additional advantages of the SCODA technique for planetary sample processing include the lack of moving parts, excellent rejection of contaminants, and potential for miniaturization and automation for use on a rover or spacecraft. Furthermore, the technique could be adjusted to detect *any* long charged polymers, meaning a common ancestry with terrestrial life is not a requirement.

In addition to Mars there are other proposed habitable planetary zones including the lower cloud level of Venus and the ocean and overlying ice shell of Europa [4]. The temperatures and pressures of the lower cloud level of Venus are about 300 K and 1 bar and water content is highest (~few hundred ppm). [5,6] We show here that the sample preparation processes we performed for DNA extraction are ideal for producing high DNA yield and for modification to a spacecraft rover or probe for exploration of three distinct planetary environments.

Objective: To purify, concentrate, extract and sequence DNA from three distinct planetary analog scenarios for Mars, the Venusian troposphere, and Europa.

Samples: Here we prepared, analyzed, quantified, and sequenced samples for three planetary analogs: (1) Mars – Antarctic Dry Valleys or Atacama desert, (2) Venus– aerosol samples at the Johns Hopkins University Applied Physics Laboratory (JHU/APL), Maryland and (3) Europa– Greenland ice sheet.

The Atacama desert and Antarctic Dry Valleys have been studied as Martian analogs by a number of researchers [e.g. 7,8,9]. In this project we analyzed samples collected by [Chrits-Cristoph], in the Atacama desert. The Atacama has been described as an excellent analog for present day Mars with its extreme aridity, low biomass, accumulation of salts, and similar oxidizing conditions [7,10]. Samples were analyzed from two locations near the Yungay area: Bea Hill (BEA) and Charanal (CH).Antarctic Dry valleys samples were collected from the University Valley (UV) and Pearse Valleys, which provide a relevant Martian analog with a cold, hyper-arid soil where any water is transient, episodic and ice is the favored state.Greenland ice sheet samples were collected in 2012 and provided by NASA Goddard The samples were chosen as a Europa analog due to long term deposition and stasis.

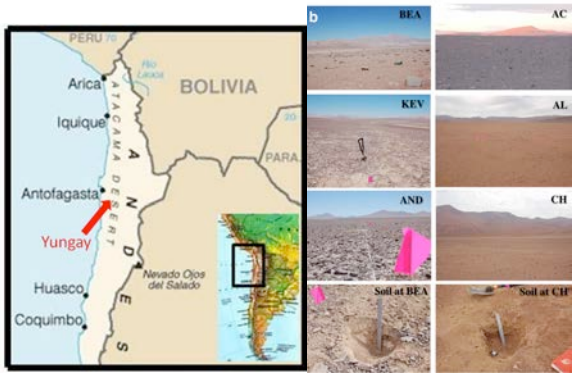

Figure 1. Sample locations in the Atacama Desert, Chile (adapted from [Crits-Christoph 2013]).

Aerosol samples: Terrestrial aerosol samples were collected on the roof at JHU/APL, a suburban site located between Washington, DC and Baltimore, MD. A Portable Sampling Unit (PSU, Hi-Q model PSU-3) collected ambient aerosols onto Teflon filters over 24-hour intervals for 30 days. A Vaisala MAWS201 meteorological station was co-located to collect relevant meteorological data, including temperature, relative humidity, barometric pressure, wind speed and wind direction. A subset of the sampled filters were spiked with *Bacillus thuringiensus var. Kurstaki* (Btk) spores. The spores were aerosolized from a slurry via a Sonotek Ultrasonic Nozzle into dried, conditioned air.

Dry spore particles were captured on top of ambient aerosol particles by loading the sampled filters into a BGI filter holder and drawing the spores onto the filter with a metered vacuum pump (Gast, Inc.). Each filter was cut into quarters and subjected to the particle extraction process outlined by Palmgren et al (1986) to prepare for DNA extraction.

DNA Extraction Process:

Salinity Cleansing: In order to process a sample using SCODA, the salinity level must be such that conductivity is below about 300 µS/cm in 5 mL [11]. For the Martian and Europan analog samples containing high salinity, we perform a method of removing salts with minimal DNA loss. This process involves submersing the sample in a high salinity solution containing carboxyl-modified polystyrene nanoparticles with a superparamagnetic magnetite core. Nucleic acids are temporarily bound to the nanoparticle matrix and collected with a magnet, allowing the removal of high salt liquids and resuspension in liquids suitable for downstream analysis.

DNA Extraction: Next, for all samples, the DNA/sample is concentrated and purified using the SCODA system within a Boreal Auroa system that applies the magnetic fields. [1,2,3] Figure 2 depicts the SCODA system and process of sample injection, application of electric fields for concentration, and final extraction.

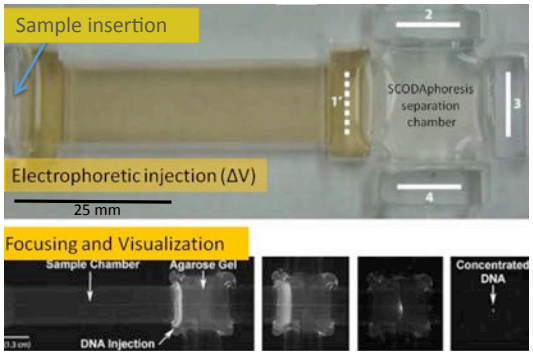

Figure 2. SCODA system showing sample insertion, direction of DNA and any contaminants down the sample chamber (left to right) and then concentration of DNA and separation from contaminants in the chamber on right (Adapted from Bradburne et. al., 2012, and Boreal Genomics 2012).

DNA Quantification and Sequencing: The resulting DNA is quantified using spectrophotometry and DNA-specific fluorescent dyes, then prepared for sequencing on the Illumina miSeq instrument. Bioinformatic characterization of organisms in the resulting data is performed using the metagenomic software package Kraken [12], which assigns identity to each read by comparing to all complete annotated genomes currently available in RefSeq. In addition to assigning identity to individual reads, the data is *de novo* assembled into contiguous fragments for comparative genomics analysis.

Discussion: A previous study applying the SCODA extraction method obtained increased DNA yield over a Powersoil extraction performed on samples of identical origin in the Atacama desert [*2*]. Through the salinity cleansing method performed here, further increase in DNA yield is expected. The higher the DNA yield capability, the greater our chances of discovering life on a planetary body that may only harbor miniscule biomass. Additional advantages to the methods tested here include the automation of the salinity cleansing method for use on in-situ instruments on future spacecraft rovers and probes.

Future work will involve devoloping these sample prep processes for operation as flight instruments. Further testing for sample collection in the Venusian environment will also occur through application of appropriate atmospheric gas ratios inside a chamber.

References: [1]Neish et al. (2012), *AbSciCon,*Abstract #. [2]Bradburne et al. (2012), *LPSC* abstract # . [3]Pel et al. (2009) *PNAS, 106, no. 35*, 14796–14801. [4] Pappalardo et al 2013, *Astrobiology, 13*, *no. 8*. [5]Fegley and Treiman (1992), In *Geophysical Monograph* 66: Venus and Mars: Atmospheres, Ionospheres and Solar Wind Interactions. [6]Schulze-Makuch and Irwin (2002), *Astrobiology, 2, no. 2.* [7]Crits-Christoph et al. (2013), *Microbiome*, *1*:28 [8]Marchant and Head (2007), *Icarus, 192* [9]Wentworth et al. (2005), *Icarus, 174*. [10]Fairén et al. (2010), *Astrobiology, 10, no. 8;* [11]Boreal Genomics Aurora User Manual (BG-2002-07-004 v2.09) [12] Wood et. al., 2013