THE USE OF RESEQUENCING MICROARRAYS FOR MICROBIAL MONITORING FOR HUMAN SPACE EXPLORATION. Verena Starke[1], Lisa Monaco[2], Ginger Flores[3], and Andrew Steele[1].[1]Carnegie Institution of Washington, Geophysical Laboratory Washington DC 20015 (e-mail: vstarke@gl.ciw.edu). [2]Jacobs Sverdrup, ESTS Group, Huntsville Alabama. [3]NASA Marshall Space Flight Center, Huntsville, AL.

Introduction: In preparation for space missions, NASA has refocused research toward testing technology that will be required by human exploration beyond low Earth orbit. These systems/technology include bioregenerative environmental control and life support systems (ECLSS), health screening, microbial pathogen monitoring technologies, in situ resource utilization (ISRU) and techniques for the purposes of life detection (LD) and planetary protection (PP). It is evident that microorganisms affect many of the goals and systems listed above and that rapid, onboard microbial monitoring will contribute toward mission safety and success. In order to monitor microbial communities, we developed a high-density oligonucleotide resequencing microarray for the identification of microorganisms relevant to human space exploration. This initial chip will be a first demonstration and proof of principle for this. The project team includes the Lab-on-a-Chip Application Development (LOCAD) project at NASA Marshall Space Flight Center, Carnegie Institution of Washington, and work was supported by a collaborative agreement with Affymetrix, Inc. (Santa Clara, CA).

Resequencing Microarray Technology And Design: High-density oligonucleotide microarrays allow researchers to simultaneously interrogate large quantities of genetic information in a single experiment. DNA microarrays have been used with success to conduct whole-genome analysis of single-nucleotide polymorphisms (SNPs) [1], generate maps of transcriptional activity across the entire human genome [2], and resequence non-contiguous regions of genomic DNA [3]. Resequencing microarrays are capable of interrogating more than 300,000 bp of sequence on a single array [4] (see figure 1 for resequencing principle). Previous studies have demonstrated that genomic DNA from pathogens can be resequenced using high-density oligonucleotide microarrays [5, 6]. Through the analysis of DNA sequence variation, multiple pathogens can be identified in a single assay, with high sensitivity and specificity.

The use of Affymetrix GeneChip® resequencing arrays allows large-scale resequencing of 16S and 18S rRNA sequences for the identification of microorganisms in diverse environments. Additionally, the microarray contains the DNA-directed RNA polymerase subunit beta (*rpoB)* gene sequence to provide further genetic identification within a species of gram-positive bacteria. The microarray design includes specific gene sequences for pathogens, extremophiles, and other microorganisms of all three domains of life (bacteria, archaea, and eukaryota), and the design is applicable to each of the areas mentioned above. At this time, 157 organisms have been outlined for initial demonstration and proof of concept experiments.

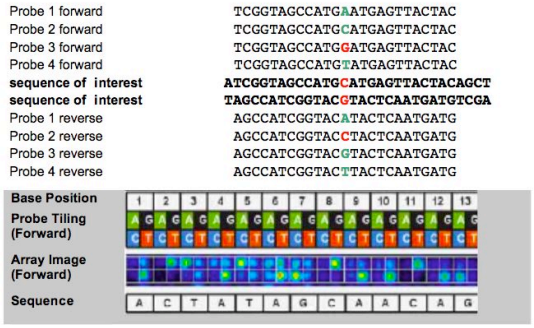

Figure 1: *Resequencing principle*. Oligonucleotide probes are synthesized using tiling strategy with eight unique 25-mer probes per base position. Each 25-mer probe is varied at the central position to incorporate each possible nucleotide —A, G, C, or T — allowing for the detection of both known and novel Single-nucleotide polymorphisms. (Figure from Affymetrix [7])

Experimental design: For the initial testing and proof of principle of the microarray we used purified genomic DNA of 10 microorganisms from ATCC (7 bacteria, 2 eukaryotes, 1 archaea) as template to test for hybridization efficiency, cross-hybridization of close related organisms, detection efficiency as well as detection limits. Purified genomic DNA provide a clean quality standard for the initial testing to eliminate any additional factors that could arise from the DNA extraction procedure, which could interfere with the PCR and microarray procedure. We used the genomic DNA of those microorganisms as template for the amplification of the 16S / 18S rRNA region, which was added to microarray for hybridization.

Results and Discussion: In this experiment 36 resequencing microarrays, comprising 19 different hybridization scenarios, were used for analysis. Our study

demonstrates that the microarrays were able to distinguish among different 16S rRNA gene sequences from different organisms. The figures 2 and 3 show the visual output of the microarrays after hybridization with the target template.

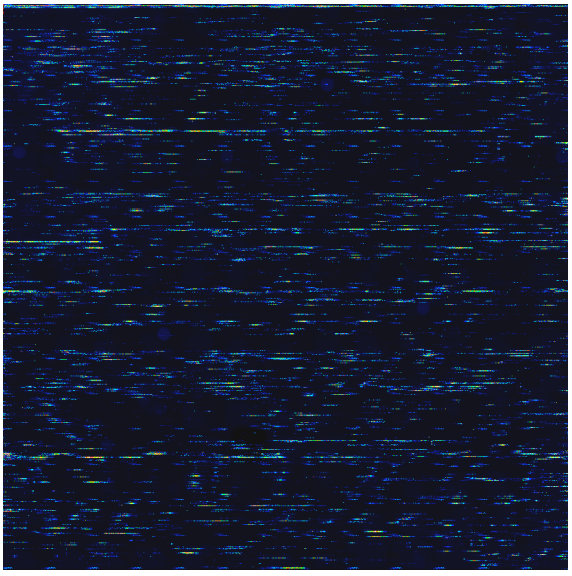

Figure 2: *Image of microarray* (false color): In this scenario the 16S rRNA gene PCR product from the single organism *Bacillus subtilis* was hybridized to the array (plus internal Affymetrix controls).

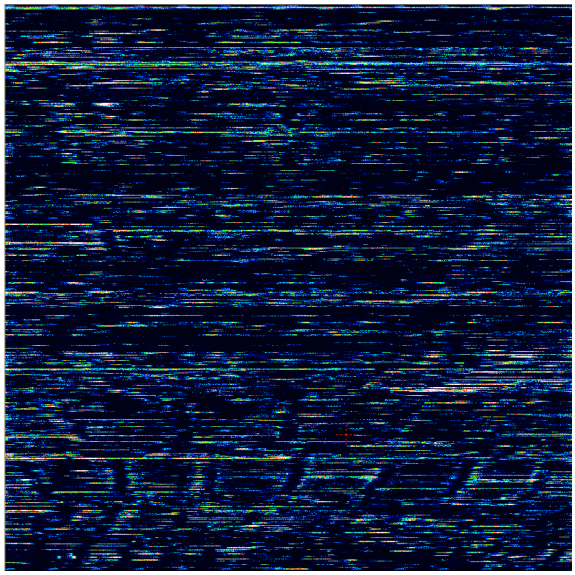

Figure 3: *Image of microarray* (false color): In this scenario the 16S or 18S rRNA gene PCR product from 10 different organisms (7 bacteria, 2 eukaryotes, 1 archaea) was hybridize to the array (plus internal Affymetrix controls).

Different hybridization scenarios, such as a using DNA of one single or of several different organisms, resulted in different hybridization patterns / signals (see figure 2 and 3). After gridding of the array, the GeneChip Sequence Analysis software analyzes signals from the array. The software assigns signals to their associated probe and constructs a sequence, resulting in potential microbe identification. Subsequently, sequence analysis of obtained sequences can help in interpreting the inferred phylogenetic relationship between sampled organisms.

Microorganisms interfere with or are crucial to the goals and systems in human exploration, and, consequently, the development of effective microbial monitoring technologies is critical for mission safety and success. It can be envisioned that resequencing-based microbial detection microarrays will ultimately support crews of future human exploration missions.

Further work: After the initial testing and protocol establishment, environmental samples need to be applied to the microarray to test for microbial diversity. Additionally, the tested environmental sample will be sequenced to provide a control and to calculate the efficiency and accuracy of the microarray in regards to unknown sample.

References: [1] Warrington J.A. et al. (2002) *Human mutation, 19*, 402-409. [2] Rinn J. L. et al. (2003) *Genes & Development 17*, 529-540. [3] Zwick M.E. et al. (2004) *Genome Biology, 6*, R10. [4] Hacia J. (1999) *Nature, 21,* 42-47. [5] Lin B. et al.(2006) *Genome Research, 16,* 527-535. [6] Wilson W.J. et al. (2002) *Molecular and Cell Probes, 16*, 119-127. [7] Affymetrix. GeneChip CustomSeq Resequencing Array Program. Data Sheet http://www.affymetrix.com/support/technical/datasheets/customseq_program_datasheet.pdf