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Letter to editor Open Access

Next Generation Sequencing and the Extreme Microbiome Project (XMP)

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Letter to Editor

In response to the rapidly growing field of met genomics, the Association of Bimolecular Resource Facilities (ABRF) has organized a new Met genomics Research Group (MGRG), a team of scientists with backgrounds in microbiology, genetics and genomics, bioinformatics, oceanography, geochemistry, planetary sciences, climate research, and extremophile research. The MGRG has two goals: development of reagents and methods that include standardized control samples, and met genomic characterization of samples from extreme environments.

Improvements in metagenomic methods will benefit from the availability of standardized reference samples that represent the range of organisms potentially present in samples from the field. Reference samples can be used in metagenomics sequencing to assess the performance of a platform and help individual laboratories compare their local results to those of the larger research community. Reference standards will also assist in the development of much needed peripheral reagents including high performance DNA extraction kits, complex enzyme mixes for microbial lysis, nucleic acid-free sample concentrators, and bioinformatic pipelines.

The development of the MGRG Class I and Class I+ bacterial standard include 6 and 11 species, respectively, of bacteria with varying GC content and Class I genomes (low sequence redundancy). Species have been selected from different taxonomic groups, including Gram positive, Gram negative, and Archaea, and are being distributed through the American Type Culture Collection (ATCC). More complex reference standards containing Class II and Class III bacteria (moderately or highly repeated sequences) and mixed eukaryotic species are being designed for future development. Standardized reference samples will be available as quantified whole-cell mixtures and purified metagenomic DNA.

One of the first applications of the Class I standard is to assist in the production of a complex enzyme mix for microbial lysis to be assembled in collaboration with Sigma Chemical. This blend of enzymes includes lytic enzymes such as Mutanolysin, Lysozyme, Lyticase, Chitinase, and Achromopeptidase, along with several other proprietary enzymes. Furthermore, high performance DNA extraction kits are being engineered through collaborations with Omega Bio-Tek and other manufacturers that will use this enzyme blend combined with chemical, thermal, and bead beading techniques. Sample collection devices are also being developed by the XMP team and Steritech Corp including a special nucleic acid free metagenomics filtration device equipped with a polycarbonate NTE membrane to allow efficient microbial release.

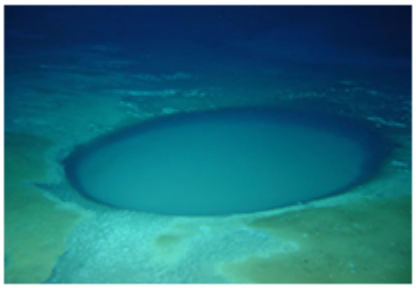

Figure 1: Deep Ocean Brine Lake Gulf of Mexico.

Figure 2: "Door to Hell" Gas Crater.

The MGRG has also initiated a novel microbiome project to characterize organisms from extreme environments by deploying three whole-genome shotgun sequencing platforms: Illumina HiSeq (2x250), MiSeq (2x300), and Ion Torrent PGM (400bp). Amplicon and targeted sequencing, such as for 16S ribosomal RNA, will not be included in these studies.

This projected has been designated the Extreme Microbiome Project (XMP), and characterizes samples from many unique sites around the world including Lake Hillier in Western Australia (Ken McGrath) (Figure 1), the "Door to Hell" gas crater (Figure 2) in Turkmenistan, which has been burning continuously since the 70s, (George Kourounis expedition), deep ocean (>3,000 mbsl) brine lakes

(Samantha Joye expeditions), deep ocean sites of Western Greenland (Diana Krawczyk, Greenland) (Figure 3), Alaskan permafrost, acidic brine ponds (Sarah Johnson), and a range of sample types from Antarctica (Joye and Mikucki). Extreme animal microbiomes include feces of penguin and hummingbird.

Figure 3: Lake Hiller Western.

Not only are the sampling sites of the XMP unusual and remote and therefore new to shotgun metagenomics research, but the studies will also utilize the new reagents and methods outline above. Sequencing libraries are being synthesized using BioO, New England Biolabs, and Life Technologies reagents.

Data for whole genome shotgun sequencing of DNA and RNA has been analyzed by the XMP bioinformatics team using open source and custom software. Results for the Door from Hell gas crater were recently presented at the annual ABRF meeting in St. Louis and showed that *Norcardioides sp., Pimelopbacter simplex, Propionibacterium avidum, Catenulispora acidiphila, Stackebrandtia nassauensis, Streptosporangium roseum, Leifsonia xyli, Streptomyces cattleya,* and *Kitasatospora setae* were detected. DNA-Seq and RNA-Seq data for the penguin microbiome and the pink lake, Lake Hillier, Australia will be presented at the New York City MetaSub meeting in June 2015. Additional information can be found at www.ABRF.org and www.extrememicrobiome.org.