

Scott Tighe (Univ of Vermont), Kelley Thomas (UNH), Matt Settles (Univ of Idaho), Ken McGraft (AGRF-Brisbane), Shawn Levy (Hudson Alpha), Nathan Biven (Univ of Missouri), Chris Mason (Cornell), Darryl Reeves (Cornell), Stefan Green (UIC), Russ Carmical (BCM), Don Baldwin (Pathonomics), Samantha Joye (Univ of Georgia), Nadim Ajami (BCM), Ebrahim Afshinnekoo (Cornell), Jodie Lee (ATCC) and Tim Hunter (Univ of Vermont)

## Mission Statement

The goal of the Metagenomic Research Group is to evaluate, study, and refine methodologies related to any aspect of metagenomics and microbiome studies, including study design, controls, detection methods, and bioinformatics pipelines with hopes of standardizing methods and increasing detection efficiency

## Abstract

The ABRF MGRG membership is composed of microbiologists, geneticists, bioinformaticist, climate researchers and oceanographers all with the common goal to advance the methods of metagenomics as well as describe new and interesting microbiome environments.

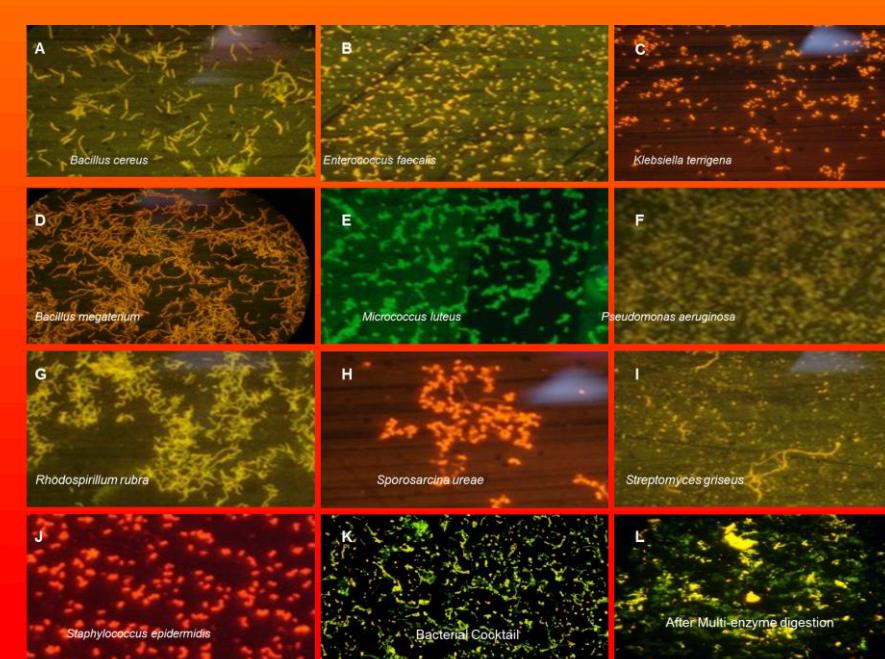
Two studies are underway by the ABRF MGRG. The first is the Microbial Standards Project in which we are synthesizing a microbial cocktail containing specific numbers of cells with various types of ATCC bacterial strains. This can be used for control studies, extraction efficiency studies, data analysis, and Matrix spiking. The second is the Extreme Microbiome Project (XMP) which will study microbial communities only from extremophilic and unique environments.

## Microbial Standards Project

The ABRF MGRG is working to develop a microbial reference standard to be used as a control similar to the ERCC controls. This standard will be composed of 6-10 microbes which are "fixed" and with a known cell number and can be added to samples as a matrix spike for recovery assays. The MGRG has established relationships with NIST and the ATCC.

## Methods

Six to eight bacteria with low, medium, and high GC content belonging to both the Gram Positive and Negative groups. Class I genomes will be the focus for the first Standard.



ATCC microbial strains will be grown in a shaker to ensure unicellular suspensions. Ethanol will be added during vortexing to a concentration of 40%. Cells are washed and resuspended in PBS Ethanol.

Counting of cells will be performed using manual microscopy Miltenyi Biotec, MACSQuant VYB, Logos Bio Mojo Counter and others to determine exact cell number.

1

## EXtreme Microbiome Project (XMP)

The MGRG has initiated a new microbiome project. The project will focus on developing and surveying metagenomic methods to help facilitate the recovery of DNA and RNA from unique sample types as well as develop bioinformatics tools for *De novo* assembly

### Current Locations

#### Lakes of Northern Greenland

Greenland Institute of Natural Resources  
Climate Research Centre  
Diana Krawczyk



Novel Centric diatoms from Greenland  
Diana Krawczyk

#### Stefan Green (MGRG member) Sampling the "Door to Hell" Gas Crater in Turkmenistan



#### Deep Ocean Brine Lake Mandy Joye's Expeditions



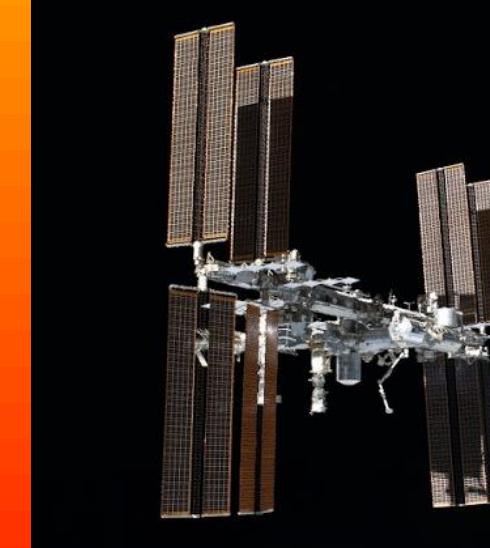
At the bottom of the sea floor at a depth of 10,000 feet there are lakes that appear to be something you would see on land. These underwater lakes are rich in microbial life



#### Deep Ocean Hydrothermal Vents



#### Space Station Microbial Flora



#### Pink Lakes of Western Australia



Metagenomic samples will be extracted using a variety of techniques and will be the subject, in part, of study. Both DNA and RNA will be recovered for downstream analysis for shotgun sequencing using both long and short read sequencing.

RNA-Seq, DNA-Seq, and Methy-Seq will be used for the XMP project. Due to high [and varying] GC content, evaluation of library synthesis techniques and reagents will be performed.

Bioinformatics approaches will be a strong focus during the XMP. Evaluation of current software as well as assembling new pipelines.

### Sequencing Partners

Relationships with Illumina, Pacific Biosciences, and Genapsys are being pursued. Sequencing library chemistry will include Illumina, BioO, and Rubicon.

### Contributors and other Possible Collaborations

ATCC and NIST  
Woods Hole Oceanographic  
Greenland Institute

## Acknowledgements

The ABRF MGRG would like to thank the ABRF executive board for supporting the formation of the new research group. Special thanks to Tim Hunter, our EB liaison for supporting such a unique study plan