Single Cell Genomics: Reaching the Limits of Life Detection Sensitivity. R. Stepanauskas, Bigelow Laboratory for Ocean Sciences (McKown Point 180, West Boothbay Harbor, ME 04575).

Abstract: Reading genetic information encoded in individual, uncultured microbial cells represents the ultimate level of life detection sensitivity and genetic data richness. It also allows, for the first time, the study of multiple genes and entire genomes of uncultured microorganisms, independent of the complexity of their communities and compatible with extremely low sample quantities.

Single cell genomics relies on the physical separation of individual cells, followed by their lysis, whole genome amplification, and subsequent DNA sequencing. In 2009, Bigelow Laboratory for Ocean Sciences established the first high-throughput facility in this field, providing single cell genomics services to the broad scientific community. During its first two years in operation, Bigelow Laboratory Single Cell Genomics Center contributed to cutting-edge research projects at over 30 organizations around the globe. Over 300,000 individual cells have been analyzed by the Center so far, providing unique access to genomic DNA, without cultivation biases, from microorganisms representing over 60 phyla of bacteria, archaea and protists. The types of samples processed by the Center range from marine to deep subsurface to mammalian gut content, and the type of research questions addressed range from microbial ecology and evolution to human health and bioprospecting. Center's research accomplishments include discoveries of inorganic carbon fixation pathways in abundant bacterial groups in the dark ocean, in situ trophic interactions of uncultured protists, identification of novel phototrophs, etc. [1-10]. An example of exobiology-relevant results include genomic sequences representing multiple microbial candidate divisions from a subsurface sample collected at the Deep Underground Science and Education Laboratory (DUSEL) (Fig. 1). Our findings of bacteria-like Sigma factors and RuBisCO genes in some of the discovered novel archaeal lineages provide new insights into the early evolution of life on Earth and indicate sources of energy utilized by subsurface microorganisms.

In summary, single cell genomics is no longer a theoretical possibility but rather a rapidly expanding research field that offers unprecedented opportunities for the search of extraterrestrial life at the ultimate level of sensitivity – single cell.

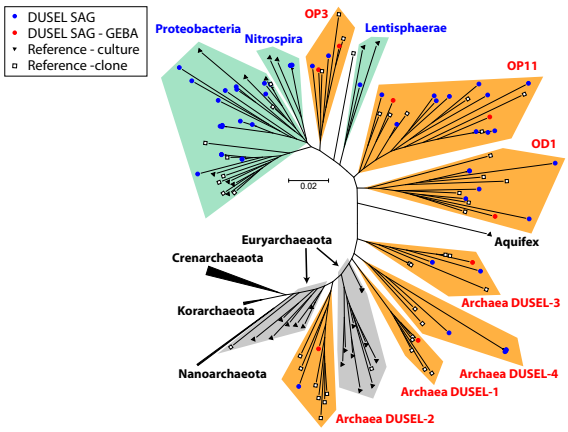

Figure 1. Phylogenetic composition of microbial single amplified genomes (SAGs) recovered from the Deep Underground Science and Education Laboratory (DUSEL).

References:

1. Swan BK, Martinez-Garcia M, Preston CM, Sczyrba A, Woyke T, Lamy D, Reinthaler T, Poulton NJ, Masland EDP, Gomez ML, Sieracki ME, DeLong EF, Herndl GJ, Stepanauskas R (2011) Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. Science 333:1296-1300
2. Yoon HS, Price DC, Stepanauskas R, Rajah VD, Sieracki ME, Wilson WH, Yang EC, Duffy S, Bhattacharya D (2011) Single-cell genomics reveals organismal interactions in uncultivated marine protists. Science 332:714-717
3. Hess M, Sczyrba A, Egan R, Kim TW, Chokhawala H, Schroth G, Luo S, Clark DS, Chen F, Zhang T, Mackie RI, Pennacchio LA, Tringe SG, Visel A, Woyke T, Wang Z, Rubin EM (2011) Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331:463-467
4. Heywood JL, Sieracki ME, Bellows W, Poulton NJ, Stepanauskas R (2011) Capturing diversity of marine heterotrophic protists: One cell at a time. ISME Journal 5:674-684
5. Martinez-Garcia M, Swan BK, Poulton NJ, Lluesma Gomez M, Masland D, Sieracki ME, Stepanauskas R (2011) High throughput single cell sequencing identifies photoheterotrophs and

chemoautotrophs in freshwater bacterioplankton. ISME Journal, advance online publication

6. Martinez-Garcia M, Brazel DM, Poulton N, Swan BK, Lluesma Gomez M, Masland D, Sieracki ME, Stepanauskas R (2011) Unveiling in situ interactions between marine protists and bacteria through single cell sequencing. ISME Journal, advance online publication
7. Woyke T, Sczyrba A, Lee J, Rinke C, Tighe D, Clingenpeel S, Malmstrom R, Stepanauskas R, Cheng J-F (2011) Decontamination of MDA reagents for single cell whole genome amplification. PLoS ONE 10:e26161
8. Fleming EJ, Langdon AE, Martinez-Garcia M, Stepanauskas R, Poulton NJ, Masland EDP, Emerson D (2011) What's new is old: Resolving the identity of *Leptothrix ochracea* using single cell genomics, pyrosequencing and FISH. PLoS ONE 6:e17769
9. Martinez JM, Poulton NJ, Stepanauskas R, Sieracki ME, Wilson WH (2011) Targeted sorting of single Virus-Infected cells of the coccolithophore *Emiliania huxleyi*. PLoS ONE 6:e22520
10. Ghai R, Pasic L, Fernandez AB, Martin-Cuadrado AB, Mizuno CM, McMahon KD, Papke RT, Stepanauskas R, Rodriguez-Brito B, Rohwer F, Sanchez-Porro C, Ventosa A, Rodriguez-Valera F (2011) New abundant microbial groups in aquatic hypersaline environments. Scientific Reports 1:srep00135