Proposal to use 454 technology to track microevolution within a single species of Lost City Archaea

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Addendum: DNA Extraction Procedure

DNA has already been extracted from the carbonate chimneys according to a protocol modified from that of Barton *et al*. (2006) and Brazelton *et al*. (2006) and described in full here: Frozen chimney samples were thawed and crushed with a sterile mortar and pestle. Approximately 0.25-0.5 g of chimney material is gently mixed into 250 µL of 2x Buffer AE (200 mM Tris, 50 mM EDTA, 300 mM EGTA, 200 mM NaCl, pH 8) and 2 µg of poly-dIdC (Sigma) and incubated at 4ºC overnight. Proteinase K (to a final concentration of 1.2 mg/mL) and 10 µL of 20% SDS were added to the mixture and incubated at 37ºC for 30 min. A further 150 µL of SDS was added, followed by two rounds of phenol:chloroform:isoamyl alcohol (25:24:1 ratio by volume) extractions. The supernatants from all tubes (36-48 tubes are processed in parallel) were carefully pipetted into SnakeSkin dialysis tubing (Pierce) and dialyzed against 20 mM EGTA overnight. This large scale dialysis step proved to be very efficient in removing inorganic minerals as well as organic contaminants. After dialysis, DNA was precipitated with 0.1 volumes of 3M sodium acetate and 1 volume of isopropanol at -20ºC for 2-4 hours. Pellets were collected by centrifugation at 16,000 g for 20 min at 4ºC and washed once in cold 70% ethanol. Pellets were dried in a vacuum centrifuge and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8).

References

Barton, H.A., N.M. Taylor, B.R. Lubbers, A.C Pemberton. (2006) DNA extraction from low-biomass carbonate rock: An improved method with reduced contamination and the low-biomass contaminant database. J. of Micro. Bio. Meth. 66:21-31.

Brazelton, W. J., M. O. Schrenk, D. S. Kelley and J. A. Baross. 2006. Methane and sulfur metabolizing microbial communities dominate in the Lost City hydrothermal vent ecosystem. Appl. Environ. Microbiol. 72:6257-6270.