DNA Detection In Salt

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Abstract:

Biological material surviving in modern halite (salt) on Earth may point to a method for detection of potential or former life in salt deposits on Mars. This project attempts to find an efficient method extracting cells and DNA from modern halite crystals to gain more insight into efficient method of extracting DNA from ancient salt. Our method considers the limitations of Mars Rover techniques in terms of reagents and simplicity. Halite was collected from the north shore of Great Salt Lake, Utah. Through direct experimentation, we designed a filtration system to isolate DNA from salt samplesand refined this process to provide the highest yields of clean DNA. To determine quantities of DNA, we experimented with several highly-sensitive detection methods. Our most promising method utilized the dye, pico-green, which is detectable by a ultraviolet spectrophotometer. Methods for the best yield and detection will be presented as well as a design that adapts this work to remote techniques.

Introduction:

Halite crystals contain fluid inclusions that can harbor life. The microorganisms found within these extreme conditions are extremophiles that can live in saturated salt conditions. Fluid inclusions can also preserve ancient biological molecules which can shed light into the history of organisms that data back millions of years. The ancient DNA that is contained within halite fluid inclusions is sensitive to degradation upon the use of extraction chemicals. The aim of our project was to develop a DNA extraction protocol that eliminated the use of extraction chemicals and instead used centrifugation as the main extraction method. DNA is often quantified by UV spectrophotometry, but there are two problems with this approach. Our experiments showed that DNA from salt was contaminated with environmental material, and it was difficult to use an optical system. The second problem was that the amount of DNA was beneath the detection limits of the instrument. To solve these problems, we turned to the dye, PicoGreen, which should detect quantities of DNA too small for traditional DNA detection methods. In addition, it should bind to DNA directly, avoiding problems with contamination by debris. Our protocol used modern halite crystals to determine the sensitivity of the procedure and the amount of starting product (ancient DNA) that will be optimal. Also, we developed a filtration technique to produce a high quality samples.

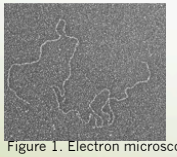

Figure 1. Electron microscopy of ancient DNA fragments from liquid inclusions of salt crystals (Baxter and Griffith, unpublished).

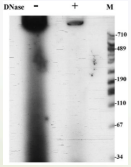

Figure 2: DNA from 250 million year old sample treated with DNase to verify the presence of DNA material. (Griffith and Baxter, unpublished)

Methods:

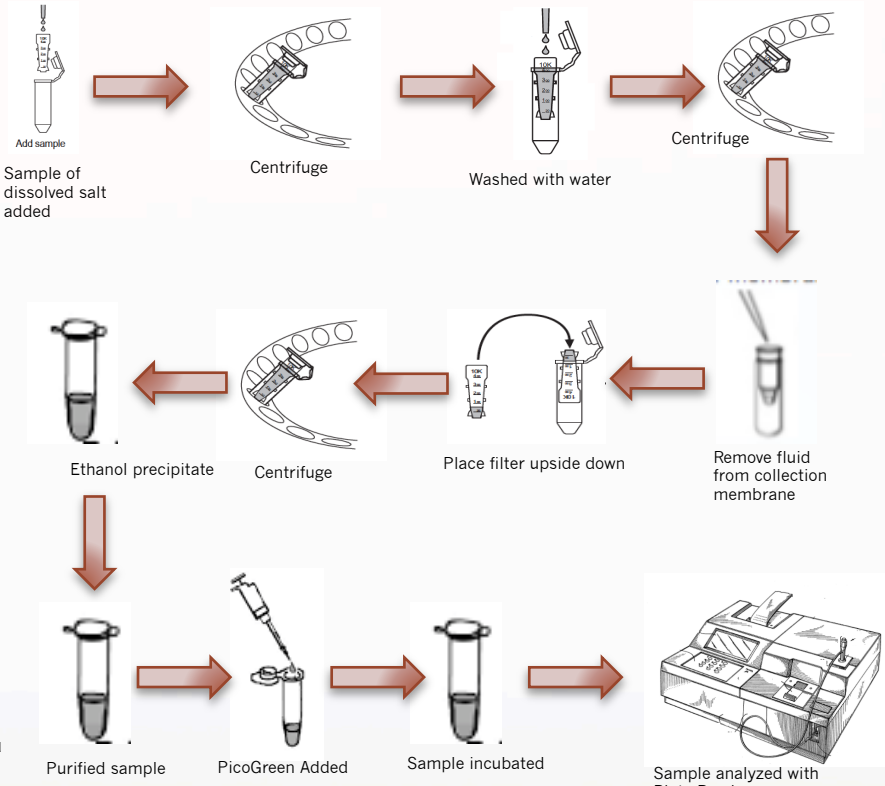

Figure 3: Amicon centrifugal filter and Qiagen spin column. Images from Amicon Ultra 500 μL filter and Qiagen spin column manual. Along with PicoGreen Assay and Biotek Plate Reader.

Results & Discussion:

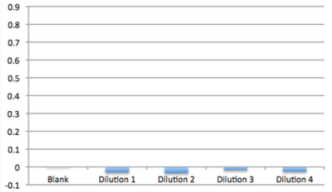

Figure 5:PicoGreen Assay with Plate Reader

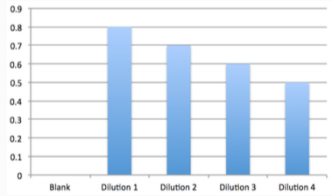

Figure 6: NanoDrop Expected

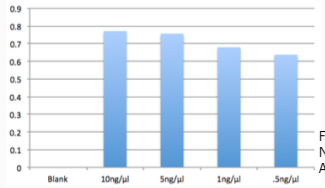

Figure 7: NanoDrop Actual

Conclusion:

The PicoGreen assay using the plate reader was deemed unreliable due to the extremely low quantities of DNA recorded as well as negative values. Given the claimed lowest possible quantity of DNA that PicoGreen can detect, 25pg/mL, the observed values did not seem plausible. A nanodrop test with standard dilutions of herring sperm DNA with and without PicoGreen confirmed that the plate reader was faulty. Samples have been stored for use with a new plate reader to re-measure DNA quantities from each filtration method. This tests will provide data on the optimal filtration technique, and the next step of the experiment will be to start testing progressively older salt samples. Based on the DNA quantities of these samples, the maximum DNA yield for different ages of salt will show if DNA can be detected from samples up to 250 million years old.

Salt Samples Timeline

460 million years from Detroit mine

253 million year (Permian) sample is from Waste Isolation Pilot Plant (WIPP) DOE facility.

66 million years (Jurassic) sample is from Redmond, Utah (Real Salt Mine)

Modern salt from Great Salt Lake

Figure 4: Timeline of available salt samples for testing.

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References:

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