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 samples and refined this process to provide the highest yields of clean DNA. To determine quantities of DNA, we utilized the dye, PicoGreen, which is detectable by fluorimetry. Methods for the best yield and detection will be presented as well as a design that adapts this work to remote techniques.

Introduction:
Ancient sodium chloride salt (halite) crystals contain fluid inclusions that can harbor life (e.g. Vreeland et al., 2000). 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 (Spathis et al., 2008). The aim of our project was to develop a DNA extraction protocol was to develop efficient extraction and quantification methods of DNA at amounts as low as 5 ng/mL. DNA is often quantified by UV spectrophotometry, but there are two detection methods using a fluorimeter, resulting in intensity readings shown in figure 6. As the DNA concentration increased (x axis), the intensity increased (y axis) linearly. This shows that the readings correlated with the expected standard quantities and that the technique using PicoGreen works. Quantification of minute amounts of molecular grade DNA works using fluorimetry.

Methods:

DNA Extraction

<table>
<thead>
<tr>
<th>Sample of dissolved salt added</th>
<th>Ethanol precipitate</th>
<th>Purified sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
<td>Place filter upside down</td>
<td>Remove fluid from collection membrane</td>
</tr>
<tr>
<td>Washed with water</td>
<td>DNA</td>
<td>Sample quantified by fluorimetry</td>
</tr>
</tbody>
</table>

Quantification of DNA

Filtration methods and DNA extraction developed for future halite salt samples are illustrated in figure 3. DNA Samples were prepared from molecular grade DNA. A serial dilution was performed to get a range from 5 ng/mL to 1000 ng/mL. The PicoGreen and DNA with both diluted in 1x TE buffer. The DNA and PicoGreen dilution were combined at a ratio of 1:1 to a final volume of 10 µL, then analyzed in the fluorimeter as shown in figure 4. DNA, PicoGreen, and TE buffer provided by Kiti Quant-IT PicoGreen dsDNA Reagent, from Invitrogen (Carlsbad, California).

Results & Discussion:

DNA standards were prepared as described above. Samples were examined with a fluorimeter, resulting in intensity readings shown in figure 6. As the DNA concentration increased (x axis), the intensity increased (y axis) linearly. This shows that the readings correlated with the expected standard quantities and that the technique using PicoGreen works. Quantification of minute amounts of molecular grade DNA works using fluorimetry.

Conclusion:
Molecular grade DNA can be detected at amounts as low as 5 ng/mL using our PicoGreen protocol and fluorimeter. Given the claimed lowest possible quantity of DNA that PicoGreen can detect, 25 pg/mL, better equipment could yield lower measurable quantities than what we found. Currently, our lab is preparing to compare the standard curve created from purified DNA with DNA from Halobacterium. After this, we will use environmental samples of DNA from modern Great Salt Lake to ensure that this quantification method will work from extracted DNA. Finally, we will test this method on ancient salt samples (Figure 5) that are up to 250 million years old. The maximum DNA yield for different ages of salt will show if DNA can be detected from samples up to 250 million years old. This has broader implications for studying halite deposits on Mars with Rover technology, in the search for molecules of life that may be preserved there.

References:

Acknowledgments:
This project was funded by a NASA Space Grant research infrastructure competitive award. We would also like to thank Somi Yoo for setting the foundation of the project.

Salt Samples Timeline

- 460 million years from Detrohmine
- 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 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).

Figure 3: Amicon centrifugal filter and Qiagen spin column. Images from Amicon Ultra 50051 filter and Qiagen spin column manual.

Figure 4: Images from Amicon Ultra 50051 filter and Qiagen spin column manual.

Figure 5: Timeline of available salt samples for testing.

Figure 6: Standard curve generated using molecular grade DNA, PicoGreen assay, and fluorimetry. Excitation set at 480 nm, Emissions were read at 524 ± 2 nm, and the fluorimeter was set on high sensitivity.

Extracting DNA From Salt: Using PicoGreen to Explore Detection Limits
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