



Extracting DNA From Salt: Using PicoGreen to Explore Detection Limits

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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 samples and refined this process to provide the highest yields of clean DNA. To determine quantities of DNA, we utilized the dye, pico-green, 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 date back millions of years (Griffith et al., 2008). The aim of our project was to develop a DNA extraction protocol well 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 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 using a fluorimeter (Sankaranarayanan et al., 2011). In addition, it should bind to DNA directly, avoiding problems with contamination by debris. We are developing a protocol using standard DNA, and then modern halite crystals from Great Salt Lake to determine the sensitivity of the procedure and the amount of starting product that will be optimal. Also, we developed a filtration technique to produce a high quality samples. The next step will be utilizing ancient salt samples, such as that collected from the Permian Salado Formation (Griffith et al., 2008).

Methods:

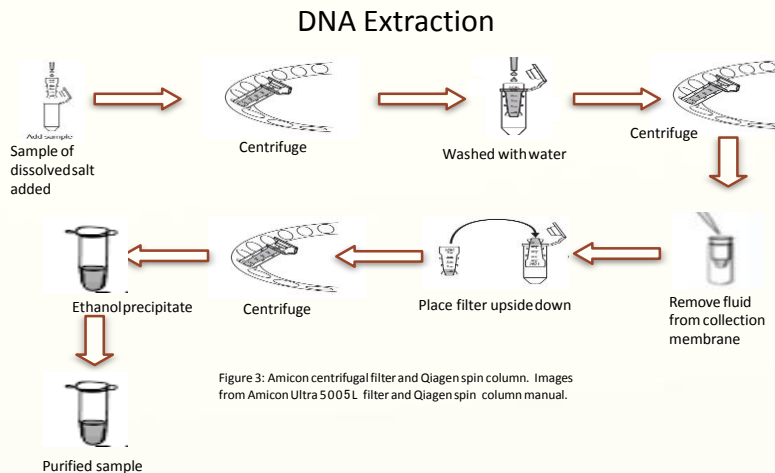


Figure 3: Amicon centrifugal filter and Qiagen spin column. Images from Amicon Ultra 5005L filter and Qiagen spin column manual.

Quantification of DNA

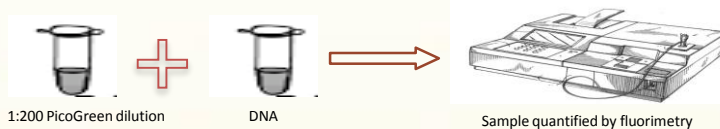


Figure 4: Images from Amicon Ultra 5005L filter and Qiagen spin column manual.

Filtration methods and DNA extraction developed for future halite 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 kit Quant-iT PicoGreen dsDNA Reagent, from Invitrogen (Carlsbad, California).

Results & Discussion:

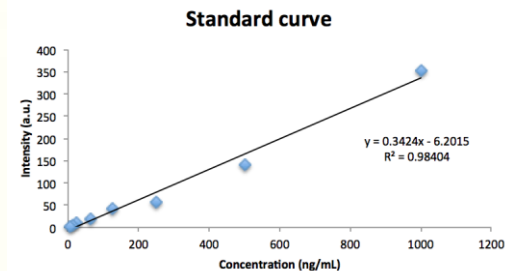


Figure 6: Standard curve generated using molecular grade DNA, PicoGreen assay, and fluorimetry. Excitation set at 490 nm, Emissions were read at 524.02 nm, and the fluorimeter was set on high sensitivity.

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, 25pg/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 Herring sperm. After this, we will use environmental samples of DNA from modern Great Salt Lake halite 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:

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Sankaranarayanan K, Timofeeff MN, Spathis R, Lowenstein TK & Lum JK (2011) Ancient Microbes from Halite Fluid Inclusions: Optimized Surface Sterilization and DNA Extraction. *PLoS ONE* 6(6): e20683. doi :10.1371/journal.pone.0020683

Vreeland, R. H., Rosenzweig, W. D., & Powers, D. W. (2000). Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*, 407(6806), 897-900.

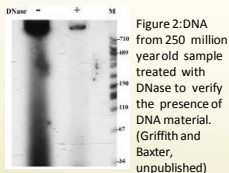
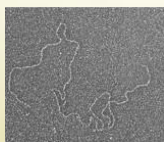


Figure 1. Electron microscopy of ancient DNA fragments from liquid inclusions of salt crystals (Baxter and Griffith, unpublished).

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 5: Timeline of available salt samples for testing.

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