A More Effective & Efficient Method of Barcoding Tardigrades

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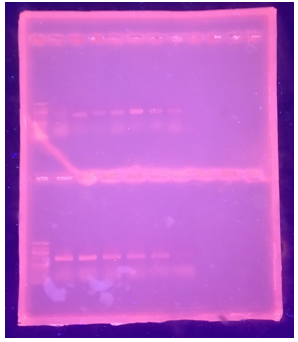
Abstract:
Tardigrades are microscopic organisms found in various diverse ecosystems stretching across the world, and are known for their resilience as well as versatility of the tardigrade which allows for survival in extreme conditions and environmental stress. Their resilience against exposure to environmental pressures allows for them to survive and exist in all different types of regions. Our objective was to determine the most efficient method to barcode such a diverse group of organisms and to determine a methodical approach to preparing the DNA sent off to be sequenced. We tested different DNA isolation processes such as bead-beating and silica based extraction. We also created our own set of primers and tested whether the amount of tardigrades affected DNA yield. Overall, we concluded that the use of the invertebrate primers from Cold Spring Harbor were far too different from the DNA sequences in tardigrades for it to sit-down on the gene it is supposed to anneal to.

Introduction:

The goal of our project was to find the most effective and efficient way(s) to extract tardigrade DNA through two aspects of DNA isolation. First, because of Tardigrades' microscopic size, we wanted to find out how many tardigrades are needed at minimum in order to get sufficient DNA extracted. Second, we were interested in testing a few different extraction methods in order to find out whether some produced better results than others.

We encountered an issue with Cold Spring Harbor invertebrate primer provided, which did not match with the tardigrade DNA, so PCR did not occur. Our mentor, Dr. Ellen Jorgensen, created a matching primer by using the NCBI and BLAST databases to compare the DNA of two tardigrade species we suspected ours to be *Hypsibius dujardini*, and ordering primers that matched their common sequences.

Materials and Methods:
Extract different amounts (5 & 10) of tardigrades using a transfer pipette. We used different methods of DNA isolation to separate the DNA from the tardigrades collected. These methods and protocols include silica DNA isolation provided by Cold Spring Harbor Laboratory, the Qiagen DNeasy Blood & Tissue Kit, and the *Quick*-DNA Fungal/Bacterial Microprep Kit (bead beating). The cold spring harbor primer (which didn't produce results) binds to the cox1 gene. So, getting the gene sequences from *Ramazzottius varieornatus* and *Hypsibius dujardini* tardigrades, we compared them to the CSHL invertebrate primer, finding that *Ramazzottius varieornatus* partially aligned, while *Hypsibius dujardini* didn't. Because the primer didn't seem to work, we decided that we probably had *Hypsibius dujardini* tardigrades, and started working on making a primer for that. We matched the the two tardigrade species and chose two regions around the CSHL primer site in the cox1 gene, and ordered primers with those sequences.



Results:
Originally using the Cold Spring Harbor invertebrate primer, we received no results. We created two new primers designed to match the tardigrade DNA at two different regions, which produced positive results. Overall, we also discovered that one tardigrade could produce enough DNA for gel electrophoresis.

Discussion:
We discovered that the invertebrate primers from Cold Spring Harbor were unable to sit down properly on the intended gene, which made the amplification unsuccessful. To remedy this problem, we compared the gene sequences of two different species of tardigrades, Hypsibius dujardini and *Ramazzottius varieornatus*. By comparing these sequences, we were able to come up with two potential primers that could work better than the ones originally given to us. One of the primers were superior to the others, as the gel showed more light bands compared to DNA amplified with the other primers. This means that the Cold Spring Harbor primers are not able to amplify DNA as effectively as they can. If it is difficult to barcode tardigrades which are such common angles, it must be even more difficult to barcode other microscopic organisms. By discovering the problems primers have on barcoding DNA, we can inform other experiments that some primers are made for too large of a group, and thus, to obtain desired results, it would be more effective to make primers that are more specific towards the species.

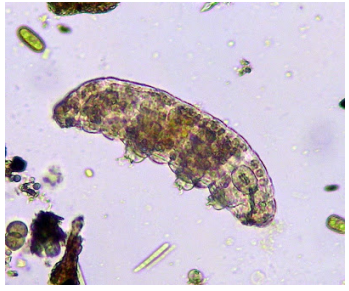


Image: Phil. (1970, January 01). Water bears in space - and on my garage roof. Retrieved from http://beyondthehumaneye.blogspot.com/2009/04/water-bears-in-space.html

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