RECLAMATION
Managing Water in the West

U.S. Department of the Interior
Bureau of Reclamation

Molecular Methods for the Ecological Research Laboratory

Using eDNA to detect invasive species

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The amplification of environmental DNA allows researchers to detect invasive species from water samples. There are several different DNA amplification methods that can be used including polymerase chain reaction, quantitative PCR, and loop mediated isothermal amplification (LAMP).

Mission Issue

Molecular methods can be used for the early detection of invasive species, such as quagga and zebra mussels, which important for giving Reclamation facilities early warning of their presence.

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Problem

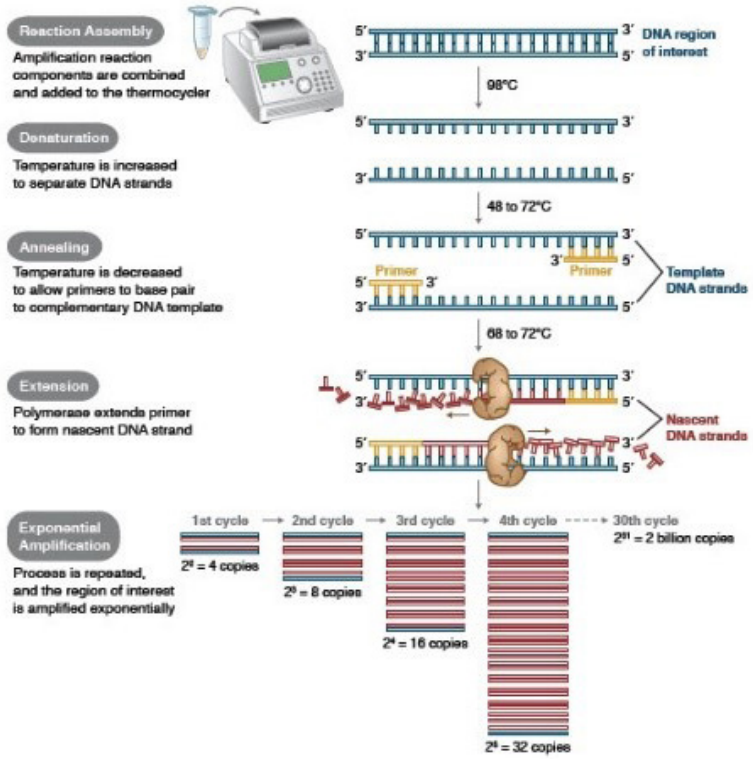
Advancing molecular methods for the detection of invasive species is an ongoing process. Detecting environmental DNA by polymerase chain reaction (PCR) continues to change as new methods and technologies are developed. The recent purchase of a quantitative polymerase chain reaction (qPCR) instrument has increased the sensitivity of the assays performed by the Ecological Research Laboratory. Understanding both the limitations and advantages of these amplification technologies is important if Reclamation researchers are going to stay at the forefront of eDNA analysis.

This research looked at three different DNA amplification methods: conventional PCR, qPCR, and LAMP assays to determine which one would work best for the detection of quagga and zebra mussel eDNA. Early detection of these two organisms is important because it gives Reclamation facilities time to plan for a potential mussel infestation. Quagga and zebra mussels can impact dam facilities by clogging pipes and trash racks. They also cause significant environmental damages.

Solution

The Ecological Research Laboratory has for years performed PCR on bulk water samples for the early detection of quagga and zebra mussel environmental DNA. Over the last three years, well over a thousand bulk water samples have been analyzed for the presence of eDNA from quagga and zebra mussels. The method has been well developed and gives reliable results. DNA results can be obtained from single suspects using the methods that have been developed. The recent purchase of a qPCR instrument means that new primers and methods must be developed for the analysis of the eDNA samples. There have been several published methods for qPCR analysis of quagga and zebra mussel eDNA.

The bulk of the work done for this project was to determine which master mix to use for the qPCR analysis. The best method for setting up and the running the analysis on the qPCR instrument. And finally, how to interpret the results. Determining the controls, number of replicated, and standards to use for with this instrument will be an ongoing process. Using qPCR, it was possible to analyze single suspects and get positive findings. Using LAMP assays for the early detection of quagga and zebra mussels had mixed results. Some of the primers and reagents used did not consistently give reliable results. Also, the positive results can not be sequenced to provide conformation of a finding. Only a few of the single suspects were positive by LAMP assay. By exploring the LAMP assay we were able to assess both its limitations and advantages.



Conventional PCR components and process showing the steps. Source: New England Biolabs.

"Using DNA amplification methods such as qPCR and LAMP assays it is possible to detect eDNA from invasive species which allows managers to have more time to plan for the impacts of organisms that could cause damage to both facilities and the environment."

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More Information

https://www.usbr.gov/research/projects/detail.cfm?id=1748

Application and Results

For the last three years conventional PCR has been used to analyze bulk water samples for the presence of quagga and zebra mussel eDNA. This has been performed on well over a thousand samples. With the purchase of a qPCR instrument a switch over to this new technology is underway. Experiments were performed to determine which master mix buffer to use in with the qPCR instrument. The analysis of eDNA samples from water bodies that are known to be positive to quagga mussels showed that the qPCR method could detect more positives than conventional PCR. A third amplification method, LAMP assay, was also compared to conventional PCR.

The LAMP assay was did not perform as well as conventional PCR and qPCR with dilution curves of quagga and zebra mussel DNA. Another issue with LAMP assays is that the resulting amplification product cannot be sequenced. Having DNA sequencing results is a second conformation for positive PCR results. The move to using qPCR will be ongoing for the Ecological Research Laboratory as our knowledge of this method continue to expand.

Future Plans

For next year, additional research will be done to assess using environmental RNA to detect quagga and zebra mussels. The research that started in this project will continue to be advanced as the qPCR instrument will be used to detect quagga and zebra mussels in bulk water samples from across the western United States.