Using the Primers ITS4, ITS5, Bd1a, and Bd2a to confirm the identity of Samples of *Batrachochytrium dendrobatidis*

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INTRODUCTION

Chytridiomycosis is a disease that is lethal to the amphibian population that is caused by the fungus *Batrachochytrium dendrobatidis (Bd).* The upcoming research that will be performed to determine if there is a correlation between the quality of a stream and the amount of *Bd* present in the amphibian population of the connected stream will need us to be able to confirm that the results obtained are not altered in any way from mistakes or outside contaminant.

A contaminant could yield a false negative which, if present, would cause there to be no way to know as it would be assumed that since no positive results were obtained that there wasn't any Bd present on the amphibians that were the source of our samples. Since it would be assumed that there wasn't any Bd present on the amphibians in a certain stream it would damage the accuracy of our results. The quality of the stream should have a correlation with how many afflicted amphibians are present in the stream and if a false negative was obtained from a poor stream while other poor streams had high levels of Bd within them then the results wouldn't be definitive as there was an aberration in the results. This section of the research was preformed to be sure that the two groups could produce the same results that were known to be the correct outcomes.

MATERIALS & METHODS

Using isolated Bd DNA a process was created that will be utilized to assist in confirming the results of tests on samples that will be taken from amphibians in various streams in north Georgia. An agarose gel was made that contained ethidium bromide within it. Samples of Bd DNA of 2µL were combined with 3.75µL of magnesium chloride, 2.5µL of dNTP's, 2.5µL of taq buffer, as well as, 6.125µL of distilled water, 1.25µL of taq polymerase and 2.5µL of each of the primers in their pairs being sure to add only the Bd primers to a tube and not adding the ITS primers to the same tubes that contain the Bd primers. Once the tubes had everything added to them they were placed into a PCR thermocycler to amplify the amount of Bd DNA.

The obtained amount of DNA then had 10µL of loading dye added to the tubes. After the loading dye was added they would be vortexed and centrifuged to mix the loading dye and DNA together, then the mixture would be loaded into the gel that was previously made. This gel would then be run in an electrophoresis chamber for 45 minutes on 102 volts. Previously the gel was ran for 30 minutes but the bands on the resulting gel were too close in proximity and increasing the run time would be favorable in regards to being able to measure the distance of the bands. Once the gel was ran we placed the gel on an ultraviolet lightbox to observe the bands created by the ethidium bromide staining. The band distances were then measured and recorded.

RESULTS

After editing our procedure for running the gel multiple times the final results were produced. In our gel we saw that the bands for Bd1a/2a were the same for each of the loads of Bd1a/2a. For the ITS4/5 bands both were alike in distance, just like the bands for the Bd1a/2a bands. The bands for ITS4/5 and Bd1a/2a were at separate distances with the Bd1a/2a bands traveling further from the wells than the ITS4/5 bands had traveled.

The bands for the molecular marker were much more separated than when we only had the gel run for thirty minutes instead of forty-five minutes. In the lane where the sterile water was loaded into there wasn't a band present, this was expected since there was no Bd DNA in that load that the ethidium bromide could bind to. Since no ethidium bromide was bound in that lane, when we put the gel on the ultraviolet lightbox nothing was visibly changed from the original gel. If there was something that the ethidium bromide was bound to, when placed on the UV lightbox it would glow a pink color that indicates there is DNA in that sample. In the upcoming research ethidium bromide will be extremely useful in assisting in making the bands more defined so it is possible to measure the resulting gel more easily.

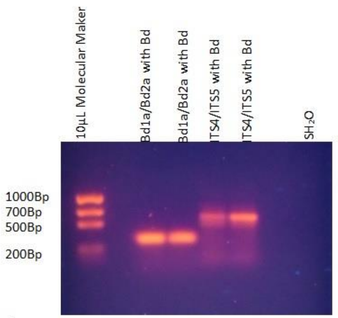

Figure 1:
This gel was run at 102 volts for 45 minutes. The gel contained ethidium bromide that was used to bind to the DNA that was run through the gel, once under ultraviolet light the bands would show up a pink color that would be more distinguishable than if we had just run the samples with Bromophenol blue dye.

RESULTS

Load	Lane	Distance (in millimeters)
10µL molecular marker	1	25, 28.5, 30.5, 36
Empty	2	Empty
Bd1a/2a with Bd DNA	3	34.8
Bd1a/2a with Bd DNA	4	34.8
ITS4/5 with Bd DNA	5	29.5
ITS4/5 with Bd DNA	6	29.5
Empty	7	Empty
Sterile water	8	No bands present

Figure 2:
The above measurements were taken after the various samples of Bd DNA were run through the electrophoresis chamber. The table goes with the picture of the gel. The left side of the picture corresponds to lane one on the table, the far right side of the picture corresponds with lane eight.

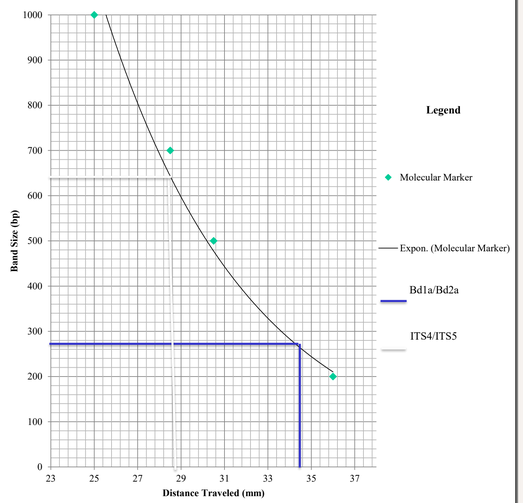

Figure 3:
The provided graph presents the distance of the bands from the molecular marker and their known size in base pairs. The graph can be used to determine how many base pairs the bands of the Bd1a/Bd2a and ITS4/ITS5 had.

CONCLUSIONS

The final gel was very promising, the goal was to develop the best possible technique to obtain bands on the gel. This procedure brought results that were optimal for the upcoming research on the Bd fungus. If the future research was to yield any results that provided us with a false positive, it may cause conflicts with the results of gels from other streams that we collected from. Then the conflicting results would raise more questions about the research as well as prolong our period of time that the research was performed.

By looking at the distance traveled by each of the primers used it is seen that the ITS4/5 primers traveled less distance than the Bd1a/2a primers. This suggests that the amount of base pairs the ITS4/5 primers contain is a higher amount than the Bd1a/2a primers. This can also be confirmed by graphing the distance of every band of the molecular marker their corresponding distance and amount of base pairs the band contains. After graphing the known values of the molecular marker see how far the bands traveled and then trace the graph to that distance and record the number of base pairs that correspond to that distance of the bands.

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