# Detecting *Bridgeoporus nobilissimus* in Wood Cores Using a Genetic Test: A Test of a Core Processing Method and Method Sensitivity

Matt Gordon, M.S.
Molecular Solutions, LLC
May 8, 2009

## Introduction

A genetic marker for the rare fungus *Bridgeoporus nobilissimus* has been used to detect this fungus in wood cores taken from stumps, snags, and living trees (Gordon 2008). Several questions were raised by this work. In order to sample a tree, at least four core samples are needed (Gordon, 2008). Testing would be more effective and efficient if all core samples from one tree could be mixed, sampled, and tested, rather than testing each sample individually from single points within the core samples. However, if only one core is positive, the dilution of the fungal DNA with uncolonized wood may cause the test to produce a false negative result. Another issue is mixing. If the wood from four cores is mixed, the fresh weight will be about 17g (dry weight about 12g). In a typical DNA extraction protocol, only 0.2 to 0.4 g of material is extracted at a time. If mixing is not thorough, some 0.2 g subsamples may not contain the *B. nobilissimus* DNA even though it is present in the main sample. Additionally there were questions about the amount of processing necessary to prepare the wood samples for DNA extraction.

In this study we defined a sample processing protocol for wood cores and tested its sensitivity and consistency among subsamples. We found that the method tested here works well, uses inexpensive equipment, and requires less time than other published methods.

## Materials and Methods

The core sample preparation protocol used in this study is given in the Appendix to this report. We tested this protocol by making up batches of cores with a known amount of *B. nobilissimus* colonized wood. In May of 2007 a small piece of moist rotten wood (~5g) adjacent to a *B. nobilissimus* sporocarp was removed from a large stump. Visually the wood appeared to be colonized by a fungus and genetic testing proved that *B. nobilissimus* was present in the wood. After testing, this piece of wood was stored in a zip-lock plastic bag in a refrigerator. In October of 2008 another subsample of this wood was tested, and it was found to still be positive, and its appearance had changed very little from when it was collected. There were no visual signs or smells of bacterial or mold growth.

Since this wood seemed to be well-colonized by *B. nobilissimus*, we used this as the known amount of colonized wood in our test batches. Uncolonized wood was taken as 0.25” x 12” cores from two *Abies amabilis* and two *A. procera* trees growing in an area where no *B. nobilissimus* was known to be present. Subsequent testing showed that the core material did not contain *B. nobilissimus*. Core samples from both trees were mixed together, and were weighed

and stored in paper envelopes in a refrigerator. The cores were weighed 8 days later, just before they were used, and were found to have lost about 30% of their weight while stored in the refrigerator.

Five test batches were prepared by placing the desired weight of wood cores and colonized wood into a 100 W electric coffee grinder. The concentration of colonized wood in each batch was varied, and ranged from 0.9% to 15% on a fresh wood basis. The weight of wood used in the 15% batch was equivalent to the weight of one wood core. Multiples of this weight were used to make up the more dilute batches. Each batch was ground for 60 s or 90 s. Grinding was done in 30 s intervals with 30 s of rest in between to prevent heat build-up in the sample. No heat build-up beyond a mild warming was detected after 90 s of grinding (with two rest periods), and this was enough time to thoroughly grind the largest samples (8 cores, 34g). The grinder was washed with soap and water and dried between samples. To test the effectiveness of the cleaning method, a negative control batch of wood (no colonized wood) was ground immediately after the batch with the highest concentration of colonized wood.

For each batch, four subsamples of ground wood were removed from the grinder and placed in 2 ml microcentrifuge tubes. Two subsamples were taken from the negative control batch. One sample of colonized wood, with no added wood core material (the positive control) was taken. DNA was extracted from each subsample with a standard Chelex method and all DNA samples were cleaned with the UltraClean DNA purification system (MoBio Laboratories, Carlsbad, CA). DNA samples were then run in PCRs with the *B. nobilissimus*-specific primers developed by Redberg et al. (2003). Reaction products were electrophoresed on an agarose gel at 1.5V/cm for 1 hr, and the gel photographed while illuminated by UV light.

**Results**

Table 1 shows the concentration of colonized wood used in each batch and the results of testing each subsample from each batch. All subsamples of all batches were positive, except for the two subsamples of the negative control batch. These results indicate that the methods used provide a very sensitive test for the presence of *B. nobilissimus*, and that the sample preparation method tested here provides a uniform distribution of fungal DNA in the final sample. One subsample (~0.22 g) was sufficient to detect 0.30 g of *B. nobilissimus* infected wood in 8 combined cores (~34 g). The results also indicate that the equipment cleaning method used in this study is sufficient to avoid cross contamination of samples.

Although dry cores were used in this study, fresh cores can also be used. In fact, trials with fresh cores showed that they are ground to a uniform consistency more quickly than dried cores. The fact that a piece of *B. nobilissimus*-colonized wood has maintained a significant level

of *B. nobilissimus* DNA after two years of storage in the fresh state indicates that this is an excellent storage method for cores to be tested for *B. nobilissimus* DNA.

Table I Composition of samples tested for *B. nobilissimus* DNA, and results of the testing.

| Batch Number | Colonized Wood wt (fresh) (g) | Core Wood wt (fresh basis*) (g) | Dilution (fresh basis) | Sub-sample | Sub-sample wt (g) | DNA Test Result |
|---|---|---|---|---|---|---|
| 1 | .62 | 4.3 | .15 | 1-1 | .20 | + |
| 1 | | | | 1-2 | .22 | + |
| 1 | | | | 1-3 | .20 | + |
| 1 | | | | 1-4 | .19 | + |
| 2 | .60 | 8.6 | .07 | 2-1 | .26 | + |
| 2 | | | | 2-2 | .25 | + |
| 2 | | | | 2-3 | .30 | + |
| 2 | | | | 2-4 | .20 | + |
| 3 | .60 | 17.2 | .035 | 3-1 | .21 | + |
| 3 | | | | 3-2 | .23 | + |
| 3 | | | | 3-3 | .20 | + |
| 3 | | | | 3-4 | .19 | + |
| 4 | .60 | 34.4 | .017 | 4-1 | .21 | + |
| 4 | | | | 4-2 | .23 | + |
| 4 | | | | 4-3 | .20 | + |
| 4 | | | | 4-4 | .20 | + |
| 5 | .30 | 34.4 | .009 | 5-1 | .23 | + |
| 5 | | | | 5-2 | .23 | + |
| 5 | | | | 5-3 | .21 | + |
| 5 | | | | 5-4 | .22 | + |
| 6 | 0 | 4.3 | 0 | 6-1 | .22 | - |
| 6 | | | 0 | 6-2 | .22 | - |
| 7 | .10 | 0 | 1.0 | 7-1 | .10 | + |

*Dry wood was used for wood cores, but the equivalent weight of fresh wood is given here.

Figure 1 and Table II show the results of individual PCRs. Subsamples 7-1, 2-1, 2-2, and 2-3 were run on a preliminary gel and are not shown in Figure 1, however the results for these subsamples are given in Table I.

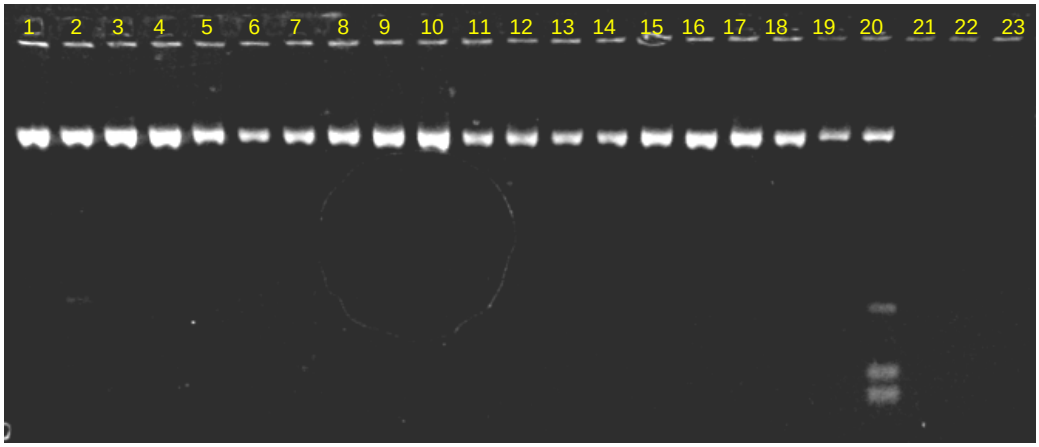


Figure I Photograph of the gel of reaction products after PCR with *B. nobilissimus*-specific primers. Subsample identities are given in Table II.

Table II Subsamples shown in Figure 1.

| Lane | Subsample |
|---|---|
| 1 | 1-1 |
| 2 | 1-2 |
| 3 | 1-3 |
| 4 | 1-4 |
| 5 | 2-4 |
| 6 | 3-1 |
| 7 | 3-2 |
| 8 | 3-3 |
| 9 | 3-4 |
| 10 | +C * |
| 11 | 4-1 |
| 12 | 4-2 |
| 13 | 4-3 |
| 14 | 4-4 |
| 15 | 5-1 |
| 16 | 5-2 |
| 17 | 5-3 |
| 18 | 5-4 |
| 19 | 5-3 # |
| 20 | 5-4 # |
| 21 | 6-1 |
| 22 | 6-2 |
| 23 | PCR blank |

*+C is a positive PCR control. The DNA in this reaction was from a *B. nobilissimus* sporocarp.

#All reactions used 2 microliters of DNA except reactions 19 and 20 which used 4 microliters.

## Discussion

In this study we tested a relatively simple method for preparing wood cores for genetic testing for *B. nobilissimus* DNA, and then we tested the dilution detectability of the *B. nobilissimus*. The method produced a sample in which the *B. nobilissimus* DNA was evenly distributed so that one subsample (~0.22 g) taken from 4 combined cores (~17 g) of one tree was sufficient for determining the presence of *B. nobilissimus*. The PCR method used to determine the presence of *B. nobilissimus* was very sensitive. This dilution study shows the method can detect 0.9% of B. nobilissimus colonized wood in a sample of non-colonized wood. The lower limit of detection by this method was not established because all samples tested were positive. Since the *B. nobilissimus* likely makes up a small proportion of the total weight of the colonized wood, the method appears to be very sensitive to the presence of *B. nobilissimus*.

## Acknowledgements

Carol Apple, a biometrician from the USDA Region 6 Forest Service, Regional Office in Portland, Oregon designed the testing plan and provided the sample test matrix used (with modification) in this study.

Kelli Van Norman, Inventory Coordinator Interagency Special Status/Sensitive Species Program OR/WA BLM & R6 Forest Service, and C. Apple provided valuable suggestions for the improvement of the manuscript.

## References

Gordon, M. 2008. Field methods for the detection of *Bridgeoporus nobilissimus* DNA in trees, stumps, and snags. Final report submitted to USDI Bureau of Land Management, Oregon State Office. Contract number HAP084563. On file with: Interagency Special Status/Sensitive Species Program, USDI Bureau of Land Management, Oregon State Office, Portland, Oregon; USDA Region 6 Forest Service, Regional Office, Portland, Oregon. http://www.fs.fed.us/r6/sfpnw/issssp/documents/inventories/inv-rpt-fu-brno-field-methods-for-detection-2008-12-09.pdf

Redberg, G.L., D.S. Hibbett, J.F. Ammirati, and R.J. Rodriguez. 2003. Phylogeny and genetic diversity of *Bridgeoporus nobilissimus* inferred using mitochondrial and nuclear rDNA sequences. *Mycologia,* 95(5): 836–845.

## Appendix

### Wood Core Sample Preparation for Genetic Testing

Sample Storage

After collection, wood cores should be stored in a zip-lock plastic bag and kept in a cool, dark place. A refrigerator or cooler is ideal. If this is not possible, air drying of cores and storing in an envelope in a dry location is a second storage option.

Sample Processing

Place all cores for one sample in a 100W (or greater) electric coffee grinder. Grind the cores until the wood is a uniform consistency with no recognizable chunks of core remaining. Grind for at least 60 s to insure uniform mixing. If heating of the sample is noticed, break the grinding time into shorter intervals with 30 s of resting time between grinding intervals.

For DNA extraction remove at least .28 g of wood material per test (.20 g for dry cores).

Clean the grinder thoroughly between samples. One method that works is to add enough soapy water to cover the blades, run the grinder for a few seconds, then pour out the water and rinse with fresh water and dry thoroughly. After drying, run the grinder for 10 s and a small amount of water will be spun out of the rotor area. Soak this up with a paper towel.