Diagnostics of Armillaria Root Disease

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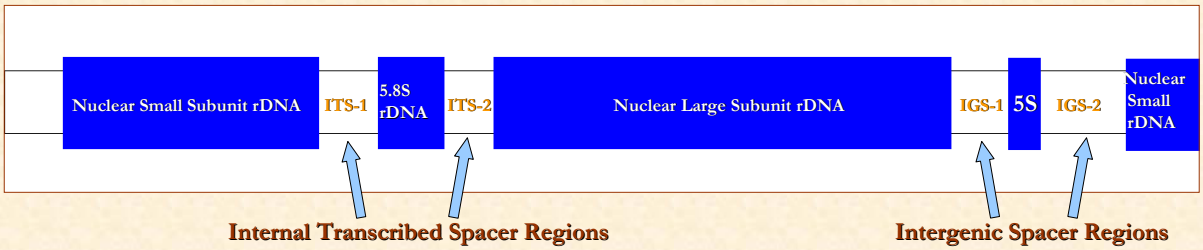
- STEP 3 -

Molecular Techniques for Identifying Pathogenic Species of *Armillaria*

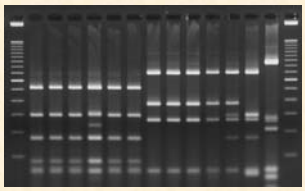
Armillaria species identification using DNA Sequencing and PCR (Polymerase Chain Reaction)

The chart below shows the different ribosomal **DNA regions** we use to help **identify species of *Armillaria*** using **DNA sequencing.**



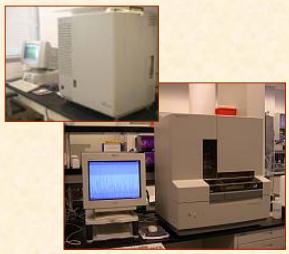
These regions are copied millions of times using PCR and then read by a machine that determines the DNA sequence for a particular isolate. We can identify the species of the isolate by examining the DNA sequence.

We follow a recipe to prepare the PCR mixture. The mixture for PCR is prepared using sterile equipment and exact recipe of reagents.



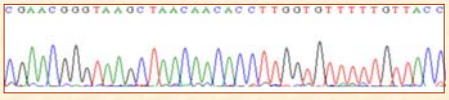
PCR samples are placed into a thermocycler. With this machine, we can program different temperature cycles that are needed to perform PCR. The DNA is amplified during this step. Gel electrophoresis is used to confirm that PCR worked.

To perform PCR, a sample of DNA must be added to the PCR mixture. Samples can be retrieved by scraping a mycelial bark fan, rhizomorph, or a young *Armillaria* culture with a pipette tip. Either way, the DNA sample must be free of contaminating organisms.



Samples are sent to a commercial sequencing center, where the DNA sequencing is performed.

We put the scraping into the PCR mixture using a pipette tip. The amount of DNA needed is so small that the tissue can barely be seen by the naked eye.



This is an example of sequence data returned to us. DNA sequencing is usually completed in 2-4 days. (This includes the whole process!)

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