DNA sampling protocol.

The method used to capture the DNA for a fungi specimen is very simple and robust. We use Whatman FTA PlantSaver cards. The FTA cards are stable for years at room temperature which makes them very easy to store. You do not have to wear gloves when carrying out this procedure but it certainly won't hurt. The main source of contamination is sample to sample contamination so making sure that any board used for cutting and the razor blade are fresh/clean is advisable.

We recommend selecting fresh young specimens for sampling without any obvious signs of mold. The longer the fruiting body has been "up" the more likely it has picked up some mold or bacteria contamination. The idea is that there is enough DNA captured by the card that it is present in great abundance compared to any contaminates. However DNA technology is very sensitive so don't place to much material on the card, less is more!

After sampling the DNA prepare a voucher with the remaining material. Send the DNA barcoding assistant entries, which contain an image in the field and GPS coordinates, completed FTA plant Saver card, paper work, digital pictures and vouchers to Richard Jacob. The FTA plantsaver cards are then stored and sent in a batch to Duke University for sequencing. Vouchers from the successful samples are sent to Dukes herbarium.

Step by step procedure

1. Cut a small slice, one or two gills or a thin section of a cup mushroom, from the sample with a clean razor blade.
2. Move the piece of mushroom to an empty square on a FTA plant saver card.
3. Close the flap of the card and either hit with a small hammer or squash with a hard object like the edge of a phone.
4. Lift up the flap and check to see if liquid from the sample has penetrated through to the riverside of the card.
5. If so carefully remove any remain material from the FTA card cover and the filter paper. Try not to scratch or otherwise damage the filter paper on the card.
6. Label the cover of the card with the sample number. And place the card back in to a plastic bag.
7. Dry the remaining sample as a voucher; see the "Preparing Mushrooms for Herbarium Storage" protocol for more information.
8. Send completed cards with paper work and images to Richard Jacob.

Western Pennsylvania Mushroom Club
Fungi, fun and friends!

Pictures of specific steps

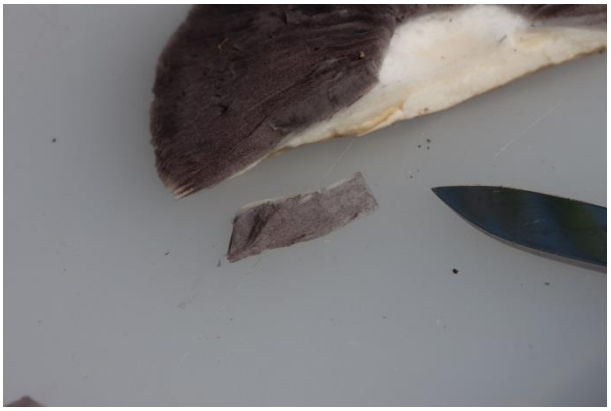

Figure 1 Cut a small slice one or two gills think.

Figure 2 Place the sample of the FTA PlantSaver card

Figure 3 Squash the sample either with a hammer blow or by squeezing a hard object over it.

Figure 4 After squashing the material check to see that the juices have soaked right through the card.

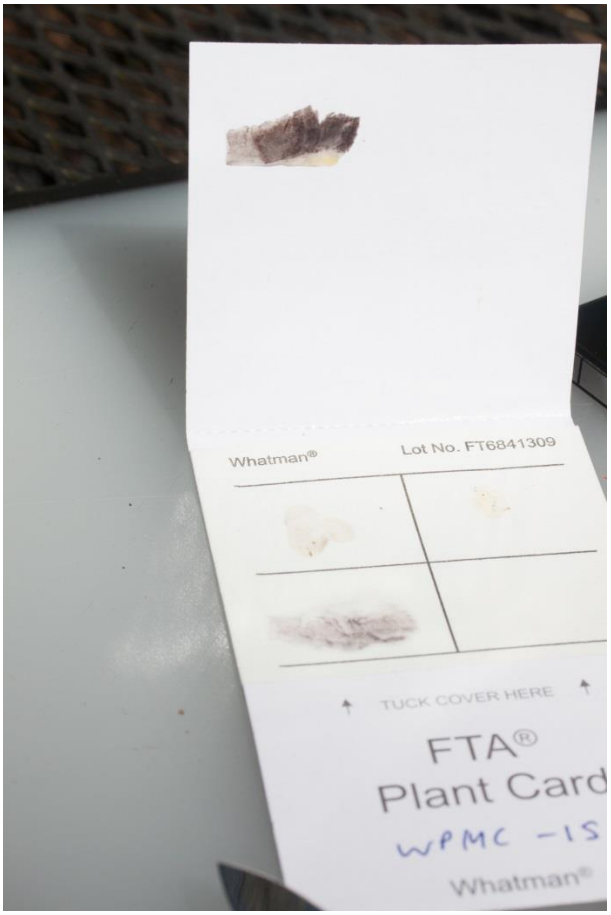

Figure 5 Remove any remaining material from the cover and very carefully from the filter paper.