These protocols are designed for fungal DNA sequencing being conducted by MAWDC volunteers.

1. Fieldwork
   1. We intend to use iNaturalist to log our observations in the field, and a good working knowledge of this app is essential to our mission.
   2. At the time of this writing there is only one active sequencing project, our collaborative work with the Audubon Naturalist Society at their Woodend facility in Chevy Chase MD. However many other projects will come online soon. When collecting **please be sure** to submit your observation to the correct iNat project.
      1. ANS Woodend: ‘Mycology of Woodend Nature Sanctuary’.
      2. Sequanota ’22: tbd
   3. The iNaturalist number will be used as the unique identifier for tracking throughout all processing steps.
   4. **Create Observation and Photograph**
      1. Use your smartphone in the field to create the iNat observation.
      2. Be sure to have GPS turned on to store location information.
      3. Avoid excessive handling of the specimen if possible.
      4. Multiple pictures are ideal, top, side and the hymenium (gill, pore, tooth, etc) at a minimum.
         1. Take photos of the mushroom in situ before digging it up.
         2. Clear away any debris, if possible, to get an unobstructed view.
         3. Once photographed in situ, carefully remove the fungi, making sure to get the subterranean bulb/volva/root too (if present).
         4. For some genera, a halved stipe and/or pileus is very helpful.
         5. If possible, include multiple growth stages, preferably in one image.
         6. If possible, fill out the field data slip and place it alongside the sample in a photograph. Note characteristics/details that may be lost during subsequent drying/processing.
            1. Substrate the mushroom was growing from (ground, wood, etc).
            2. Trees nearby for fungi on soil.
            3. Changes to flesh color upon handling.
            4. If it oozes a liquid.
            5. Results of chemical tests.
            6. Odor.
            7. Taste, when appropriate.
            8. Texture (fragile, crumbly)
         7. The photo of the field data slip is useful for capturing scale. If a field data slip is not available, try using another object such as a wristwatch or a pen.
         8. The following short [youTube video](https://youtu.be/_SaG4sIx8R0) by Sigrid Jakob is a helpful tutorial.
      5. Be sure to include the observation in the appropriate iNat project.
      6. Write the iNat number on the field data slip. The field data slip will stay with the sample throughout processing and onto long term storage, so having the iNat number documented will link the observation to the physical sample. The iNat number is the sequence number at the end of the URL (under Metadata on the info tab). *Note: a sequence number can be appended to the URL substring ‘inaturalist.org/observations/’ to quickly look-up an observation.*
      7. Place specimen and field data slip in (preferably) wax paper for short term storage.
      8. Optional: It’s possible to take higher quality photos with the camera and append them to the observation at a later time.
      9. Optional: Double extra credit for appending microscopy photos of spores and other microscopic structures.
2. Process sample(s) via Polymerase Chain Reaction (PCR)
3. Determine if PCR amplification was successful.
4. Dry sample for long term preservation.
   1. This process requires a dehydrator with temperature control.
   2. Place sample in a 110-120 degree F dehydrator until they’re cracker dry. Higher temperatures may damage DNA. This will typically take 10 or more hours.
   3. To limit cross contamination don’t overcrowd your samples. Fungi will continue to sporulate during drying.
   4. Hard ascomycetes may be airdried to limit ascospores from being discharged onto other samples.
   5. If there are many samples, place the field data slip in the dehydrator with the mushroom.
   6. Once dried, store samples and field data slips individually in Ziploc bags.
5. Send sample to lab for sequencing.
6. Analyze sequence results for potential GenBank matches.
7. Submit sample to herbarium.