



## Sample collection protocol

One of the most important aspects of the project is collecting a sample correctly. When you see a mushroom out on a walk or foray **do not** rush straight in and pick it. Instead take pictures first and note the habitat. If you have a smartphone, iPhone or Android, you can use the DNA barcoding assistant app to both take a picture and capture the GPS coordinates. It is very helpful if you can take a picture with the index card and sample number on it in the field as this then clearly links the sample to the number. Then pick the mushroom but rather than plucking it from the substrate dig it up, roots and all, or cut a piece of substrate off with the mushroom. This way you collect a complete sample as any root features that the mushroom has maybe the key diagnostic feature. Once you have dug up or otherwise collected the sample you may want to take some additional photos of delicate features that might be lost or damaged before annotation later on. Finally place the sample in a paper bag or wrap in tinfoil along with the index card/sample number.

Our numbering system looks like this:

WPMC20131220-1-1  
WPMCyymmdd-k-s

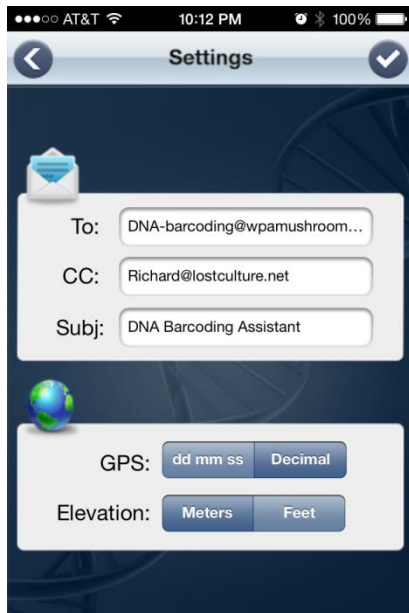
yyyy = year  
mm = month  
dd = day  
k = kit number or collector number  
s = sample number from 1 to x

The above example was collected on December 20<sup>th</sup>, 2013 using kit 1 and was the first sample of the day.

Sticker sheets can be prepared before you go collecting using the Avery online software ([http://www.avery.com/avery/en\\_us/Templates-&-Software/Software/Avery-Design--Print-Online.htm](http://www.avery.com/avery/en_us/Templates-&-Software/Software/Avery-Design--Print-Online.htm)), Use the SampleSheetGenerator.pl script to create a template for the sample Avery software. The stickers are printed on Avery sticker sheets No 08167. See the Sticker and Label printing protocol for more information.



## DNA barcoding assistant app setup



Install the application on your phone using the iTunes store or for Android devices go to <http://www.dnabarcodingassistant.org/> for download links. On the settings page set the “To” email address to [dna-barcoding@wpamushroomclub.org](mailto:dna-barcoding@wpamushroomclub.org) and the “CC” address to your own email address. Leave the subject as the default value “DNA barcoding Assistant”. GPS should be set to “Decimal” and Elevation to “Meters”. Save the new settings. At the end of the walk you can send the records using the send button. Choose actual size or equivalent for the pictures.

## Step by step procedure

1. Write the sample number on the index card and sample collection form. Or if using stickers attach them to an index card and a sample collection form.
2. If possible place the index card next to the mushroom on the ground or on the wood and take a picture with the mushroom and card. If you are using the DNA barcoding assistant app use that to take a picture too and then scan the barcode to assign a sample number.
  - o If you do not have pre-printed sample number stickers write the sample number on to the index card and sample collection form.
3. If you can tell the spore color from spores on surfaces below or on the mushroom itself note it on the sample collection sheet.
4. Take any additional pictures that you feel necessary to document the mushroom. You can use your phone or a separate camera for this. Taking at least two avoids disappointment when you find out the one picture is blurred.
5. Fill out the sample collection document. You do not need to add GPS coordinates the walk name or number is normally sufficient. You do not need to enter a species name if you do not know it. Do enter your own name though.
6. On return to the sample tables place the specimen, index card and sample collection sheet on a separate table.



7. Please either give the pictures of the mushrooms to Richard Jacob or email them to [dna-barcoding@wpamushroomclub.org](mailto:dna-barcoding@wpamushroomclub.org). If you have a lot of files we recommend using the file transfer service "We Transfer" (<https://www.wetransfer.com/>).