Fungal DNA Preparation

Background.

Extracting and analysing DNA for identification is straightforward today but still requires some precautions. In essence a pure sample as fresh as possible with minimal environmental contamination is most likely to produce an accurate identification. A full cap and stem is ideal and can be fresh or dried under a low heat (<50^{o}C) but if tissue is limited remove a sample as follows.

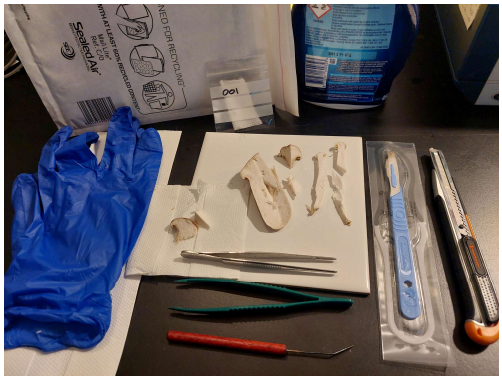
Excise a small amount of tissue. Only 100 – 200mg is needed for extraction but this is best obtained at the lab from within the tissue not the surface material. As such select a reasonable part (2-5 grams) of solid tissue to allow a clean and representative sample to be dissected away from contamination.

Gills, pores, cap and stem tissue generally work well for agarics while solid parts of other fungal structures can be productive but avoiding contamination from soil and debris. Fresh tissue is preferred but lightly dried tissue is acceptable.

While clear, visual contamination can be avoided microbiological contamination may be present in the tissues, particularly with older and decaying specimens. Samples and tissues with yeast and mould growth in particular should be avoided as the identification will be incorrect or confused. Similarly, contamination by flakes of skin may lead to a *Homo sapiens* identification so gloves are advisable when handling tissue.

Materials.

- Clean, working surface which can be swabbed with disinfectant, rinsed and dried – eg, a tile or a flat plastic lid.
- Laboratory or protective gloves.
- Scalpel or craft knife, swabbed.
- Tweezers or mounting needle, swabbed.
- Foil/kitchen tissue to wrap, unused plastic zip bag/sterile bottle for transit.
- Small, padded bag for posting.



Procedure.

1. Select a suitable sample (as noted above), ideally one which is fresh, and without residues of soil, debris or other fungal samples.
2. If processing a sample place on clean surface and cut into flesh with sanitised blade to expose tissue.
3. Remove duplicate 2 – 5 gram (~2 cm^3) samples, wrap in clean kitchen tissue to minimise condensation and place in transit container – zip bag or small plastic bottle. Include label with code for identification.
4. Forward by post to Keith Thomas, 42, Frenchgate, Richmond, North Yorks. DL10 7AG.

Notes.

- Process sample as soon as feasible. If working with a difficult sample requiring extensive key observations and microscopy consider drying a sample at early stage using low temperature (< 50°C).
- Maintain hygienic conditions and work in clean conditions when preparing, avoiding dust and airborne contamination.
- Store samples in dry conditions to minimise degradation.

What can go wrong?

- Contamination with mould, yeast or bacteria– green or black growth on surface, tissue softens. Discard and restart with fresh sample if available. Condensation from enclosed containers enhance deterioration.
- Contamination from surfaces, instruments, skin, environment.
- Tissue lacks enough DNA or is difficult to extract – eg, spores from *Gasteromycetes*, *Myxomycete* tissue.

Result formats.

1. **Simple taxonomic name** and likely ID against type sequences previously submitted to data base. >98% similarity would be a confident level, above 99% preferable.
2. **Listing of similarities** to the range present in the data base.
3. **DNA Sequence** to paste into data base for further analysis and comparison. (https://blast.ncbi.nlm.nih.gov/Blast.cgi?LINK_LOC=blasthome&PAGE_TYPE=BlastSearch&PROGRAM=blastn).

   For directions on interpretation of BLAST search see: https://fundis.org/component/sppagebuilder/41-examining-your-blast-results