UiO : Natural History Museum
University of Oslo

DNA Bank
PO Box 1172 Blindern
0318 Oslo
Norway

Phone: (+47) 22 85 18 01
nhm-dnabank@nhm.uio.no
www.nhm.uio.no

ADVICE FOR FIELD COLLECTION OF DNA SAMPLES

PREAMBLE

When collecting samples for the purpose of genetic analyses, it is important to treat the samples in a best possible way to maximize the chances of sucsessful extraction and further analyses of DNA. The current document provides some advice and guidelines for how to achieve this.

The advice have been compiled based on notably two external sources;

- Protokoll for prøvemateriale for DNA strekkoding (in Norwegian), Norwegian Barcode of Life (NorBOL)
- *Instructions: How to collect specimens and preserve their DNA*, National Center for Insect Biodiversity, NHM

See also the *Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring*, freely available at http://www.abctaxa.be/volumes/volume-8-manual-atbi.

PROCEDURE

Animals

- Use 96% ethanol (80–85% for fragile arthropods; avoid denatured alcohols) or RNAlater
- If the samples contain much water, replace the original ethanol after 3–6 hours to maintain its concentration. Depending on the size and amount of water in the animal, consider replacing the ethanol several times during the first day or two. For very large animals, take a subsample for DNA analysis
- Avoid formalin; if necessary for fixation, replace with ethanol as soon as possible and make a note about the use of formalin
- In general, if any other chemicals than ethanol have been used for fixation or other purposes, make a note about this
- For smaller animals, like smaller insects, it may also be an alternative to dry the specimens quickly, using e.g. silica gel. Use sufficient silica to completely dry the animal within 24 hours. Keep the samples at low humidity until DNA can be extracted
- Keep samples dark and cold until they can be properly frozen to prevent DNA fragmentation

Fungi

- Sample a fresh and clean part of the fungal sporocarp (e.g., basidiocarp-gills or ascocarp interior) in a breathing container (e.g., a teabag) together with the collection number
- 2-3 cm^2 will normally suffice for multiple DNA extractions
- Dry the sample at max 35°C (a slow drying process is better for DNA preservation) or in a container with silica as soon as possible after collection
- If needed, the collection can be kept in a fridge for up to 48 hours prior to drying
- In lack of a proper fungal drier or silica gel, the collection can be dried in a warm place (e.g., on a floor with heating or at the top of a fridge)
- Fungi should not be dried in direct sunlight
- Dried fungi are susceptible to insect attach
- Place the dried collection (along with the teabag) in a zip-lock plastic bag
- If you feel unsure the collection is completely dry, you may place a few grains of silica-gel alongside the fungus

Plants

- Use silica gel to ensure rapid drying of the samples; the ratio of silica:plant material should be 5–10:1. Mix silica and plant material well. The sample should be completely dry within 24 hours
- Sample fresh, green parts of the plant
- 2–3 cm^2 will normally be sufficient for 1–2 DNA extractions – make sure not to collect too litle!
- The drying will be more efficient if the sample is broken into smaller pieces, especially for succulent plants
- Keep samples dark and in plastic tubes or sealed zip-lock bags to keep them dry

Labelling of samples in the field

Although conditions may be rough and time limited during field collection of samples, it is of utter importance to label the samples adequately. Using e.g. just a single number for identification of samples leaves absolutely no room for subsequent cross-checking and correction of any errors. It is therefore strongly advisable to label the samples with several independent pieces of information, to facilitate subsequent error detection and hopefully correction.
Field samples should therefore include at least the following information:

- Taxon
- Sample ID
- Locality
- Coordinates
- Collection date
- Collector

Pre-labelling of sample containers with some of the required information (e.g. running numbers/IDs, locality, collector, …) can save some time in the field.

Geographical coordinates – what to include

Everyone would agree that it is important to register the position (geographical coordinates) of sampling localities, yet many fail to do so properly.

For a set of coordinates to be complete, it is not enough to include just the two groups of numbers in a UTM or MGRS reference, or in fact not even the degrees, minutes and seconds in a Lat/Long reference. Important information such as grid zone, 100,000-meter square identification and geodetic datum is often missing.

Detailed information about coordinate systems, datums etc. can be found elsewhere; for coordinate systems links to Wikipedia are included in the table below, and for datums here.

Complete geographical references/coordinates should contain the following information (all examples and precision figures referring to the southern end of the Zoological Museum building, NHM):

Required data	Examples	Precision	Comments
Lat/Long (Latitude & Longitude)			**Wikipedia**
Latitude & longitude	59.91948N, 10.77111E	4 decimals: < 11 m 3 decimals: < 111 m	Alternative notations: Decimal degrees (D), Degrees & decimal minutes (DM) and Degrees, minutes & decimal seconds (DMS). Always remember to include N or S AND E or W
	59 55.169N, 10 46.267E	2 decimals: < 20 m 1 decimal: < 185 m	
	59 55 10.1N, 10 46 16.0E	1 decimal: < 3 m	
Geodetic datum	WGS84		
MGRS (Military Grid Reference System)			**Wikipedia**
Grid zone indicator	32V		
100.000-meter square identifier	NM		
Easting & Northing	99021, 43768 990, 438	1 m 100 m	Number of digits determines precision level
Geodetic datum	WGS84		
UTM (Universal Transverse Mercator)			**Wikipedia**
Grid zone indicator	32V		
Easting & Northing	599021, 6643768		Number of digits is fixed – *i.e. do not manipulate this!* - and has no relation to precision level
Geodetic datum	WGS84		

The preferred coordinate format of the DNA Bank is Lat/long, but other formats are also accepted **given that they are complete**. Whichever format you choose, be sure to be aware of your choice and include this information in the field data! On GPS devices you may usually choose both which coordinate system and datum to use, and you may also later change this to show your previously recorded data in another system.

Geographical coordinates – trueness and precision

Another relevant topic is trueness (also referred to as accuracy) versus precision. Precision refers to the precision level of the measured coordinates, as explained in the table above. On a GPS device, this will typically be represented by a number saying that the precision of the position presented is e.g. +/- 3 m. Trueness refers to how close the measured coordinates are to the true location or sampling point.

If you know that a sample has been taken from a certain small island, but you don't know exactly where, you may register the precise coordinates of e.g. the centre of the island to represent the sampling locality, while still knowing that the true sampling locality may be somewhere else on the island. In this case the precision may be high (e.g. 3 m) but the trueness rather low (e.g. the radius of the island).

The opposite example would be if you knew exactly where the true sampling locality was, but for some reason the precision of the coordinates registered was low (e.g. due to bad satellite coverage or a bad or too large-scale map).

Some time you might also want the precision to be low, for instance if you want to register a locality with a certain geographical extent, e.g. the whole of an island. However, the best option would then be to register the precise coordinates of e.g. the centre of the island or the sampling area, but indicate the extent of the island as trueness = e.g. 500 m (i.e. the radius of the island or sampling area).

Both trueness and precision should be included in the sample data.