COLLECTION PROTOCOLS – BIOPSY/ TISSUE SAMPLES

Part I: Protocols for Field Storage and Transport to the Frozen Ark Laboratories

Number of specimens:

Ideally, each species should be represented in the frozen collection by *fifteen* to *twenty* separate individuals (with each individual if big enough represented by several separate tissue samples from different organs). If there are samples of the species from several localities, it is desirable to have the localities equally represented in the frozen collection. This arrangement will give valuable information about variation within the species.

In cases where the specimen is too small to allow several separate samples to be taken from one individual, then a proportionately larger number of individuals should be preserved. We realise that it will not always be possible to obtain the ideal numbers of species and samples. A single sample from an endangered species is better than none at all, but multiple samples are highly desirable.

Specimen Preparation:

Ideally, specimens should be transported whole. If this is not possible (e.g. excessive size) enough material should be provided to allow for 12 tissue slices (or more) to be taken. The minimum size for each tissue slice is 1mm x 2mm but we recommend tissue slices of 2mm x 4mm where possible.

It is essential that aseptic conditions are used during sample preparation. In particular, it is extremely important that fresh sterile scalpel blades, etc. are used for each specimen when taking samples of tissue in order to avoid cross contamination.

Labelling Specimens:

All Specimens collected in the field should be labelled in two ways:
1) Using a permanent marker pen on the exterior of the collection tube
2) Using a pencil (or Indian Ink marker) with the label written on waterproof paper and stored with the specimen inside the tube.

Note that permanent marker will wash off in ethanol!

Field Preservation and Transport:

1) Live Collections:

Where possible, specimens should be transported live to the Frozen Ark laboratory. If there is any uncertainty over the survival of specimens then it is essential that specimens are preserved to prevent the degradation of DNA using one of the protocols given below.

2) Freezing Samples:

Specimens can be frozen in the field (either in liquid nitrogen or with dry ice) and then transported frozen to the laboratory. As dry liquid nitrogen shippers use a vapour-phase freezing process and contain no liquid, they are accepted for travel on aeroplanes. The dry shipper will remain at -150 ◦C for approximately three weeks and accommodate a variety of containers. Alternatively, specimens can be transported on dry ice, but must be labelled appropriately and shipped via an acceptable courier service.

Note: This method is not suitable for the preservation of live cells. For cell preservation, samples must either be returned live to the Frozen Ark laboratory or the Frozen Ark tissue cell preservation protocols (Part II, Appendix 1) must be followed in the field prior to freezing.

3) Collecting in Ethanol:

Although not quite as effective as freezing, ethanol provides an extremely useful field alternative for DNA preservation. Specimens should be preserved in pure 95% - 100% ethanol (not IMS or alternatives). It is very important that the ethanol is changed several times. The specimens should be rinsed twice in ethanol and again after 24 hours and until the ethanol is clear (not cloudy). For shipment of ethanol preserved specimens, place a wad of cotton wool in the tube, allow to soak with ethanol, then drain off the remaining liquid ethanol immediately prior to transit. In order for preservation to occur, it is imperative that the ethanol penetrates the tissues. Particular care must therefore be taken when the organism is enclosed (e.g. although pulmonate land snails preserve very well in ethanol, prosobranchs are problematic as the opening of the shell is blocked by an operculum).

Ethanol preservation is not static and DNA will degrade in ethanol at room temperature over time. If a freezer or refrigerator is available, store specimens in ethanol at either -20 ◦C (better) or at 4 ◦C.

Note: This method is not suitable for the preservation of live cells. For cell preservation, samples must either be returned live to the Frozen Ark laboratory or the Frozen Ark tissue cell preservation protocols (Part II, Appendix 1) must be followed in the field.

For further advice please contact info@frozenark.org