

Divisions of Mammals, Amphibian and Reptiles, and Birds – Procedures for Genetic Sampling and Tracking.

GENETIC SAMPLING

Sampling Guidelines

1. Collect tissues as soon as soon as possible after death (preferably < 1 hour).
2. Use separate 2 ml cryo-vials for each tissue type
3. If the collection is to be split, collect duplicate tubes for all samples.

Mammal Specific Protocols

- Collect samples of skeletal muscle and liver regardless of preparation type.
- If liquid nitrogen or ultralow freezers are available, store tissues in tubes without chemical preservatives.
 - a. Fill tubes to mark on vial and secure cap.
 - b. Ink Biorepository (BR) # on tube.
- If cold storage is not immediately available (i.e. when in the field) use chemical preservatives (DMSO, EtOH, RNA later).
 - a. Do not take too much tissue, cut samples into small pieces
 - b. Etch BR# on prefilled tube with scribe

Amphibian and Reptile Specific Protocols

- Collect samples of skeletal muscle, liver, and/or heart regardless of preparation type.
- Toe-clips, scale-clips and tail-clips can be take if bag limits are exceeded (following permit regulations). Take photos to use as eVoucher if possible before releasing individuals.
- If liquid nitrogen or ultralow freezers are available, store tissues in tubes, if chemical preservatives are used (e.g. DMSO/EDTA) allow appropriate time for saturation (~1 hr) before freezing.
 - a. Fill tubes to mark on vial and secure cap.
 - b. Place Biorepository (BR) # sticker on tube.
- If cold storage is not immediately available (i.e. when in the field) use chemical preservatives (DMSO, EtOH, RNA later).
 - a. Do not take too much tissue, cut samples into small pieces
 - b. Etch Field ID # on prefilled tube with scribe

Bird Specific Protocols

- Collect different tissues depending on the type of specimen, and store them as follows:

- 1) Fresh specimens
 - a. Tissues removed as soon as possible after death (preferably <1 hr)
 - b. Tissue types
 - i. Muscle (if preparing fluid specimen only take this type)
 - ii. Heart
 - iii. Liver
 - iv. Kidney
 1. If small bird and more tissue needed
 2. If requested by researcher
 - v. Blood (special circumstances)
 - c. At least one 2 ml cryo-vial per specimen
 - d. Preservation methods
 - i. Liquid nitrogen / Ultra Cold
 1. Fill tubes to fill mark on vial
 2. Secure cap
 3. ETCH BioR# on tube with scribe
 - ii. DMSO buffer / RNA Later / ETOH
 1. care should be taken not to take too much tissue
 2. tissue should be cut into small pieces
 3. ETCH BioR# on tube with scribe
- 2) Other specimens (salvaged, zoo, etc) from freezer
 - a. Tissues removed at time of prep
 - b. Tissue types
 - i. Muscle (always)
 - ii. Heart (sometimes)
 - iii. Liver (sometimes)
 - c. At least one 2 ml cryo-vial per specimen
 - d. Preservation Method
 - i. Freezer
 1. Store initially in Prep lab freezer
 2. Transfer to Ultra cold at end of day
 - ii. Other?

GENETIC SAMPLE TRACKING

Scenario 1 – Genetic sample with voucher at USNM. This is the ideal situation of a voucher and tissue for retention in the Smithsonian.

- a. Genetic samples in single or multiple cryo-vials
- b. Each vial is assigned a unique BioRepository (BR) number and marked with labels provided from BR. The tubes are also inked or etched.
- c. BR# is entered in field catalog
- d. Voucher is assigned a USNM number and is entered as Specimen/Lot record in EMu
- e. A GS record is created in EMu for each vial/BR# from the voucher EMu Record

Scenario 2 – Genetic sample with voucher that may be returned to country of origin. Oftentimes collections are made with the understanding that a certain portion of the voucher collection will be returned to the country of origin; however it is not always known in advance how the specimens will be split, and years may pass before any transfers are made. In this instance, the entire collection may be catalogued into the Smithsonian for the purposes of identifying specimens and publishing results. After identification and publication, a portion of the collection is then transferred to another museum.

- a. Genetic samples in single or multiple cryo-vials.
- b. Each vial is assigned a unique BR# and marked with labels provided from BR [tubes also inked or etched]
- c. BR# is entered in field catalog.
- d. Voucher is assigned a USNM number and is entered as Specimen/Lot record in EMu
- e. A GS record in EMu is created for each vial/BR# from the voucher EMu Record
- f. Voucher specimens to be returned are processed as follows:
 - i. Status of voucher is changed to Exchanged, and noted where voucher resides and associated other museum catalog number
 - ii. Status of tissue is changed to Collection, and noted as tissue only.

Scenario 3 – Genetic samples with voucher remaining in host country. Host countries may request that a collection of vouchers be split prior to export. This usually means that half the voucher specimens remain in the country of origin while the other half is permitted to be exported and transferred to the Smithsonian, with both parties retaining duplicate tissue aliquots.

- a. Genetic samples in single or multiple cryo-vials
- b. Each vial is assigned a unique BR# and marked with labels provided from BR
- c. Tissue(s) are entered as Genetic Sample record(s) in EMu and assigned one USNM number regardless of whether it is one or several tubes.
- d. A note is added to the Emu record that a voucher exists in another museum.

Scenario 4 – Genetic sample only, no voucher. While it is always preferable to have some form of voucher for every genetic sample, we do have vouchered legacy tissues that we intend to keep, and there are other circumstances (endangered species, for example) where an unvouchered genetic sample may be collected.

- a. Sample in single or multiple cryo-vials
- b. Each vial is assigned a unique BioR# and marked with labels provided from BR
- c. Tissue(s) are entered as Genetic Sample record(s) in EMu and assigned one USNM number for one or more samples.