BAT1K Tissue Protocols

Permits and permissions

For BAT1K, it will be necessary to acquire fresh tissue samples for many species. For submission of tissue samples to BAT1K, it is mandatory that all samples have the relevant documentation and permits. All relevant permits and licenses should be prepared and submitted well in advance of sample collection to allow for review and processing time. All procedures for samples that have been or will be collected must conform to appropriate local, regional and national guidelines. All procedures should be compliant with international agreements such as the Nagoya Protocol-Convention on Biological Diversity. Rules and regulations regarding air travel should be checked to determine if specimens are considered "Restricted Articles" with appropriate certificates acquired. Certain endangered species will require special export and import permits. CITES (the Convention on International Trade in Endangered Species) has appendices of protected species for which special permits are required. Bat species found in the CITES appendices are listed in Table 1.

Table 1: Bat species listed under the CITES Appendices (as listed 2018).

Appendix I	Appendix II	Appendix III
Acerodon jubatus	*Acerodon* spp. (4 species excluding species included in Appendix I)	*Platyrrhinus lineatus* (Protected in Uruguay)
Pteropus insularis	*Pteropus* spp. (58 species excluding species included in Appendix I)	
Pteropus loochoensis		
Pteropus mariannus		
Pteropus molossinus		
Pteropus pelewensis		
Pteropus pilosus		
Pteropus samoensis		
Pteropus tonganus		
Pteropus ualanus		
Pteropus yapensis		

Whole specimen sampling and storage

Once a bat has been euthanised, a selection of tissues from the bat should be sampled and preserved immediately. This has shown to be necessary for high molecular weight DNA preservation which is a requirement for Bat1K quality genomes. Having a selection of tissues immediately flash frozen is necessary to achieve the quality and quantity of DNA required for genome sequencing and to preserve the RNA needed for annotation. Tissues in order of preference are listed below in Table 2. If sampling from a whole individual is not possible, wing punches can be taken to establish cell cultures (see below). Tissue samples for Bat1K need to be obtained in isolation of other samples using sterile equipment to avoid cross contamination and stored separately. All equipment and working surfaces must be cleaned thoroughly between each different specimen sampled to avoid contamination. The stomach and intestine should be dissected and removed to avoid any potential contamination of DNA from food items that have been consumed.

Table 2: Tissues for genome sequencing listed in roughly preferred order. If taking multiple tissue types is possible, that is highly preferred. Different tissue types must be placed in separate tubes an all tissues must be clearly identified and the tissue type clearly labelled on the tube. Samples from different animals must **never** be mixed into the same tube!

Tissue Type	Notes
Liver Spleen Lung Muscle Heart	For more information see Wong *et al.* (2012) The submitting of tissues to Bat1k requires that all tissues are clearly defined and labelled.
Wing biopsy sampling and cell culture preparation	3mm wing tissue biopsies for creating a cell culture are suitable if sampling from a whole individual is not possible (i.e. endangered/protected species). A full protocol for cell culture preparation is found separately, below.

Tissue for DNA sequencing: To achieve maximum freezing efficiency, once dissected tissues should be placed into sterile cyrotubes and placed immediately into liquid nitrogen (or when not possible dry ice). Ensure that there is space in the tubes for expansion. Once

transferred, tubes should be stored in liquid nitrogen or dry ice. Samples that are flash-frozen should be shipped in liquid nitrogen or dry ice. Once back in the laboratory, samples should be maintained in either liquid nitrogen or in -80^{o}C freezers to maximise preservation

Tissue for RNA expression analysis: Subsamples of each tissue collected (including the stomach once opened and cleaned) should be preserved in the same way detailed above.

Sample transport

Frozen samples should be shipped on dry ice. Please take care to pack enough dry ice such that it does not evaporate in transit. Please also get in touch with Bat1K before shipping to ensure that all necessary paperwork and customs forms have been completed.

Documentation of samples

After tissue collection, the carcass should be prepared as a voucher specimen (for more details, see Simmons & Voss (2009), which can be found under the tissue protocol section on the website (http://www.bat1k.com)), when possible. Tissue samples and the voucher sample which they were taken from should be labelled with the same field number. Tissue samples and voucher specimen should be lodged in a public research collection with all associated permits and field notes. Digital images taken while alive or shortly after euthanasia should also be lodged for specimen colouration purposes. In the case of endangered species when only wing biopsies can be taken, photographic voucher images are acceptable as long as positive identification is possible. For more information regarding field notes, see Wong *et al*., (2012), which can be found under the tissue protocol section on the website (http://www.bat1k.com).

Submission to BAT1K

As we are still in the initial stages, we would like anyone who is donating tissue sources to keep them frozen at <-80 degrees until funding is acquired to initiate the first phase of Bat1K. Once Phase 1 is initiated, submission documents will be made available under the tissue protocol section on the website (http://www.bat1k.com). In the meantime, please contact us at bat1kconsortium@gmail.com to let us know which samples have been collected and are available.

WING TISSUE/BIOPSY COLLECTION FOR GROWING PRIMARY FIBROBLAST CELLS

To establish cell cultures from wing tissue biopsies, it is vital to practise sterile methods to ensure no introduction of fungi and bacteria, which can inhibit growth and prevent the culture from establishing.

Growth medium

High-glucose DMEM, 20% FBS plus 1% Penicillin-Streptomycin-Fungizone and 50 µg/ml gentamicin. Medium can be stored for up to 6 weeks at 4^{o}C (antibiotic stability) or for several months at -20^{o}C. Media should be transported at 4^{o}C, and temperature fluctuations are to be avoided. While taking medium to the field for biopsies collection, keep them in small polystyrene box with ice packs to make sure it stays cold.

Wing biopsies collection (from living animal):

1. If possible, wear gloves while collecting the wing punches.

2. Spray hands/gloves with 70% IMS (industrial-methylated-spirits) or 70% ethanol before collecting the samples.

3. Preferably, use new biopsy punch and sterile forceps for each bat. Cleaning them with 70 % IMS also works.

4. The less you handle the wing punch the better.

5. After collection, put the wing punch to cold growth medium (in 2 ml tubes), and store it at 4^{o}C until shipping.

Note: The more starting material the better. Collection from living animal is restricted to wing punches, but when collecting the material from a dead/sacrificed animal, the more wing tissue the better. It is possible to grow fibroblasts from tissue collected up to several days after death, however that depends on number of factors (mainly the temperature at which the body has been stored, preferred is 4^{o}C). While collecting larger pieces of wing membrane, use 15 ml tubes filled with growth medium or, if it is not available, wrap the wing with wet tissue, put in a zip-lock bag and ship to the lab directly.

Sample transport

Wing punches samples should be transported in a polystyrene box filled with ice packs for chilling, via courier. Ice packs should be stored in -20^{o}C, and taken out to room temperature

30 min before preparing the parcel to be posted so that samples do not freeze. We have been growing fibroblasts from samples delivered to the lab up to 7-9 days after collection, but the earlier they get to the lab the better chances of success, with up to 3 days post-collection being preferred.

Sample freezing

Prepare freezing medium (growth medium supplemented with 10% DMSO - mixing DMSO with medium is an exothermic reaction so make sure it is chilled before putting samples in the medium). Place biopsies/tissue chopped in 0.5x0.5 cm fragments in 2 ml cryovials filled with freezing medium and immediately put the tubes at -80^{o}C, inside Mr.Frosty or other slow-cooling device. Slow rate cooling is essential for preserving the viability of the cells.