

Collecting Necropsy Samples for Genetic Profiling at Trent University (<http://narightwhale.nrdpfc.ca/>)

Below is an outline of the ideal sampling and storage conditions for samples submitted to Trent. If you are unable to follow this protocol please contact Trent for advice on the best way forward.

Sample Collection:

When skin/muscle tissue is present:

- Collect 10 pieces of skin (if possible) and store them each in a separate tube (see below)
 - Should be from various locations of the body (if possible), where few cyamids are present
 - Avoid areas where skin lesions are present to minimize bacterial contamination
 - From cooler regions of the body (flipper, flukes, regions under water)
- Collect 2 pieces of muscle tissue from flipper or fluke region
- Collect 2 whole digit bones from the flipper. To prevent bacterial contamination, do not cut/grind samples.
 - Following sample collection, clean and dry bones to help prevent bacteria growth. Wash and softly scrub bones using a small amount of 'Dawn' dish soap and lots of water. Dry them off and put in a fume hood or oven with a pilot light on paper towels for a few days to dry well. If possible, freeze at -20 before shipping.
- Store each sample in a separate vial or bag (labeled as per below). For example, skin sample 1 is cut into 3 separate pea-sized pieces and stored in same vial. However, skin sample 1 is stored separately from the pea-sized pieces of skin sample 2.

When skin is not present:

- Collect 4 whole digit bones from the flipper (as outlined above).

Sample Storage:

- Soft tissue samples (skin, muscle) should be stored in a cool place in tubes containing a salt saturated DMSO solution (0.25M EDTA pH 8.0, 20% DMSO, NaCl saturated). There must be enough solution in the tube to completely submerge and penetrate the sample. Cut samples into pea-sized pieces to maximize DMSO penetration. Wrap a piece of parafilm around the lid to prevent leakage.
- Bone samples should be stored dry, in a clean, unused Ziploc bag and sealed tightly to prevent moisture and contaminate entry. If you think the bone may not be dry and contains residual moisture (which can lead to bacterial breakdown) it should be shipped frozen, or contact us before sending.

Labeling Tubes and Bags:

Tubes and bags containing necropsy samples should be clearly labeled and a piece of clear scotch tape or packing tape should cover the label to ensure labels do not come off or bleed. Include the following on label:

- If possible, a unique barcode (your own or one from Trent University)
- Collection date (to be written as 01-Jan-2013) and observer code (initials of the collecting institution)
- Necropsy sample number (when multiple samples are collected; muscle 1 of 2, skin 1 of 10)
- Necropsy field code if known (e.g. MJM9604)
- Storage solution (e.g. DMSO, ETOH)
- Tissue type (e.g. skin, muscle, bone)

On a separate piece of paper, include a spreadsheet with the information above AND: 1) Area of the body sample was collected from (head, fluke, tailstock, etc.); 2) Location (lat/long preferable, geographic description acceptable); 3) All the places where the sample is being sent (e.g. Trent or Trent and NMFS); and 4) in the case of bone, sample preparation (dried under fume hood etc.)

*For each sample, all information should be included **verbatim** in the online sample submission form (<http://whales.wildlifegenomictracker.com/samples/new>) along with any additional information required on that form.

It is critical that information on the tubes matches that entered into the database to prevent confusion linking samples to database records.