

Collecting Right Whale Necropsy Samples for Genetic Profiling Saint Mary's University (SMU)

Below is an outline of ideal sampling and storage conditions for samples from dead right whales submitted to SMU. If you are unable to follow this protocol, please contact Brenna or Tim Frasier at SMU (details below) for advice on the best way forward.

*Please note(!) that the priority tissue type from dead whales is **bone**, with skin and then muscle being the next best options for DNA analysis. For each animal, where possible please collect both bone and skin samples.*

Sample Collection:

When bone is present:

- Collect 1- 2 whole small digit bones from the flipper (as outlined above). To prevent bacterial (and other) contamination, avoid cutting or grinding the samples.
 - Following sample collection, cut away as much soft tissue as possible until only bone remains. 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. The drier the sample the better! Store double bagged. If possible, freeze at -20 before shipping.
 - In the event that the bones are large, and no small finger bones are available, drilled bone shavings from dense, compact bone (such as the back of the skull) are preferred, as opposed to cancellous bone (e.g. the interior spongy regions of vertebrae or large limb bones). Clean away an area of the bone, cutting away all soft tissue. Drill a small surface (~1/4 inch) hole and discard outer shavings, then change the drill bit and collect shavings from deeper within the hole, again with a preference for compact bone over cancellous/spongy bone. Ideally, collect the equivalent of 2-3 14ml tubes of bone shavings. Do NOT put in DMSO. Before storage or shipping, dry the shavings out thoroughly on a piece of aluminum foil or on a weigh boat and return to dry tube or bag. Before and after use of each drill bit, clean with 30% bleach solution and rinse with 95% ethanol.

When skin is present:

- Collect 4 pieces of skin (if possible) and store them each in a separate tube (see below)
 - Each sample should be from a different location of the body (if possible), where few cyamids are present.
 - Avoid areas with skin lesions to minimize bacterial contamination.
 - Collect from cooler regions of the body (flipper, flukes, regions under water).
 - Cut into pea-sized portions before adding to tube filled with salt saturated 20% DMSO solution.
 - Store each sample in a separate vial (labeled as per below). For example, skin sample #1 is cut into 3 separate pea-sized pieces and stored in same vial. Skin sample #2 is stored separately from the pea-sized pieces of skin sample #1.

When only muscle is present:

Note: muscle is sub-optimal and a last-resort tissue type for DNA analysis of dead whales)

- Collect 2 pieces of muscle tissue from flipper or fluke region.
- Avoid areas where skin lesions are present to minimize bacterial contamination.
- Collect from cooler regions of the body (flipper, flukes, regions under water)(areas that are most likely to be cooler, therefore less degraded).

- Cut into pea-sized portions before adding to tube filled with salt saturated 20% DMSO solution.
- Store each sample in a separate vial (labeled as per below).

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. Ideally, double-bag each sample. 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
- Collection date (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 3)
- 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. SMU, NMFS etc.); 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 at <http://whales.wildlifegenomictracker.com/samples/new> along with any additional information required on that form. Because the submission form requests shipping date and shipping permit numbers, please submit once you have that information available. ***It is critical that information on the tubes matches that entered into the database to prevent confusion linking samples to database records.***

SMU Shipping and Contact Information:

Mailing:

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