A Status Update on IWC Samples Held in the Southwest Fisheries Science Center's Marine Mammal and Turtle Molecular Research Sample Collection

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The Southwest Fisheries Science Center (SWFSC) is the repository of the IWC's scientific biopsy samples collected on their research cruises. These samples are permanently stored for molecular genetic analyses. All are frozen at -20C in either a 20% salt saturated solution of DMSO (Dimethyl sulfoxide) or 100% ETOH. Samples are available to researchers through a Sample Request Program at SWFSC only after their research proposal has been approved by the IWC Scientific Committee.

These samples are valuable due to the uniqueness of their collection location (Antarctic and Southern Hemisphere), the timing of the collection of the samples considering that most whale populations are in early stages of recovery from severe depletion and the likelihood that very few additional samples will be collected from these areas in the near future. To avoid depletion of these samples, a project was undertaken to prolong the life of the samples so they could be used in future genetic projects using whole genome amplification.

In conjunction with the IWC, it was decided to perform whole genome amplifications (WGAs) on all 877 IWC samples held in the SWFSC's Collection (see Table 1 for species list). WGA is a procedure that takes genomic DNA (gDNA) and multiplies it by amplification creating up to one million-fold gDNA copies. The product produced can then be used for both mitochondrial and nuclear genetic work. We chose to use the Qiagen REPLI-g Ultrafast Mini Kit (Qiagen, Valencia, CA, USA), to generate the WGA product. Before processing all of the IWC samples, we wanted to test the protocol on to ensure we were obtaining enough DNA product to produce both mitochondrial and nuclear markers.

For testing the protocol, we selected 25 false killer whale (*Pseudorca crassidens*) gDNA samples for which we had previously generated mitochondrial control region sequences and microsatellite data. In all 25 cases, successful WGA product was produced and yielded identical control region sequences and matched the microsatellite data across the eight selected loci that had been generated previously.

All IWC samples were extracted using either the Qiagen DNEasy Kit or the QiaXtractor, a robotic DNA extraction station (Qiagen, Valencia, CA, USA). WGAs were generated from these gDNAs using the Qiagen REPLI-g Ultrafast Mini Kit (Qiagen, Valencia, CA, USA). A subset of WGAs was tested for amplification of both mt control region and nuclear material. The samples tested included those that were considered to be in immediate danger of depletion along with randomly chosen samples. A 400 basepair region of the 5' end of the hypervariable mtDNA control region was amplified using primers D (5'- cctgaagtaagaaccagatg- 3'; Rosel *et al*. (1994)) and TRO (5′- cctccctaagactcaagg-3′; developed at SWFSC). Both strands of the amplified DNA product were sequenced independently as mutual controls on the Applied Biosystems Inc. (ABI, Carlsbad, CA, USA) model 3730 sequencer. All sequences were aligned using Sequencer, v4.1 software (Gene Codes Corp., Ann Arbor, MI, USA). For testing the nuclear gene, the real time qPCR sexing assay developed by Morin *et al.* (2005) was employed.

A total of 143 WGA samples were tested. Of these, 136 samples were successful in generating WGAs and tested positive for both mitochondrial and nuclear genes. The few samples that failed were likely due to the receipt of small amounts of tissue and, therefore, not enough gDNA could be generated to produce good quality WGAs. Total numbers of all IWC samples by species and the material available is summarized in Table 1.

Table 1. Total number of IWC samples by type stored in the Southwest Fisheries Science Center's Marine Mammal and Turtle Molecular Research Sample Collection as of April 2012.

Species	No. Tissue Samples Accessioned	No. Tissue Samples Remaining	No. gDNA Samples	No. WGA Samples Completed
Balaenoptera musculus	245	242	243	241
Balaenoptera physalus	46	46	46	46
Balaenoptera acutorostrata un-named subsp.	7	7	7	7
Megaptera novaeangliae	468	465	466	462
Eubalaena australis	69	69	69	69
Orcinus orca	31	30	30	29
Delphinus delphis	1	0	1	1
Stenella coerueleoalba	2	1	2	2
Stenella attenuata	1	1	1	1
Tursiops truncatus	4	4	4	4
Lagenorhynchus cruciger	3	3	3	3
TOTAL	877	868	872	865

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Literature Cited

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