**International Whaling Commission**
**135 Station Road, Impington, Cambridge, UK, CB24 9NP;**
**Tel: +44  1233971  Fax: +44 1223 232876  E-mail:iwc@iwcoffice.org**

# RESEARCH PROPOSAL: REQUEST FOR USE OF IDCR/SOWER SAMPLES

## 1. TITLE OF PROJECT(DO NOT EXCEED 30 WORDS)

**Global killer whale phylogeography part II: mitogenome and nuclear genetic analysis using next-generation DNA sequencing and SNP genotypes**

## 2. DETAILS OF NAMED INVESTIGATORS (PRINCIPAL INVESTIGATOR FIRST)

| | |
|---|---|
| Name | Phillip A. Morin, PhD |
| Address | Southwest Fisheries Science Center<br>3333 N. Torrey Pines Ct<br>La Jolla, CA, 92120, USA |
| Email | Phillip.morin@noaa.gov |
| Nationality | USA |
| Domicile | USA |

| | |
|---|---|
| Name | Andrew Foote, PhD |
| Address | Centre for GeoGenetics<br>The Natural History Museum of Denmark<br>Øster Voldgade 5 - 7<br>1350 Copenhagen Denmark |
| Email | footead@gmail.com |
| Nationality | UK |
| Domicile | Denmark |

| | |
|---|---|
| Name | John Durban, PhD |
| Address | Southwest Fisheries Science Center (see adress above) |
| Email | John.durban@noaa.gov |
| Nationality | UK |
| Domicile | USA |

| | |
|---|---|
| Name | Robert Pitman |
| Address | Southwest Fisheries Science Center (see adress above) |
| Email | Robert.pitman@noaa.gov |
| Nationality | USA |
| Domicile | USA |

## 3. DESCRIPTION OF PROJECT (DO NOT EXCEED 3000 WORDS)

*(i) Background:*

Killer whales (Orcinus orca) are apex predators found in all the world's oceans (Forney and Wade, 2006). Currently considered a single species (Rice, 1998), local variation in a number of characteristics, including body size, color patterning, social structure, vocalization pattern and behavior has led to the recognition of several named killer whale types (often referred to as "ecotypes") (Baird, 2000; Barrett-Lennard et al., 1996; Deecke et al., 2005; Foote et al., 2009; Ford et al., 1998; Parsons et al., 2009; Pitman and Ensor, 2003; Pitman et al., 2007). In particular, prey specialization appears to be a defining characteristic of these types, with partially or fully sympatric populations having specific, sometimes non-overlapping prey preferences (e.g., fish vs. marine mammals) (Ford et al., 1998; Herman et al., 2005; Krahn et al., 2007; Pitman and Ensor, 2003; Saulitis et al., 2000). Although ecological specialization is not uncommon (Gavrilets and Losos, 2009; Schluter, 2009), the fact that killer whales exhibit specialization within an ecosystem that is largely based on social mechanisms is of great interest, suggesting that speciation may have occurred in the absence of physical barriers to gene flow. Many killer whale populations are being negatively impacted by human activities such as over fishing and pollution, and such threats are likely to vary substantially between types (e.g., Ross et al., 2000; Ylitalo et al., 2001). Effective management therefore requires the delineation of conservation units (Moritz, 1994) within the genus Orcinus to facilitate different management strategies.

Despite a worldwide distribution and phenotypic differences among killer whale types, genetic diversity of mitochondrial DNA (mtDNA) is low, and the control region and other mtDNA loci have been used with limited success to determine population structure and phylogeography. In a survey of approximately 1000bp of the control region from over 100 samples from various locations around the world, Hoelzel et al. (2002) found only 13 haplotypes and no clear pattern of genetic association with ocean basin or type. They concluded that killer whales had gone through a worldwide bottleneck approximately 145,000-210,000 years ago (i.e., during the Pleistocene), and that the genetic patterns reflected stochastic distribution of mitochondrial haplotypes following the post-bottleneck expansion, rather than phylogenetic lineages reflecting the evolution of ecotypes. Analysis of an expanded set of mtDNA control region sequences by LeDuc et al. (2008), including 80 samples from three subsequently described ecotypes in the Southern Ocean around Antarctica, found similar patterns, but also found that two Antarctic types associated with the ice edge were each monophyletic, albeit with very low levels of differentiation. Indeed, levels of differentiation among types worldwide have been marked by only one to six fixed differences and total genetic distances of <0.3% for the most divergent control region lineages. This low level of mtDNA diversity has resulted in only weak inference of phylogeographic patterns and divergence times in killer whales, limiting our ability to understand their evolution and taxonomy. Studies of nuclear microsatellites have begun to clarify population structure within ecotypes, and propose even more recent divergence of regional ecotypes within the last 20,000 - 40,000 years (Hoelzel et al., 2007; Pilot et al., 2010).

To test these hypotheses, we used whole mitogenomes to infer killer whale evolutionary patterns, and our results indicate complete genetic separation of recognized ecotypes in the North Pacific and Antarctic (Morin et al., 2010). We found much deeper initial separation (either geographic or ecological) between the mammal-eating Transient clade in the North Pacific and a second clade in the Atlantic or lower latitudes approximately 700,000 years ago, followed by ecological and/or geographical diversification of the second clade into the present day types at high latitudes, including secondary contact with Transients. These splits between types date from approximately 150,000 to 700,000 years ago rather than 20-40,000 years ago, consistent with species or subspecies level designations. Given the clear lack of phylogenetic information in mitochondrial control region sequences, and the high mutation rates that could cause microsatellites to reach saturation over the time periods that we have inferred from mitogenomes, we believe the mitogenome data provide much stronger support for inference of divergence times in killer whales.

Despite these striking results, large geographic gaps remain in our understanding of killer whale diversity. It remains to be seen whether there are additional types (e.g., subantarctic Type D (Pitman et al., 2010)), whether we can detect evidence of diversifying selection in the mitochondrial genomes of additional types (e.g., Foote et al., 2011), and how killer whales at lower latitudes fit into the evolutionary picture. We propose to expand mitogenome sampling to address these questions, and to develop nuclear SNPs to analyze the corresponding nuclear genome patterns within and among killer whale types globally (e.g., Mesnick et al., 2011; Morin et al., in prep; Morin et al., 2004; Morin and McCarthy, 2007).

**Relevance to IWC:**

The taxonomy of the killer whales is in a state of flux. The previous prevailing concept of a single species has recently evolved into a diversity of views that include the possibility of multiple species, supported by many types of data (e.g., Foote et al., 2009; Ford et al., 1998; Herman et al., 2005; Morin et al., 2010; Pitman et al., 2010; Pitman and Ensor, 2003; Pitman et al., 2007). The 2004 biological review team convened by NOAA to assess the status of the Southern Resident killer whale population as a DPS under the Endangered Species Act "unanimously concluded that the Southern Residents are discrete from other North Pacific resident killer whale populations. In particular, all available data—including behavior, demography, and core and summer ranges, as well as genetics—indicated that the Southern Residents are an independent population that is distinct from other populations" (Krahn et al., 2004). One of the primary impacts of this conclusion was to highlight that our knowledge of killer whale taxonomy is still extremely limited, and as a consequence, population assessments and management plans for killer whales are inadequate. For the purposes of improving management of the ESA listed Southern Resident killer whale DPS, and improving the taxonomy and population structure information for assessment and management of other killer whale types, it is critical that both mitogenome and nuclear genetic analyses continue on both the regional and global scales.

*(ii) Specific objectives*

This proposed research has 2 primary goals:

1) To further elucidate killer whale phylogeography using approximately 100 mitogenome sequences from geographically widely distributed killer whales, with emphasis on obtaining broader coverage of lower latitudes in the Atlantic and Pacific oceans, and samples from previously unsampled areas or ecotypes (e.g., the Indian Ocean, western Pacific, and type D and other potential types in the Antarctic).
2) To develop a set of SNPs that can be used for global analysis of killer whale nuclear phylogeography, and genotype those SNPs on a set of approximately 200 samples that combine the diversity and distribution of samples from this study and the previous mitogenomic study.

This proposed study complements currently funded research that targets regional diversity within the Transient and Resident killer whale types in the North Pacific (Details available on request). Together, the studies will include almost 300 new mitogenomes that will provide detailed data on killer whale population and evolutionary patterns from the local to the global scale.

*(iii) Scientific methodology and approach*

Samples:

We will use tissue samples obtained from dart-biopsy of animals at sea, stranded animals, and museum specimens. Tissue samples from approximately 100 killer whales will be selected from existing collections to represent all of the broadest geographic distribution across their known range.

Enriched genomic library preparation and sequencing:

DNA will be extracted from soft tissues using standard silica-binding methods (Qiagen). Approximately 100 ng of DNA will be used for each genomic library preparation. Hard tissues (bone, tooth) will be extracted using previously described methods (Morin et al., 2006). Individually indexed genomic libraries will be prepared as described by Meyer & Kircher (2010). Indexed libraries will be pooled and hybridized to a capture array (Agilent Technologies, Inc.) to enrich the pooled library for the mitochondrial genome and approximately 75 nuclear loci, following the methods of Hodges et al. (2009). The enriched pooled library will be sequenced using an Illumina Genome Analyzer, to produce an expected ~25 million single-end read sequences of about 80bp each. Expected mitochondrial average coverage is >100x, and expected nuclear average coverage is >10x. This is sufficient for highly accurate and complete sequencing of the mitochondrial genomes of all 100 samples in a single experiment, and for discovery of nuclear SNPs that can be used in the future for nuclear genome analysis.

Analytical methods:
Mitogenome haplotypes will be analyzed for diversity, demographic history, geographic and population-specific patterns and evolutionary relationships to other killer whale types, using Bayesian phylogenetic and skyline methods (e.g., as implemented in the program BEAST (Drummond and Rambaut, 2007)), and inference of levels and directions of gene flow (e.g., MCMC methods as implemented in the program MDIV (Nielsen and Wakeley, 2001)).

*(iv) Programme or plan of research.*

| | |
|---|---|
| Sample selection and transfer to SWFSC (if needed) | Jan.-June 2012 |
| Sample preparation and sequencing | June-Sept. 2012 |
| Data analysis and manuscript preparation | Sept. 2012 – March 2013 |

*(v) Requirement for resources sought in this application.*
No funding is sought for this project, as all funding is already in place. We seek permission to use genomic DNA and/or whole genome amplification (WGA) products from killer whale tissue biopsies obtained on IWC SOWER cruises, and currently held at the SWFSC.

*(vi) Any wider justification for the project.*
We expect to prepare at least one manuscript for publication that further describes the diversity, phylogeography, and evolutionary history of killer whales based on whole mitochondrial genomes and nuclear SNP genotypes. The primary areas of interest are the level of genetic isolation of killer whale types, diversity within and between populations, evolutionary relationships and pattern of speciation of killer whales globally. This analysis will be based on a combination of whole mitogenomes and nuclear SNPs, with reference to other genetic, geographic, behavioral and acoustic analyses that have been previously published (e.g., Baird and Dill, 1995; Barrett-Lennard, 2000; Barrett-Lennard and Ellis, 2001; Bigg et al., 1990; Foote et al., 2009; Foote et al., in press; Ford, 1991; Ford and Ellis, 1999; Herman et al., 2005; Hoelzel et al., 2007; Parsons et al., 2009; Yurk et al., 2002)

Two other projects on population structure and evolutionary relationships within and between North Pacific Resident and Transient killer whales will benefit from the global analysis, as the larger and broader sampling of killer whale types and diversity will help to place the diversity in these putative species in context.

Data from nuclear locus sequencing will be useful for detecting variation that is meaningful for local adaptation. Sequences will be mapped to the killer whale genome, currently being sequenced by Andy Foote and collaborators.

## *Project description*

## 4. CURRICULUM VITAE OF NAMED INVESTIGATORS (1 PAGE PER INVESTIGATOR)

**Phillip A. Morin, PhD**
Research Molecular Geneticist, Marine Mamma Genetics Group, Southwest Fisheries Science Center, La Jolla, CA 92037

Ph.D. in Biology from the University of California, San Diego. October, 1992.
Bachelor of Science, with Honors, in Biology/Chemistry, from the University of Puget Sound, Tacoma, Washington.

Relevant Publications:

Duchene S., Frey A., Alfaro-Núñez A., Dutton P.H., Gilbert M.T.P. and Morin P.A. in revision. Marine turtle mitogenome phylogenetics and evolution. *Molecular Phylogenetics and Evolution*.

Hancock-Hanser B., Frey A., Leslie M., Dutton P.H., Archer E.I. and Morin P.A. in revision. Targeted multiplex next-generation sequencing: Advances in techniques of mitochondrial and nuclear sequencing for population genomics *Mol Ecol Res*.

Martien K., Archer F.I., Rosel P., Cipriano F., Taylor B.L., Leslie M., Hancock-Hanser B., Vollmer N., Viricel A. and Duchene S. in prep. Analytical approaches to subspecies delimitation with genetic data. *Marine Mammal Science*.

Vilstrup J.T., Ho S.Y.W., Foote A.D., Morin P.A., Kreb D., Krützen M., Parra G.J., Robertson K.M., de Stephanis R., Verborgh P., Willerslev E., Orlando L. and Gilbert M.T.P. 2011. Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. *BMC Evolutionary Biology* 11:65.

Mesnick S., Taylor B., Archer E.I., Martien K., Escorza Treviño S., Hancock B.L., Moreno Medina S.C., Pease V.L., Robertson K.M., Straley J.M., Baird R., Calambokidis J., Wade P., Burkanov V., Lunsford C.R., Rendell L., Whitehead H. and Morin P.A. 2011. Sperm whale population structure in the eastern North Pacific inferred by the use of single nucleotide polymorphisms (SNPs), microsatellites and mitochondrial DNA. *Mol Ecol Res* 11 (suppl. 1):278-298.

Foote A.D., Morin P.A., Durban J.W., Willerslev E., Orlando L. and Gilbert M.T.P. 2011. Out of the Pacific and back again: insights into the matrilineal history of Pacific killer whale ecotypes. *PLoS ONE* 6:e24980.

Duchene S., Archer F.I., Vilstrup J., Caballero S. and Morin P.A. 2011. Mitogenome phylogenetics: The impact of using single regions and partitioning schemes on topology, substitution rate and divergence time estimation. *PLoS ONE* 6:e27138.

Taylor B.L., Martien K.K. and Morin P.A. 2010. Identifying units to conserve using genetic data. *In*: I. Boyd, D. Bowen, S. Iverson eds.) *Marine Mammal Ecology and Conservation: A handbook of techniques*. Oxford University Press, Oxford. 306-324.

Morin P.A., Archer F.I., Foote A.D., Vilstrup J., Allen E.E., Wade P., Durban J., Parsons K., Pitman R., Li L., Bouffard P., Nielsen S.A., Rasmussen M., Willerslev E., Gilbert M.T.P. and Harkins T. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research* 20:908-916.

Foote A.D., Morin P.A., Durban J.W., Pitman R.L., Wade P., Willerslev E., Gilbert M.T.P. and da Fonseca R.R. 2010. Positive selection on the killer whale mitogenome. *Biology Letters* 7:116-118.

**Andrew Foote, PhD**
Centre for GeoGenetics, The Natural History Museum of Denmark, Øster Voldgade 5 – 7,
1350 Copenhagen Denmark

Education:
PhD in Zoology, University of Aberdeen, UK, 2010
Marie Curie Post-doctoral fellow, The Natural History Museum of Denmark, 2010-present

Selected publications:

Foote, AD , Morin, PA, Durban, JW, Pitman, RL, Wade, P , Willerslev, E , Gilbert, MTP & da Fonseca, RR 2011, ' Positive selection on the killer whale mitogenome ', Biology Letters , vol 7, pp. 116-118.

Vilstrup, JT , Ho, SY , Foote, AD , Morin, PA, Kreb, D, Krützen, M, Parra, GJ, Robertson, KM, de Stephanis, R, Verborgh, P , Willerslev, E , Orlando, LAA & Gilbert, MTP 2011, ' Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae ', B M C Evolutionary Biology , vol 11, pp. 65.

Foote, AD , Vilstrup, JT , De Stephanis, R, Verborgh, P , Abel Nielsen, SC , Deaville, R, Kleivane, L, Martin, V, Miller, PJO, Oien, N, Pérez-Gil, M , Rasmussen, M , Reid, RJ , Robertson, KM , Rogan, E, Similä, T, Tejedor, ML, Vester, H, Vikingsson, GA , Willerslev, E , Gilbert, MTP , Piertney, SB & Rasmussen, M 2011, ' Genetic differentiation among North Atlantic killer whale populations ', Molecular Ecology , vol 20, no. 3, pp. 629-641.

Beck, S, Esteban, R, Kuningas, S & Foote, AD 2011, ' The influence of ecology on sociality in the killer whale (Orcinus orca). ', Behavioral Ecology .

Foote, AD , Morin, P, JW, D , Willerslev, E , Orlando, LAA & Gilbert, MTP 2011, ' Out of the Pacific and back again: the matrilineal history of Pacific killer whale ecotypes. ', PLoS ONE , pp. e24980.

Deecke, V, Nykänen, M , Foote, AD & Janik, V 2011, ' Vocal behavior and feeding ecology of killer whales ( Orcinus orca ) around Shetland, UK ', Aquatic Biology , vol 13, pp. 79-88.

Rehn, N, Filatova, O, Durban, J & Foote, AD 2011, ' Cross-cultural and cross-ecotype production of a killer whale ‘excitement’ call suggests universality ', Naturwissenshaften , vol 98, pp. 1-6.

Foote, AD , Simila, T, Vikingsson, G & Stevick, P 2010, ' Movement, site fidelity and connectivity in a top marine predator, the killer whale ', Evolutionary Ecology , vol 24, pp. 803-814.

Morin, PA, Archer, FI , Foote, AD , Vilstrup, J , Allen, EE, Wade, P, Durban, J, Parsons, K, Pitman, R, Li, L, Bouffard, P , Abel Nielsen, SC , Rasmussen, M , Willerslev, E , Gilbert, MTP & Harkins, T 2010, ' Complete mitochondrial genome phylogeographic analysis of killer whales (Orcinus orca) indicates multiple species ', Genome Research , vol 20, no. 7, pp. 908-16.

Foote, AD , Newton, J, Piertney, SB , Willerslev, E & Gilbert, MTP 2009, ' Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations ', Molecular Ecology , vol 18, no. 24, pp. 5207-17.

**John Durban, Ph.D.**
Fisheries Biologist, Southwest Fisheries Science Center, La Jolla, CA, USA

Education:
B.Sc. in Animal Ecology from the University of Aberdeen (U.K.)
Ph.D. in Zoology from the University of Aberdeen (U.K)
Post-Doctoral Research Associate in Biostatistics, National Research Council (U.S.)

Selected Publications:

Durban, J.W., Pitman, R.L. 2011. Antarctic killer whales make rapid, round-trip movements to subtropical waters: evidence for physiological maintenance migrations? Biology Letters. doi:10.1098 / rsbl.2011.0875.

Matkin C. et al. in press. Contrasting abundance and residency patterns of two sympatric populations of transient killer whales in the northern Gulf of Alaska. Fisheries Bulletin.

Foote, A. D. et al. 2011 Out of the Pacific and Back Again: Insights into the Matrilineal History of Pacific Killer Whale Ecotypes. PLoS ONE. 6, e24980.

Durban, J., Deecke, V. 2011. How do we study killer whales? Journal of the American Cetacean Society, 40: 6-14.

Tyack, P. L, et al. 2011 Beaked Whales Respond to Simulated and Actual Navy Sonar. PLoS ONE. 6, e17009.

Durban, J. et al. 2011. Abundance indices of eastern North Pacific gray whales from southbound migration counts, 2007-2011 Report to the Scientific Committee of the International Whaling Commission SC/63/BRG7.

Pitman, R. L., Durban, J. W. 2011 Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (Orcinus orca), type B, in Antarctic Peninsula waters. Marine Mammal Science. (DOI: 10.1111/j.1748-7692.2010.00453.x)

Fearnbach, H., et al. 2011 Size and long-term growth trends of Endangered fish-eating killer whales. Endangered Species Research. 13, 173-180.

Barrett-Lennard, et al. 2011 Predation on gray whales and prolonged feeding on submerged carcasses by transient killer whales at Unimak Island, Alaska. Mar Ecol Prog Ser. 421, 229-241.

Foote, A. D., et al. 2011 Positive selection on the killer whale mitogenome. Biology Letters. 7, 116.

Pitman, R. L. et al. 2010. Observations of a distinctive morphotype of killer whale (Orcinus orca), type D, from subantarctic waters. Polar Biology 34: 303-306. (DOI 10.1007/s00300-010-0871-3.

Pitman, R. L., Durban, J. W. 2010 Killer whale predation on penguins in Antarctica. Polar Biology. 33, 1589-1594. (10.1007/s00300-010-0853-5)

Durban, J. et al. 2010. Comparing two methods of shore-based counts of eastern North Pacific gray whales. Report to the Scientific Committee of the International Whaling Commission SC/62/BRG8.

Durban, J. et al. 2010. Clustered mark-recapture analysis of mammal-eating killer whales around the Aleutian Islands and Gulf of Alaska. Marine Biology 157(7): 1591-1604.

Morin, P. A., et al. 2010. Complete mitochondrial genome analysis of killer whales (Orcinus orca) indicates multiple species. Genome Research 20:908–916.

Parsons, K. et al. 2009. The social dynamics of southern resident killer whales and conservation implications for this endangered population. Animal Behaviour 77(4): 963-971.

Johnson, D.S. et al. 2008. Continuous-time correlated random walk models for animal movement data. Ecology 89(5): 1208-1215.

**Robert Pitman**
Marine Ecologist, Southwest Fisheries Science Center, La Jolla, CA, 1988 to date

Selected Publications:

**Pitman, R. L.** & Durban, J. W. 2011 Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (*Orcinus orca*), type B, in Antarctic Peninsula waters. *Marine Mammal Science* 28:16-36.

**Pitman, R. L.** (ed.). 2011. Killer whale: the top, top predator. Whalewatcher (Journal of the American Cetacean Society) 40:1-67.

Durban, J. W., and **R. L. Pitman.** 2011. Antarctic killer whales make rapid, round-trip movements to sub-tropical waters: evidence for physiological maintenance migrations? Biology Letters doi: 10.1098/rsbl.2011.0875.

**Pitman, R. L.,** and J. W. Durban. 2010. Killer whale predation on penguins in Antarctica. Polar Biology. doi: 10.1007/s00300-010-0853-5.

Foote, A. D., P. A. Morin, J. W. Durban, **R. L. Pitman**, P. Wade, E. Willerslev, M. T. P. Gilbert and R. R. da Fonseca. 2010. Positive selection on the killer whale mitogenome. Biology Letters doi: 10.1098/rsbl.2010.0638

Morin, P. A., F. I. Archer, A. D. Foote, M. Thomas, P. Gilbert, E. E. Allen, P. Wade, J. Durban, K. Parsons, **R. L. Pitman**, L. Li, P. Bouffard, J. Vilstrup, S. A. Nielsen, E. Willerslev and T. Harkins. 2010. Complete mitochondrial genome analysis of killer whales (Orcinus orca) indicates multiple species. Genome Research 20:908-916.

LeDuc, R.G., K.M. Robertson, and **R.L. Pitman**. 2008. Mitochondrial sequence divergence among Antarctic killer whale ecotypes is consistent with multiple species. Biology Letters 4:426-429.

Jefferson, T.A., M. A. Webber, and **R. L. Pitman**. 2008. Marine Mammals of the World. Academic Press, San Diego, CA. 573 pp.

**Pitman, R.L.,** W.L. Perryman, D. LeRoi, and E. Eilers. 2007. A dwarf form of killer whale in Antarctica. Journal of Mammalogy 88:43-48.

**Pitman, R.L**. and P. Ensor. 2003. Three different forms of killer whales in Antarctic waters. Journal of Cetacean Research and Management 5:131-139.

## 5. SAMPLES

### (I) NO., SPECIES, AREA, SIZE (E.G. 0.5CM$^3$) AND TYPE (E.G. BLUBBER, SKIN, BLUBBER+SKIN) OF SAMPLES REQUIRED

Genomic DNA or whole genome amplification (WGA) products from tissue biopsies collected on SOWER cruises, and currently held in the SWFSC genetics tissue collection. Approximately 100 ng needed for mitogenome analysis, and 200-1000ng needed for Genotyping By Sequencing (GBS) nuclear locus sequencing.

| SWFSC ID | IWC Field ID | Sample source |
|---|---|---|
| 17221 | IWCSM2990119.03 | BIOPSY-IWC-SOWER |
| 17222 | IWCSM2990221.01 | BIOPSY-IWC-SOWER |
| 17224 | IWCSM1990119.23 | BIOPSY-IWC-SOWER |
| 17227 | IWCSM1990205.57 | BIOPSY-IWC-SOWER |
| 17228 | IWCSM1990205.58 | BIOPSY-IWC-SOWER |
| 17229 | IWCSM1990221.67 | BIOPSY-IWC-SOWER |
| 17230 | IWC.0010106 | BIOPSY-IWC-SOWER |
| 17231 | IWC000117.02 | BIOPSY-IWC-SOWER |
| 17232 | IWC000126.15 | BIOPSY-IWC-SOWER |
| 73077 | 7101131 | BIOPSY-IWC-SOWER |
| 88341 | 410014 | BIOPSY-IWC-SOWER |
| 88342 | 110130 | BIOPSY-IWC-SOWER |
| 88344 | 410103 | BIOPSY-IWC-SOWER |
| 88345 | 610215 | BIOPSY-IWC-SOWER |
| 88347 | 610228 | BIOPSY-IWC-SOWER |
| 88350 | 610242 | BIOPSY-IWC-SOWER |
| 88352 | 8101008 | BIOPSY-IWC-SOWER |
| 88353 | 9101024 | BIOPSY-IWC-SOWER |

### (II) STORAGE/PACKING REQUIREMENTS

NA

### (III) TRAVEL COSTS (DO YOU UNDERTAKE TO PAY ALL TRANSPORTATION AND HANDLING COSTS?)

NA

| | |
|---|---|
| X | I undertake to pay all transportation and handling costs |

## 5. PERMITS

### (I) DO YOU HAVE THE APPROPRIATE PERMITS (E.G. CITES) FOR THE IMPORTATION OF THE SAMPLES

*Give details*

No international transfer of samples is needed, so CITES permits are not required. We are permitted to hold and perform research on samples under MMPA permit 14097-01.

## 6. SCHEDULE OF WORK, REPORTINGAND USE OF RESULTS

*(i) ) Expected completion of final report* **(note that an annual progress report is required)**

June 2013

*(ii) Will you submit a manuscript on the results to the Journal of Cetacean Research and Management upon completion of the work? (Whilst this is not a pre-requisite of a successful application, it will be taken into account). If not state your publication plans*

Manuscript(s) will be submitted to appropriate journals depending on results and journal subject (e.g., Molecular Ecology, Molecular Phylogeny and Evolution, PLoS-one, Nature, Science)

*(iii) Will you agree to the use of the results of your study, if requested by the IWC Scientific Committee under its Data Availability Agreement that protects first publication rights of the researchers?*

| | | | |
|---|---|---|---|
| X | Yes | | No |

## 7. TWO REFEREES WHO COULD BE APPROACHED

| | |
|---|---|
| Name | Paul Wade, PhD |
| Address | National Marine Mammal Laboratory<br>Alaska Fisheries Science Center<br>7600 Sand Point Way N.E. F/AKC3<br>Seattle, WA 98115-6349 |
| Email | Paul.wade@noaa.gov |

| | |
|---|---|
| Name | Frank Cipriano, PhD |
| Address | San Francisco State University<br>San Francisco, CA, 94132 USA |
| Email | cipriano@sfsu.edu |

References:

Baird, R.W., 2000. The killer whale—foraging specializations and group hunting. In: Mann, J., Connor, R.C., Tyack, P.L., Whitehead, H. (Eds.), Cetacean societies: field studies of dolphins and whales. Univ. Chicago Press, Chicago, IL, pp. 127-153.

Baird, R.W., Dill, L.M., 1995. Occurrence and behaviour of transient killer whales: seasonal and pod-specific variability, foraging, and prey handling. Can J Zool 73, 1300-1311.

Barrett-Lennard, L.G., 2000. Population structure and mating patterns of killer whales (*Orcinus orca*) as revealed by DNA analysis. Univ. British Columbia, Vancouver, B.C., Canada.

Barrett-Lennard, L.G., Ellis, G.M., 2001. Population structure and genetic variability in northeastern Pacific killer whales: Towards an assessment of population viability. Canadian Science Advisory Secretariat, Ottawa, Canada.

Barrett-Lennard, L.G., Ford, J.K.B., Heise, K., 1996. The mixed blessing of echolocation: differences in sonar use by fish-eating and mammal-eating killer whales. Anim Behav 51, 553-565.

Bigg, M.A., Olesiuk, G.M., Ellis, G.M., Ford, J.K.B., Balcomb, K.C., 1990. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Report to the International Whaling Commission (Special Issue) 12, 386-406.

Deecke, V.B., Ford, J.K.B., Slater, P.J.B., 2005. The vocal behaviour of mammal-eating killer whales: communicating with costly calls. Anim Behav 69, 395-405.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7, 214.

Foote, A.D., Morin, P.A., Durban, J.W., Pitman, R.L., Wade, P., Willerslev, E., Gilbert, M.T.P., da Fonseca, R.R., 2011. Positive selection on the killer whale mitogenome. Biology letters 7, 116-118.

Foote, A.D., Newton, J., Piertney, S.B., Willerslev, E., Gilbert, M.T.P., 2009. Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. Molecular Ecology 18, 5207-5217.

Foote, A.D., Similä, T., Vikingsson, G.A., Stevick, P.T., in press. Movement, site fidelity and connectivity in a top marine predator, the killer whale. Evolutionary Ecology.

Ford, J.K.B., 1991. Vocal Traditions among Resident Killer Whales (*Orcinus-Orca*) in Coastal Waters of British-Columbia. Canadian Journal of Zoology-Revue Canadienne De Zoologie 69, 1454-1483.

Ford, J.K.B., Ellis, G.M., 1999. Transients: Mammal-Hunting Killer Whales of British Columbia, Washington, and Southeastern Alaska. UBC Press, Vancouver.

Ford, J.K.B., Ellis, G.M., Barrett-Lennard, L.G., Morton, A.B., Palm, R.S., Balcomb, K.C., 1998. Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. Can J Zool 76, 1456-1471.

Forney, K.A., Wade, P.R., 2006. Worldwide distribution and abundance of killer whales. In: Estes, J.A., DeMaster, D.P., Doak, D.F., Williams, T.M., Brownell, R.L., Jr. (Eds.), Whales, Whaling and Ocean Ecosystems. University of California Press, pp. 145-162.

Gavrilets, S., Losos, J.B., 2009. Adaptive radiation: contrasting theory with data. Science 323, 732-737.

Herman, D.P., Burrows, D.G., Wade, P.R., Durban, J.W., Matkin, C.O., LeDuc, R.G., Barrett-Lennard, L.G., Krahn, M.M., 2005. Feeding ecology of eastern North Pacific killer whales Orcinus orca from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Mar Ecol-Prog Ser 302, 275-291.

Hodges, E., Rooks, M., Xuan, Z., Bhattacharjee, A., Benjamin Gordon, D., Brizuela, L., Richard McCombie, W., Hannon, G.J., 2009. Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing. Nat Protoc 4, 960-974.

Hoelzel, A.R., Hey, J., Dahlheim, M.E., Nicholson, C., Burkanov, V., Black, N., 2007. Evolution of population structure in a highly social top predator, the killer whale. Mol Biol Evol 24, 1407-1415.

Hoelzel, A.R., Natoli, A., Dahlheim, M.E., Olavarria, C., Baird, R.W., Black, N.A., 2002. Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. Proceedings of the Royal Society of London, B 269, 1467-1473.

Krahn, M.M., Ford, M.J., Perrin, W.F., Wade, P.R., Angliss, R.P., Hanson, M.B., Taylor, B.L., Ylitalo, G.M., Dahlheim, M.E., Stein, J.E., Waples, R.S., 2004. 2004 Status review of Southern Resident killer whales (*Orcinus orca*) under the Endangered Species Act. NOAA Technical Memorandum NMFS-NWFSC-62.

Krahn, M.M., Herman, D.P., Matkin, C.O., Durban, J.W., Barrett-Lennard, L., Burrows, D.G., Dahlheim, M.E., Black, N., LeDuc, R.G., Wade, P.R., 2007. Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Mar Environ Res 63, 91-114.

LeDuc, R.G., Robertson, K.M., Pitman, R.L., 2008. Mitochondrial sequence divergence among Antarctic killer whale ecotypes is consistent with multiple species. Biology letters 4, 426-429.

Mesnick, S., Taylor, B., Archer, E.I., Martien, K., Escorza Treviño, S., Hancock, B.L., Moreno Medina, S.C., Pease, V.L., Robertson, K.M., Straley, J.M., Baird, R., Calambokidis, J., Wade, P., Burkanov, V., Lunsford, C.R., Rendell, L., Whitehead, H., Morin, P.A., 2011. Sperm whale population structure in the eastern North Pacific inferred by the use of single nucleotide polymorphisms (SNPs), microsatellites and mitochondrial DNA. Mol Ecol Res 11 (suppl. 1), 278-298.

Meyer, M., Kircher, M., 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb Protoc 2010, pdb prot5448.

Morin, P.A., Archer, F.I., Foote, A.D., Vilstrup, J., Allen, E.E., Wade, P., Durban, J., Parsons, K., Pitman, R., Li, L., Bouffard, P., Nielsen, S.A., Rasmussen, M., Willerslev, E., Gilbert, M.T.P., Harkins, T., 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Research 20, 908-916.

Morin, P.A., Archer, F.I., Huebinger, R.M., Martien, K., Bickham, J.W., George, J.C., Postma, L.D., Taylor, B.L., in prep. An empirical comparison of SNPs and microsatellites for population structure, assignment, and demographic analyses of bowhead whale populations. Molecular Ecology.

Morin, P.A., LeDuc, R.G., Robertson, K.M., Hedrick, N.M., Perrin, W.F., Etnier, M., Wade, P., Taylor, B.L., 2006. Genetic analysis of killer whale (*Orcinus orca*) historical bone and tooth samples to identify western U.S. ecotypes. Marine Mammal Science 22, 897-909.

Morin, P.A., Luikart, G., Wayne, R.K., Grp, S.W., 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution 19, 208-216.

Morin, P.A., McCarthy, M., 2007. Highly accurate SNP genotyping from historical and low-quality samples. Molecular Ecology Notes 7, 937-946.

Moritz, C., 1994. Defining 'Evolutionarily Significant Units' for conservation. Trends in Ecology & Evolution 9, 373-375.

Nielsen, R., Wakeley, J., 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158, 885-896.

Parsons, K., Balcomb, K.C., Ford, J.K.B., Durban, J.W., 2009. The social dynamics of southern resident killer whales and conservation implications for this endangered population. Animal Behaviour. Anim Behav 77, 963-971.

Pilot, M., Dahlheim, M.E., Hoelzel, A.R., 2010. Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (Orcinus orca). J Evol Biol 23, 20-31.

Pitman, R.L., Durban, J.W., Greenfelder, M., Guinet, C., Jorgensen, M., Olson, P.A., Plana, J., Tixier, P., Towers, J.R., 2010. Observations of a distinctive morphotype of killer whale (Orcinus orca), type D, from subantarctic waters. Polar Biology 34, 303-306.

Pitman, R.L., Ensor, P., 2003. Three forms of killer whales (*Orcinus orca*) in Antarctic waters. Journal of Cetacean Research and Management 5, 131-139.

Pitman, R.L., Perryman, W.L., LeRoi, D., Eilers, E., 2007. A dwarf form of killer whale in the Antarctic. Journal of Mammalogy 88, 43-48.

Rice, D.W., 1998. Marine Mammals of the World. Systematics and Distribution. Special Publication No. 4, The Society for Marine Mammalogy.

Ross, P.S., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F., 2000. High PCB concentrations in free-ranging Pacific killer whales, Orcinus orca: Effects of age, sex and dietary preference. Marine Pollution Bulletin 40, 504-515.

Saulitis, E., Matkin, C., Barrett-Lennard, L.G., Heise, K., Ellis, G.M., 2000. Foraging strategies of sympatric killer whale (*Orcinus orca*) populations in Prince William Sound, Alaska. Mar Mamm Sci 16, 94-109.

Schluter, D., 2009. Evidence for ecological speciation and its alternative. Science 323, 737-741.

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E., 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (Orcinus orca) from Prince William Sound, AK. Sci Total Environ 281, 183-203.

Yurk, H., Barrett-Lennard, L.G., Ford, J.K.B., Matkin, C., 2002. Cultural transmission within maternal lineages: Vocal clans in resident killer whales in southern Alaska. Anim Behav 63, 1103-1119.