Annex O

Report of the Working Group on DNA Identification and Tracking of Whale Products

Members: Zeh (Chair), Baker, Berggren, Cipriano, Clark, Dalebout, Dizon, Goto, Grønvik, Hovelsrud-Broda, Hatanaka, Kasuya, Komatsu, Lento, Morishita, Pastene, Perrin, Perry, Rose, Rosenbaum, Sakamoto, Simmonds, Taylor, Walløe.

1. TERMS OF REFERENCE

Terms of reference as received from the Working Group on Stock Definition (Annex I) are given as Appendix 1. These were developed in accordance with a plenary decision by the Chair of the Scientific Committee that attention would be given only to scientific matters consonant with the competence of the Committee, including questions of genetics and other aspects of biology but excluding technical matters relating to design, establishment and operations of market monitoring and analysis systems that would be better left to a different, more suitable technical group designated by the Commission.

2. APPOINTMENT OF CHAIR AND RAPPORTEURS

Zeh chaired the Working Group. Perrin and Dizon served as rapporteurs.

3. AGENDA

The agenda was based on the Terms of Reference and is given as Appendix 2. Agenda Item 5 relates to points 1 and 4 of the Terms of Reference; Items 6.1 and 6.3 relate to point 2; and Item 6.2 relates to point 3.

4. REVIEW OF DOCUMENTS

Relevant information was contained in SC/52/SD1, 5-8, 11, 17, SC/52/RMP19, SC/52/AS8, Dizon *et al.* (2000) and Baker *et al.* (2000). The Chair noted that in accordance with the plenary decision, only material in these documents relevant to the adopted agenda would be discussed. Several members expressed disappointment at this ruling and the constraints of the agenda, considering that the 'tracking of products derived from whales' necessarily involved market analysis. While recognising that further expertise would be required on the issue of how to conduct unbiased market surveys, these members believed that the Committee did have the expertise to develop a list of questions for the Commission's use concerning what market surveys should address to contribute to the management of whales. They also believed that the Committee could provide useful information on the technical difficulties that need to be addressed in order to monitor whale-product markets. Some of these difficulties relate to genetic techniques, while other difficulties relate to analytical procedures, such as how to better develop tests to assign individuals to stock origin.

Future discussions and preparations for Committee meetings would be facilitated by a review by the Commission of the intent of their Resolution 1999-8. Specifically, it would be useful for the Committee if the Commission would provide detailed objectives of what an identification/tracking scheme would be expected to achieve.

5. METHODS OF GENETIC ANALYSIS

Much of the following is drawn from SC/52/SD11, which summarised the report of a Workshop on Forensic Genetics held in La Jolla, California, 14-16 June 1999 (Dizon *et al.*, 2000). The Workshop reviewed the current state of genetic methodologies useful for the identification of species, stocks and individuals, and the tissue and DNA databases held by the participants.

It was emphasised that different markers and databases are needed for different tasks: highly variable markers (nuclear loci, e.g. STRs such as microsatellites) and diagnostic register databases (containing all whales from all legal sources) for identification to individual (genetic profiling), and less variable markers (such as mtDNA sequences) and geographically broad reference libraries for identification to species and stock. However, it was also noted that a single tissue sample or sample of extracted DNA can serve as the source for DNA elements required for all the analyses, i.e. that special or separate samples need not be collected for the different purposes.

There are two approaches to using DNA to test the provenance of tissue samples suspected as being from a cetacean: (1) 'DNA profiling' is the process of establishing the source of the sample by comparing the meat product's genetic profile with that of a harvested individual - a 'DNA register' of the genetic profiles of whale products intended for the market is necessary; (2) a 'reference library' is needed for 'lineage testing', the process of inferring the species or stock origin of a tissue sample by comparing its DNA to reference DNA from a library of known individuals.

The use and composition of a register of known individuals and a reference library are different. A DNA register as discussed below is a limited database of cetacean DNA profiles for cetacean products that are intended for the market. The reference library is simply an extensive database that strives to catalogue a large and representative

collection of cetacean DNA from known species and stocks. The DNA library is used to infer species or stock of a whale product for which this information is not known, e.g. one that does not match any of the individuals in the DNA register of whales from documented sources.

5.1 Identification of species and stocks

The underlying assumption of a DNA register is that the stock (and obviously the species) of the registered animal is known with a high degree of certainty. For stock designation, the sampling position with the date of sampling is usually assumed a reliable determiner of stock membership of a permitted cetacean. If for a variety of reasons, sampling position and date are unknown, DNA methods can infer the lineage of these samples with varying degrees of success.

Lineage testing at the species level is based on the systematic assumption that a suite of morphological or genetic characters (i.e. fixed differences) unites all members of a bona fide species. These characters unambiguously differentiate that species from others. Because such taxa theoretically can be characterised with certainty, an individual from a particular taxon can be assigned with certainty. A number of groups have exploited this operational definition of species to establish species identities of market samples, bycatch and beach-cast cetaceans via the comparison of homologous mtDNA sequences of test and known samples (Dizon *et al.*, 2000). In the vast majority of forensic situations, genetic establishment of species is well accepted. For all the baleen whales, all of the common beaked whales and the sperm whale, the IWC can assume that species-level identification based on genetic sequence comparisons can currently be reliably performed. Further developments are needed only to make the process faster and cheaper (for examples of promising new techniques, see Dizon *et al.*, 2000).

Lineage testing at the stock level is altogether different. Stocks share a high proportion of genetic markers. Thus, differences between stocks are modal rather than absolute. Generally, the ability to successfully diagnose taxa declines continuously as one moves down the hierarchy from ocean basins or hemispheres, to highly distinct population segments, and, finally to less well-defined local stocks (Dizon *et al.*, 2000). And indeed, there are only a few situations of immediate interest to the IWC where stock identifications can be made for an individual with reasonable confidence. Analyses of mtDNA sequences reveal diagnostic differences (i.e. fixed differences) between ocean basin stocks of North Pacific and North Atlantic minke whales (Hori *et al.*, 1994; Pastene *et al.*, 1996). Diagnostic differences also characterise the dwarf and the other common minke whales (Hori *et al.*, 1994; Pastene *et al.*, 1994; 1996) and the large- and small-form Bryde's whales (Yoshida and Kato, 1999). However, all three of these situations could arguably have more in common with making species-level inferences, rather than stock-level ones. The gene flow between these conspecific pairs has been so low for so long that fixed differences between the pairs have accumulated. In IWC documents, the most cited example of within-ocean-basin mtDNA differentiation is that observed in the J and O stocks of minke whales in the North Pacific (Goto and Pastene, 1997; Goto *et al.*, 2000). Genetic differences are dramatic and arguably as high as anything observed between sympatric or partially sympatric stocks within the same hemisphere and ocean basin. Yet, no fixed diagnostic character has been discovered that unambiguously differentiates an *individual* minke whale as being from J or O stock. Perhaps this will be discovered as more of the minke whale genome is examined. For now the stock identity of J and O stock individuals cannot be determined in the same straightforward manner as specifying the species of an unknown individual. If it cannot be done on the highly differentiated J and O taxon pair, it is not likely to be done in other situations of interest to the IWC. Examples include distinguishing whether a cetacean sample is from a central/northeast Atlantic minke whale or another Atlantic minke whale stock, from an eastern or western Pacific gray whale, or from a pygmy or true blue whale. Not even bowhead whales from different ocean basins can be individually assigned to stock at this point (SC/52/OS7). The reality of the situation is that while lineage testing at the species level involves examining fixed differences, lineage testing at the stock level usually involves examining modal differences, making it difficult to unambiguously assign the individual sample to stock.

Uncertain taxonomy remains a barrier to species identification and determination of relationships for some whales. An example is that of the Bryde's whale complex (SC/52/SD17); resolution of the taxonomic problem and development of the ability to identify these whales to species will depend on availability of more reference sequences from various parts of the ranges of the forms involved.

Attention was drawn to a paper from last year's meeting (Cipriano and Palumbi, 1999), which contained a review of methodology for high-through-put screening of samples and associated techniques. Note was also taken of a spectrophotometric method, in development by D. Duffield, that may prove to be an alternative to sequence matching for identification to species.

5.2 Identification of individuals

Although technically demanding, genetically matching a harvested individual, via DNA profiles, to its parts in commerce is straightforward compared with lineage testing of stock origins. Because matching DNA profiles is now the primary procedure used by criminal forensic laboratories to link a suspect to the crime scene, its chain-of-custody procedures, technician and laboratory certifications, and analytic protocols are well established.

To check for a match between a suspected whale product and a registered-animal sample, the length of the allelic pair (maternal and paternal) in both samples is measured at a number of independent, highly variable nuclear markers, i.e. STR/microsatellite loci (for glossary of the genetic terminology see Dizon *et al.*, 2000). If each sample pair has identical alleles at each locus, the two samples can be assumed to have originated from the same individual. As long as sufficient numbers of sufficiently polymorphic STR loci are used, the probability of a false match due to chance will be vanishingly small (and quantifiable), and if good laboratory practices are followed to avoid contamination, the chance of a false match due to laboratory error can be minimised. The confidence in any discovered matches will likely be more than adequate for IWC management.

Unlike in human forensics, a DNA register of whales from a regulated hunt is intended to allow verification of any sample or collection of samples to all individuals from the hunt. This requires multiple pair-wise matches, where the probability of a match by chance is greatly increased. Further, confidence in probability of identity will differ depending on the population or stock of interest due to differing allelic frequencies (e.g. Paetkau and Strobeck, 1994). This will require an understanding of stock structure

for non-target as well as target stocks. These issues may require further development to establish robust statistical methods for individual identification.

For a given species of management interest and in a given trade situation, a DNA register can be considered 'diagnostic' when all of the registered individuals are defined as permitted and any others are defined as not permitted. This is the preferred system, as it is fully definitive. In the case of a non-diagnostic register, registered individuals would be defined as permitted, but unregistered conspecific individuals would be of uncertain status.

6. REFERENCE DATABASES AND REGISTERS

6.1 Collection and archiving of samples from catches and bycatches

Specific information on techniques and procedures for collection and archiving of samples is given in Dizon *et al.* (1997).

It was agreed that having a large and representative collection of cetacean tissues or DNA from known species and stocks was desirable for purposes of inferring species and stock origins of unknown tissue samples. One example of the need for such broad geographic representation in a reference database is that of lack of available fin whale DNA samples that could contribute to the identification of samples of unknown origin to stock/area (SC/52/SD17). Such data are also necessary to advance current understanding of species taxonomy and stock structure. Institutions within member nations having substantial tissue/DNA holdings should be encouraged to establish publicly accessible databases describing samples they are willing to share for bona fide scientific studies relating to lineage testing, species taxonomy and stock structure. Information should be furnished for each individual sample, including the species if known, source of the sample, sampling position and date, availability as tissue or DNA, etc. The group welcomed an offer by Dizon to contact interested parties intersessionally to conduct efforts to establish consistent data and web-page formats and to establish terms of the exchange regarding use, publication and further distribution of the sample.

Dizon *et al.* (2000) summarised the cetacean tissue archives held, as of June 1999, by the Southwest Fisheries Center, USA; Auckland University, NZ; Marine Research Institute, Iceland; and University of Wales, UK. For the future, data needs and formats will have to be developed and agreed upon so that IWC members can present information on an annual basis in a useful and consistent fashion on tissue holdings. Information should be furnished for each individual sample rather than as summaries of species as was done in Dizon *et al.* (2000). The sampling position and date of sampling is usually assumed a reliable determiner of stock membership.

For making recommendations regarding this Commission request, it would also be useful to have a clear statement for whose use the register is intended. If matches are to be sought by laboratories other than the original laboratory, access to the DNA register and calibrating allelic ladders is obviously necessary.

6.2 Status of and condition for access to reference databases

Walløe described the status of the Norwegian DNA register for minke whales (Appendix 3), following on a proposal to the Committee made in 1997 (Olaisen, 1997). In the development phase, tissue samples from 50 minke whales from the Norwegian catch in 1996 were analysed to identify a set of robust STR markers, to develop a protocol for mitochondrial sequencing and to try to identify Y-chromosome polymorphisms in minke whales. The results (Dupuy and Olaisen, 1998a) were discussed at an international Workshop held in Oslo in March 1998 and attended by several members of the Committee. Based on the results of the pilot study and the recommendations of the Workshop, 12 STR markers were selected and a protocol for mtDNA sequencing was finalised (details are given in Dupuy and Olaisen, 1998b).

The search for Y-chromosome markers was unsuccessful. A contract was let to a Canadian firm, VITA-Tech, in 1999 (Appendix 3). Samples from all whales from the 1996 catch (excluding four) have been analysed by the Department of Forensic Medicine of the University of Oslo. All samples from 1999 have been analysed and quality-checked/validated in Canada and Norway. All samples from the catches in 1997 and 1998 have been analysed by VITA-tech, and quality checking and validation will be completed in a few weeks.

The Norwegian DNA 'register' will be located in the Directorate of Fisheries, Bergen. While called a 'register', it will actually be a combination of an individual-whale register designed for forensic use and a reference library for both forensic and research use, containing two types of datafiles, one for STR and gender profiles and one for mtDNA sequences. The data are stored at present as Excel files, where one line represents one whale (Dupuy and Olaisen, 1998b). Database software and software for searching the STR part of the register are available from human forensic laboratories. When the register database has been completed, plans are to make it available in part on the Internet. Planned for the future are archives of tissue samples and extracted DNA. Also planned is sampling and register posting of foetuses from harvested minke whales. Walløe noted that Greenland has approached Norway about the possibility of using the same system for minke whales taken in Greenland, and discussions are being held with Iceland concerning the possible inclusion of data from stored whale products. He also noted that the databases will be used not only for forensic purposes but also for scientific research, including stock-identification problems in the North Atlantic and stock-abundance research. They will possibly include mtDNA from stranded baleen whales and whale data from Japan. In addition, allelic ladders for the loci employed in profiling will be provided on request for purposes of standardisation across laboratories.

The group thanked its Norwegian members for the presentation of this information and noted that the Norwegian DNA register is based on standards established for human forensic investigation (Dupuy and Olaisen, 1998a). As such, the technical specifications of the work (as described in the request for proposals for the commercial contract) are of high technical quality. These technical specifications would be a useful model for other countries intending to establish DNA registers.

Goto reported on progress on a system in Japan. It is not yet complete but will be modelled on the Norwegian system and will contain microsatellite, mtDNA and Y-chromosome data. Specifications will be finalised soon. So far, analysis has been completed of 498 northern minke whales (*Balaenoptera acutorostrata*) taken in JARPN and approximately 900 Antarctic minke whales (*B. bonaerensis*) taken in JARPA. Suitable microsatellite loci have been identified for sperm, Bryde's and fin whales. Microsatellite and mtDNA data will be entered for frozen stocks and for strandings and bycatches to the extent possible.

6.3 Standardisation of analyses

Allelic dropout is more likely to occur with very small or degraded samples which contain very little DNA, such as processed market samples. For this reason, size 'binning' of alleles must be standardised across gels and among laboratories. Methods for precise sizing, automated binning of alleles, and reduction of error rates in large-scale genotyping programmes have been standardised for humans after extensive development and cross-laboratory validation using a common set of DNA reference or 'voucher' samples (Ghosh *et al.*, 1997) or 'allelic ladders' (SC/52/RMP19; Appendix 3).

These problems of standardisation are addressed in the Norwegian DNA register with a 'voucher' set of extracted genomic DNA for individuals with known genotypes (B. Olaisen, pers. comm. to Baker). This solution also provides for some standardisation of PCR conditions, as well as subsequent sizing of the profiles.

6.4 Other issues

It was noted that expeditious international transfer of reference samples, DNA extracts, etc. will be important to efficient international cooperation in standardisation of methods. Such transfer requires CITES permitting and certification. It is **recommended** that the Commission urge member nations to expedite issuance of CITES permits where appropriate for transfer of samples for scientific research.

7. ADVICE TO THE COMMISSION

The Working Group **recommends** that the Commission consider the conditions and requirements described above as necessary for useful, reliable and efficient identification of origins of whale products through DNA sampling and analysis. The **recommendation** that a register be fully diagnostic, for whatever trade situation is envisaged, is especially important. Also important is the fact that conditions for access, replication and standardisation must be specified if the system is to be transparent and verifiable.

The Committee could offer further advice if the Commission would provide detailed objectives of what an identification/tracking scheme would be expected to achieve.

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Appendix 1

TERMS OF REFERENCE OF WORKING GROUP ON DNA IDENTIFICATION AND TRACKING OF WHALE PRODUCTS

The following resolution (IWC Resolution 1999-8) was passed by the Commission last year (IWC, 1999, p.40):

RESOLUTION ON DNA TESTING

RECALLING that the Commission is developing a Revised Management Scheme that will require regular updates on relevant new methods and technologies for the inspection and monitoring of commercial whaling operations;

NOTING that one of the most promising of these technologies is DNA-based identification of market products and genetic typing of known catches;

NOW, THEREFORE, the Commission:

REQUESTS the Scientific Committee to establish an agenda item to provide annual reports on progress in the following areas:

(1) Genetic methods for species, stock and individual identification;
(2) Collection and archiving of tissue samples from catches and bycatches;
(3) Status of and conditions for access to reference databases of DNA sequences or microsatellite profiles derived from directed takes, bycatch, frozen stockpiles and products impounded or seized because of suspected infractions; and

FURTHER REQUESTS the Scientific Committee to provide advice to the Commission on the development and implementation of a transparent and verifiable system of identification and tracking of products derived from whales taken under the RMP, and to provide a means to differentiate such products from those taken outside the RMP.

Terms of reference for the Working Group appointed to this task were:

(1) Describe useful current methods of genetic analysis, and methods that are likely to become implementable soon (if any). Description should include whether the method identifies specimens to species, stock or individual level, accuracy of identification given current and potential data, and feasibility, including logistics and cost implications.
(2) Describe or refer to established scientific protocols for collection and techniques for storage of samples that will allow subsequent repeatable analysis; identify conditions under which samples cannot be analysed.
(3) Document known reference databases and conditions of access, and describe which species and geographic areas are included. Identify other reference databases for which holdings and/or access conditions cannot be determined during this meeting.
(4) Describe the succession of tests that might be applied to a sample from a whale product, to determine as far as possible its species, stock or individual identity. Identify cases where this procedure might provide only limited information, and associated statistical issues.

REFERENCE

International Whaling Commission. 1999. Chairman's Report of the Fiftieth Annual Meeting. *Ann. Rep. Int. Whaling Comm.* 1998:3-61.

Appendix 2

AGENDA

1. Terms of reference
2. Appointment of Chair and rapporteurs
3. Adoption of agenda
4. Review of documents
5. Methods of genetic analysis
   5.1 Identification of species and stocks
   5.2 Identification of individuals
6. Reference databases and registers
   6.1 Collection and archiving of samples from catches and bycatches
   6.2 Status of and conditions for access to reference databases
   6.3 Standardisation of analyses
   6.4 Other issues
7. Advice to Commission

Appendix 3

THE NORWEGIAN DNA REGISTRY FOR MINKE WHALES – UPDATED INFORMATION/CURRENT STATUS

Lars Walløe and Sidsel Grønvik

The proposed specifications for a Norwegian database register for minke whales were presented to the Scientific Committee in 1997 (Olaisen, 1997). The Scientific Committee established a Working Group to discuss detailed aspects of the genetic information proposed to be registered. Their report is given as Annex Q to the Report of the Scientific Committee (IWC, 1998).

Tissue samples from 50 minke whales from the Norwegian catch in 1996 were analysed by the Department of Forensic Medicine, University of Oslo, following the proposals given in Olaisen (Olaisen, 1997). The aim of this pilot project was to find a set of robust STR markers, to make a protocol for the mitochondrial DNA sequencing and to try and identify Y-chromosome polymorphisms in minke whales. The results from the analyses were presented in Dupuy and Olaisen (1998a) and were the basis for discussions at an international Workshop held in Oslo in March 1998. The Workshop was attended by a number of international experts on whale genetics. An invitation to participate in the workshop was also extended to all members of the IWC Scientific Committee.

Based on the results from the pilot project and the discussions in the March 1998 workshop it was decided on a set of 12 STR markers and a protocol for mtDNA sequencing. From the pilot project it was concluded that the approach for detecting Y-chromosome polymorphisms was unsuccessful. The detailed specifications for the typing procedure are given in Dupuy and Olaisen (1998c).

In early 1999 the Norwegian government invited tenders for a three-year contract for genetic analysis of minke whale tissue. Adjunct 1 includes an announcement, criteria for evaluation of submitted tenders, and a document giving instructions for DNA profiling for the Norwegian whale DNA database. Tenders were submitted from eight laboratories including two from the UK, one from the USA, one Belgian, one Canadian and three Norwegian laboratories. VITA-Tech, Ontario, Canada got the contract. The decision was based on a total consideration of the criteria given in Adjunct 1.

Samples from all whales from the 1996 catch (excluding four) have been analysed by the Department of Forensic Medicine, University of Oslo (Dupuy and Olaisen, 1998b). All samples from 1999 have been analysed by VITA-Tech and quality checked/validated both in Canada and Norway. The quality control included independent analysis of 5% of the samples by a third laboratory (a Norwegian laboratory). All samples from the catches in 1997 and 1998 have also been analysed by VITA-Tech. A few of these are to be reanalysed and a validation of all the 1997-1998 data will be done within a few weeks.

The Norwegian DNA registry will be located in the Directorate of Fisheries, Bergen. It will contain two types of data files, one for STR and gender profiles and one for mtDNA sequences, as specified in Dupuy and Olaisen (1998c). Database software and software for searching in the STR part of the registry are available (from human forensic laboratories), but the database register itself has not yet been established. We are planning to make a copy of parts of the database available on the web, but the formal decision has not yet been made by the appropriate Norwegian authority. Examples of these types of data files are also given in Adjunct 1. At present these are Excel files, where each line represents one whale.

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Adjunct 1. Contract for genetic analysis

The Norwegian government wishes to announce a three-year laboratory contract for genetic analysis of minke whale (*Balaenoptera acutorostrata*) tissue.

Norway at present conducts a limited harvest of Northeast Atlantic minke whales. A DNA register, covering each individually harvested whale, has been established in connection with this harvest. The aim of the register is *inter alia* to detect and prevent illegal trade in minke whale products.

The whale DNA profile to be entered in the register is composed of three parts for which a typing procedure has already been developed:

(1) a set of 10 DNA markers (STRs) which together will identify each individual whale;
(2) determination of gender (based on size differences between pseudohomologous parts of the X and Y chromosomes);
(3) a species specific test based on maternal inherited mitochondrial DNA.

Scope of contract: This contract covers analysis of a starting sample of up to 1,200 and thereafter an annual sample size of up to 800.

Scientific institutions and laboratories wishing to compete for the contract must have national accreditation or document that they are taking steps to obtain such within the contract period. Last date for requesting tender documents is 14 May at 15:00 hours. Last date for submission of tender is 14 June 1999 at 15:00 hours. For details about the contract and typing procedure please contact:

The Royal Norwegian Ministry of Fisheries
Dept. of Resources and Planning
P.O. Box 8118 Dep
N-0032 Oslo
Norway
Telephone: (47) 22246441
Fax: (47) 22249585
E-mail*: else-marie.horn@fid.dep.telemax.no*

The following criteria will be given emphasis when deciding upon the contract

Price
It is a primary target to cut costs, and to obtain the required laboratory results as reasonable as possible. However, a number of other criteria will be given emphasis when determining the contract.

Merit
The laboratory/institution's ability to perform high-grade laboratory analysis will be considered based on the laboratory/institution's previous merit in this field. Furthermore the laboratory/institution will be judged based on the merit of the senior scientist in charge.

Security
To obtain high quality control and guaranteed continuity in the analysis the laboratory/institution's security control will be emphasised.

Quality control/accreditation
Accreditation delivers confidence in certificates and reports by assessing the competence of providers based on widely accepted criteria set by the European or international standardisation bodies. It is a requirement for the fulfilment of this contract that the laboratory/institution be accredited by its national standardisation body, which must be recognised by the EA (European cooperation for Accreditation). If not accredited at the start of this contract the laboratory/institution must prove that steps are being taken to obtain accreditation, and that this can be obtained within the span of the contract. If accreditation is lost within the contract period, or the laboratory/institution fails to obtain accreditation, or is found to be unlikely to obtain accreditation by the national body then this may lead to a cancellation of the contract.

Continuity
The purpose of the analysis is to feed the results into an established register. This register will only function well if it is continuously updated. To ensure that this is the case emphasis is put on the laboratory/institution's ability to conduct continuous analysis. This will be considered based on size, equipment and the number of qualified personnel in the staff.

Guarantee
The laboratory/institution must be able to produce a governmental guarantee from its home country that no restrictions will be imposed on the import of the whale samples or laboratory work.

Instructions for DNA profiling for the Norwegian whale DNA database

(1) The laboratory should adhere to high quality standards (such as those defined by forensic organisations like TWGDAM, ENFSI or EDNAP) and should be or make efforts to be accredited for DNA work.

Quality control and quality assurance features should assure that:

(a) analysts have acceptable education, training and experience for the task;
(b) reagents and equipment are properly maintained and monitored;
(c) procedures used are generally accepted in the field; and
(d) appropriate controls are used (as specified in procedures).

If requested by the Ministry, the laboratory should accept to open the laboratory for an evaluation (e.g. by site visit, inspection, peer review, or external audit).
(2) The laboratory should (start to) participate regularly in proficiency tests, and the results should – upon request – be available for the Ministry.
(3) Portions of the tissue samples and DNA extracts of the duplicate samples (A and B) should be retained (stored in freezer) and made available for the Ministry whenever required.
(4) Laboratory records (protocols, notes, worksheets, etc.) are prepared, retained by the laboratory and made available for inspection when required by the Ministry.
(5) The genetic markers included in the Norwegian whale DNA database should be those 12 defined in Dupuy and Olaisen 'Typing procedure for the Norwegian Minke Whale DNA Register'.
(6) The choice of basic equipment as well as of the typing procedure should be according to that described by Dupuy and Olaisen. If alternatives are to be used, documentation to show that they have a quality at least at the level of the equipment/procedure described, should – upon the Ministry's request – be produced. The Ministry shall decide if a change is acceptable.
(7) To minimise the probability of errors, each whale is typed twice, one tissue sample in the complete set of the 12 markers, another tissue sample with a selected set of these markers (see 'Typing procedure'). These typings are performed blindly in relation to each other, and the results are compared when both typings are finished. The DNA profile is accepted if both typings show a complete set of acceptable and identical results. Whenever any discrepancy, new samplings and complete typings are performed until acceptable and identical results – and a complete profile – are obtained. DNA type quality/acceptability is decided in accordance with generally accepted rules (e.g. in STR and gender marker analyses, SDs of allele fragment length measurements should not exceed 0.15 bp, allele peak heights should exceed 50, and stuttering should be low enough to allow safe distinction between hetero- and homo-zygotes). A reference set of sequenced allelic ladders will be provided for by the Institute of Forensic Medicine, University of Oslo.
(8) STR-, gender- and mtDNA types/names should be in accordance with 'Typing procedure'. This means that alleles are designated in accordance with the true (sequenced) length of the PCR product, and that the relation to type designation based on repeat number is as it is shown in tables 7.1 to 7.10 in Dupuy and Olaisen 'Typing procedure'. Gender 'alleles' are named 212 (the Y-chromosome PCR product) and 245 (the X-chromosome PCR product), respectively; mtDNA designation is related to the reference sequence GenBank accession No: X61145 as described.
(9) DNA profiles should be presented as follows.

(a) Two Excel files are generated, one for STR and gender profiles, the other for the mtDNA sequence.
(b) In each of these, consecutive whales are numbered (e.g. for the 1996 catch, the whales were numbered from 96001 to 96338). In the STR/gender file, each whale profile is given in one row, with one column for each allele (two columns for each STR marker and for the gender locus). In the mtDNA profile file, each profile has one row, and one column for each site where the sequence deviates from the reference sequence. Examples from the 1996 of hard copies from each of these two file types are shown in enclosures. The mtDNA file should include one column for each site showing deviation from the reference sequence in any of the whales caught that year.
(c) Hard copies – designed as shown in enclosures – are also made available.

DNA profiles – presented as described above – should be delivered to the Ministry within 6 months after the receipt of tissue samples from a given year's catch.

[Table follows on next page]

MtDNA profiles of the Norwegian 1996 catch quota.

	MtDNA bp no.																																										
	-21	0	1	2	3	4	5	6	7	8	9	10	11	12	13	13I	13H	13IH	13IHI	14	15	16	19	62	78	89	107	117	134	145	172	205	207	208	214	217	219	250	265	271	272	273	289	298
	Reference sequence																																											
Whale no.	G	G	A	A	A	A	A	T	A	T	A	T	A	T	A	(T)	(A)	(T)	(A)	T	T	G	C	T	C	C	C	A	C	G	G	A	C	C	T	A	T	T	C	T	T	C	C	C
96001	A	G	A	A	A	A		T	A	T	A	T	A							T	T	G						C			A												T	
96002	A	G	A	A	A	A		T	A	T	A	T	A							T	T	G								A														A
96003		G	A	A	A	A	A	T	A	T	A	T	A	T	A					T	T	G										G												
96004	A	G	A	A	A	A		T	A	T	A	T	A	T	A					T	T	G																						T
96005		G	A	A	A	A	A	T	A	T	A	T	A							T	T	G																						

STR and gender DNA profiles of the Norwegian 1996 catch quota.

	GATA098		GT509		EV1		EV37		GT310		GT023		GATA028		GT211		GT575		GATA417		ZFYX	
Whale no.	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender	STR	Gender
96001	87	99	193	201	153	153	203	203	111	117	99	99	203	215	110	112	148	156	217	224	212	245
96002	95	95	193	201	153	155	199	203	117	121	99	103	157	211	94	108	148	158	213	228	212	245
96003	91	95	207	213	153	153	201	203	115	115	99	99	207	211	102	104	152	154	213	238	212	245
96004	95	95	207	211	149	153	205	207	117	117	99	103	203	215	104	110	156	158	228	228	212	245
96005	95	95	205	207	151	155	203	205	111	115	105	103	211	211	102	104	156	158	213	220	212	245