# Annex T

# Final Proposed Annexes for Item 20

**Annex {DNA} dated *day/month/year***

**Specifications and requirements for diagnostic[17] DNA registers and market sampling schemes**

## 1. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF A DIAGNOSTIC DNA REGISTER/TISSUE ARCHIVE

### 1.1 Laboratories

#### 1.1.1 Minimum laboratory requirements

(1) Laboratories performing DNA analysis shall be recognised by the Contracting Government under whose jurisdiction whales are harvested.
(2) Quality control and quality assurance features shall ensure 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 have been approved by the IWC Scientific Committee (see Items 1.2-1.5); and
   (d) appropriate controls are used.
(3) Thorough laboratory records (protocols, notes, worksheets, etc.) shall be maintained and archived for possible inspection (see Item 1.7).
(4) Changes in equipment and approved methods shall be recorded and reported annually to the IWC to allow ongoing standardisation among registers (see Item 1.7).
(5) A suitable inventory management system shall be in place so that the whereabouts and use of each sample/ aliquot over time during storage and analysis can be traced.
(6) Portions of the tissue samples and DNA extracts should be retained and stored indefinitely or until advised by the Scientific Committee, using an appropriate preservation method (see Item 1.2.2).
(7) The probability of genotyping errors occurring should be estimated and minimised, using standard procedures and also including provisions for detection of mis-labelling, duplicate samples, data entry errors, etc. DNA sample quality should be checked routinely prior to genetic analysis to ensure adequate accuracy in the genotyping of degraded samples (as recommended in IWC (2009), and subsequent updates to the genetic analysis guidelines). DNA data quality/acceptability should be addressed in accordance with generally accepted rules and reported annually where possible (e.g. PHRED scores for sequences, SDs of fragment length measurements for microsatellite alleles, means and SDs of peak heights for microsatellites, some evaluation of stutter for each microsatellite locus). This information should be reported annually to the IWC (see Items 1.5 and 1.7).
(8) A reference set of samples should be designated for allelic standards and an equimolar allelic ladder should be constructed by cloning and sequencing a range of alleles for each microsatellite locus.
(9) The laboratory shall participate in calibration exercises with other laboratories if requested to do so by the IWC (see Item 1.1.2), and taking into account both the analysts involved, the methods and/or software used for binning alleles, and the type of equipment used for genotyping.
(10) The laboratory should be available for external evaluation and participate regularly in proficiency tests such as double-blind comparisons (e.g. see Item 1.7).

#### 1.1.2 Calibration of laboratories if more than one is used

Where more than one laboratory is used to generate a single register or a group of registers, or for the comparison of samples (e.g. under Item 1.8 or Item 2), appropriate calibration of microsatellite genotype scoring (e.g. absolute size or binning) must be undertaken and the results reported to the IWC. The details of the calibration exercise shall be determined by the international expert group (see Item 1.7). The calibration exercise will primarily comprise a double blind experiment with known individuals. Cloned alleles should be used to construct an allelic ladder for calibration purposes. The results of calibration exercises must be reported to the IWC. In designing calibration exercises and reviewing the results, it must be remembered that the primary function of diagnostic DNA registers is to determine whether illegal activity is taking place and that the default position is no match=illegal activity. In this regard it is important to estimate the likelihood of:

- erroneously failing to match products to an animal in the register when it is actually there – i.e. falsely implying an infraction;
- erroneously matching products to an individual in the register when it is not actually there – i.e. missing an infraction when one has occurred.

[17]A diagnostic DNA register is one that contains DNA profiles of all animals from which products might legally appear on the market (e.g. from legal direct catches, legal imports, bycatches, ship strikes etc.). DNA profiles from legally imported whales should thus be included in the importing country's registry as one of the conditions for importation. On this basis, any products found on the market that were from whales not included in the register will be from illegally taken or illegally imported whales.

[18]Contracting Governments under whose jurisdiction bycaught/stranded whales and their products may be legally marketed are responsible to develop a technical manual for collecting samples and ancillary data for inclusion in DNA registries, and for disseminating such materials and training to others who may be involved in the collection of genetic samples for such use.

**1.2 Sample collection**
Samples for DNA registry should be collected by trained personnel[18] before products from them can enter the market.

**1.2.1 Size of samples**
At least two samples of skin/muscle of at least 5x5x5mm must be collected from each animal for each register/archive. In addition, where possible, at least four muscle samples of 20x20x20mm should be taken. Where possible, a sample of tissue from any foetuses detected should be collected. All samples should be taken as quickly as possible and immediately placed in an appropriate preservative, and then frozen as quickly as possible at or below -20°C.

**1.2.2 Preservation**
Samples should initially be preserved in 95% ethanol (in at least five times the volume of the sample, due to potential problems of dilution and evaporation) or in five times the volume of NaCl-saturated DMSO (dimethyl-sulfoxide). If not able to be frozen immediately, the samples should be shipped as soon as possible (preferably within 7 days) to the analysing laboratory. This temporary storage and shipping should be in temperatures <25°C to minimise the possibility of degradation of the sample.

Long-term storage of skin/muscle samples should be in 95% ethanol or NaCl-DMSO at or below -20°C. The additional muscle samples should be frozen in liquid nitrogen; transport should be with dry ice. For best preservation long-term storage of frozen tissue samples should be at or below -80°C or if that is not possible at or below -20°C.

**1.2.3 Labelling**
Reliable labelling of the sample is essential. The container should be labelled on both the inside and the outside with a unique identifying code that can be related directly to the biological and other information collected for the individual (see Item 1.2.4). The label on the inside must be indelible and insoluble in alcohol to ensure that the number remains legible after storage in ethanol. The label on the outside must also be robust and remain legible if exposed to ethanol or water.

**1.2.4 Information to be collected**
In addition to the information noted in Annex {SI} dated *day/month/year* to be collected for each whale (including date, locality, species, sex, and body length), the unique identifier (see Item 1.2.3) and the name (plus address if non-nominated person, e.g. in the case of bycatch) of sampling person must be recorded.

**1.3 Tissue analysis**
**1.3.1 Extraction of DNA**
Extraction of DNA should be carried out using standard methods which have been reviewed and approved by the IWC Scientific Committee. Extracted DNA aliquots should be stored in freezers at or below -80°C.

**1.4 Markers and methods of analysis**
Analysis of samples should be undertaken without knowledge of the biological and other information available for the whale from which the sample was taken.

Samples should be analysed for (at least):

(1) mitochondrial DNA - primarily for identification to species and population but also contributes to profiling;
(2) microsatellites (or Short Tandem Repeats, STRs) - for DNA profiling; and
(3) Y chromosomes - sex identification which also contributes to profiling.

**1.4.1 Mitochondrial DNA**
Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009 or subsequent updates) must be approved by the international expert group (see Item 1.7). Species identification should be accomplished with an approximately 500bp fragment of the 5'-end of the control region and sequencing should occur in both directions.

**1.4.2 Microsatellites**
Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009 or subsequent updates) must be approved and reviewed annually by the international expert group (see Item 1.7). Fluorescent techniques that allow electronic records to be kept should be used. This group will ensure that the number and degree of variability of loci used in DNA registers will be sufficient to allow for an acceptable level of average probability of correctly identifying an individual.

**1.4.3 Sex identification**
Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009 or subsequent updates) must be approved by the international expert group (see Item 1.7). Sex is an additional genotype that may prove useful to identify market samples and may also serve as a check on field data. Error rates (obtained by comparison with reliable field identification of sex) should be estimated and reported to the international expert group (see Item 1.7).

**1.5 Format of individual records**
Each whale is given a unique identifier that can be cross-referenced back to the biological and associated data for that animal. Records must contain:

(a) a microsatellites and sex profile, in which each whale profile is given one row, with one column for each allele (two columns for each microsatellite marker and the sex locus); and
(b) a mtDNA sequence file, in which each profile has one row, and one column for each site where the sequence deviates from the reference sequence.

In addition, the following must be archived:

**General information for each sample:**
- genotyping system; and
- software system.

*'Raw' data:*
- electropherograms;
- quality scores;
- raw allele sizes;
- peak heights;
- gel image (depending on platform used); and
- number of times the genotype replicated.

*Summary data on each locus:*
- error rate and how determined;
- allele frequencies in a given population;
- deviations from Hardy-Weinberg equilibrium; and
- evidence of null-alleles, short-allele dominance (or short-allele bias due to preferential amplification) or other artefacts.

**1.6 Matching**
The international expert group (see Item 1.7) will agree on software packages to be used for matching purposes.

**1.7 External audit of DNA registers**
An international expert group established pursuant to paragraph 42 shall:

- review and approve the initial technical specifications for the register(s) and any changes to those protocols;
- where necessary, decide on appropriate laboratories;
- where necessary, design calibration exercises for laboratories and review the results of those exercises;
- review annually specific information and statistics formally reported by the register(s) under Items 1.4-1.6;
- design and undertake periodic technical audits including the provision for trials using 'blind' control samples; and
- design and arrange for periodic site visits to examine whether the agreed protocols (under Items 1.2-1.5) are being followed.

The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to Contracting Governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.

**1.8 Mechanism for comparing samples to the IWC's central register, further to domestic market survey systems**
A Contracting Government may request the IWC to compare any appropriately-documented tissue sample against the IWC's electronic register, regardless of where the sample was collected. The tissue sample should be sent to a qualified laboratory[19], not necessarily associated with the national registry. The associated documentation, which is specified below, should be sent to the Secretariat. The laboratory should send the DNA profiles (see Item 1.5) to the Secretariat as soon as possible, and the sample should be kept in long-term storage (see Items 1.1.1 and 1.2.3).

The associated documentation shall describe chain of custody from time of collection to submission, including the following information:

- name and address of 'collector';
- location obtained;
- type of vendor;
- date and time of collection;
- label, if present (or verbal description of nature and origin of product offered by vendor);
- where possible, photographs; and
- comments by the Contracting Government where the market sample was collected.

Analysis of the samples shall be carried out following the same quality control, sample handling and calibration procedures specified above in Items 1.1-1.4 by a qualified laboratory[19]. Officially-attested documentation of chain of custody must be established for the period between submission and provision of analytical results.

The comparison of the DNA profile against the IWC's central register shall be made using agreed software (see Item 1.6) [Option 1: after the annual update from the relevant national register.] [Option 2: Profiles that do not match would be held in a database that would be checked against the annually-updated registry each year.] The Secretariat shall make public the results within one week.

**1.9 Submission of DNA profiles to the IWC's central registry**
Contracting Governments under whose jurisdiction whales and whale products may be legally marketed shall maintain a diagnostic DNA register and tissue bank. Before any products from a whale enter the market, samples for the DNA registry shall be collected from that whale, and submitted for inclusion in the domestic registry. DNA profiles shall be transmitted annually to a centralised archive maintained by the Secretariat.

## 2. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF MARKET SAMPLING SCHEMES

The purpose of market sampling is twofold: to act as a deterrent to illegal activity and to detect whether such activity is occurring. Market sampling in its initial stage is not intended to determine the precise number of animals that may be involved. Rather, if illegal products are discovered, a targeted method of detecting the origin of the products and the extent of the illegal operation specific to the case should be developed.

**2.1 Design principles**

(1) Market sampling schemes shall be case-specific. Their design shall be based on the best available information on the temporal and geographical nature of the particular market(s) and product pathways. Power to detect/deter will increase with the geographical and temporal scope of the surveys.
(2) The design of market sampling schemes will be iterative and schemes should be reviewed periodically. Experimental testing of their potential to detect illegal products should be undertaken and reported. This should include estimation of the possibility of falsely suggesting illegal activity and missing illegal activity when it occurs.
(3) Appropriate (e.g. not highly processed products from which it is difficult to obtain reliable microsatellite profiles) products should be chosen.
(4) A balance between deterrence (sampling carried out openly and with publicity) and detection (undercover sampling) shall be maintained and reported.
(5) The full range of cetacean products shall be sampled in case mislabelling occurs.
(6) An officially-attested documentation of chain of custody from time of collection to results of matching must be collected and archived, including the information given in Item 2.3.
(7) Analysis and matching must be carried out in an IWC-approved laboratory (with appropriate calibration if necessary) following the procedures given in Item 1 above.

**2.2 Development of appropriate market sampling schemes including audit**
The international expert group (see Item 1.7) under the auspices of the IWC shall:

(1) co-operate in the design of and approve any market sampling scheme before it is implemented and review the associated results;

[19]A qualified laboratory is one recognised by a Contracting Government that meets the standards of Items 1.1.1 and 1.1.2 as specified by the international expert group.

(2) co-operate in the design of and approve experimental work and review results referring to Item 2.1 (2) above;
(3) design and arrange for periodic site visits to ensure that the approved scheme is being implemented; and
(4) experimental procedures should reflect the need for a standardised set of markers suited to the generation of accurate data from degraded source materials.

**2.3 Data to be collected**

- Product or sample of product of sufficient size to obtain DNA sample (see Item 1.2.2).
- Location obtained.
- Date and time.
- Label (or verbal description of nature and origin of product offered by vendor).
- Source (e.g. wholesale market, shop, dockside etc.).
- Photograph of product before sub-sampling.
- Name and contact information of person collecting.

This information should be archived in an appropriate electronic manner.

**2.4 Reporting**

The authorities responsible for undertaking the market sampling schemes in accordance with Paragraph 42 of the Schedule shall submit an annual report of their market sampling activities to the international expert group via the IWC Secretariat at the end of February of each year. That report shall include: details of the methods used; a summary of the number and nature of the products sampled, and the geographical and temporal spread of sampling; the results of the matching exercise.

The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to contracting governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.

REFERENCE

International Whaling Commission. 2009. Report of the Scientific Committee. Annex I. Report of the Working Group on Stock Definition. Appendix 2. Guidelines for DNA data quality control for genetic studies relevant to IWC management advice. *J. Cetacean Res. Manage. (Suppl.)* 11: 252-256.

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## Annex {SI} dated *day/month/year*

## Scientific Information

1. The following information shall be provided by Contracting Governments for all whaling operations and, where possible, for mortalities due to bycatches and ship strikes:
   (a) date of capture, striking or discovery;
   (b) species;
   (c) sex;
   (d) position of capture or striking or discovery to the nearest minute of latitude and longitude[1]; and
   (e) number of whales struck but lost.

   A set of verified records shall be submitted to the Secretariat within 30 days of the end of each season, in an electronic format to be provided by the Secretariat. These records shall be publicly available.
2. In addition, the following samples and/or information shall be collected/reported in formats to be provided by the Secretariat.
   (a) The length of all whales caught shall be obtained, measured in a straight line parallel to the whale from the tip of the upper jaw to the notch of the flukes to the nearest 0.5 feet or nearest 0.1m[2]. These data shall be reported to the Secretariat within 30 days of the end of each season and included in the IWC database. These data shall be publicly available.
   (b) Where possible, at least one earplug shall be collected from each whale caught. The resultant age estimations and the identity of the reader(s) shall be reported to the Secretariat in a timely fashion, normally within one year of collection and included in the IWC database for use under the Scientific Committee's Data Availability Agreement.
   (c) Where possible, both ovaries shall be collected from each female caught. Corpora counts shall be reported to the Secretariat normally within one year of collection and included in the IWC database for use under the Scientific Committee's Data Availability Agreement.
   (d) If sufficiently trained personnel are present, the presence, length and sex of foetuses shall be recorded, assigned to the appropriate female. If it is not possible for such personnel to be present, these data should still be recorded where possible, and the lack of trained personnel noted. These data shall be forwarded to the Secretariat within 30 days of the end of the season and included in the IWC database. These data shall be publicly available.
   (e) Lactation shall be recorded, assigned to the appropriate female and reported to the Secretariat within 30 days after the close of the season and included in the IWC database[3]. This information shall be publicly available.
   (f) At least 5cm$^3$ of skin shall be collected from each whale caught and, where possible, a sample of tissue from the foetus should be collected. Long term archiving of all samples with appropriate identifying information is the responsibility of the harvesting nation. A list of archived samples shall be forwarded to the Secretariat within 30 days of the end of each season. This information shall be publicly available.

[1]For whales taken under paragraph 13, position shall be given at least to the nearest settlement and, where possible, to the nearest minute of latitude and longitude.
[2]Onboard small coastal whaling vessels such as those participating in Norwegian and Icelandic operations, it may be difficult to obtain accurate length measurements because whales are handled on a limited space. It is recognised that measurements in these cases may not be as accurate as those taken in ideal situations.
[3]For whales taken under paragraph 13, this information shall be provided where possible and an indication given of the experience of the data collector.

## Annex {OI} dated *day/month/year*

## Operational Information

1. All Contracting Governments under whose jurisdiction whales are harvested shall report to the Commission the following information:
   (a) the name and gross tonnage of each factory ship; and
   (b) a list of the land stations that were in operation during the period concerned.
2. All Contracting Governments shall report to the Commission for each whale catcher attached to a factory ship or land station:
   (a) the dates on which each is commissioned and ceases whaling for the season;
   (b) the number of days on which each is at sea on the whaling grounds each season; and
   (c) the gross tonnage, horsepower, length and other characteristics of each.
3. The information required under paragraphs 1(a) and (b) shall also be recorded together with the operational information specific in a log book format similar to that shown in Table 1. A set of verified records shall be submitted to the Secretariat within 30 days of the end of each season, in an electronic format to be provided by the Secretariat. These records shall be publicly available.