ACCOBAMS-MOP9/2025/Inf34

GENETIC PASSPORT FOR CETACEANS KEPT IN CAPTIVITY

GENETIC PASSPORT FOR CETACEANS KEPT IN CAPTIVITY

prepared by: Sandro Mazzariol, DVM, PhD

1. Scope of the document

The purpose of this document is to propose a "genetic passport" in order to certify the identity of cetaceans kept in captivity, to keep a record of each individual and track their parentage in the event of reproduction.

This document will include an annex (Annex 1) containing a form where the animal's physical characteristics and genetic information to be collected in accordance with the ACCOBAMS Best Practice on Cetacean population genetics. Furthermore, the document will also describe the various stages involved in setting up the identification system of the animal, including recommendations for the creation and management of a centralized repository in the NETCCOBAMS platform.

2. Legal framework

In the following paragraphs, a background on the existing legislation is provided considering not only the legal framework related to environmental issues related to conservation but also what is known in terms of animal identification in order to implement what is already in place and to avoid discrepancies with the existing rules.

2.1. ACCOBAMS

In 2012, the ACCOBAMS Scientific Committee noted "*the illegality of live removals of cetaceans from the Black Sea*" and called for "an inventory and thorough assessment of individual identity of all bottlenose dolphins kept in captivity by means of genetic, morphological and photo-ID methods", *as well as for the provision of "appropriate administrative measures in order to prevent substitution of dolphins that die in captivity from animals taken from the wild*" (Recommendation 8.2, adopted in November 2012). Following the recommendations by the Scientific Committee, in November 2013 the Fifth Meeting of ACCOBAMS Parties adopted Resolution 5.14, entitled Live Removals of Bottlenose Dolphins in the Black Sea (*Tursiops truncatus*).

In the recent years, some experts proposed the necessity of creating facilities posing an alternative to dolphinaria, by creating a semi-captive environment; the so-called cetacean sanctuaries or refuges. In 2017, the document ACCOBAMS-MOP7/2019/Inf 09 "Taking of cetaceans dolphinaria and quasi-dolphinaria-legal analysis" provided an overview of the legal framework regarding the existence of these facilities and recalled that ACCOBAMS Parties adopted Resolution 3.20, providing Guidelines for the Release of Cetaceans into the Wild: all these document stress a particular attention that should be paid in relocating captive animals in a wild or semi-wild condition with one of the main concern being identified in genetically contamination of the local population(s) in case of escape from the pen. This concern was also expressed within the document ACCOBAMS MOP8/2022/Inf52, "Scientific perspective on "potential marine semi-enclosed facilities" in the ACCOBAMS Area", prepared in 2021 by dr. Joan Gonzalvo.

2.2 CITES

With the adoption of Resolution Conf. 8.13 (Kyoto, 1992), the Parties were encouraged to use microchips for individual marking of live animals whenever they felt this was appropriate. The potential of this method for the regulation of trade in specimens of species listed in the appendices to the Convention was also recognized. The revised and updated version of the Reference Guide to the European Union Wildlife Trade Regulations (2020) specifies that all the live vertebrates subject to marking requirements should be marked with an unalterable microchip transponder

conforming to ISO Standards 11784:1996 (E) and 11785:1996 (E). In cases where this is not possible due to physical or behavioural characteristics of the animal, a ring, band, tag, tattoo or another appropriate method should be used.

This information is usually included in different CITES Certificates as the Live Specimen, Certificate of Captive Breedings and Certificate of ownership. All these documents include all the relevant information for the identification of the animals, including a complete description of the animals reporting distinctive marks, ID type and number, place and date of birth and information regarding father and mother with all the relevant documentation. Since all the establishment should declare the list of specimens being hosted in their facilities, the certificate should be obtained in case of trade, exchange or movement of the live animals.

This document should be obtained by the owner in case of trade, exchange or travel of the specimen At its 17th meeting, the Conference of the Parties to CITES (Johannesburg, 2016) further dealt with the above mentioned species (Recommendations 17.299-301). Parties are now encouraged to use genetic analysis to confirm the origin of the animals prior to the issuance of export permits. Furthermore, Parties are encouraged to establish national or regional repositories where relevant genetic identification data are stored and to make them accessible on-line, as well as to report to the Animals Committee on exports of *Tursiops truncatus ponticus* and their origins.

2.3 EU Legislation

The EU endorsed the CITES Convention with the Council Regulation EC 338/97. More recently, the EU implement the Directive 1999/22/EC, also known as Zoo Directive. The Directive defines obligation and relevant features for facilities keeping zoo and aquaria animals including cetaceans. The Zoos Directive Good Practice Document prepared for the European Commission in 2015 gives relevant instruction on how to apply the Directive to the facilities in Europe. One of the more relevant indications is the need of animal records. Under the scope of the Zoos Directive, animal records serve two well-defined functions: i) they are a source of information for competent authorities during inspection and authorization, and; ii) they are essential for a zoo when planning and executing conservation, education and veterinary care programmes. A record includes information about an individual animal or groups of animals.

An individual animal record may contain information about its provenance, history, daily care and health condition. Examples of animal records are: transaction documents (including proof of legal ownership, purchase contracts, permits or certificates from authorities like CITES, etc.), identification information, reports of collection changes (including in-house moves), pedigrees/lineages, veterinary information, including images, test results, etc., nutrition and body condition information, information on sampling and parts/products distribution, etc.

If specific legislation exists, the system must be the one prescribed by law, and by no means use an identification method that is forbidden by its local/national legislation, even if it is commonly accepted in other countries or by the zoo community. The inspector should make sure such regulations are taken into account and followed by the zoo. Certain specimens of species listed in Council Regulation (EC) No 338/97 have to be uniquely marked or labelled, for example, for internal EU trade control purposes (e.g. live animals listed in Annex A). These marking requirements have been developed to prevent fraud and to curtail illegal trade in specimens and products that are controlled by the EU Trade Regulations Wildlife

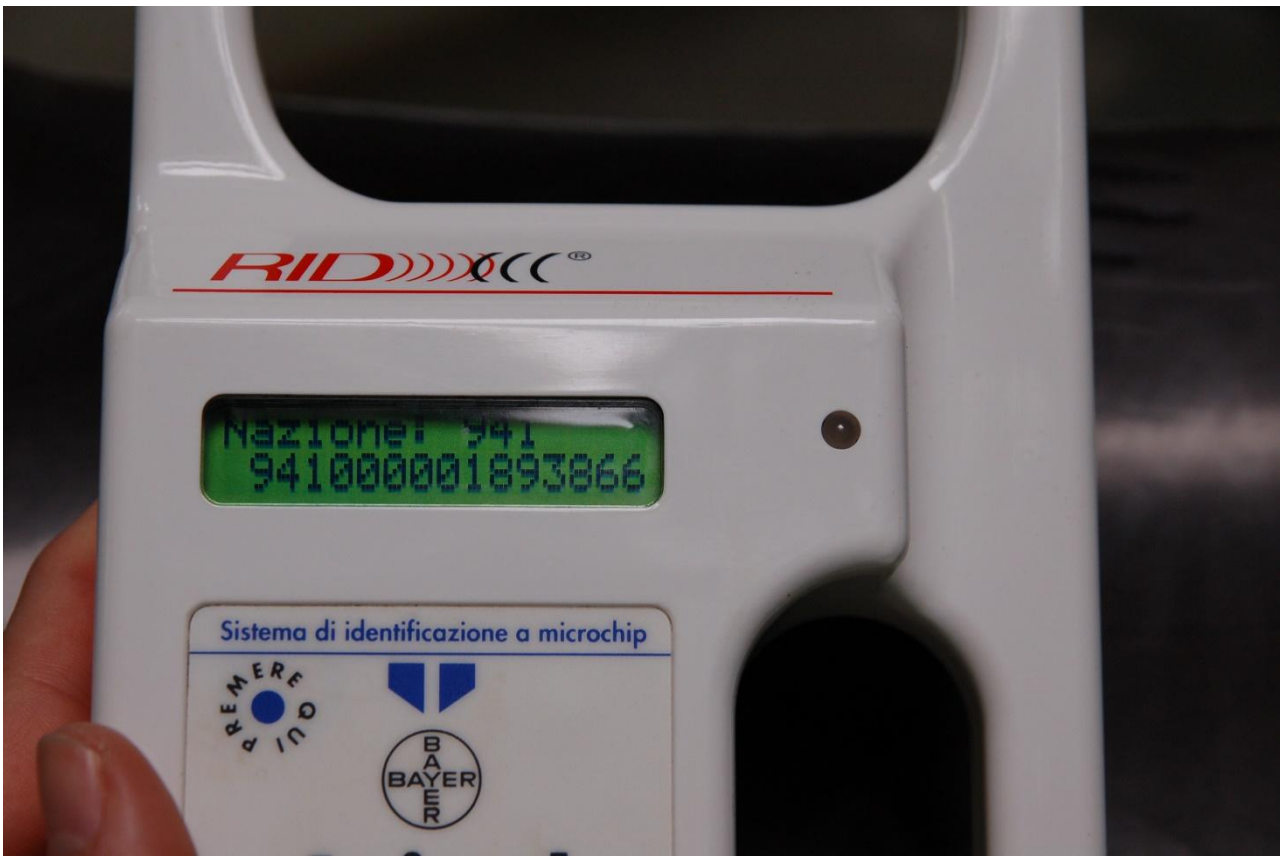


Fig. 1. Microchip detection with CITES ID number in a dolphin

A summary of relevant legislation regarding ID and marking in the EU can be found here after. All live vertebrates (mammals, birds, reptiles, amphibians and fish) listed in Annex A that are exempt from the prohibition on commercial use [Art. 8.3 of Council Regulation (EC) No 338/97], for example captive-bred specimens, must be uniquely marked in accordance with Art. 66 of Commission Regulation (EC) No 865/2006 before an internal trade certificate can be granted for their commercial use. The full details of the mark have to be provided on the permit or certificate of the specimen (Art. 68.2 of Commission Regulation (EC) No 865/2006).

EU Regulation 2016/429 on transmissible animal diseases, also known as 'EU Animal Health Law', was adopted by the European Parliament and the Council in March 2016, and became applicable in all EU Member States on 21 April 2021. Animal Health Law (AHL) is a single comprehensive framework law designed to support the EU animal sector towards competitiveness and safe and efficient movement of animals and their products. AHL repeals, streamlines, and replaces a large number of legal acts into a single law. Through this streamlining, the AHL aims to provide, among others, rules that allow greater use of new technologies for animal health activities e.g., surveillance of pathogens, electronic identification and registration of animals. In this regard, the EU Animal Health Law Guidance Handbook published in March 2024 confirms the need of animal identification in the confined establishments. Record keeping obligations for confined establishments are also in place through Article 32 of Commission Delegated Regulation (EU) 2019/2035. These record keeping requirements are linked to the approval requirements of the confined establishment, and therefore focus on the biosecurity, animal traceability and disease surveillance planning in place at the confined establishment.

3. EAZA

Most of the zoo and aquaria in Europe and Middle East are associated members of the European Association of Zoo and Aquaria (EAZA). EAZA has the main goal of putting together different facilities with the aim of standardizing procedures, ensuring animal health and welfare, provide common strategies for the management of kept population excluding animals exchange among different facilities (www.eaza.org).

EAZA has set up Taxon Advisory Group (TAG) and TAG members are professional zoo and aquarium people who work in EAZA member institutions and have specialist knowledge and a keen interest in the group of species covered by the specific TAG. EAZA has also put together all relevant policies, guidelines, statements and rules regarding breeding programme management in one concise document: the EAZA Population Management Manual. This document is a strong tool for EAZA's management of species and provides the breeding programme managers with all relevant background information as well as templates and forms to ensure high quality of breeding programme management. Additionally, EAZA established the Ex-situ programmes (EEP) which aim at conserving healthy populations of animals in captivity. Since the acquisition of new animals and the regular exchange of animals between and beyond EAZA Members is considered essential when realizing healthy, demographically and genetically sustainable populations, they include also molecular investigations for assessing different relationships, likely parenthood or population genetics, according to specific guidelines like those found in the EAZA Tissue Bank (https://www.eaza.net/assets/Uploads/Biobank/Biobank-docs/2021/EAZA-Biobank-sampling-protocol-English-final-2021.pdf).

EAZA has published in 2024 a Guidance Handbook for the implementation of the Animal Health Law (AHL) in zoos, produced in cooperation with the European Association of Zoo and Wildlife Veterinarians, with the main goal to give proper support in the applying the new EU Directive 2016/429 to zoos and aquaria as establishments. This guidance give ID requirements of animals kept in a confined establishment targeting terrestrial species. The definition of a terrestrial animal in AHL covers all species of terrestrial mammals, all avian species as well as bumblebees and bees. However, this definition does not cover mammals living entirely within aquatic environments (e.g., Cetacea, Sirenia), or reptilian or amphibian species.

Finally, EAZA has set up a common online centralized database "Species360 ZIMS" that provides a globally recognized solution to record keeping for non-domestic species in human care. Data from ZIMS can easily be extracted for audits by the competent authority and provide up to date information on the movement records into the confined establishment as well as an individual's age and gender and health status.

4. Forensic genetics.

In 2022, the ACCOBAMS Best Practices on Cetaceans Population Genetics. Even if the primary focus of this document was on matters related to understanding population structure, abundance and movements to meet its conservation and management objectives, it also confirmed that genetics can be used for a large number of issues, including also the forensic use for species identification for trade and individual identification of captive individuals. In fact, in the analysis of genetic material from an animal source, the identification of a species and an individual represents the objectives to be achieved. The ability to accept or reject a family relationship represents the final application of wildlife DNA forensic techniques.

In 2008, during the International Society for Animal Genetics (ISAG; the Animal Forensic Genetics Standing Committee) conference, members of the committee defined animal forensic genetics as "the application of genetic techniques and theories relevant to legal or law enforcement issues concerning animal biological material". DNA profiling consists of

targeting genetic markers (microsatellites or SNPs) that are highly variable within species and can be excellent tools for highlighting genetic differences between individuals.

4.1 Genetic markers

The first type of genetic marker are mini-satellites, as the difference in length of these repeated sequences between individuals provided the basis for the use of polymorphisms in identification testing. They are the hyper-variable regions of introns in the animal myoglobin gene, demonstrating the presence of highly variable regions in nuclear DNA. The analysis of minisatellite sequences combined with hybridization allows for obtaining an individual genetic fingerprint.

More recently, micro-satellites, also known as short tandem repeats (STRs), are preferred to mini-satellites due to their advantage in the analysis of degraded material and their outstanding discriminatory power that provides a virtually unique combination. Micro-satellites gained popularity due to their high level of polymorphism, producing results that constituted strong evidence and their capability to predict the phenotype.

Variants of short repetitive DNA sequences are observed in both sex chromosomes, Y-STRs and X-STRs, and autosomes. Autosomal STRs allow individual identification, while sex STRs are useful for determining sex. Given the uniparental inheritance of the Y chromosome, the Y-STR profile of related individuals turns out to be identical, and when combined with the analysis of familial searching and an investigative genetic genealogy, it allows the identification of donor relatives. Consequently, a suspect group can be narrowed down to individuals who share the same Y chromosome haplotype, and among them, the individual whose autosomal profile matches the evidence can be identified as a possible suspect.

Finally, although single nucleotide polymorphisms (SNPs) have a smaller discriminatory power, they represent an additional tool to complement STRs rather than a replacement, useful in the analysis of degraded biological material. SNP markers are successfully used in the analysis of samples which contain a small amount of degraded genetic material. Analysis of SNPs in biological traces often involves mitochondrial DNA (mtDNA) when nuclear DNA (nDNA) is not available or exploitable. Due to greater resistance to degradation and the presence of numerous copies per cell, mitochondrial DNA can be successfully analyzed in biological samples subjected to physical and chemical degradation. In accordance with guidelines concerning mtDNA typing issued by the DNA Commission of the International Society of Forensic Genetics, the entire control region must be sequenced for maximum data reliability.

The advent of new molecular biology techniques has facilitated the resolution of many forensic cases otherwise unsolvable through classical approaches (morphometric and behavioral analysis, etc.). Currently, genetic markers and specific regions targeted by the Sanger sequencing method are largely employed to corroborate and confirm morphological evidence for species identification and/or phylogenetic reconstructions.

4.2 Species identification

Wildlife forensic genetics frequently use mitochondrial polymorphisms in conjunction with DNA barcoding by analyzing the cytochrome b gene, extremely conserved within the same species. This loci, which are the preferred gene locus for taxonomic analysis and species identification, and cytochrome oxidase subunit 1 (COI), for which the sequencing of 600 base pairs (bp) was proposed as a method to record the terrestrial biodiversity. When differentiation between closely related species is required, the analysis of the mtDNA D-loop (control region; CR) will be more appropriate. Additionally, the use of a single genetic marker cannot produce high levels of confidence in taxonomic identification. To perform validating tests, the biological material of a reference sample must come from a

known source (i.e., zoological institutes), but in the absence of sample specimens, the authentication of species by sequencing a gene locus (cyt b or COI) compared to sequences in a database is equally acceptable.

It should be further emphasized that SNPs play a relevant role in forensic genetics, not only for the identification of individuals but also for reconstructing the phenotypic profile of a subject from its DNA sample. The development of genetic markers also finds an important application in the analysis of laboratory animals by ensuring the purity of breeding lines, good monitoring of model species, and the identification and variation of genes acting on specific traits. A reasonable number of samples must be considered to estimate allele frequencies, and individuals must be representative of the population from which the unknown sample might come. Often a cluster of 200 individuals from a representative population is considered a de facto standard, although the sample size depends on the number of potential contributors and the level of locus diversity. A widely used factor in forensic genetics influencing the probability of sharing an allele between two individuals with a common genetic ancestor is the kinship factor, also called "Fst". In the modern human population, the degree of common ancestry is typically low (between 0.01 and 0.03), contrary to animal populations, which present a higher Fst value.

As stated in the ACCOBAMS Best Practices on population genetics, several genetic studies identified in the Northeast Atlantic and Mediterranean Sea a clear population structuring based on mitochondrial (control region), nuclear microsatellites (9-25 loci) and Single Nucleotide Polymorphisms (~26000 SNPs) with varying geographical scales. Several studies were also carried out using mitochondrial control region DNA sequences (404-630 bp), complete mitogenomes and microsatellite loci (9 loci) to assess population genetic structure in the Black Sea, confirming it is a distinct clade compared to the Mediterranean one. In the ACCOBAMS Best Practices document, all the more relevant papers were reviewed including all the relevant markers used for the different cetacean species living in the Mediterranean Sea, Black Sea and North-east Atlantic Ocean.

4.3 Kinship relationships and sex

Defining kinship relationships is crucial both in conservation and forensic genetics, with the main application in the discrimination of wild animals from those bred in captivity. Since genetic markers are inherited from one generation to the next, DNA profiles allow us to verify the parent–offspring relationship. If alleles observed in a sample do not match those of a putative parental DNA profile, the possibility that the test subject represents his offspring can be excluded. This exclusion method to reject a parentage claim does not require profile data from the wider population and is consequently easier to be performed. However, the variability at a genetic marker level is represented by the occurrence of an inheritable mutational event, where one allele changes to another between generations.

To resolve kinship structures and determine the sex, variants of short repetitive DNA sequences in sex chromosomes are the preferred genetic markers. For instance, Y-marker typing has been used for identifying paternal lineages in some animals. Because Y-like systems are haploid, they typically display only one allele per locus. Sometimes, more than one allele is observed (due to gene duplication, translocation, gene conversion, etc.). Unfortunately, they have a great limitation in analyses concerning animals, as they are uncharacterized for many species. When X- or Y-STR typing is used to establish a relationship between individuals within a forensic analysis, the probability of mutations and the linkage must be considered. Sex can be identified by amplifying the chromosome fragments ZFX and ZFY using properly designed primers.

4.4 Geographical origin

From a forensic perspective, determining the geographic origin of a specimen is equivalent to establishing its breeding population of origin. Biological populations exhibit varying levels of genetic variation, from extended families to

subspecies, making them difficult to be defined. Populations often share genetic material; thus, genetic markers are less useful in defining discrete differences between groups. The identification of geographical origin is based on the ability to assign one sample to a particular population and rely on the existence of population data from multiple areas. Within some species, populations may be isolated from others, and they could not be subject to gene flow. In this scenario, genetic differences are accumulated over evolutionary time until members of a population living in an isolated region will display similar types of genetic markers.

Markers showing such discrete variations are extremely useful in identifying a population and thus applicable in determining the geographic origin of a given individual with a high confidence interval. The hypervariable control region of mtDNA is often used as a marker to identify the geographic origin, with individual types of control region sequences (haplotypes) corresponding to specific populations. In the event of low mitochondrial variability, it is necessary to analyze nuclear DNA markers, which display a genetic variation between regions. Although some mini-satellite and SNP markers show discrete differences, individual alleles are often distributed across populations; thus, the degree of differentiation can be assessed only through different allele frequencies in order to characterize the genetic structure and estimate the probability of a sample belonging to a certain geographic area

4.5 Single animal identification

In 2005, guidelines for animal DNA forensic and identity testing were published (Budowle et al., 2005). Based on the model for human DNA forensic analyses, a basic discussion of the issues and guidelines was provided for animal testing to include all the details, including the employment basic analytical equipment and the establishment of standard operating protocols (SOPs). The SOP should contain a description of the criteria to be used to assess a genetic profile, including identifying an allele (such as a peak or band for a STR allele), accepting an allele as typeable (such as off-scale or low-level data assessments), addressing artifacts, addressing mixtures, and addressing contamination.

Locus	Gene Bank Access Number	Tandem Repeat		Sequence primer (5′–3′)	Allele Size	Annealing T
D08	NA	(TG)n	F R	GATCCATCATATTGTCAAGTT TCCTGGGTGATGAGTCTTC	94–122	58
EV37	NA	(AC)n	F R	AGCTTGATTTGGAAGTCATGA TAGTAGAGCCGTGATAAAGTGC	189–241	56
KWM2	NA	(AC)n	F R	GCTGTGAAAATTAAATGT CACTGTGGACAAATGTAA	138–160	47
KWM9	NA	(AC)n	F R	TGTCACCAGGCAGGACCC GGGAGGGGCATGTTTCTG	170–188	59
KWM12	NA	(AC)n	F R	CCATACAATCCAGCAGTC CACTGCAGAATGATGACC	160–186	50
MK6	AF237891	(GT)n	F R	GTCCTCTTTCCAGGTGTAGCC GCCCACTAAGTATGTTGCAGC	147–187	51
MK8	AF237892	(CA)n	F R	TCCTGGAGCATCTTATAGTGGC CTCTTTGACATGCCCTCACC	80–114	58
MK9	AF237893	(CA)n	F R	CATAACAAAGTGGGATGACTCC TTATCCTGTTGGCTGCAGTG	161–175	54
Ttr04	DQ018982	(CA)n	F R	CTGACCAGGCACTTTCCAC GTTTGTTTCCCAGGATTTTAGTGC	103–127	65
Ttr11	DQ018981	(CA)n	F R	CTTTCAACCTGGCCTTTCTG GTTTGGCCACTACAAGGGAGTGAA	193–219	61
Ttr19	DQ018980	(CA)n	F R	TGGGTGGACCTCATCAAATC GTTTAAGGGCTGTAAGAGG	182–200	61
Ttr58	DQ018985	(CA)n	F R	TGGGTCTTGAGGGGTCTG GTTTGCTGAGGCTCCTTGTTGG	166–194	62
Ttr63	DQ018986	(CA)n	F R	CAGCTTACAGCCAAATGAGAG GTTTCTCCATGGCTGAGTCATCA	83–149	59
TexVet5	AF004905	(CA)n	F R	GATTGTGCAAATGGAGACA TTGAGATGACTCCTGTGGG	196–216	55
TexVet7	AF004907	(CA)n	F R	TGCACTGTAGGGTGTTCAGCAG CTTAATTGGGGGCGATTTCAC	155–169	64

Note: NA = Not available in the Gene Bank database.

Fig. 2 - Table of genetic markers used for captive dolphins population. Gomez et al., 2022

For cetaceans few genetical studies were published with forensic aims. Gomez and colleagues (2022) were able to investigate the genetic diversity and population structure of 210 captive dolphins (*T. truncatus*) from 18 Mexican dolphinariums were characterized using 15 STRs. This set of markers, reported in fig. 2 and extrapolated by the existing literature, allowed them to genetically distinguish each dolphin with remarkable efficiency. This information can be useful also for identifying paternal and maternal relationship and building a pedigree (Fig. 3)

Table 1. PCR primer sequences for the microsatellite DNA analysis

Locus	Repeat sequences (5' to 3')	Primer sequence (5' to 3')	Fragment size (bp)
D-18	$(CA)_3$-TA-$(CA)_{21}$	CCCAAAACCGACAGACAGAC GATCTGGGGATGCAGG	90
D-22	$(CA)_3$-TA-$(CA)_{21}$	GGAAATGCTCTGAGAAGGTC CCAGAGCACCTATGTGGAC	135
EV37Mn	$(AC)_{24}$	AGCTTGATTTGGAAGTCATGA TAGTAGTGCCGTGATAAAGTGC	178-224

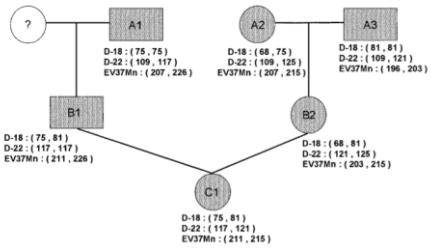


Fig. 3 - Pedigree for captive dolphins. Sumiyama et al., 2008

5. Samples and tissue for genetic analyses.

Samples and tissues to be used in genetic analysis vary if the animal is alive or dead. It should be noted that, in both cases, sampling should be conducted by trained personnel authorized by local, national and international legislation. Additionally, samples storage and delivery should be done under CITES rules. The ACCOBAMS Best Practices on Population Genetics suggest all the most relevant tissues and preservation methods to perform genetic analyses. An additional guidance is provided by the EAZA Sampling protocol which explain how to get samples and delivered them towards the EAZA Biobanks.

6. Genetic passport

Wildlife DNA registries, in which legally traded specimens can be individually recognized via their DNA profile, represent the way to ensure that illegal wildlife captured cannot be traded via the supply chain. While it has been implemented for some species as for African cheetahs (*Acinonyx jubatus*) populations, it has not yet been implemented for cetaceans kept by humans. For these reasons and to evaluate genetic pollution due to animal's release in wild or semi-captive conditions, the ACCOBAMS Scientific Committee has proposed to establish a common repository of genetic sequences and a genetic passport for each individual maintained in aquaria and dolphinaria.

A genetic passport for animals is a comprehensive genetic profile created from an animal's DNA. It contains detailed information about the animal's genetic makeup, which can be used for various purposes such as health management, breeding programs, and conservation efforts. Currently, genetic passport has been issued in some countries for dog and cats, horses, pigs, cattle and genetically altered animals. In general it could include information on:

Genetic Markers: Specific DNA sequences associated with various traits and conditions. These markers help identify breed-specific characteristics, hereditary health issues, and genetic diversity.

Health Information: Details about genetic predispositions to diseases or disorders that are common within a particular species or breed.

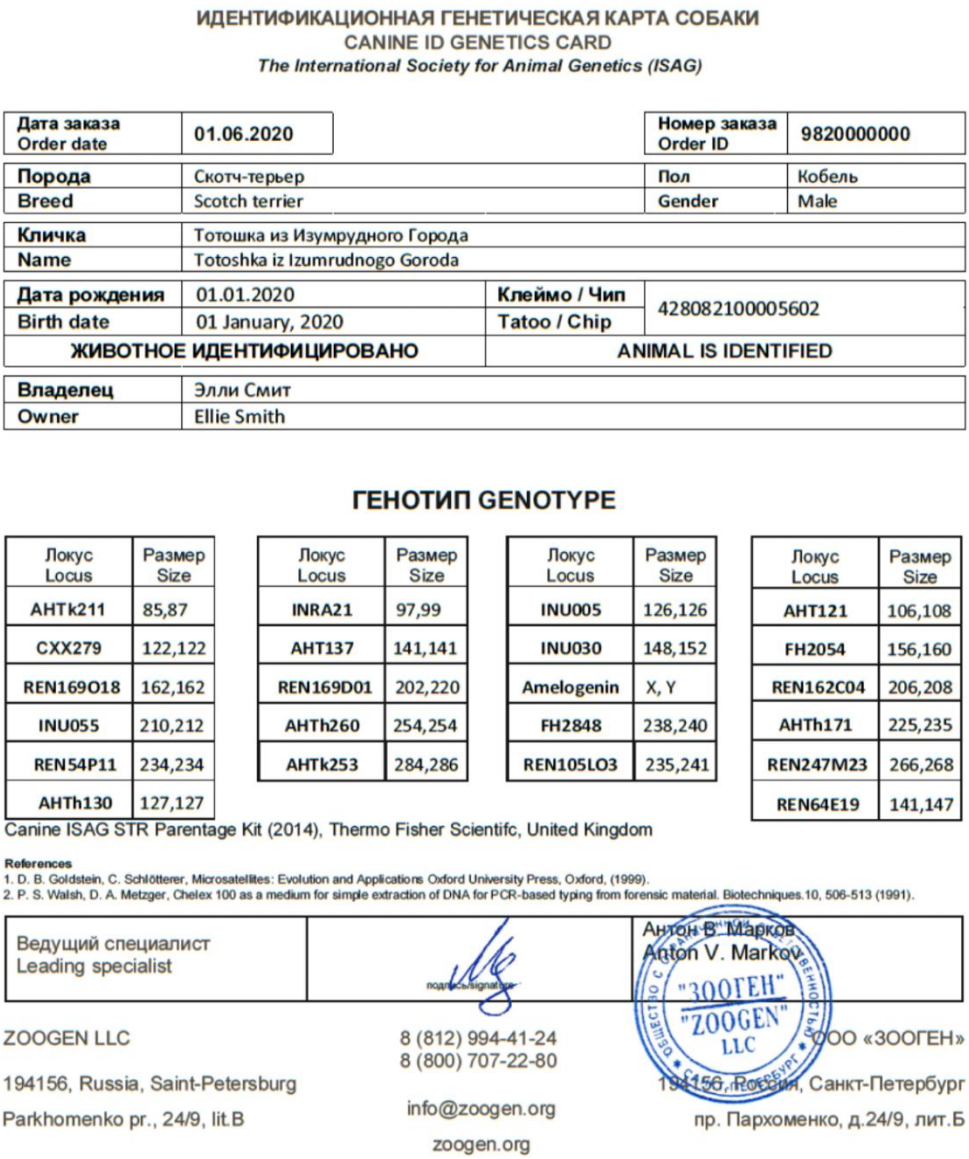
Breed Verification: Confirmation of the animal's breed or lineage, useful for purebred certification and pedigree verification.

Physical Traits: Genetic factors influencing physical attributes like coat color, size, and other breed-specific characteristics.

Behavioral Traits: Insights into genetic components that may influence behavior, temperament, and trainability.

This information can flow in a genetic pedigree, a chart or diagram that represents the genetic lineage and relationships among individuals within a species, typically used to track the inheritance of specific traits or diseases.

Few examples are available on-line, mainly for domestic animals as dogs or horses, as an example of what it could be implemented for cetaceans. Fig. 4 shows a canine ID genetic passport proposed by a company according to the International Society for Animal Genetics reporting dog information (i.e. breed, sex, name and microchip no., place of birth), owner data and a genotype section containing information about loci used for the identification which represent the unique genetic information inherited from its parents.



ИДЕНТИФИКАЦИОННАЯ ГЕНЕТИЧЕСКАЯ КАРТА СОБАКИ
CANINE ID GENETICS CARD
The International Society for Animal Genetics (ISAG)

Дата заказа Order date	01.06.2020	**Номер заказа** Order ID	9820000000
Порода Breed	Скотч-терьер Scotch terrier	**Пол** Gender	Кобель Male
Кличка Name	Тотошка из Изумрудного Города Totoshka iz Izumrudnogo Goroda		
Дата рождения Birth date	01.01.2020 01 January, 2020	**Клеймо / Чип** Tatoo / Chip	428082100005602
ЖИВОТНОЕ ИДЕНТИФИЦИРОВАНО		**ANIMAL IS IDENTIFIED**	
Владелец Owner	Элли Смит Ellie Smith		

ГЕНОТИП GENOTYPE

Локус Locus	Размер Size
AHTk211	85,87
CXX279	122,122
REN169O18	162,162
INU055	210,212
REN54P11	234,234
AHTh130	127,127

Локус Locus	Размер Size
INRA21	97,99
AHT137	141,141
REN169D01	202,220
AHTh260	254,254
AHTk253	284,286

Локус Locus	Размер Size
INU005	126,126
INU030	148,152
Amelogenin	X, Y
FH2848	238,240
REN105LO3	235,241

Локус Locus	Размер Size
AHT121	106,108
FH2054	156,160
REN162C04	206,208
AHTh171	225,235
REN247M23	266,268
REN64E19	141,147

Canine ISAG STR Parentage Kit (2014), Thermo Fisher Scientifc, United Kingdom

References
1. D. B. Goldstein, C. Schlötterer, Microsatellites: Evolution and Applications Oxford University Press, Oxford, (1999).
2. P. S. Walsh, D. A. Metzger, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques.10, 506-513 (1991).

Ведущий специалист Leading specialist	подпись/signature	Антон В. Марков Anton V. Markov

ZOOGEN LLC
194156, Russia, Saint-Petersburg
Parkhomenko pr., 24/9, lit.B

8 (812) 994-41-24
8 (800) 707-22-80
info@zoogen.org
zoogen.org

ООО «ЗООГЕН»
194156, Россия, Санкт-Петербург
пр. Пархоменко, д.24/9, лит.Б

"ЗООГЕН" "ZOOGEN" LLC

Fig. 4 - Canine ID genetic passport

6.1 Genetic passport for cetaceans kept under human care

As mentioned above (par. 2.2 and 2.3), an individual identification system including all the relevant information is requested by ACCOBAMS Parties during MOP5 with Resolution 5.14 and recalled in following documents as in the MOP8/2022/Inf52. The dolphin ID genetic card should be composed by 3 different parts/documents which should include ID information, physical marks and results from genetic analyses. The three parts should contain the following information and can be 3 different documents or a single form resulting from the combination of the 3. This form is proposed in Annex 1.

a. Information on the animal

The CITES certificate, which has to be obtained from the original facility before moving, includes all the relevant information declared by the owner and issued by CITES national authorities. In Europe and candidate countries, this information is additionally requested by the Directives 1999/22/EC, 92/43/CEE and EU/2016/429 which prohibit taking

animals from the wild environment and impose traceability and identification systems for animals like cetaceans. Minimum set of information are:

1. name of the animal
2. Species (including scientific and common name)
3. sex
4. Place and date of birth.
5. Type and number of the identification (i.e. microchip, etc.)
6. Reference of the CITES document (Certificate/permit number, date and country of issue
7. Origin: wild vs establishment defined according to EU Directive 2016/429
8. If applicable, list of the previous establishments
9. Detail of the parents (M&F) including ID (type and number), species, origin

All this information should correspond to the information filled in the CITES certificates that should be attached to the document.

b. Physical marks

Identification marks could be included in the CITES certificate, as an additional document reporting physical external traits which can help the univocal identification of the animal, similarly for the equine passports shown here below in Fig. 5.

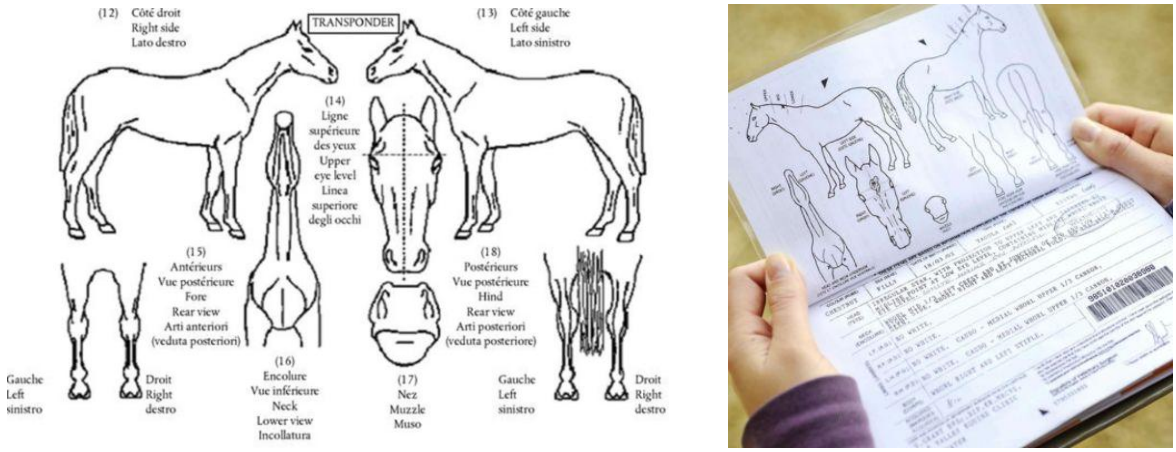


Fig. 5 - Graphic description according to Italian legislation on the equine passport

In cetaceans, very few physical features can be used for univocally identify an animal: morphometrics analysis can be used only in adults while skin marks can change during life. External color patterns are not enough informative considering that the most representative species kept by humans in the ACCOBAMS area is *Tursiops truncatus.*

The most relevant and reliable external features are the features of the dorsal fin and/or the caudal fin, besides any relevant scar (not rack-marks), relevant injuries and physical abnormalities. Pictures should be collected using the same rules for photo-identification for the species, like the one proposed by the LIFE DELFI - Basic Protocol for Field Work, Data Handling and Matching.

This information would be included in the same document of the identification sheet. The following information should be collected:

1. total length and weight

2. general picture of the animal
3. pictures (right and left) of the dorsal fin and/or caudal fin
4. pictures, description and localization of relevant morphological anomalies, diseases or injuries

If possible, x-ray of the pectoral fins should be performed for age estimation.

c. Genetic analyses

Regarding genetics information, the main purposes is providing references for animal identification and kinship analyses in order to prevent genetic pollution in case of animals' release in a wild or semi-wild conditions: the information to be coagulated should be limited to species, origin, sex and likely parental confirmation. Considering the definition of a genetic passport currently used for human beings, the document is more consistent with the animal ID genetic card rather than a real genetic passport which should also consider other markers as including also those regulating phenotypes, health and behavioral traits. This information is in line with the current knowledge on dolphins' genetics summarized in the above-mentioned literature.

6.2 Recommendations for the creation and management of a centralized repository

In order to compare the genotyping obtained from single animals, reference genomes and population reference databases is needed in a centralized repository. In forensic sciences, the establishment of reference databases and platforms helps to carry out studies focused on genetics and conservation biology. As for humans, there are many sources that forensic scientists and scholars can refer to for the analysis of animal species and a better sample localization of rare and endangered species. All this is in line with the ACCOBAMS Resolution 8.7 suggesting the use of a centralized repository in the NETCCOBAMS platform.

A first steps towards this result could be starting creating a library using specimens already existing in tissue banks: this can allow a first set of genetically characterized individuals as comparison for any analyses. This can be done when ACCOBAMS Scientific Committee could identify reference laboratories. In fact, forensic genetics guidelines recommend validation process for performing the necessary analyses to standardize procedures and avoid contamination or difficult interpretation. Laboratories should have CITES authorization, proper facilities and equipment including sequencing capacity and big data storage and analysis.

In order to proceed in this direction, some issues, mainly concerning with the animal ownership, should be solved: in fact, while free ranging cetaceans generally have no owners (res publica), the dolphins kept by humans are legally the property of some private entities and no analyzes or procedures can be imposed except those regarding single identification (already regulated by other laws) and health issues. For this reason, a first step could be to recommend that competent Authorities should adopt genetics as a technique for cetaceans identification along with other methodology imposed by CITES and/or national rules.

Furthermore, most of the facilities keeping cetaceans belongs to EAZA circuits which have an independent system of identification and management of the captive animals population aimed to manage breedings and animals' exchanges. It is not yet clear if the EEP for marine mammals is using genetics for managing the populations but an interaction with EAZA should be attempt to join efforts and evaluate common strategies. If this way is not successful aquaria and dolphinaria could choose their own circuit limiting the amount of available data limiting most of the genetic analyses. In any case, if the repository will be hosted in the NETCCOBAMS platform policies foreseen some restricted access to samples and sequences should be considered, restricting access with specific criteria.

To summarize, to build and manage a centralized repository in the NETCCOBAMS platform, it is necessary:

a. identify 1-2 laboratories that can act as a reference for the genetic analyses;
b. create a library as reference using tissues archived in tissue banks and reference areas;
c. build a system for the data entry and repository
d. define if it is necessary create a pedigree that could help the identification process.
e. discuss with EAZA regarding the access to live specimens and sharing of existing data;
f. recommend to ACCOBAMS parties that the identification system for cetaceans should be done using microchip AND genetics characterization
g. discuss and decide the level of accessibility of the repository

7. References

ACCOBAMS 2022. ACCOBAMS Best Practices on Cetacean Population Genetics. Version 1, October 2022. By Gauffier, P., Schleimer, A., Carvalho, I., Chaieb, O., Donovan, G., Fontaine, M., Fraija, N., Genov, T., Gol'din, P., Lupše, N., Mazzariol, S., Méndez Fernández, P., Panti, C., Salivas, M., Tardy, C., Tonay, A., Vishnyakova, K. 94 pp.

ACCOBAMS MOP8/2022/Inf52, "Scientific perspective on "potential marine semi-enclosed facilities" in the ACCOBAMS Area", prepared in 2021 by dr. Joan Gonzalvo

EAZA Population Management Manual: Standards, procedures and guidelines for population management within EAZA. Amsterdam April 2019 (6) Last update: June 2024

EAZA Biobank Sampling Protocol. Last update: 2021 https://www.eaza.net/assets/Uploads/Biobank/Biobank-docs/2021/EAZA-Biobank-sampling-protocol-English-final-2021.pdf

EU ANIMAL HEALTH LAW AND CONFINED ESTABLISHMENTS: A Guidance Handbook to AHL Implementation in Zoos. First Edition February 2024

Budowle B, Garofano P, Hellman A, Ketchum M, Kanthaswamy S, Parson W, van Haeringen W, Fain S, Broad T. Recommendations for animal DNA forensic and identity testing. Int J Legal Med. 2005 Sep;119(5):295-302.

Cardinali, I.; Tancredi, D.; Lancioni, H. The Revolution of Animal Genomics in Forensic Sciences. *Int. J. Mol. Sci.* 2023, *24*, 8821.

Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR. Habitat structure and the dispersal of male and female bottlenose dolphins (Tursiops truncatus). Proc Biol Sci. 2005 Jun 22;272(1569):1217-26. doi: 10.1098/rspb.2005.3076.

Kraft, S.; Rodríguez, F.; Olavarría, C.; Poulin, E.; Pérez-Álvarez, M.J. Genetic Analysis as a Tool to Improve the Monitoring of Stranded Cetaceans in Chile. *Biology*2023,*12*,748

Gómez, R.; Neri-Bazán, R.M.; Posadas-Mondragon, A.; Vizcaíno-Dorado, P.A.; Magaña, J.J.; Aguilar-Faisal, J.L. Molecular Assessments, Statistical Effectiveness Parameters and Genetic Structure of Captive Populations of *Tursiops truncatus* Using 15 STRs

Krützen, M.; Valsecchi, E.; Connor, R.C.; Sherwin, W.B. Characterization of microsatellite loci in *Tursiops aduncus*. *Mol. Ecol. Notes* 2001, *1*, 170–172.

Rosel, P.E.; Forgetta, V.; Dewark, K. Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). *Mol. Ecol. Notes* 2005, *5*, 830–833.

Rosel, P.E.; Hansen, L.; Hohn, A.A. Restricted dispersal in a continuously distributed marine species: Common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Mol. Ecol.* 2009, *18*, 5030–5045

Rooney, A.P.; Honeycutt, R.L.; Davis, S.K.; Derr, J.N. Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. *J. Mol. Evol.* 1999, *49*, 682–690.

Shinohara, M.; Domingo-Roura, X.; Takenaka, O. Microsatellites in the bottlenose dolphin *Tursiops truncatus*. *Mol. Ecol.* 1997, *6*, 695–696.

Sumiyama D, Kitamura S, Terasawa F, Hori Y, Murata K, Kulski JK, Inoko H. Paternity determination of captive bottlenose dolphins (Tursiops truncatus) using microsatellite DNA analysis. J Vet Med Sci. 2008 Jul;70(7):711-3.

Valsecchi, E.; Amos, W. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 1996, *5*, 151–156.

Hoelzel, A.R.; Potter, C.W.; Best, P.B. Genetic differentiation between parapatric “nearshore” and “offshore” populations of the bottlenose dolphin. *Proc. Biol. Sci.* 1998, *265*, 1177–1183.

ANNEX I - Genetic Passport Template

Individual information			
1. Name		**5. ID Type**	
2. Species	*Latin name*	**6. ID number**	
	Common name		
3. Sex	*M/F*	**7. Place of birth**	*Specify complete address*
4. Origin	*wild/zoo/other*	**8. Date of birth**	
9**. Previous facilities**	*1.*	*2.*	*3.*
CITES information			
10. Type of document		**12. Date of Issue**	
11. No. of the certificate		**13. Country of origin**	
14. Permit*		**16. Date of Issue**	
15. No. of permit		**17. Country of import**	
Parents information			
Father ID details**		**Mother ID details****	
18. Name		**23. Name**	
19 **.Species**		**24 .Species**	
20. ID Type		**25. ID Type**	
21. ID number		**26. ID number**	
22. Origin		**27. Origin**	
Physical marks and features			
28. Total length (cm)		29**. Total weight (kg)**	
30. General picture from the left***			

31. General picture from the right* **			
32. General picture from the top* **			
33. Picture of the dorsal fin (from the right and from the left)* **			
34. Picture of the caudal fin (from the top)* **			
35. Description and pictures of morphological peculiarities, abnormalities, scars and injures (left)** **			
a.	b.	c.	d.
e.	f.	g.	h.
36. Description and pictures of morphological peculiarities, abnormalities, scars and injures (right)** **			
a.	b.	c.	d.
e.	f.	g.	h.
X-ray for age determination	*Y/N*		
** If imported*		**** include pictures in the form and attached originals*	
*** Include all documentation for parents*		***** Refer with letter different marks on the drawings*	
Genotype			
37. Laboratory			
38. ID of the sample in the laboratory		39. Date of the analysis	

40. Description of the sample (blood/swab/tissue)		41. Date of sampling, methods or preservation and name of the sampler	
42. Species confirmation		43. Targeted genes	
44. Locus	*Fragment Size*	*Primer Sequence*	
1. D08			
2. EV37			
3. KWM2			
4. KWM9			
5. KWM12			
6. MK6			
7. MK8			
8. MK9			
9. Ttr04			
10. Ttr11			
11. Ttr19			
12.Ttr58			
13. Ttr63			
14. TexVet05			
15. TexVet07			
16.			
17.			
18.			