

Biomaterial and DNA Bank Organization for Animal Population Genetic Research

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Received May 8, 2024; revised May 17, 2024; accepted May 27, 2024

Abstract—Biobanks play an important role in population genetic studies of animals as a valuable resource for *ex situ* conservation of genetic diversity and research in evolution, zoology, ecology, and genetics. One of the main objectives of biobanks is to preserve samples of genetic material from different animal species, thus preserving information on genetic diversity and conserving *in situ* populations. This is particularly important for rare and endangered species, animal breeds, and plant varieties, where genetic diversity may be declining because of population loss. Biobanks enable the exchange of specimens and data, which plays an important role in the study of the evolution and origins of different species, helping scientists to investigate the processes of divergence and adaptation. They also serve as a source for work in the study of genetic diseases, behavioral traits, and interaction of species in ecosystems. Biobanks provide the basis for various types of genetic research, such as genome sequencing, phylogeny, DNA variability analysis, and functional genomics, which in turn provide the opportunity to develop new methods for detection of genetic diseases, genomic selection, and conservation and restoration of animal populations. Biobanking thus plays an important role in population genetic research of animals, providing scientists with access to a wide range of genetic information that is essential for understanding and conserving our planet's biodiversity. The issue of environmentally sound and efficient storage of biomaterial is more relevant than ever. In this review, we consider different approaches to the organization of a bank of biomaterials and DNA in the field of population genetic studies of animals and features of their collection, transport, processing, and storage.

Keywords: biobank, biomaterial and DNA storage, DNA extraction, transport of biomaterials, dry blood spot technology

DOI: 10.1134/S2079086424600759

INTRODUCTION

Rapid progress in the field of genomic technologies makes it possible to obtain huge amounts of information in a short time, as well as to effectively use biomaterial with varying degrees of DNA preservation. Requirements for the quality of starting material vary depending on the objectives of research and the technologies used (Love Stowell et al., 2018). The approach to storage of samples needs to be modernized and optimized to meet the modern needs of researchers (Caenazzo et al., 2020). In the scientific community, biobanks have been established for over 100 years by various institutions around the world (De Souza et al., 2013). Biobanks are large repositories of biological samples, ranging from animal samples to plant and microbial samples, which are used for research purposes. There are over 100 national animal gene banks worldwide, and some of them have significant collections; for example, the US and Brazilian collections contain 1.3 and 1.9 million samples from 64932 and 30168 animals, respectively (Blackburn

et al., 2024). According to the GGBN (Global Genome Biodiversity Network), only 13 records belong to domesticated animal species (Groeneveld et al., 2016). In Europe, according to EUGENA (European Genebank Network for Animal Genetic Resources), as of 2023, 14 biobanks of animal samples are registered (Table 1, <https://www.eugena-erfp.net/en/>).

Several small animal biobanks and less formal specimen collections can be found in many countries. The institutions where they are housed range from veterinary clinics, zoos, breeding and diagnostic companies, and national gene banks of farm animals to research institutes and universities (Groeneveld et al., 2016). Let us list the most famous of the large biobanks (Table 2). The NMNH Biobank (USA), part of the National Museum of Natural History in Washington, is considered one of the largest museum biorepositories of natural history specimens. Currently, its capacity exceeds 42 million cryogenic samples. Frozen Ark (UK) is an international project organized on the basis

Table 1. List of biobanks included in EUGENA (European Genebank Network for Animal Genetic Resources) as of 2023

Name	Country	Year of joining EUGENA	Number of samples	Number species	Number breeds
CGN	Holland	2018	547886	11	154
AREC Raumberg-Gumpenstein	Austria	2017	421389	4	46
MCB Sp. z o.o.	Poland	2022	382055	1	8
INIAV	Portugal	2023	265929	6	40
Temerin	Serbia	2018	261133	1	3
BNGA	Spain	2019	107066	6	66
NBBM	Poland	2019	54215	3	12
NCBGC	Hungary	2022	18757	10	31
GB NPPC-VÚŽV	Slovakia	2021	4250	5	15
LBTU	Hungary	2021	3318	5	6
IMGGE	Serbia	2021	1788	3	14
IAH	Serbia	2023	1109	4	14
SVCVP	Serbia	2018	585	1	1
UMo, BTF	Montenegro	2017	568	3	8

of several dozen research laboratories, which store frozen cellular material of rare and endangered species. San Diego Zoo's Frozen Zoo (USA) is a biobank that contains frozen tissue and cell samples from various animal species from the San Diego Zoo. It is used for research, conservation of species, and breeding programs. Copenhagen Zoo's Genome Bank (Denmark) is a biobank that contains genetic samples of various animal species from the Copenhagen Zoo. It is used for research in the field of conservation and genetics of animals. The National Resource Center for Non-Human Primates (USA) is a biobank that contains a wide range of samples from monkeys and other primates. It provides researchers with access to materials to study various aspects of primate behavior, health, and genetics. An example of a large biobank in the Russian Federation is the USI Bank of Genetic Material of Domestic and Wild Species of Animals and Birds of the Ernst Federal Research Center for Animal Husbandry, which stores a collection of tissue and DNA of more than 200000 animals of various species and on the basis of which large-scale population genetic studies of animals are carried out (Abdelmanova et al., 2021; Kharzinova et al., 2022). A cryobank was created at the Moscow Zoological Park to preserve the genetic resources of rare animal species for the purpose of obtaining offspring using artificial insemination, to study the features of reproduction and the morphophysiological characteristics of gametes and embryos, and to study and preserve genetic diversity (Maksudov et al., 2009, 2014).

All types of biosamples are stored in the biobank for a long but limited time. In traditional biobanks, samples are stored in large freezers and other storage facil-

ities until needed. In 1998, recommendations suggested collecting and storing in gene banks an effective population of 50 unrelated species of animals for each breed, which would allow an average level of inbreeding of 1% per generation during restoration (Blackburn, 2018). To store maximally unrelated animal species in biobanks, samples are usually selected through genetic analysis. The most common method is to compare the genetic code using polymorphic markers such as microsatellites (STR) or SNP markers. These studies make it possible to determine the degree of relationship between animals and select the most unrelated individuals for storage in a biobank. Another important criterion when selecting animals is their genetic diversity, so preference is given to individuals with the most unique genotype. The most commonly used samples in animal population genetic studies are blood, tissue, hair follicles, semen, milk, feces, feathers, and occasionally buccal epithelial scrapings (Muller et al., 2016). Let us examine in more detail the different types biomaterials and the features of their storage, transport, and DNA extraction from them.

STORAGE OF BIOMATERIALS

To store liquid samples, special freezers are required that are capable of maintaining specified parameters (-20 , -80 , -196°C), but they take up a lot of space and consume a significant amount of energy, which, together with the release of heat and the difficulty of processing freon, make this method the least environmentally friendly. It is often difficult to take into account all the associated costs. Backup freezers, generators, 24-h staffing, maintenance, security, quality management, and air conditioning are just

Table 2. Examples of biobanks storing different types of frozen animal biomaterial

Name	Number of samples	Number of species	Country
National Museum of Natural History	4200000	18000	United States
National Animal Germplasm Program	1281819	40	United States
Frozen Ark	48000	5500	United Kingdom
BioBankSA	80000	500	South Africa
Australian Frozen Zoo	50000	298	Australia
The National CryoBank	30000	—	Canada
Conservation Genome Resource Bank for Korean Wildlife	13475	407	Korea
San Diego Zoo's Frozen Zoo	11000	1300	United States
Kunming Wild Animal Cell Bank	1455	289	China
URI Bank of Genetic Material of Domestic and Wild Species of Animals and Birds of the Ernst Federal Research Center for Animal Husbandry	200000	22	Russia

some of the costs associated with storing biospecimens in refrigeration equipment (Muller et al., 2016). Blood collection by venipuncture requires timely processing by centrifugation and aliquoting, which is very time-consuming and labor-intensive (McClendon-Weary et al., 2020). An alternative method for storing biospecimens that is cost-effective and logistically attractive is the organization of storage of samples at ambient temperature (Lou et al., 2014; Muller et al., 2016). The ability to stabilize biospecimens at ambient temperatures for extended periods of time may present operational advantages during both long-term storage and transport of samples. One of the challenges of biobanks is backing up samples to a geographically separate location to reduce the risk of freezer damage in the event of an emergency. The ability to create backup samples stored at room temperature can reduce the need for additional storage space. However, despite the overall attractiveness of ambient temperature sample storage, there is a pressing need for long-term sample and nucleic acid stabilization technologies that provide cost-effective and simple storage of biological samples at ambient temperature and do not require cold chain transport or complex sample recovery protocols (Lou et al., 2014; Muller et al., 2016).

Fabrics can be stored in a dried form at room temperature in separate ventilated packaging to prevent the growth of fungi and bacteria. This method has a minimal negative impact on the external environment, but DNA degradation occurs quite quickly, and the process of extracting DNA from dry tissue is more labor-intensive and time-consuming than, for example, for blood. For best DNA preservation, it is optimal to freeze the obtained material as quickly as possible, since storage at temperatures above 4°C for several days deprives researchers of the opportunity to isolate complete DNA from samples (Al-Griw et al., 2017). Fixation in alcohol allows tissue samples to be trans-

ported at room temperature without prior drying, but further storage is best done in refrigeration equipment. Before isolating nucleic acids, the material requires thorough drying from alcohol, but lysis occurs faster than with dried samples. In animal husbandry, the method of one-time marking with ear tags and collecting tissue from the puncture site into a sterile numbered tube with a preservative is currently gaining popularity, which helps to avoid contamination, marking errors, and rapid degradation of biomaterial (Beketov et al., 2024). Depending on the type of preservative, such samples can be stored at room temperature for one week to a month.

A less invasive and more gentle approach to pets is to use somatic milk cells to obtain genetic material. Standard methods do not provide sufficient DNA from milk for genomic studies, only for amplification of mitochondrial genes or short microsatellite loci. Therefore, modification of traditional large volume (12–14 mL) milk pre-centrifugation extraction protocols is required to collect sufficient cells to subsequently obtain 12–45 ng/μL DNA (Liu et al., 2014). Currently, there are kits available that allow milk to be stored at room temperature for a month before DNA extraction (Norgen's Milk DNA Preservation and Isolation Kit).

For breeding animals, sperm is successfully used as biomaterial. The unique packaging of sperm DNA makes it resistant to DNA extraction methods used for somatic cells. All existing protocols for accessing sperm DNA use a combination of three components: detergents and/or chaotropic salts to facilitate cell lysis; proteinase K to digest nuclear proteins; and reducing agents to break disulfide bonds between protamines (Wu et al., 2015). In general, traditional commercial kits such as Chelex-100 (Bio-Rad, USA) and DNeasy Blood & Tissue Kit (Qiagen, USA) are suitable for DNA extraction from animal sperm. The for-

mer allows higher DNA concentrations to be obtained, while the latter's columns are more successful in removing contaminants (Silva et al., 2014).

Hair follicles are often preferred as a source of nucleic acids owing to their ease of collection, as they have the advantage of storage of dry biomaterials; however, the quantity and quality of the DNA obtained is only sufficient for microsatellite analysis and genotyping for several dozen SNPs. To obtain higher DNA concentrations, at least 40 follicles must be used and stored at 4°C (Gurau et al., 2021).

When studying wild animals that are not the object of commercial hunting, it is often impossible to obtain biomaterial directly from the individual. In such cases One option is to collect feces, which can be fixed in alcohol or dried for transport. The procedure for extracting DNA from fecal samples is very labor-intensive, and the yield and quality of DNA are extremely low with high levels of contamination. The DNA obtained in this way can be used only for a limited number of experiments that do not require large quantities and the preservation of chains, such as, for example, microsatellite analysis. However, for some STR loci, the synthesis of alternative primers is still required to ensure the amplification of highly degraded DNA within 100–150 bp (Dimsoski et al., 2017).

Transport of Samples

For population genetic studies, the issue of obtaining and transporting samples is of particular importance. In conditions of expedition and field work, there is often no access to a constant power source, which makes it impossible to maintain the required temperature conditions. In addition, the optimal time period between collection, storage, and analysis to maintain sample integrity may not be observed, reducing the reliability of the data (Fouts et al., 2020).

Certain difficulties when working with animals, especially wild ones, lie directly in obtaining biomaterials, since not every animal will allow a person to approach and, even more so, take samples. To collect animal tissue samples remotely, a remote veterinary injector or crossbow equipped with a dart with a biopsy needle is used. When a biopsy needle hits an animal, it cuts out a piece of skin, which then falls out on its own or is pulled out using a cable attached to the dart (Spasskaya et al., 2022). Animals harvested by hunters or traps, as well as samples obtained using combs to collect hair from animal passages (trails, burrow exits, etc.) and home range marking sites in territorial species, can be a useful resource for studying the gene pools of wild animals (Yu et al., 2007; Sintasath et al., 2009; Curry et al., 2011; Aston et al., 2014).

Certain fixation methods are suitable for preserving nucleic acids: the most commonly used are 96% ethyl alcohol or drying of samples. To prevent blood clot-

ting, special vacuum tubes are used with K2 or K3 EDTA salts applied to the walls, which bind calcium ions; however, without refrigeration equipment, hemolysis will only be delayed for 24 hours. Liquid samples of blood, saliva, and other biological fluids can be easily applied to a narrow glass fiber membrane in the field, dried for 1–2 h without the need for special equipment, and then transported to laboratories in a paper envelope by mail or courier service (Samsonova et al., 2022). This method does not require special temperature conditions; the samples take up little space and are stored for a long time. To reduce the risk of nucleic acid degradation, it is possible to use membrane materials pretreated with nuclease inhibitors and other preservatives. For example, commercial FTA cards (Flinders Technology Association) contain reagents for cell lysis, protein denaturation, and protection of nucleic acids from nucleases, oxidation, and UV degradation. It is also possible to use bactericidal and fungicidal agents to prevent contamination of samples.

Positive experiences have been reported in extracting animal DNA from dried blood spots (Fowler et al., 2012; Samsonova et al., 2022), milk (Venkatesh and Gopal, 2018), and insect homogenate (Desloire et al., 2006) and conducting genetic studies after several years of storing such samples on cellulose cards at ambient temperature. An approach to collecting and storing dry insect DNA samples on a membrane carrier at room temperature was also presented (Miller et al., 2013), in which the storage stability of such samples was at least two years. An analysis of published works that touched upon various aspects of transportation and storage of dried spots of animal biological fluids showed that, in most cases, dried samples with target analytes can be transported to the laboratory under ambient conditions or even at elevated temperatures (37°C) without loss of stability for at least 7 days, although such samples can often be stored much longer (Samsonova et al., 2022). However, as some authors note, humidity is probably the most critical parameter at the time of collection and during storage of dried samples, including for genetic studies (Love Stowell et al., 2018). In any case, dry storage is necessary not only for the stability of the target analyte in the dry sample but also to prevent mold growth and potential membrane/sample contamination. In this regard, the use of bioassay carriers made from nonnatural materials, such as polymer or glass fibers that are poorly biodegradable, is promising.

Using Dried Blood Spot Technology for Storing Biological Material and Conducting Genetic Research

Dried blood spot (DBS) technology offers many advantages over other blood storage methods owing to the reduction in time and equipment required to collect samples, as well as ease of handling, storage, and transport (McClendon-Weary et al., 2020). In addi-

tion, the small sample size required makes this method attractive in cases where sampling is limited, such as in neonates or small animals. The use of filter paper or a special fiberglass membrane allows capillary blood to be collected without venipuncture and samples to be quickly dried and stored in small volumes at different temperatures—from room temperature to 80°C. This method is widely used in medical research, in particular, for the detection of infectious and hereditary diseases, but the prospects of the method in population genetic studies are underestimated (Carpentieri et al., 2021). However, DNA samples collected, for example, during epidemiological studies, can be stable on filter paper for many years and decades when stored under dry conditions (Chaisomchit et al., 2005; Gauffin et al., 2009). DNA extraction from dry samples can be performed using either express protocols, in which after a short temperature lysis (at 70–95°C for 5–15 min) the samples can be immediately used for PCR (Express-DNA-bio, Alkor Bio-M LLC, Moscow; Gordiz Sprint, Gordiz LLC, Moscow; etc.), or standard protocols for liquid blood or dry spots (salt reprecipitation method, phenol-chloroform method, sorption on magnetic and fiberglass carriers, spin columns, etc.). The procedure for nucleic acid extraction from membrane-dried samples may require optimization to recover the highest possible level of extractant, as each method has its own advantages and disadvantages (Ali et al., 2017). Optimization of nucleic acid extraction has, for example, been described for protocols of DNA/RNA extraction from ticks (Desloire et al., 2006), from canine DBS (Tani et al., 2008), from dried brain tissue (Sakai et al., 2015), and from human DBS (Molteni et al., 2013; Kumar et al., 2019). Rapid extraction methods are often used to perform micro-satellite analysis because there is no need to further normalize and dilute the resulting DNA. However, such methods are not suitable for protocols of sequencing and whole genome analysis sensitive to DNA quality, quantity, and purity (Steinberg et al., 2002), which should be kept in mind when planning the structure of a biomaterial and DNA bank. It has been shown that whole genome (WGS) and whole exome (WES) sequences can be obtained from DBSs collected during neonatal screening and archived for decades which match the sequences DNA from freshly obtained blood from the same individual with an error rate of no more than 1.5%. From dry samples stored for 27 years at $T = -20^{\circ}\text{C}$, DNA was successfully isolated and whole-genome amplification was performed, followed by analysis of single-nucleotide polymorphisms (SNPs) (Hollegaard et al., 2013). The advent of commercially available Phi29 polymerase and multiple displacement isothermal amplification (MDA) kits has greatly expanded the possibilities for using small amounts of DNA for whole genome studies (Sjöholm et al., 2007). It has been noted that cellulose cards are a convenient tool for long-term storage of archival samples under normal

environmental conditions for subsequent DNA and RNA extraction; however, for optimal performance, low relative humidity must be maintained during storage of such samples (Smith and Burgoyne, 2004; Owens and Szalanski, 2005; Lou et al., 2014; Muller et al., 2016). The use of membrane-dried biospecimens for collecting, archiving, and storing genetic material from mammals and insects has been described (Smith and Burgoyne, 2004; Owens and Szalanski, 2005; Lall et al., 2010; Miller et al., 2013). For example, after storing bird blood spot samples on FTA cards at room temperature (18 to 43°C) for 44 months, DNA was successfully isolated from them and the ILRC locus was amplified (Smith and Burgoyne, 2004). A variety of membrane-dried samples of biological fluids and animal tissues have found application in population genetics research as a convenient tool for collecting and studying samples. Numerous examples of such use are summarized in a recently published review on the use of SPC technology for veterinary applications and biological research (Samsonova et al., 2022).

ISOLATION OF DNA FROM VARIOUS BIOLOGICAL MATERIAL

One of the important aspects of creating a DNA biobank is the high-quality extraction of nucleic acids. High DNA concentration and the absence of protein impurities and residues of buffer mixtures and alcohols used in the extraction process directly depend on the selected extraction method. Currently, the most common methods used are the reprecipitation method, the method using a sorbent or magnetic particles, and the column method (Lubennikova et al., 2020). All methods are characterized by the main stages of nucleic acid extraction: lysis of samples, during which the destruction of cell membranes and proteins occurs owing to the addition of a cell lysis buffer containing detergents and chaotropic agents, and the enzyme proteinase (Antonova et al., 2010). The process of lysis of samples may vary depending on the biological material used and its preparation. Thus, if for blood 15–60 min of lysis of samples at a temperature of 56–60°C is sufficient, then for flesh, muscle tissue, and antlers it may be necessary to leave the samples in a thermostat overnight with a temperature decrease to 37°C for more successful destruction of cellular structures.

A number of authors note the high yield and purity of the DNA samples obtained when isolated by the reprecipitation method, as well as by the commercial QIAamp® Blood Mini Kit column kit (Chacon-Cortes and Griffiths, 2014; Schiebelhut et al., 2017). However, it is worth noting that DNA reprecipitation requires highly qualified laboratory staff and experience with routine methods, as this method is time-consuming and there is a risk of removing precipitated nucleic acids if work is not done carefully. One of the

Table 3. Efficiency of selection methods according to four criteria (where the highest score means the best efficiency)

Method	DNA yield	Purity	PCR efficiency	Cost	Average value
Phenol-chloroform	1	0.97	1	0.27	0.81
PAL1 (Fiber Plate DNA Extraction Kit)	0.61	0.66	0.83	0.81	0.73
TKFS (magnetic particles)	0.58	0.97	0.95	0.4	0.72
QIAG (Qiagen DNeasy™) columns	0.58	0.5	1	0.44	0.63
PMAX—Promega Maxwell®	0.33	0.5	0.91	0	0.58

DNA yield is the average amount of ng DNA/mg tissue, where the maximum value is 1. Purity is presented as a proportion of samples with a minimum amount of impurities defined by the ratio $A_{260}/A_{280} \geq 1.80$, and the efficiency is the proportion of samples that were amplified during PCR. Cost is the difference between the unit and the dollar cost per sample. The average value is proportional to the average score for the four criteria for each method (according to Schiebelhut et al., 2017).

undoubted advantages is the low cost of reagents. In comparison, commercial methods allow for shorter working times with large amounts of material and also reduce the impact of the human factor. Thus, it is necessary to conduct our own research on a comparative analysis of the kits presented in Russia. The magnetic particle extraction method shows moderate values of both purity and concentration of DNA and is one of the simplest methodologies. Results may vary depending on the material and commercial kit selected. The column method, according to the authors of the article (Schiebelhut et al., 2017), is most favorable for DNA extraction from tissues; when extracting from blood, lower concentration values are shown, but nevertheless with satisfactory purity of the extracted samples (Table 3).

A special case is the extraction of DNA from ancient biomaterials. Paleontological and archaeological samples contain very small amounts of DNA, which is usually highly fragmented. Therefore, the success of further molecular genetic analysis depends on the quality of DNA extraction (number of authentic matrices, absence of contamination and impurities). (Grigorenko et al., 2009). Typically, genomic DNA is isolated from bone powder obtained from teeth. Before obtaining the powder, the teeth are removed from the skulls, treated with hydrogen peroxide, and irradiated with ultraviolet light (254 nm). DNA is extracted from dentin powder using commercial kits Prep Filer™ BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific Inc., USA), QIAamp DNA Investigator Kit (Qiagen, USA), or COrDIS Extract decalcine (GORDIZ LLC, Russia) (Andreeva et al., 2022).

Thus, the success of effective use of a biobank depends on the isolation methods used. The quality and capabilities of a biobank depend on the correct selection of the type of biomaterial used to form the bank, taking into account the possibilities provided by modern extraction methods. However, a more detailed analysis of the commercial kits available on the market and their success when applied to different biological materials is required.

Storage and Quality Control of Isolated DNA

Storing isolated DNA helps minimize the risks associated with biomaterial degradation. Purified DNA stored in liquid phase can be subject to degradation by a variety of factors: water, UV radiation, ozone, oxygen, metabolites, and various associated contaminants (e.g., traces of metal ions, lipids, and polyphenols). These factors lead to DNA damage such as depurination (the main degradation process), depyrimidination and deamination, oxidation of bases or sugars, crosslinking of strands, and DNA single-strand breaks, which pose a serious threat to DNA integrity. Some of these factors are the source of errors that ultimately appear during the sequencing and amplification processes commonly used in DNA analysis (Lindahl, 1993).

In blood samples, the concentration of isolated DNA decreases by an average of 3–5% per year, while solutions of well-purified and concentrated DNA can be stored at $T < -32^{\circ}\text{C}$ without reducing its quality and quantity (Skirko et al., 2020; Kalinin et al., 2022). Another advantage is the smaller volume occupied in freezing equipment by DNA tubes (0.2–1.5 μL) compared to blood tubes (4–8 mL). The choice of container has a significant impact on the cost of storing one sample. DNA can be stored in either a 200–300 μL or 1.5–2 mL tube, which increases the cost of storage by at least 3 times simply because the container takes up more space in the freezing equipment (Muller et al., 2016).

Storage of isolated DNA can be carried out under various conditions. The primary storage mode is considered to be low temperature storage, but it is possible to store DNA in dried form in the presence of stabilizers or as spots on paper/fiberglass at room temperature (Muller et al., 2016). Long-term storage of extracted DNA samples is recommended at -80 or -196°C (liquid nitrogen). To assess the quality and quantity of DNA, electrophoretic separation in a gel is widely used together with molecular weight markers (data on the lengths and concentration of fragments are provided by the manufacturers). Visualization of a sample

in the high molecular weight region (>30 kb) without smear (coloring along the entire length of the gel without a clear strip) indicates its high state of preservation. For monomolecular sequencing on the PacBio platform, it is recommended to use DNA with a size of >100 kb, which can be visualized using pulsed electrophoresis, fractionating high-molecular DNA fragments from 10000 bp to 10 Mb under conditions of a (pulsating) electric field periodically changing in direction (Chef-DR II System, Bio-Rad) or an automated pulsed field source (Femto Pulse system, Agilent), allowing separation of DNA fragments up to 165 kb in length.

The purity and concentration of DNA in samples can be assessed using a spectrophotometer, including in microvolumes (e.g., NanoDrop—1–2 μL) (Doludin et al., 2020). However, more reliable concentration data can be obtained using a fluorimeter (Qubit), which allows one to estimate only the concentration of double-stranded nucleic acids without the influence of contaminants. Given that the risks of genotyping errors in degraded DNA decrease with increasing amounts of good quality DNA (Arandjelovic et al., 2020), DNA preparations with a dsDNA concentration of at least 1 ng/ μL , an A260/A280 ratio within 1.6–2.0, and an A260/230 ratio of 1.5–2.6 are considered suitable for conducting microsatellite studies; for conducting studies on SNP markers, values of at least 3 ng/ μL , 1.8–2.0, and 1.5–2.6, respectively, are suitable. To perform whole genome sequencing, the requirements for the source material are even higher: DNA concentration of at least 10–15 ng/ μL , OD260/OD280 ratio in the range of 1.8–2.0, and A260/230 = 1.5–2.6, fragment lengths from 30 kb.

MARKING AND CATALOG OF SAMPLES STORED IN THE BANK

Labeling of animal biomaterial samples in a biobank is an important procedure that allows for the unambiguous identification and tracking of each sample in the repository. Labeling of samples ensures the accuracy and reliability of their identification, which is critical for the proper operation of the biobank. Here are several methods for labeling animal biomaterial samples in a biobank (Zagorovskaya, 2013):

(1) Barcodes are one of the most common methods of labeling samples. Each sample is assigned a unique barcode containing information about it. Barcodes can be applied to labels that are attached to sample containers or directly to the containers themselves.

(2) RFID (Radio Frequency Identification) tags are a technology that enables contactless identification and tracking of samples. Each sample is equipped with a special RFID tag that contains information about it. Using radio frequency readers, it is easy to locate a sample and access its descriptive data.

(3) QR codes (Quick Response) are two-dimensional barcodes that can contain a large amount of information. They can be used to label samples, similar to barcodes. QR codes can be scanned using mobile devices or dedicated scanners to access information about each sample.

(4) Unique numbers: each sample may be assigned a unique number or code that allows it to be identified. This number may be written on labels or printed on sample containers.

Requirements for labeling samples are determined by the conditions and duration of their storage. The most stringent requirements apply to cryogenic labels. In addition to resistance to temperature changes, their adhesive layer and printed surface must be resistant to condensation, solvents, and mechanical abrasion. Manufacturers of laboratory equipment offer labels and printers specially designed for labeling samples for cryopreservation. They maintain high quality marking throughout the entire storage period. Their cost is about 150–250 rubles (\$1.6–\$2.7) per tube (Cryo-DirectTAG™, NitroTAG™, Thermo Scientific). Biobanking-specific materials and labeling printing tools are not always available and they can be costly to reorganize existing biobanks containing thousands of samples. An alternative is to select materials and methods for printing labels taking into account storage conditions and characteristics of containers (cryotubes). Some production areas have high labeling requirements and the corresponding materials have specifications. For example, labels with suitable parameters are used to identify cables in the far north, as well as when storing frozen foods. Most areas use thermal and thermal transfer labels. Thermal labels are made from cellulose and impregnation that darkens when heated. They are not resistant to moisture and abrasion, so they can only be used for short-term marking of samples. On thermal transfer labels, data is printed by heating the ink ribbon with the printer's thermal head. They are significantly more durable and can be made from sustainable materials, including synthetic polymers. It is known that materials that are identical or similar in chemical nature provide the most reliable contact during gluing. For example, vinyl labels with an acrylic adhesive layer are well suited for plastic Eppendorf-type test tubes. The sizes of the labels are also selected on the basis of the types of test tubes. Additional reliability of fixation can be achieved by using labels whose length is slightly longer than the outer circumference of the test tube, due to overlapping gluing. The height of the labels should be convenient for their placement on a flat (cylindrical) surface of a test tube or other container. For example, on the basis of these requirements, labels measuring 12.5 × 32 mm are suitable for the above-mentioned Eppendorf tubes. Cryovials often have an independently separating cap—the markings on it are duplicated to avoid cross-contamination when taking aliquots.

Each sample in the database is accompanied by descriptive data, including information about the animal species, location and date of collection, sex, age, and other characteristics that may be useful for analysis. The importance of detailed, high-quality phenotypic data for genome-wide studies cannot be overstated. Of great interest is also the inclusion of geographic information on sampling locations in the catalog, allowing for the creation of maps of the range of species, the identification of areas of high biodiversity, and the assessment of the impact of environmental change on populations, and the planning of conservation measures by identifying genetically distinct populations that may require special conservation strategies. This allows conservationists to identify priority areas for protection on the basis of genetic diversity and geographic distribution, ensuring the conservation of unique genetic lineages within a species (Comizzoli and Wildt, 2017). Genetic data can provide insights into the health and viability of populations, such as levels of inbreeding, genetic diversity, and adaptive potential (Spasskaya et al., 2022). By combining this information with georeferenced data, researchers can track changes in population status over time and evaluate the effectiveness of conservation measures. Integrating georeferenced and genetic data is critical to detecting invasive species and understanding their spread. Genetic data can help identify the source of invasive populations, while georeferenced data can help track their movements and develop management strategies to combat their spread. In the case of studies of domestic animal species, integration with geographic information systems can be used to identify sampling locations and indicate existing potential gaps in collection points. A spatial assessment of the coverage and completeness of the biobank (Brazilian Germplasm bank) with information on populations of different sheep breeds in Brazil revealed a lack of samples of individual populations and even a complete absence of some breeds, as well as patterns of changes in genetic distances depending on the distance from the breeding core (McManus et al., 2021).

The purpose of the cataloging system is to compile and update the list of samples and data on their location in the biobank. The sample is assigned a number which corresponds to a catalog entry and a location in the repository. The ability to quickly obtain up-to-date and reliable information about the location of a sample determines the convenience and quality of a biobank's work. The less accurate the information about the position of a sample, the longer it takes to find it, during which time other samples are exposed to temperature fluctuations and condensation, as well as the risk of contamination. Updating the data on the position of a sample reduces the risk of its loss, especially with repeated access (Dagher, G. and Dagher, A., 2018).

The position of a sample in the biobank, as well as its retrieval from storage, can be tracked automatically,

for example, using a scanner that reads the label on the bottom of tubes located in cryostalls with a transparent bottom each time they are placed in a refrigerator (Henderson et al., 2019). Thus, such a device, coupled with a spreadsheet on a computer or tablet, solves the main task of automating a biobank. Fully robotic systems allow samples to be retrieved remotely, sent for testing, and returned to storage, while recording the outcome of the manipulations, such as the number of defrosting cycles and the residual sample volume (Linsen et al., 2020).

Software and engineering automation tools for biobanks are not a standard product and have a high cost, which is also formed by demand in high-budget areas of medicine (Linsen et al., 2020). At the same time, automation tools are also used in routine clinical laboratory practice, and their primary function—tracking samples—coincides with the main task of cataloging in biobanks. Sample handling, analysis, quality assessment and volume measurement, and storage and retrieval are the capabilities of such systems that are most in demand in biobanking (Linsen et al., 2020). Automation tools in clinical laboratories emerged earlier than in other biomedical fields and are now in demand and widely used, so they can be highly reliable at low cost (Linsen et al., 2020). However, they may lack biobanking-specific features.

The need for automation increases with the increase in volume of the biobank and frequency of access to samples. Questions of convenience, economic feasibility, and reliability of electronic systems compared to physical catalogs remain open and deserve special attention in each specific case. Rational choices can be made by understanding the advantages and disadvantages of these biobanking methods. Thus, the NAGP (National Animal Germplasm Program, USA) required an update of the database program in connection with the addition of new animal species, which revealed the need for a more flexible and scientist-friendly database configuration, in particular, because these diverse species and breeds do not have a common taxonomic structure (Irwin et al., 2012).

Automated systems improve preservation of samples by reducing variations in storage conditions (Powell et al., 2019). In particular, the acceleration of search and manipulation reduces the “penguin effect”—uneven freezing of samples in the center and at the edges of the rack. Automatic recording significantly reduces the risk of loss of samples (Powell et al., 2019). Despite the high cost, automated systems can reduce some costs, primarily owing to more compact sample storage (Powell et al., 2019), which reduces the number of refrigerators and the need for personnel.

The disadvantages of automatic systems include their requirements for the standardization of materials for storing samples. Most automated systems cannot handle all types of tubes, labels, and cryoboxes (Pow-

ell et al., 2019). Automated systems are more demanding in terms of energy supply conditions and personnel qualifications. Thus, in addition to the price of the system itself, operating costs and consumables costs with automation can be significantly higher than with manual biobank management. Thus, automation is most relevant when there is a large number of samples of the same type, high cost and stability of energy and human resources, long storage periods of samples and the need for repeated access to them. However, even the largest biobanks may be manually managed, like national book libraries in the pre-electric era.

Cataloguing samples in animal population genetic studies helps to create a database that serves as a basis for conducting analyses, identifying genetic trends, assessing levels of genetic diversity, and making decisions about population conservation. It is an important tool for understanding the genetic structure of animals and developing measures to conserve their biological diversity. The wide range of genetic resources in gene bank collections, derived from diverse environments, can help accelerate the identification of genes or combinations of genes that will improve adaptation, productivity, and other aspects of importance to humanity. Together with geographic information systems, high-throughput genotyping, and high-quality phenotyping, such collections can serve as a source for highly informative databases and for determining potential uses depending on environmental or economic factors (Blackburn et al., 2024).

ETHICAL ASPECTS OF BIOBANKING IN ANIMAL GENETICS

The lives of humans and animals are closely linked. This is especially true for species in which humans have scientific interest. Population genetic studies can have significant implications for both humans and animals. In the modern world, there is a gap between increasing technical capabilities and knowledge and a less active understanding of the impact of this progress on other areas of life and universal human values. Therefore, attention to the ethical aspects of biobanking is increasing significantly, and the development of an appropriate legislative framework is becoming a necessity (Smith and Johnson, 2021). The FAO has developed guidelines that address the physical structure, minimum human resources, and equipment requirements (FAO Animal Production and Health Guidelines No. 12 (2012). The country's response to the above elements and these recommendations is a matter of national policy and support for the activities of the gene bank by foundations. In Russia, there is practically no regulatory framework in this area compared to other developed countries (Maleina, 2020). Meanwhile, the formation of a regulatory framework for working with biological collections is absolutely necessary, primarily because of the close connection between biological collections and the concept of

“national biological resources” (Kamensky et al., 2016). In the absence of strict legal regulation, the ethical assessment of the researcher completely determines the consequences of his work. This is especially important in countries with rich biodiversity, including Russia (Petrova, 2020).

In 2018, the National Association of Biobanks and Biobanking Specialists (NASBIO) was founded with the aim of uniting specialists and research centers to create and develop a network of biobanks in Russia. Work is currently under way to create the National Information Platform of Biobanks of the Russian Federation (NIPB RF), which will provide access to information on the activities of biobanks, their collections, and the conditions for scientific cooperation and the provision of biosamples for research (Meshkov et al., 2022). This information system may contain geographic information system (GIS) data on the location of collection and storage of biomaterial, as well as descriptions of phenotypes and results of genetic analysis. GIS can be used to monitor the distribution of rare animal species and populations and to plan expeditions for collecting samples. Using biocollections with a detailed description of phenotypic data, it is possible to conduct research to identify the genetic mechanisms of their formation, as well as to search for new associations and markers. Biobanks contain information on genetic variations associated with adaptation to different geoclimatic conditions and resistance and with productive qualities of farm animals. These data represent an economic resource that can be harnessed to address new breed developments, changing climates, food demands, and changing consumer tastes (Adams and Getz, 2022).

Aboriginal (local) animal breeds often play an important role in the lives of the ethnic groups associated with them, for example, in reindeer herding. Information about the genotypes of these animals can be used to preserve the population, search for economically valuable adaptive qualities, and create breeds with desired properties (Rijal and Sharma, 2022). However, such use of data may give rise to conflicts between private and public interests and may affect the autonomy of individual populations. For example, the created productive breeds compete with native animals, which can play an important role in the lives of the local population. The very choice of the object of population genetic research carries an ethical burden, as it affects the distribution of limited resources aimed at preserving species and populations in need (Johnson, 2020).

When creating biobanks, the key factor determining the possibilities of their use for various purposes is the characterization of animal phenotypes. For example, the description of productive qualities creates the possibility of commercialization of biobank data, and the registration of physiological or behavioral features

is the basis for conducting fundamental scientific research (Chen and Li, 2021).

Thus, when creating a biobank and determining the conditions for its use, it is necessary to analyze various aspects of the possible use of the genetic data contained in it, including the economic resource, as well as their importance for the preservation of aboriginal populations and related ethnic groups, the issue of respect for their autonomy, and the priority in choosing the object of population genetic research.

CONCLUSIONS

The optimal biobanking strategy is to conserve different types of materials for their most environmentally friendly and sustainable use. Immediately after delivery of samples from the collection site to the laboratory, high molecular weight and high-quality purified DNA can be isolated from part of the material and stored at low temperatures as a highly concentrated solution for the most sensitive and complex whole-genome research protocols. Some of the material can be stored in liquid aliquot form in freezers; some of the material in the form of liquids or suspensions can be applied to a membrane carrier and dried and then store at room temperature and in refrigeration units. Creating duplicates of a sample in the form of materials with different storage conditions will help to avoid loss in the event of unexpected power grid failures or disasters. Thus, depending on the purpose of the experiment and the requirements for the material, it is always possible to select the optimal variant of a sample from the biomaterial bank. Currently, biobanks as a source of genetic information are becoming a universal platform for numerous interdisciplinary studies. The creation of a reliable unified system for storage, exchange, and research of biosamples or an international and domestic biobanking industry, undoubtedly, has a great future in solving the fundamental problem of humanity—the conservation and rational use of biodiversity.

FUNDING

This work was supported by the Russian Science Foundation (project no. 22-76-10053).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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