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# Innovations in cryoconservation of animal genetic resources

## Practical guide

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# SECTION 1

# Building a gene banking strategy

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## 1.1 INTRODUCTION

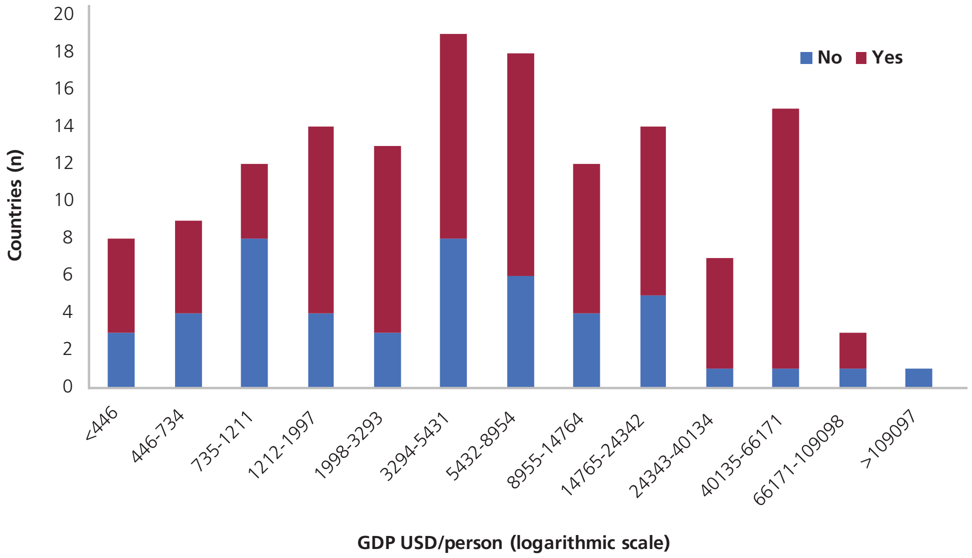
Gene banking is a powerful tool for the management of animal genetic resources for food and agriculture (AnGR). Although gene banking is technically demanding and can require substantial initial investments, once genetic materials are cryopreserved, properly stored and documented, they can remain viable for a practically endless amount of time. Many countries have invested in national gene banks as part of their strategy for AnGR management. The primary goals of gene banks are to have "insurance" to protect against breed extinction or catastrophic loss, and to support the *in situ* populations across species and breeds. Additional goals are being identified because of a growing interest in active management of genetic diversity.

The *Global Plan of Action for Animal Genetic Resources* (Global Plan of Action; FAO, 2007) has a single Strategic Priority (Number 9) devoted specifically to *ex situ* conservation, but gene banking can contribute to other strategic priorities and actions for the sustainable management of AnGR. For example, Strategic Priority 4 is to "establish species and breed development strategies and programmes." An *ex situ* collection can complement the *in situ* population, and increase options for genetic improvement or adaptation to changing environmental or economic conditions. Strategic Priority 11 is to "develop approaches and technical standards for conservation." Operation of a gene bank allows for the development, adaptation, and refinement of approaches to build collections and cryopreserve genetic material. To reflect the growing role of gene banks and to maximize returns on investment in them, countries should consider a wide range of approaches to utilize their *ex situ* collections.

Although it can require a substantial initial financial investment, many countries have decided to invest in gene banks despite their overall level of economic development. Figure 1.1 shows the number of countries reporting to FAO the presence or absence of an animal gene bank as a function of the size of its economy, expressed in terms of gross domestic product (GDP) per person (in USD). No strong economic influence on the presence of gene banks is shown. Although most high-income countries report gene banks, in general, a majority of countries report having gene banks across all levels of GDP.

Cryoconservation and *in situ* conservation are complementary methods to manage animal genetic diversity. A cryoconserved collection of genetic material can be invaluable for improving management of an *in vivo* population. Small populations that are economically viable will usually have a low risk of extinction from competition with international transboundary breeds. However, their small population size implies that they still risk loss of genetic diversity,

FIGURE 1.1
**Numbers of countries reporting that they have a gene bank (yes/no) according to level of economic development (GDP/person in USD)**



*Source:* Authors' own elaboration.

due to genetic drift, demographic factors, or suboptimal breeding programmes. Banked material can be used to maintain or increase the genetic variation by effectively increasing the number of animals used as parents in each generation (Eynard *et al.*, 2018).

Cryoconservation activities can also contribute to sustainable breed use and development, even for breeds that have well-functioning breeding programmes. For example, alterations in the production environment (e.g. climate change) and economic forces that may alter the market are circumstances that require a change in the selection programme. The additional genetic diversity available in a gene bank would be crucial in these situations, even for populations that are not at risk in terms of census size.

Having multiple goals for a gene bank implies a variety of stakeholders interacting with the bank, both as providers and users of material. As a result, the interests of these stakeholders must be taken into consideration when planning for gene bank operation and management.

National gene banks should formally establish a gene banking strategy to address a range of goals. Once developed, gene banking strategies should be reviewed periodically to ensure their continued relevance and appropriateness.

## 1.2 ISSUES AND CHALLENGES TO BE CONSIDERED IN A GENE BANKING STRATEGY

Gene banking is not simply a technical exercise of identifying a sufficiently large number of donor animals, and collecting and cryopreserving quantities of genetic material from these animals. Various factors need to be considered when developing the gene banking policy and strategy. These factors may influence the breeds and types and quantities of material to be gathered, complementary data to be collected along with the material, and even whether cryoconservation will be adopted within a given country as part of the overall approach to management of AnGR in the context of a national strategy.

### 1.2.1 Role of cryoconservation in dynamic management of genetic variation

A first step in developing a gene banking strategy is to develop or review national policies and management plans for AnGR in general. This step will help establish the context for gene banking and ensure that elements of the strategy are in harmony with the overall national strategy and action plan for AnGR (FAO, 2009) or similar policies and strategies developed for specific livestock sectors, species, or breeds.

Management of genetic diversity usually considers one or more of the three basic goals:

1. preventing the loss of breeds;
2. maximizing or optimizing the amount of overall genetic variation maintained within and among breeds of a species; and
3. ensuring the maintenance of important phenotypes and thus the genetic variation that underlies these phenotypes.

These basic goals are not independent from each other. Various breeds may have important phenotypes that are unique with respect to other breeds. Maximizing overall genetic variation should in general capture the alleles responsible for important traits. Emphasis placed on each of the goals may differ from one species to another. Cryoconservation can contribute to any of these goals.

### 1.2.2 Ethical issues

As mentioned previously, gene banking cannot be solely regarded as a scientific or technical activity. Sociological and ethical issues can be considered when developing a gene banking strategy, especially for banks that are supported by public funding (Zomerdijk *et al*., 2020). For example, assuming resources are not available to conserve all breeds within a country, ethical issues may arise in the process for choosing the breeds to be conserved. Also, cryoconservation relies heavily on utilization of biotechnologies, some of which (e.g. invasive germplasm collection techniques, or cloning) may be opposed for ethical reasons by members of the general public. On the other hand, even controversial biotechnologies may be considered acceptable if their application can be justified as the best, the most cost-effective or practical option to preserve a certain breed.

### 1.2.3 Cost effectiveness

Gene banks must spend their funds in the most effective manner possible. Therefore, the cost-effectiveness of gene-banking activities must be considered and accounted for in the gene banking strategy. Section 4 provides more detail on the economic aspects of cryoconservation programmes.

Many public gene banks provide stored material free of charge to users who can demonstrate a justifiable need. However, gene banks may also decide to charge for services to generate revenues from the use of gene bank material (Albert *et al.*, 2014; Van der Stijl and Eijdems, 2019). Whereas gene banks are usually expected to act as not-for-profit entities, they may need to cover some or all of their operating costs to be sustainable. Such costs include staff salaries, housing, liquid nitrogen and other consumable items, electricity and other utilities and equipment maintenance (see Section 4).

### 1.2.4 Information management

Recent advancements in computing and communications technology have greatly increased the value of data and information. That trend will only continue in the future. Cryoconservation is not exempted from this trend. Any stored material has value if it can contribute to maintenance of genetic diversity, but its value will increase in proportion to the degree with which it has been characterized and the resulting knowledge has been catalogued. Data and information about stored material should be regarded as an integral part of the resource maintained in the gene bank and accounted for in the gene banking strategy. For an increasing number of gene banks, the *resource* consists of the biological material together with its associated data. Efforts should be made to collect as much information about gene bank samples as possible. The decreasing costs of genomic analyses in recent years have increased the feasibility of genotyping donor animals for gene bank samples.

Likewise, information systems to make gene bank data available to stakeholders have increased in importance, as have information systems to link data collected by the gene bank with other relevant sources of information available on the internet. Section 8 addresses information systems for cryoconservation.

### 1.2.5 Gene banking objectives

The general aim of gene banks for livestock species is to effectively and efficiently conserve the existing genetic diversity of AnGR over time. Several plausible uses for cryoconserved material have been discussed briefly above and objectives for gene banking were presented in the previous FAO guidelines on cryoconservation (FAO, 2012), but merit a review here.

#### *1.2.5.1 Reconstitution of an extinct breed*

This objective is usually considered to be one of the most common for animal gene banks, especially for publicly funded banks. Although the continued existence of a breed is likely to be of primary importance for the livestock keepers of the breed, animal genetic diversity is generally considered a public good and breeds are the usual conservation unit. The previous FAO guidelines (FAO, 2012) generally emphasized this objective and Indicator 2.5.1b of the Sustainable Development Goals (SDG; UN, 2020) is based on quantities of stored material that are sufficient to achieve this objective.

There is a potential for significant animal losses due to pandemics or environmental disaster to occur. To address such threats, a major goal of gene banks will be to ensure that the livestock industry loses as little time as possible in recovering from the loss. This means that gene banks may have to store significant quantities of germplasm from the most important populations, and do this on a regular basis to keep up with genetic trends.

A special situation with experimental lines used in research is the choice between keeping a live population or conserving the line as a collection of cryopreserved germplasm.

The population can then be regenerated when needed for research, as illustrated by Silversides, Purdy and Blackburn (2012). While this is routinely done for model organisms used in biomedical research, it is not common for farm animals, where fertility of cryopreserved material is variable and a source of uncertainty.

#### *1.2.5.2 Support for* in vivo *conservation*

Breeds that are subject to *in vivo* conservation are usually small in terms of both real and effective population size. A collection of material in a gene bank can be used to help maintain genetic diversity, such as by alternating the utilization of parents across generations when using gene bank material (Sonesson, Goddard and Meuwissen, 2002) or extending the generation interval. Stored material may also be used to safeguard against the accumulation of deleterious recessive alleles or to redirect a breeding objective.

#### *1.2.5.3 Development of new lines or breeds*

As mentioned previously, breeds are the usual conservation unit for management of AnGR, and cryoconserved material can be used for the management of the sourced breed. However, this is not absolute. For example, cryoconserved germplasm from one breed may be used to introgress its valuable traits into another breed. Stored material from one breed may also be used to introduce genetic variation into a genetically similar breed to help prevent its genetic erosion. This approach may be especially valuable in cases where the population size of the targeted breed was dangerously small before reasonable quantities of its own genetic material could be cryoconserved. Material from multiple breeds may also be used to create a new *composite* breed (Paim *et al.*, 2019).

#### *1.2.5.4 Improved management of not-at-risk breeds*

Breeds with sufficiently large population sizes that suggest they are not at risk may still benefit from cryoconservation activities. See FAO (2013) for information about breed risk criteria. The earlier a material collection programme starts, the larger and more diverse it will be in the future. It will also be highly beneficial should unforeseen circumstances provoke a large decrease in population number or genetic diversity. In the meantime, the stored samples can be managed judiciously for other objectives, such as development of new breeds. Gene bank collections have also been proven to be a valuable resource for building reference populations for genomic selection, and tracking and tracing purposes (Blackburn, 2018; Rexroad *et al.*, 2019). Gene bank collections are also a resource to reintroduce diversity and/or re-orient breeding goals.

#### *1.2.5.5 Research*

Gene-banked material is also a valuable resource for research, such as genomic analyses to help understand the biological basis for a given breed's distinct traits. Storage of material other than germ cells (DNA or tissues) is recommended as a complement to standard gene banking of reproductive material. Such material can be used for both characterization of the stored samples and for independent research.

Cryopreservation allows germplasm and other tissues to be stored indefinitely. Therefore, the storage of material in gene banks should not necessarily be limited to material that can be utilized now, but also consider possible future developments in genetic and reproductive technologies.

## 1.3 GOVERNANCE

Effective development and implementation of a gene banking strategy will rely on the existence of a system of governance. This system will ensure that the goals of all relevant stakeholders are appropriately represented in the strategy, and that the critical elements of the strategy are implemented through the operation of the gene bank.

### 1.3.1 Stakeholder identification

Management of AnGR involves a wide range of stakeholders. In general, all stakeholders will share the basic goal of maintaining access to the widest collection of genetic variation possible. However, different stakeholders will have different reasons for wanting future access to this genetic diversity when stored in a gene bank and how to utilize it. Different stakeholders will also play different roles, as some may contribute financially to its operation, some will provide material and others will have an interest in utilizing the material. Many stakeholders will play multiple roles.

Stakeholder buy-in is a key step in the development or implementation of a gene-banking strategy. An analysis of stakeholder needs should be part of a quality management system of the gene bank. According to a recent survey on quality management of gene banks (Zomerdijk *et al.*, 2020), few banks have undertaken formal stakeholder analyses.

The first step in engagement with existing and potential stakeholders is to identify and take an inventory of the most relevant stakeholders. For countries with existing gene banks, some stakeholders will already have regular interaction with the bank. In this case an additional step is recommended to identify potential future stakeholders. Key stakeholders usually include the following.

#### *1.3.1.1 Government*

Livestock gene banks are most often government institutions or government funded institutions. In general, the actions of the government should represent the interest of the general public which include ensuring that the gene bank is operated in an ethical manner. A national gene bank can support the government's efforts to address international pledges and obligations with respect to international agreements for management of biodiversity, e.g. the Convention on Biological Diversity (CBD, 1992) or the SDGs (UN, 2020). The government may also mandate other public organizations to carry out this mission on its behalf.

#### *1.3.1.2 Breeders and breeder associations*

Livestock breeders and organizations that represent them will often be among the most important providers and users of material stored in gene banks. Therefore, garnering the support from these groups is essential for gene bank success.

#### *1.3.1.3 Breeding companies*

Interests of breeding companies will be similar to those of breeders and breeder associations. Their focus will primarily involve the populations they have under their ownership.

Therefore, gene bank governance will need to accommodate the organizational structure of each company.

#### *1.3.1.4 Other nongovernmental organizations*

Organizations that are not formal breeder associations may provide support to specific breeds or groups of breeds through advocacy and capacity building, operation of *in vivo* conservation programmes and provision of assistance in marketing, among other activities. They can often serve as a direct link to breeders of various local breeds.

#### *1.3.1.5 Research and teaching institutions*

Universities and other research or teaching institutions may wish to utilize the gene bank and its collections to support their activities. They may also provide services to the gene bank, such as data analysis and development or refinement of cryopreservation protocol. In general, these institutions and the gene bank will have similar issues involving governance.

### 1.3.2 Institutional commitment

The success of a gene banking programme will depend strongly on the commitment of stakeholders to its establishment and operation. In most cases, this will start with a strong acceptance by the government of the importance of gene banking and a commitment to provide substantial financial support, both a large initial investment for the establishment of a gene bank and then continued funding for its long-term operation. Many governments have agreed in principle to develop and implement a gene banking strategy, but have difficulties in allocating funds to build the gene bank or sustain its operations. However, as shown in Figure 1.1, many low-income countries have a gene bank, suggesting that political will is at least as important as the available budget.

Because construction and establishment of a gene bank usually requires a costly initial investment, this activity is a government responsibility and requires the appropriate government ministry to allocate funding. Regardless of the source of the funding, the government must also be prepared to commit to and plan for continued funding to support the development of collections, the maintenance and operation of the gene bank in the long term.

For success, non-government stakeholders must also be committed. They must be convinced about the benefits of providing access to their AnGR for the development and subsequent enhancement of collections and of regular and wise utilization of the material in the collections.

A firm commitment by international financial institutions may also be reasonable. Although many developing countries have already established gene banks, the initial investment can be substantial, and many countries would benefit from financial assistance.

### 1.3.3 Governance structure and decision-making process

Most gene banks will have a manager who is responsible for the management and day-to-day decisions on operation of the bank, but he or she may have to consult with various stakeholders for the long-term decisions. The establishment of a multiactor stakeholder committee to provide input and support to the gene bank is recommended.

This stakeholder committee could have several responsibilities, in particular, in assisting the development of gene banking strategy, and monitoring its implementation and rationalization as needed. As appropriate, the committee might also advise on annual budgets, capital and infrastructure advancements.

Regarding the composition of the committee, FAO guidelines recommend the establishment of a National Advisory Committee for AnGR (FAO, 2011). Given the need for synergy between the gene banking strategy and the overall national strategy and action plan for AnGR, the potential exists for the National Advisory Committee to also participate as the gene bank's stakeholder group.

### 1.3.4 Data policy

As mentioned previously, information about samples in the gene bank collection should be regarded as an integral part of the gene bank. Therefore, a data policy must be developed as part of the overall gene banking strategy. This topic will be elaborated in more detail in Section 8. The following are potential issues that may be addressed in a data policy:

- types of data to be collected and managed;
- system for organization of data, and standards for documentation and metadata;
- protection of privacy, security, confidentiality, intellectual property or other rights;
- access to and sharing of data: how and when to share data and with whom; and
- data storage: where to be maintained, and how to be secured.

## 1.4 ELEMENTS OF THE GENE BANKING STRATEGY

Gene banking of AnGR is a comprehensive and dynamic process where flexibility in collection development is key. It is a long-term process that spans decades of continued sample curation and evaluation of the collection, while projecting future needs to the extent possible. The ultimate goal of the gene bank is to provide society with a broad range of genetic options for different types of future use. In the formulation of their strategy gene bank managers should consider aspects of sampling, storage organization, documentation, utilization, rationalization, as well as communication and awareness raising.

The motivations for developing a gene bank are broad and dependent upon country needs and long-term strategies. The industry is often assumed to maintain sufficient genetic diversity for their future use, but this is not always an accurate assumption. Therefore, in deciding the scope of gene banking activities, it is essential to have comprehensive discussions with all stakeholder groups.

In essence, development of a gene bank's collection follows a hierarchical approach that consists of identifying motivations, deciding which species of livestock are important at the country level, deciding which breeds to collect, and then which animals within a breed might be selected for collection. The following provides a basis to start exploring and formulating a collection strategy.

### 1.4.1 Sampling

The gene bank may review the state of its collections against its objectives and expectations from stakeholders to identify needs for additional sampling. The main steps are listed below.

### *1.4.1.1 Species*

The strategic decision about which species to collect for the gene bank will govern the activities conducted by the gene bank, as methods and approaches for sampling and cryopreserving genetic resources will vary among species. This decision has major implications for the gene bank, as new species may be added or even deleted in specific instances depending upon the stakeholder and policy perspectives. The importance of the species to the country's economy or wealth of genetic diversity or its heritage value may all be considerations for inclusion, but the availability of appropriate cryoconservation methods will have a critical influence (see Section 6).

A wide range of approaches have been taken in deciding the species and breeds to target for collection. National gene banks often consider collecting from all livestock species present in the country. A recent survey on gene banks showed that six species (i.e. cattle, sheep, goat, horse, pig and chicken) could be found in many gene banks whereas others (e.g. rabbit, turkey) were found only in a few countries (Zomerdijk *et al.*, 2020).

### *1.4.1.2 Genetic information*

Breed is an important determinant for both the global livestock sector and collection development, and countries have taken various approaches. Regardless of species, some type of genetic assessment at the breed level will be necessary in executing whichever within-species collection goals have been established. For example, in North America and Europe, several gene banks have set a national goal of collecting all livestock breeds. Conversely, Brazil's gene banks initiated their collection efforts by focusing upon acquiring samples from rare breeds.

Due to the importance of breed as an indicator of diversity, assessments for collection purposes might include unique phenotypes or important production characteristics (e.g. milk, meat, or fiber), distinctiveness from other breeds, historical perspectives, genetic isolation based upon geography, and well recognized breeds at the national or international level (Blackburn, 2018). While some attempts (e.g. Weitzman, 1998) have been made to use genetic markers to develop subsets of breeds to capture a broad array of genetic diversity per species, such approaches do not account for the need to service the broader community of stakeholders, and have generally not been implemented because of a lack of consensus of stakeholders on the subsets (Boettcher *et al.*, 2010).

Molecular genetic studies suggest that the total variation accounted for by breeds within a species is usually less than the variation among animals within breeds (Paiva, Mariante and Blackburn, 2011). Some gene banks have successfully captured within breed genetic diversity by using different tactics, and over time the gene bank collections contained greater diversity than the *in situ* population (Danchin-Burge, Hiemstra and Blackburn, 2011). By using molecular tools such as single nucleotide polymorphism (SNP), gene banks have developed collections that have captured up to 98 percent of the variation within a breed (Wilson *et al.*, 2019), and have identified subpopulations within breeds (Hulsegge *et al.*, 2019a).

Gene banks can use a number of approaches to capture genetic diversity based upon information on hand. Box 1.1 shows how simple random sampling of donor animals can be effective in capturing a wide array of diversity.

### BOX 1.1

### Random sampling of animals for gene bank collection

Early on it was recognized that large portions of genetic diversity could be captured in collection development through random sampling of animals.[1, 2] This fact can be demonstrated by using the equation below that calculates the probability (P) of capturing a rare allele:

$P = 1 - (1 - p)^{2N}$ for semen;

or

$P = 1 - (1 - p)^{4N}$ for embryos.

For an allelic (p) frequency of 0.05 and with an N of either 50 bulls or 25 embryos collected from a breed for the gene bank, there would be a 92 percent chance of capturing the allele. Utilization of this equation is a cost-efficient approach for building collections.

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[1] **FAO.** 1983. *Animal genetic resources conservation by management, data banks and training.* Proceedings of the Joint FAO/UNEP Expert Panel Meeting, Part 1.

[2] **Smith, C.** 1984. *Genetic aspects of conservation in farm livestock. Livestock Production Science,* 11(1): 37–48. https://doi.org/10.1016/0301-6226(84)90005-8

When a breed is dispersed among production environments with no or little migration, it may be desirable to sample within and among the environments. Geographic coordinates of collection locations should be recorded and stored in the gene bank database, as they provide links to extensive information in environmental databases. Gene banks have used pedigrees to develop sampling strategies and prioritize individual animals or families, to maximize representativeness and avoid losing the less-represented families. For example, pedigrees can be used to cluster animals within a breed based upon their genetic relationships (Blackburn, 2018; Wilson *et al.*, 2019) leading to prioritization of animals. Animals within each cluster are then selected for entry into the gene bank. An advantage of this approach, relative to targeting specific individuals, is that if germplasm from a selected animal cannot be obtained, another animal from the cluster can be collected. Within breed, optimal contribution methods have been developed (Meuwissen, 2002) and used in the Netherlands in an effort to build and optimize core collections. Such an approach is useful in ensuring that the gene bank collection is genetically balanced, but requires sufficient organization and budget, and data availability can be a limitation.

Genomic information can be used in the methods previously mentioned, either instead of or in conjunction with pedigrees. In addition, genomic data can be used in other ways to select animals within a breed, including absence of known genetic abnormalities, *ex post* assessment of the collection versus *in situ* population diversity, and targeting animals for collection that have genotypes of interest for traits such as adaptability to climate change.

Utilization of estimated breeding values or phenotypes for traits of interest can also play a role in identifying animals for the collection. For instance, the gene banks in France and the United States of America have been collecting samples from animals that represent extremes for traits of interest outside the major production objective, to ensure a wide range of genetic variability has been captured and to keep the option of reorienting selection objectives. Whichever approach is used to select animals, it must be flexible (e.g., no fixed lists of animals to collect) and robust to accommodate breeding sector dynamics and time constraints.

Intensively managed breeding populations held by the private sector are also in need of the security gene banks can provide, and they represent a special case in terms of how populations might be sampled. These collections become increasingly important as company populations undergo the pressures of genetic drift and selection intensity. Approaches for these populations may range from collecting substantial numbers of males (and females in the case of poultry) from a specific generation, so that an entire cohort of individuals may be rejuvenated from cryopreservation, to collecting "snapshots" of every new generation.

#### 1.4.1.3 Continual management and updating of collections

*In situ* populations are continually changing, so gene banks need to periodically collect new samples to ensure stakeholders have a range of genetic material. This step is useful to capture genetic changes, particularly when breeders want access to genetics to correct a recent problem, such as restoring fertility or recovering a lost trait. Having access to material from varying times can accelerate the process. The time interval between sampling depends on how rapidly a population is changing. Since the optimal interval for sampling may be difficult to determine *a priori*, a regular snapshot approach is probably the easiest to implement. It also helps to maintain awareness about gene banking on a regular basis. Furthermore, the onset of genomic selection has led to considerable acceleration of genetic progress, particularly in dairy cattle, so that regular sampling is recommended. For rare breeds undergoing low selection pressure with limited use of frozen semen, where a gene bank has collected at least the minimum quantities of germplasm and animals, the sampling interval can be increased to 5 to 7 generations, considering the specific collection effort to be set-up. There may be reasons to sample these breeds more frequently, such as to mitigate genetic drift or capture unique phenotypes.

#### 1.4.1.4 Tissue types

As stated in the previous cryoconservation guidelines (FAO, 2012), reproductive efficiency varies among tissues, species and even among breeds within the same species. These factors will determine the minimum quantity of germplasm needed to perform any sort of reconstitution. How well gametes or tissues can be cryopreserved and thawed, and how many live offspring are produced are all considerations in determining the type and number of gametes or tissues to be sampled and stored in the gene bank (see Section 6). Reproductive efficiencies vary among gametes and tissues, and some gene banks have found it useful to collect a variety of sample types that provide them with more flexibility in utilizing a collection. For example, due to the homogametic male in poultry semen, ovaries and testes have been collected, cryopreserved and used to create chicks (Silversides, Purdy and Blackburn, 2012). More recently, cryoconservation of primordial germ cells has been developed and validated to revive a chicken breed (Woodcock *et al.*, 2019).

#### 1.4.1.5 Sanitary status

Sanitary issues apply to animals and to collection facilities, that may impact their suitability for gene banking. This will also vary depending upon national legislation, and gene banks will need to operate within the guidelines and regulations. Common venues for collecting germplasm include farm collection, private sector artificial insemination (AI) facilities and research institutions, which may differ in sanitary status and regulations. Section 7 provides more information.

## 1.4.2 Storage organization

The key objective of gene banks is to guarantee safe long-term storage of genetic material and associated data.

### *1.4.2.1 Centralization and distribution*

The size and capacity of a gene bank depends on its objectives, the range of species and breeds to be conserved, the financial resources available, the types and amount of genetic material to be stored, and the location of populations to be sampled. There is an optimum to find between exclusive centralization which may increase the costs of collection and distribution, and wide distribution which increases the total investment in equipment and may lead to underutilization of local storage capacity. Development of species-specific locations could increase competition among locations for financial resources from the same funding sources. Section 4 addresses economic optimization of gene banking.

In selecting the main location and facility for the national gene bank, several logistical issues should be considered, including physical safety of the collection, easy access for receiving and distributing samples by commercial carriers, accessibility and continuous access to liquid nitrogen, consistent and dependable electricity, a physically secure building, a secure room with controlled access for storing the collection, and closed circuit television or other systems to record entry and exit of people from the secured room.

Animal health concerns and other potential hazards (e.g. floods, earthquakes, fires, tornados) of a geographic area might also be criteria in determining where a gene bank should be located. Location outside an area with endemic disease issues will facilitate the entry and exit of germplasm from the gene bank.

Human resource availability may also be a consideration in choosing a location. Incentives should be in place to attract and retain qualified staff. Health, sanitary aspects and physical security must be considered when identifying the site of the gene bank.

### *1.4.2.2 Duplication and backup*

Material stored in a gene bank is a highly valuable resource and must therefore be safeguarded against loss. It is strongly recommended to maintain two separate storage facilities in different geographical locations.

A minimum of two storage locations should be identified at national level, for primary and duplicate collections. If the gene bank is already organized with distributed sites, duplication of collection is easy to organize, either by specializing one location as a duplicate according to species, or by taking advantage of existing AI centre to host mirror collections for long-term storage. In that case, distribution will take place from the central site rather than from the mirror site which acts only as a safeguard. National representatives making such a decision about backup sites need to consider the long-term (15 to 20 years) ramifications of such agreements, particularly when contracting with privately held companies to ensure the safe keeping of samples.

Exchange of duplicate material between countries may also be considered to reduce costs or promote transboundary collaboration. However, such arrangements are vulnerable to changes in national laws or disease outbreaks that may later make it difficult for a country to repatriate their samples. In addition, both locations should use the same database so that inventories can be appropriately managed, or at least agree on the same descriptors to be stored so that information can be easily exchanged between sites.

#### *1.4.2.3 Storage of material*

Gene bank managers will need to decide how to distribute samples across their liquid nitrogen storage tanks. Much of this decision will be made by the size of the liquid nitrogen tanks. The primary consideration is whether to have multiple species in a tank or to maintain separate tanks for each species. Sanitary status of samples may also impact the approach taken. Government health regulations may play a role in this decision (see Section 7). Storing multiple species per tank is usually more cost-effective and efficient, so that the liquid nitrogen tanks can be used to their maximum capacity.

#### *1.4.2.4 Associated data*

The database is essential for managing routine gene bank operations and to support management decisions. The database serves as the primary tool for receiving, storing and exchanging information about samples in the collection. Therefore, proper and accessible documentation is vital for the operational management of the gene bank, for optimization of gene bank collections, and for future use of any stored gene bank material. Gene banks need to develop and implement a database for this purpose. Basic information about gene bank collections should be easily accessible without the need for any additional information from outside the database (see Section 8).

### 1.4.3 Utilization

Gene banks are more powerful when used by a wide range of stakeholders. Stakeholder requests for gene bank samples are varied. Potential requests for gene bank samples include adding genetic variability to an *in situ* population, corrective mating for any breed, reconstituting research populations, and genomic evaluations. In addition, there is the overarching long-term objective to be able to reconstitute breed(s) in time of national crisis.

#### *1.4.3.1 Conditions for access*

Access to national gene bank's collections requires policies that ensure that all users are treated equitably, sample use is non-trivial, and access to gene bank material does not infringe upon private sector business activities (see Section 9). Depending upon the country's laws, gene banks may or may not be able to charge fees for service or germplasm. Gene bank managers need to define criteria for access and use, and may find it useful to develop a committee, comprised of persons knowledgeable about a specific species (or breed), to review requests. In situations of short supply of requested samples, the potential gain achieved by releasing samples must be weighed against potential future demands for use, and whether and how the genetic resource can be replenished.

The following are the decision points to consider when releasing samples:

- Are there any specific conditions defined in the agreement between the gene bank and the original provider of the material? If yes, then the original provider may need to be consulted.
- What is the intended use of the sample? Is it beneficial to the breed, industry, or research?
- Can or should the request be met by the private sector instead of the gene bank?
- Does the requestor have sufficient experience to make successful use of the sample?
- Does the approach proposed lend itself to successful generation of live animals (if appropriate), for example using *in vitro* vs *in vivo* fertilization?
- Is the proposed mating beneficial (e.g., in reference to genetic relationship)?
- What does the gene bank get in return when samples are released (e.g. get germplasm from the progeny to replenish the gene bank collection, genomic information and/or progeny phenotypes to document the remaining collection, cost recovery fees if permissible by law)?
- If the requested sample is used for genotyping, will the gene bank obtain a copy of the resulting genotype/sequence information?

#### *1.4.3.2 Usage scenarios*

As pointed out previously, gene banks can serve a range of objectives. Box 1.2 illustrates the usefulness of long-term preservation of germplasm for highly selected populations, beyond the classical objective of being able to reconstitute breed(s) that may become threatened or extinct.

#### *1.4.3.3 Tracking sample use and impact*

Tracking the utilization is important for gene banks. Data on utilization are evidence of the value of banks and can be used in funding activities. They are also useful in planning for the future. Figure 1.2 shows the yearly utilization of samples from the US gene bank, according to birth year of the donor. The gene bank has released samples from more than 11 000 animals since 2004. The data also shows that samples of animals of nearly all birth years are being continually used by the various stakeholders. If samples are used for genotyping, the gene bank should require the user to provide the data and other results and inform the bank about how the genotyping helped solve a problem (see Box 1.3). Furthermore, such examples are extremely useful in articulating the value of gene banking to the stakeholders, administrators, and the public at large. One option to facilitate tracking is to use the digital object identifier (doi) system for a gene bank collection, so that each user of gene bank material should refer to this doi in any publication. Terms for material utilization and information sharing should be outlined in a material transfer agreement (see Section 9).

BOX 1.2

### Using banked material to reconstitute lost Holstein sire lineages

Yue, Dechow and Liu[1] reported that the genomes of Holstein cattle in the US had only two different familial Y chromosomes in the *in vivo* population, both tracing to two important 1970s sires. Evaluating the collection in the national gene bank, the same researchers determined there were two additional paternal line Y chromosomes that could broaden the genetic diversity. However, the identified bulls were descended from the population of the 1960s and thus had relatively low genetic merit. It was decided to introgress the Y chromosomes from the two repository bulls.[2] Semen from the gene bank was used to create *in vitro* embryos from seven elite (upper 70th percentile in performance) Holstein cows. The embryos (12 and 15 per bull) yielded seven male offspring (3 and 4 per bull). At one year of age the bulls were transferred to a commercial artificial insemination (AI) centre.

Genomic evaluation of the bull calves showed that one generation of mating with an elite female would be sufficient to produce offspring with approximately the breed's current average for milk production and other economically important traits. One sire's bull calves were actually higher than breed average for net merit and milk production. Semen from the bull progeny was repatriated to the gene bank, and is also commercially available to producers. The bulls produced will be mated to highly productive cows, and it is anticipated that their progeny will be competitive with other top AI bulls. Additional studies are planned to evaluate semen differences, resequencing the bovine Y chromosome, and monitoring lifetime performance of the daughters. This experiment demonstrates that by combining advanced reproductive biotechnologies and genomic information, reintroducing gene bank genetics into a populationcan be done much more quickly and efficiently than previously thought.[3]

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[1] **Yue, X.P., Dechow, C. & Liu, W.S.** 2015. A limited number of Y chromosome lineages is present in North American Holsteins. *Journal of Dairy Science,* 98(4): 2738–2745. https://doi.org/10.3168/jds.2014-8601

[2] **Dechow, C.D., Liu, W.S., Specht L.W. & Blackburn, H.** 2020. Reconstitution and modernization of lost Holstein male lineages using samples from a gene bank. *Journal of Dairy Science,* 103(5): 4510–4516. https://doi.org/10.3168/jds.2019-17753

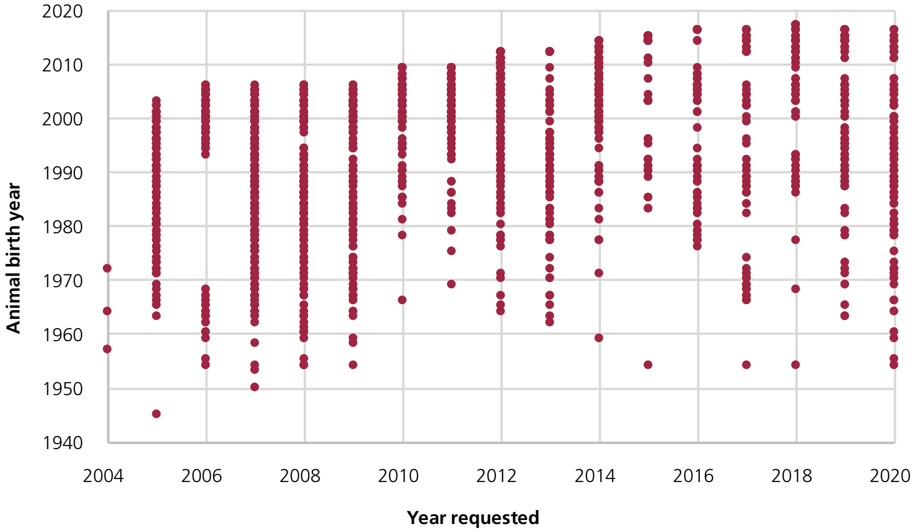
[3] **Leroy, G., Danchin-Burge, C. &Verrier, E.** 2011. Impact of the use of cryobank samples in a selected cattle breed: A simulation study. *Genetic Selection Evolution,* 43(36). https://doi.org/10.1186/1297-9686-43-36

## 1.4.4 Rationalization

Gene banks should regularly evaluate and rationalize their scope, policies, implementation strategies and protocols to optimize and further to develop the gene banking strategy. By doing so, countries will contribute to Strategic Priority 12 of the *Global Plan of Action* (FAO, 2007) to "establish and strengthen national institutions, including national focal points, for planning and implementing animal genetic resources measures, for livestock sector development." A rationalized strategy can be defined as the most cost-effective way to reach the gene bank objectives. Gene bank collections must be "fit for purpose."

FIGURE 1.2

**Samples released from the US gene bank between 2000 and 2020 among species and germplasm types**



*Source:* Authors' own elaboration.

BOX 1.3

### Genomic use – Experience from the United States of America and the Netherlands

In the United States of America, a major breed association acquired a semen sample from an important 30-year-old sire to determine if he carried a lethal mutation. The bull was not a carrier, and as a result, over 29 000 animals did not have to be genotyped, saving breeders over USD 2.2 million in genotyping costs. Also, samples from more than 400 repository dairy bulls were used to develop the first genomic breeding values for dairy cattle. This technological advancement added over USD 4 billion per year to the US dairy industry.[1]

In the Netherlands, the Holstein and Het Maas-Rijn-IJsselvee (MRIJ) gene banks and DNA collections have shown to be very valuable in the initial phase of implementation of genomic selection by Dutch cattle breeding industry. Moreover, 50 000 single nucleotide polymorphism (SNP) data of all gene bank bulls of native Dutch cattle breeds were used to establish a breed specific DNA reference population for the purpose of identifying pure-bred animals (without or with incomplete pedigree data), and to confirm breed purity.[2]

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[1] **Rexroad, C., Vallet, J., Matukumalli, L.K., Reecy, J., Bickhart, D., Blackburn, H., Boggess, *et al.*** 2019. Genome to phenome: Improving animal health, production, and well-being – A new USDA blueprint for animal genome research 2018–2027. *Frontiers in Genetics,* 10: 327. https://doi.org/10.3389/fgene.2019.00327

[2] **Hulsegge, I., Calus, M., Hoving-Bolink, R., Lopes M., Megens, H.J. & Oldenbroek K.** 2019a. Impact of merging commercial breeding lines on the genetic diversity of Landrace pigs. *Genetic Selection Evolution* 51(60). https://doi.org/10.1186/s12711-019-0502-6

For example, the notion of "sufficient" material in SDG Indicator 2.5.1b is motivated by one purpose of restoring a lost breed, but a smaller amount of material may still be useful for another purpose. As part of this rationalization analysis, gene banks should be mindful that the collections they are building might be of greatest use after 50 or 100 years.

Rationalization of livestock gene banking strategies should be done both *ex ante* and *ex post*, and involve both supply and demand related elements. Demand side elements of a rationalization framework typically include future societal and stakeholder needs related to the use of gene banks, and thus requires anticipation of drivers of change. The future demands for gene bank collections cannot be fully defined due to the unknown vagaries of the future. Gene banks may wish to evaluate the potential impact of different scenarios when developing, revising and implementing strategies to be as efficient as possible in building a collection that can meet a range of possible future demands. Supply side elements typically relate to genetic aspects, technical options, and cost effectiveness of gene bank operations.

#### *1.4.4.1 Priority setting*

**Strategy informed by characterization data.** Genomic and phenotypic characterization and storage of results in the database greatly facilitate future development and use of collections. Characterization data can also be used to compare collections with those already existing in other gene banks, and determine uniqueness of material to be stored to avoid duplication (e.g. when collecting material from transboundary breeds).

Vibrant gene banks will offer stakeholders a range of services by expanding collections to include blood, DNA, or tissue samples for further research on livestock genetic diversity. The largest use of the Dutch (over 1 000 animals of local breeds) and US collections (over 6 000 animals) has been for DNA analyses.

**Economic optimization.** For rationalization, costs of collection decisions should be optimized against their benefits. Due to the long-term horizon gene banks deal with, routine cost-benefit analysis is difficult. Given the unknown status of future collection usage, benefits are particularly hard to estimate. However, costs can be accounted for with a relatively high degree of accuracy. Gene banks can perform a full cost analysis of collection development, maintenance, and future regeneration steps. Section 4 details how gene bank operations can be optimized for a given objective.

From another viewpoint, gene bank collections represent a source of societal benefits. The advantages and disadvantages of five methods for documenting such benefits from scientific collections have been recently reviewed by Schindel *et al.* (2020).

#### *1.4.4.2 Continuous monitoring*

Continuous monitoring of the status, development and use of the collection is critical. The genetic profile of material already collected in the gene bank should be assessed in the context of genetic diversity of *in situ* populations. Allelic frequencies within commercially vibrant breeds are in a continuous process of evolution and, therefore, a gene bank needs to keep abreast with these genetic changes to keep the collection viable.

Regular gap analysis based on genomic and other relevant data can be used for adapting the strategy and its implementation. The gene bank may wish to budget for regular genomic analyses

BOX 1.4

### Gene banks serve local and mainstream breeds

The livestock breeding industry is constantly changing. Old breeds give way to new, as has been the case since Roman times.[1] The process of change is especially evident in the poultry and swine industries. The elite populations of those industries are pedigreed, and the genetics are intensively managed. While many pig breeding companies have similar breeds (e.g. Duroc, Pietrain and Landrace) and breeding goals, the finite populations mean that genetic drift will separate those populations over time, leading to unique subpopulations. The situation is similar for poultry. Gene banks should therefore engage these stakeholders to provide genetic security for these important food producing sectors. The needs of these corporations may be quite different than the normal practices for local breeds. Gene banking strategies will have to accommodate the needs of stakeholders for local breeds and commercial breeding companies. For example, maintaining *in vivo* populations is costly, so breeding companies may want the gene bank to store large numbers of animals of a unique but little used line, so that the entire line can be quickly re-established if needed. In such scenarios, the distribution of costs and benefits across the public and private actors must be duly considered.

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[1] **Wood, R. & Orel, V.** 2004. *Genetic Prehistory in selective breeding: A prelude to Mendel.* Oxford University Press.

of its key collections, or should develop partnerships with relevant stakeholders (i.e. breeders and breed associations, research institutes and breeding companies) to obtain such data.

#### *1.4.4.3 Projections toward the future*

Quantifying the different attributes of a gene banking strategy is difficult, especially in a long-term perspective. First, stakeholders' strategies are evolving (see Box 1.4). Moreover, future scenarios have a higher or lower uncertainty, while conservation decisions must be made in the short term, also taking the budget constraints into account. Gene banks should anticipate changes in future demand, e.g. motivated by possible changes in climate, production systems, markets, consumer preferences and possible calamities such as diseases and disasters, etc. Implementing surveys among stakeholders at defined intervals may be a good way for the gene bank to keep up to date with user preferences and strategies. Alternative strategies and future scenarios should be compared before the final strategy selection.

Technology breakthroughs and innovations will likely influence future use. Innovative reproductive technologies could change the value of different types of genetic material stored in gene banks. For example, genome editing could increase the value of gene bank collections as a resource base for research and development, but could ultimately also result in less use of the germplasm in gene banks for breeding. Thus, the gene bank should continually monitor advances in cryopreservation and reproductive technologies, and maintain a connection to research, for instance, through a scientific advisory board.

Different objectives may compete in terms of budget allocation and prioritization. When developing future strategies, gene bank managers or stakeholder boards may employ a strengths, weaknesses, opportunities and threats (SWOT) approach, identifying the strengths and the weaknesses from inside the gene bank, and the risks or opportunities pushed by external trends. The SWOT methodology is quite helpful to mitigate external risks and to identify opportunities that should be taken into consideration to revise the strategy about building, updating and using the collections. Regular revision of the gene banking strategy goes together with a policy for training its staff and informing its users (those who provide or utilize the material) on the latest methods and techniques.

### 1.4.5 Communication and awareness raising

Because gene bank deals with a large number and range of stakeholders, there is a need to ensure that these stakeholders are regularly informed about the activities of the gene bank and its future plans. Therefore, a communication plan should be included as part of the overall gene banking strategy. Such a communication plan will contribute to a country's implementation of Strategic Priority 18 of the *Global Plan of Action* (FAO, 2007*)*, to "raise national awareness of the roles and values of animal genetic resources." Regular communication will raise awareness about the gene bank and the importance of its activities, increasing appreciation of its importance in maintaining agrobiodiversity and sustainability of livestock production systems. Users of the gene bank will be kept abreast of the new services.

#### *1.4.5.1 Targets*

The various stakeholders will have different reasons for interaction with the gene bank and will therefore be interested in different types of information. The communication plan should account for this fact, and identify the expectations and topics of most interest to each of the various stakeholder groups. The stakeholders will vary in their amount of background knowledge and understanding of the context of gene banking, which will impact the type of language and terminology to be used.

#### *1.4.5.2 Message*

Although the explicit goal of communication will typically be to inform the various stakeholders, the implicit objective will be to produce a beneficial outcome for the gene bank. The information targeted for each stakeholder needs to be chosen by considering what they currently believe, what they need to know and how they are expected to react to the communication.

#### *1.4.5.3 Media*

In addition to different information, the various stakeholders will likely differ in the way they would like to receive the messages. The communication plan should also consider the delivery method through which the message will be transferred most effectively. For government policymakers, concise formal reports on outcomes achieved by the gene bank relative to the resources used will be of most importance. Researchers may have more confidence in material published in peer-reviewed scientific literature. Breeders and breeder associations may appreciate an online catalogue documenting the material stored in the gene bank, its characteristics and availability for access.

#### *1.4.5.4 Frequency*

The stakeholders, message and media will all influence the frequency of communication. Some forms of communication with the governmental stakeholders may have a fixed schedule. Ad hoc communication with policymakers may be strategically planned to coincide with times at which major decisions are taken. Users of the internet generally want to see updated information each time they access the gene bank's website.

## 1.5 STRATEGIC CONSIDERATIONS FOR MULTICOUNTRY GENE BANKING

Most of the world's gene banks for AnGR are national or subnational in their scope (Zomerdijk *et al*., 2020). However, under certain circumstances and for specific objectives, multicountry gene banking may be the preferred option, with increased economic efficiency or complementary technical expertise serving as key drivers in such cases.

Possible examples include the following:

- a group of countries pooling resources (which may include project funds from an outside donor) to establish a single physical gene bank that serves all countries;
- a donor country offering gene banking services to one or more less developed countries as a means of providing technical and/or financial assistance;
- several countries agreeing on a common strategy for cryoconservation of a shared transboundary breed; and
- two or more countries with operational gene banks that agree to use each other's facilities to store backup/duplicate material.

### 1.5.1 General considerations

For certain aspects, the national cryoconservation strategy of a country storing material in an externally hosted gene bank may not differ substantially from a strategy based on within-country storage. The main difference is simply the physical location of the bank. The species and breeds to be conserved and their characteristics will be mostly invariant, as will many of the stakeholders of interest. Data management will remain a critical aspect of the cryoconservation programme. Periodic monitoring of the *in situ* population and rationalization of the banked collection will continue to be necessary. Institutional commitment is no less important.

However, some additional factors must be taken into consideration:

- Cryoconservation objectives may need to be altered somewhat, depending on ease of access to the collection. Conservation of material in another country may decrease access relative to local storage, making the utilization of material to actively manage an *in situ* population more difficult. Insurance against breed extinction may thus be a more feasible objective.
- Cooperation with another country that includes technical assistance may increase the range of tissue types that can be collected and stored.
- For sanitary concerns (see Section 7), it will likely be necessary to apply the standards of the strictest country to all samples, regardless of the origin. A host country may jeopardize its collection, if the material from a country with less stringent rules is added. On the other hand, if a country stores its samples in the facility of a neighbouring country with lower standards, it may be impossible to repatriate them.

- Long-term institutional commitment remains critical. Establishing a national gene bank requires a substantial initial financial investment, which may help ensure *psychological investment* for the bank's long-term sustainability. Countries that have material hosted elsewhere will not have made this investment, and must understand that continuous institutional support will be required for the foreseeable future. Multicountry gene banking may start as part of a development project (e.g. AU-IBAR, 2020), but gene banking is an ongoing process that will require financial maintenance long after such projects end.
- Because multiple countries are involved, governance issues will be more complex.

### 1.5.2 Governance

If a multicountry gene bank consists simply of one country storing material from other countries in its bank, governance will be relatively straightforward for providers. Countries providing material will simply require a governance structure that pertains to their respective collections of stored genetic resources, which would be similar to that for a national gene bank. The host country would merely act as a service provider, and would need to agree on a cost recovery framework with the provider country or countries. (Economic aspects of gene banking are addressed in Section 4). A quality management system (see Section 2), although recommended for all gene banks, may be especially important in the multicountry context. When countries agree to host each other's backup collections, they may simply agree to undertake this on a cost-free basis, especially if the amounts of material stored by each country are similar.

Governance will be more complex, if a multicountry gene bank is geographically located in a single country, but owned and managed by a multicountry body such as a regional economic community. In this case, each country may continue to independently manage their respective collections of genetic material, but an overarching governing body with representatives from each country will likely be required to oversee the operation of the gene bank.

The cooperative cryoconservation of transboundary breeds and/or cross-country utilization of the stored material will add yet another level of intricacy. For transboundary breeds, a joint cryoconservation programme implies the necessity to establish a multicountry advisory group or similar body for management of the shared genetic resources, both *in situ* and *ex situ*. This body would need to establish a common breeding goal for the breed, as well as to manage both the development and utilization of the collection. If the gene bank is used as a mechanism to facilitate cross-country utilization of material from native breeds from participating countries, legal issues pertaining to international access and benefit sharing would need to be considered as well (see Section 9).

The potential benefits of multicountry gene banking should be given strong consideration by countries that lack national gene banks and/or countries that could substantially increase their cryoconsevatoin efficiency through international cooperation (such as through sharing backup collections). However, the added complexity of governance cannot be ignored. Through a project funded by the European Union, the African Union – Interafrican Bureau of Animal Resources (AU-IBAR, 2020) has been able to establish five subregional gene banks in strategic locations throughout the continent. However, various governance issues remain to be worked out before the banks can be made operational.

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