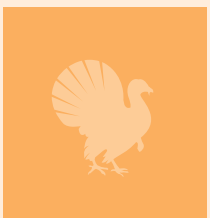
SECTION 5

Establishing a gene bank – physical structure and costs



Establishing a gene bank – physical structure and costs

Gene banking operations and facilities are likely to vary substantially from country to country, in terms of both the size and capacity of the bank and the types and amount of equipment needed. These factors are dependent upon the quantities of germplasm to be placed in the gene bank, which will in turn depend upon the objectives of the gene bank, the range of species and breeds to be conserved, and the financial resources available for the conservation programme. For the purposes of these guidelines, the types of facilities and equipment needed to operationalize a gene bank are subdivided into three size categories: small, medium and large.

In many circumstances, the elements needed to establish a gene bank will already be in place. For example, some countries have state-owned and operated AI centres, or they may have an existing gene bank for plants or wild animals. If such operations already exist, the AnGR gene bank can be incorporated into these programmes. Such an approach would certainly facilitate the development of a cryoconservation programme and the collection of material from some livestock species (i.e. those served by the AI centres). However, additions to existing facilities and equipment may be necessary. Health and sanitation issues must be considered, especially for any facilities that might be shared between wild and domestic animals.

Some features are necessary for the smooth functioning of a gene bank regardless of its size. These are the physical plant (i.e. the actual building and other structures), durable equipment, security arrangements, centralization and accessibility. Specialized human resources are also critically important.

THE PHYSICAL PLANT

The characteristics of the building housing the gene bank will depend greatly upon the total size of the facility and its operations. For example, if animal and plant gene banks are housed together, the type of buildings required will be different from those needed for a single-purpose facility for AnGR. For livestock, specific components of a single- or multiple-use facility, such as the laboratories for germplasm acquisition, processing and cryopreservation, do not need to be located in the same physical place.

Developing the gene bank will usually require the services of a professional architect. If no buildings are yet in place, professional assistance with the development of specific blueprints and construction plans will clearly be needed. If buildings exist but need to be redesigned or refurbished, this will also usually require the employment of an architect and/or engineer.

The gene banking process comprises three main activities: 1) collection of the germplasm; 2) processing and freezing of the germplasm; and 3) storage of the germplasm.

Although all of these activities can be undertaken at the same location, each requires its own separate facilities. In theory, a gene bank need only involve a place for storing germplasm (i.e. if the germplasm is provided from elsewhere). However, in most situations, a gene bank will have the infrastructure needed for at least two, if not all three activities.

Animal housing and collection facilities

Many gene banking operations will find it practical and convenient – if not absolutely necessary – to have a dedicated facility for holding animals while their germplasm is collected. Depending on the species and the type germplasm that is to be conserved, it may not be possible to collect a significant quantity of germplasm during a single intervention with an individual animal. For example, even with superovulation, only a few embryos can be obtained from a cow in a single intervention. Thus, the process will need to be repeated several times for each donor. Superovulation generally requires administering a regime of hormones over the course of several days prior to embryo collection. This is more practical if the donors are kept in a central facility. In the case of semen donors, training the animals for collection will usually increase yield and quality. For countries aiming to adhere to World Organisation for Animal Health (OIE) standards for export, holding facilities will be needed for quarantining animals prior to collection and monitoring their health after collection (for further information visit the OIE web site http://www.oie.int).

Building materials. The choice of materials for internal and external construction of the gene bank building is important. The building must be strong enough to withstand environmental challenges specific to the location as well as the normal wear and tear caused by the animals housed at the facility. The internal surfaces must be able to resist both the physical actions of the animals and the effects of regular sanitizing. Thus, the materials used must be impermeable to water and able to withstand repeated cleaning with sanitizing chemicals. The flooring must be coarse enough for the animals to maintain stable footing when germplasm is being collected (e.g. when bulls mount a teaser animal).

Multiple buildings. Preventing the spread of disease among the animals held at the facility and to the germplasm stored in the bank is critical. Therefore, the facility should have a system for quarantining all incoming animals. If possible, the collection facility should have multiple buildings. This allows the establishment of a quarantine system based on an "all in/all out" policy. Under such a system, once one group of animals leaves a given building, it can be cleaned and the next group of animals can then be brought into the empty, sanitized building and quarantined. Once the quarantine is lifted, the animals can proceed back and forth to a separate collection building, but never again enter a non-sanitized housing unit. Animals with questionable health must always be housed separately from healthy animals and never intermingle directly with them or utilize common spaces such as the collection facility. In addition, if the facility is intended to hold several species, the design must allow each species to be kept apart in different and separated buildings.

Environmental control. The facility should be designed so that the air flows in one direction through the building. This limits the potential for "dead spaces" created by air

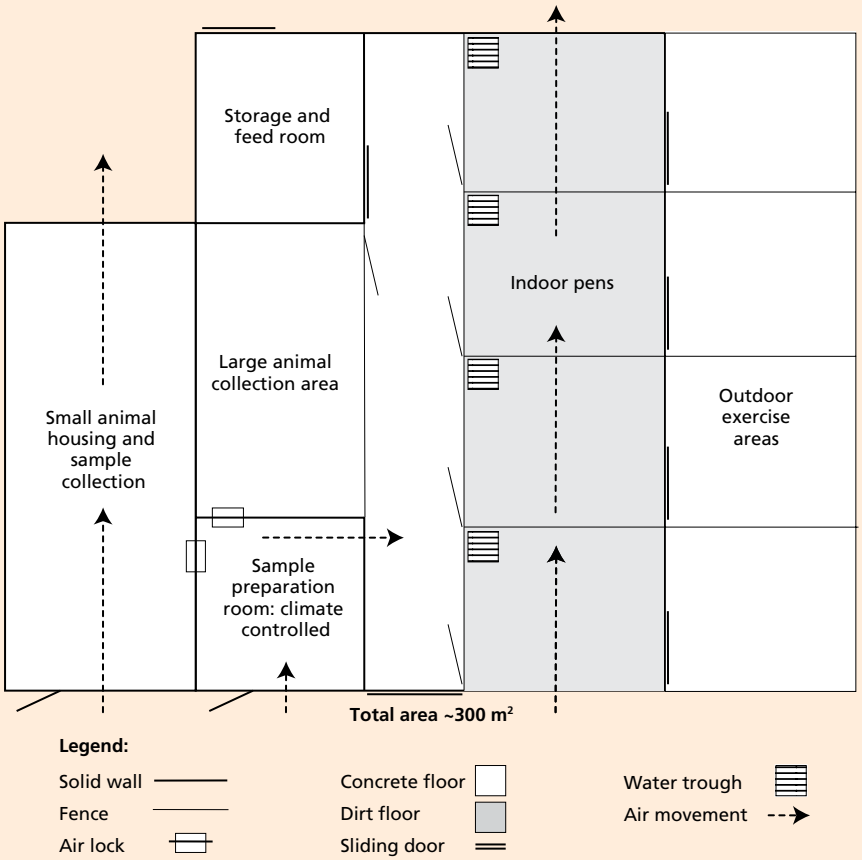
flowing in multiple directions. It also ensures that only fresh air will enter the building and that air laden with animal by-products (methane, urea, etc.) will be vented outside, thus minimizing the load of contaminants within the facility and improving the health of the housed animals. Proper ventilation will, in addition, help maintain the appropriate humidity within the facility. Temperature control is also important. Depending on the local climate, heating and/or cooling systems may be needed in order to maintain the indoor temperature within the range of thermal comfort of the species being kept. In particular, the animals must be protected against high temperatures, which can adversely affect sperm production, especially in pigs. In addition, the intensity of light and the periods of light and dark may have to be controlled in order to meet the physiological and behavioural needs of the animals.

Biosecurity. Ensuring the biosecurity of the site should undoubtedly be one of the most important concerns of a gene bank manager. The following principles should be considered:

- *Location.* The decision as to where to construct the gene bank should be based on a detailed study of potential locations. If possible, the facilities should be situated in a low animal-density area. The animal-holding buildings should be situated at least 3 km from other farms or similar sources of biological risk, and at least 1 km from the nearest main roads or railways, especially if the nearby roads and railways are frequently used for transporting livestock.
- *Perimeter fence.* The installation must have a perimeter fence to prevent the entry of non-authorized persons and domestic or wild animals.
- *Animal loading bay.* The loading bay for the arrival and departure of donor animals poses a great risk to the biosecurity of the site. It should be situated outside the perimeter fence and some distance from the animal housing units. In addition, trucks bringing feed to the facilities should unload in storage and transfer areas outside the perimeter fence.
- *Clean and dirty areas.* The facility must be clearly divided in two physically separated areas: the dirty area (buildings for animals, collection pens, and storage areas for waste materials, feed, etc.) and the clean area (laboratories, instrumentation rooms, germplasm storage facilities and offices). If these two facilities are in the same building, only one indirect connection between the clean and dirty areas should be allowed, ideally through a sanitary air lock in the wall between collection pens and laboratories. Locker rooms with changing facilities and showers should be located between the two areas.
- *Staff.* Members of staff should work exclusively in the gene bank and not have contact with other farms or facilities that house animal species from which material is stored in the gene bank. The clothes and footwear used in the laboratories should be stored in the changing facilities and not used or otherwise taken outside.
- *Waste management.* The management of waste from the facility should be organized so as to reduce the risk of disease transmission. Storage facilities from which waste is loaded and transported away must be outside the perimeter fence.

BOX 4

Example animal holding and collection facility



(cont.)

- *Pest control.* Common pests include rats, mice, birds and flies. The control of rats and mice is greatly facilitated by adequate construction and maintenance of the facilities, particularly by preventing access to feed and water. Traps and rodenticides can be used if problems are discovered. Bird-proofing nets in windows and other openings will limit the entry of birds, although controlling them in open-sided buildings or outdoor pens is very difficult. Regular cleaning of pens, strategic use of insecticides and proper ventilation are important elements of fly control.
- *Dead animals.* Even with the greatest care and precaution, it is likely that donor animals will occasionally die at the facility. Carcasses should be taken away as quickly as possible and stored outside the perimeter fence until they are collected. Wild or stray birds and other animals should be kept away from the carcasses.

The figure illustrates a basic facility designed for housing multiple types of animals, such as several rams or 20 to 30 roosters, and for collecting and processing semen samples and embryos or other genetic materials. The freezing and storage rooms will ideally be physically separated from the animal housing and handling facilities for sanitary reasons. The facility is equipped with indoor and outdoor areas for the larger animals, which enable the animals to be moved with minimal handling to a collection area. Also included are three rooms that are self-contained: the small animal room, the storage and feed room, and the sample preparation room. Having these self-contained rooms allows the creation of "micro-environments" to fit specific needs. For example, the small animal room will need to have a daylight regimen; the feed room will need to be contained so as to eliminate the risk of rodent infestation; and the sample preparation room will need to have an environmental and temperature control system that decreases the risk of cold-shocking semen samples.

The airflow of the facility is illustrated in the figure by the dotted arrows. As described above, the small animal room is separated from the rest of the facility (except for an airlock for the passage of samples into the sample preparation room) to minimize the spread of by-products (faeces, feathers, etc.). Therefore, this room has its own air-handling unit, which provides fresh air and an exhaust system. For the same reasons, the large area of the facility has its own air-handling and exhaust system. The air-handling system of the sample preparation room vents into the larger facility and the vented air is then removed by a shared exhaust system.

Note that the facility shown in the figure includes only a single large animal-housing building. Although this is not ideal, it still allows the implementation of several biosecurity and general safety measures. An all in/all out procedure still can be followed, i.e. all the animals in a group of donors arrive and leave together and the building is cleaned and left unused for a few days between each group. The system allows different species to be alternated so that multiple species are not present at the same time. Multiple pens allow for male animals to be housed in separate pens. Finally, the animal handling and sample collection areas and sample preparation room have separate entrances, so the activities in each can be the respective responsibilities of different persons. This ensures that the processing technician does not come into direct contact with the animals.

Pens. Working, handling and containment pens (indoor and outdoor) should be constructed with the following factors in mind:

- *Size.* The pens and gates should be appropriate to the size of the animals and allow them to be moved in and out with minimal handling. For example, if the pens are too large, a lot of time may be needed to herd or coax the animals to the collection site, which will cause stress in the animals and decrease the quality of the germplasm samples collected.

- *Individual pens.* Male donors should be kept in individuals pens, but they should have visual contact with other animals. The objective of this arrangement is to avoid aggression between the males and subsequent injury, but to allow the development of social relationships that help reduce stress.
- *Materials.* Pens should be of high quality and durable enough to withstand the repeated stresses caused by the animals.
- *Automatic systems.* When financially feasible, the holding pens should ideally have automatic systems for supplying water and feed to the animals and for removing waste. Reducing human contact helps to prevent the animals from becoming stressed.
- *Safety.* Pens should be free from sharp edges and jutting angles that may injure animals or their handlers. Pens should be constructed with escape routes that handlers can use if the animals become violent.

Field collection

In many countries some collection of germplasm in the field is likely to be necessary, even if the country has a complete gene banking facility including infrastructure for animal holding and germplasm collection. Field collection is likely to be more common in large countries that have a single gene bank (because transporting animals to the central facility will be more expensive) and in countries where pastoral or other pasture-based production systems are predominant. Field collection requires particular protocols and advanced planning, as well as some specialized equipment. It is advisable to prepare a specific protocol to be followed during field collection and to train technicians in best practices for off-site collection. The national gene bank at the Canadian Animal Genetic Resources Laboratory for Cryobiology has a specific set of standard operating procedures for field collection of germplasm. These procedures are summarized in Appendix A.

Equipment. The following equipment and reagents are required for efficient and sanitary collection and/or freezing of germplasm in field conditions:

- disposable coveralls
- disposable boots
- single-use examination gloves
- reusable and sealable plastic bags
- indelible permanent markers
- paper towels or absorbent paper
- frozen ice packs
- shipping container
- packaging tape and dispenser
- portable incubator
- microscope
- microscope slides
- haemocytometer
- semen straws and filling/sealing equipment
- Styrofoam boxes
- liquid nitrogen storage tank

- gloves and tools for handling liquid nitrogen
- liquid nitrogen dry-shipper
- pre-addressed shipping labels (to the gene bank)

Safety. Field collection of germplasm should whenever possible be done by a team of technicians rather than by a single technician. Interacting with large animals always involves some risk of injury, particularly when the animals are from rangeland production systems and may therefore not have regular human contact. Surgical tools and liquid nitrogen can also cause injury. Cellular phones should be carried for general communication and to contact emergency services if injuries occur. When collection is undertaken in a remote, unfamiliar area, local authorities should be notified in advance and maps and geographic information system equipment should be carried (the latter also serves for recording the location of the sampling site).

As with collection at a dedicated facility, the animals should be restrained in a manner that is safe for both animals and technicians, and care should be taken to minimize the stress caused to the animals. Biosecurity protocols should be followed in order to prevent the transmission of disease from location to location (see also Section 9).

Germplasm processing and freezing laboratories

Although the processing and freezing laboratories may be part of the same physical structure as the animal collection and holding facilities, there must be a distinct physical barrier between the two areas, as the processing and freezing area must have a higher level of sanitation. The laboratory should be designed for maximum efficiency. In particular, it should be kept as small as possible so as to reduce the amount of maintenance and cleaning required. The various workstations should be arranged in such a way that the germplasm samples move from workstation to workstation in a logical order.

Essential features of the laboratory include the following:

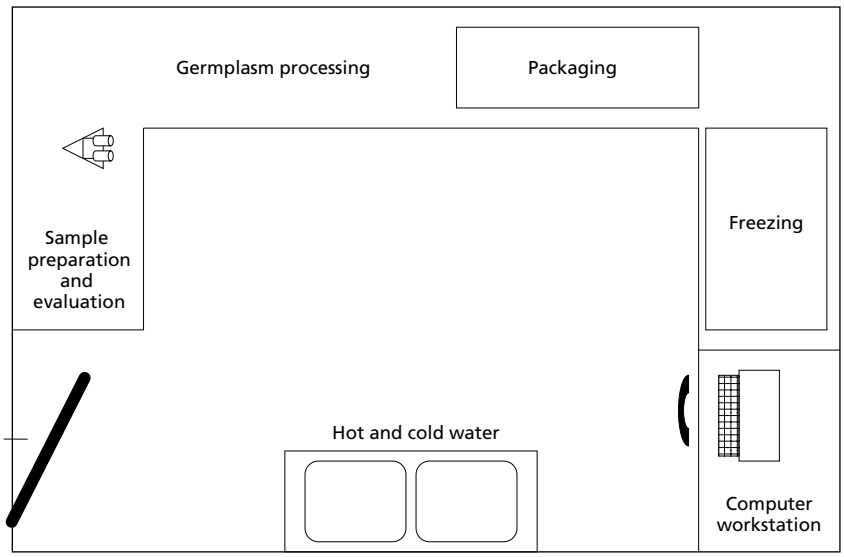
- washable work surfaces, floors (non-slip) and walls;
- sufficient lighting and ventilation;
- hot and cold running water as well as purified water;
- sufficient numbers of water-protected electrical sockets for all fixed and portable equipment; and
- adequate storage for consumable materials.

Figure 2 shows an example of a general layout for a simple germplasm processing laboratory. The various workstations are compact in order to increase the efficiency of the processing activities, which begin at the left of the diagram and then move step-by-step in a clockwise direction until the samples are finally packaged and frozen. Computing facilities are readily available (lower right of diagram), both for the operation of any software that may be necessary (e.g. for calculating dilution rates and inputting information about each sample into the central database – see Section 10).

Long-term storage room

In general, the long-term storage room for cryopreserved germplasm can and should be physically separated from any laboratory. Such an arrangement will increase the physical

FIGURE 2
Example design of a germplasm processing and freezing laboratory



Total area ~20 m²

security of the collection. By having physical barriers the potential for unauthorized access to the collection can be better controlled. Many existing gene banks have found this to be a desirable arrangement because it means that the long-term liquid nitrogen tanks do not have to be individually locked. Nonetheless, gene bank managers may, after weighing the advantages and disadvantages, decide that locking individual tanks should be a part of their standard operating procedures.

Material stored in a gene bank is a highly valuable resource and must therefore be safeguarded against loss. For this reason, it is strongly recommended to maintain two separate storage facilities in different geographic locations.

Liquid nitrogen is a dangerous material (see Box 5) and specific precautions must be taken, both in handling liquid nitrogen and in the design of storage facilities. If there is a leak of liquid nitrogen, there will be a risk that people involved in filling tanks and placing or retrieving samples, or simply working in the storage areas, will be asphyxiated. For this reason, designing the ventilation system should be a critical priority in the development of the long-term storage space. In general, nitrogen in its gaseous state is heavier than normal atmospheric air, so if liquid nitrogen spills the concentration of nitrogen will be greatest close to the floor. Therefore, ventilation ducts need to be placed at or near floor level. Conversely, air inflow ducts need to be placed high in the room (potentially at ceiling level) to help insure that nitrogen gas is forced out of the room through the lower exhaust vents.

BOX 5

Liquid nitrogen safety

Liquid nitrogen is an extremely dangerous substance to handle. Two major safety risks are:

- freezing or "burning" of skin upon contact; and
- hypoxia (lack of oxygen) and respiratory distress.

Liquid nitrogen has a temperature of -196 °C. It should never be allowed to come into contact with the skin. Protective gloves should be worn as well as standard lab coats. Shorts and open shoes or sandals should be avoided. Eye protection should be worn. Specially designed "tongs" should be used for handling the straws and containers used for storing germplasm. The low temperatures alter the physical properties of substances, so only tools specifically designed for work with liquid nitrogen should be used. If liquid nitrogen does come into contact with a person's skin, the affected area should be flooded with cold water and cold compresses applied. Medical attention should be sought immediately if eyes are affected or if the skin becomes blistered.

To prevent the possibility of hypoxia and respiratory problems, handling and storage areas should be well ventilated. Nitrogen vapour is heavier than air, so exhaust fans should be positioned so as to remove gas from near the floor. The handling and storage areas should also be fitted with oxygen meters that warn of the presence of excess nitrogen. An oxygen concentration of less than 19.5 percent is considered unsafe. If people become dizzy, everyone present must evacuate to a well-ventilated area. Victims of hypoxia must be moved to safety immediately and given artificial respiration and medical assistance.

Transport of liquid nitrogen presents special dangers. Great care must therefore be taken. Liquid nitrogen is an additional source of risk in road traffic accidents. Always ensure that tanks are safely secured. Transport of liquid nitrogen in the passenger compartment of a vehicle should be avoided. If this is not possible, one or more windows should be kept open.

It is also important to have adequate ventilation in the rooms where samples are frozen with liquid nitrogen. In both spaces, it is strongly recommended that oxygen sensors be installed and regularly tested.

SIZE AND CAPACITY REQUIREMENTS FOR GENE BANKS

The space required for gene banks can be extremely variable and may change as the mission of the gene bank changes or as the collection grows. It is important to note that the space requirements are relatively minor. Furthermore, the costs of developing the space should be amortized over appropriate depreciation timelines (e.g. more than 20 years).

Small repositories

Small repositories have limited space, but in some countries they will easily be large enough to meet the needs of national cryoconservation programmes. Facilities of this size can be expected to process and cryopreserve up to 500 straws of semen in a day. Typical dimensions of a small repository are as follows:

- wet lab (20 to 30 m^2);
- capacity to cool and refrigerate samples (to 5 to 15 °C, depending on the species) – cold room (10 m^2) or cooler cabinet (2 m^2) or Styrofoam box (0.6 m^2);
- cryotank storage room (20 m^2) (sufficient for approximately 300 000 straws);
- office for database management (12 m^2).

Medium-sized repositories

Medium-sized repositories should have a few additional features that not only increase the amount of germplasm they can store, but also the quantity of germplasm (principally semen) they can cryopreserve in a day (i.e. more than 500 straws per day). In fact, such facilities are likely to have automated equipment for straw labelling and filling and therefore be able to process and cryopreserve thousands of straws of semen per day. Typical dimensions of a medium-sized repository are as follows:

- wet lab (30 to 60 m^2);
- ability to cool and refrigerate samples – cold room (10 m^2) or cooler cabinet (2 m^2) or Styrofoam box (0.6 m^2);
- cryotank storage room (25 to 50 m^2) (approximately 400 000 to 600 000 straws);
- office for database management (12 m^2);
- other office space (12 m^2).

Large repositories

Large repositories have substantial capacity to cryopreserve semen, embryos and DNA and thereby meet their national mandates. Such facilities have the equipment needed to increase the quantity of germplasm frozen to several thousand straws per day. In addition, to the equipment necessary for cryopreserving samples, large repositories will also have equipment for analysing the quality of the germplasm cryopreserved: for example a computer-assisted sperm analysis (CASA) unit and flow cytometers. Typical dimensions of a large repository are as follows:

- wet lab (greater than 60 m^2);
- ability to cool and refrigerate samples – cold room (10 m^2) or cooler cabinet (2 m^2) or Styrofoam box (0.6 m^2);
- cryotank storage room (greater than 50 m^2) (>600 000 straws);
- office for database management (20 m^2);
- other office space (50 m^2);
- capacity to store excess liquid nitrogen or even to make liquid nitrogen.

RECOMMENDED EQUIPMENT AND ESTIMATED COSTS

The following basic equipment is needed for cryopreserving and storing samples:

- glassware for holding semen and measuring volume
- water bath
- microscope (phase-contrast for semen; stereoscope for embryos)
- centrifuge
- equipment for cooling samples (Styrofoam or plastic cooler, cooler cabinet)
- supply of pure or ultra pure (for embryos) water
- osmometer
- pH meter
- equipment for determining sperm concentration (one or more of the following):
  - spectrophotometer (fixed or portable)
  - Makler counter chamber (or disposable counting chamber)
  - haemocytometer
- temperature measuring devices
- straw labelling machine
- straw filling and sealing equipment
- freezing equipment (manual or programmable)
- carbon dioxide incubator (for embryos)
- laminar flow benches (for embryos)
- dry liquid nitrogen shipping tanks
- long-term liquid nitrogen storage tanks

Box 6 describes the types of liquid nitrogen tanks that will be needed. It is important to remember that it is not necessary to purchase all the liquid nitrogen tanks that will be needed over the long term at the same time. The tanks can be bought on a gradual, as-needed, basis.

GENE BANK SECURITY

Germplasm collections can be viewed as a national asset and thereby warrant appropriate levels of security. Building security consists of several aspects, including:

- **Safety of the germplasm from unauthorized access.** This can be achieved by controlling access to the room and the tanks themselves. In addition, gene bank managers should maintain records of which persons have access to the room(s) where collections are stored and when those persons accessed the room(s).
- **Structural integrity.** The structure of the gene banking facility should be sufficiently robust to withstand any environmental challenges that are conceivable in the local area (e.g. high winds, earthquakes, extreme temperatures, fires, floods). Construction in vulnerable areas, such as floodplains, should obviously be avoided.
- **Plans and equipment with which to continue operations in the event of major systems failures.** For example, generators for electricity and alternative sources of liquid nitrogen should be considered.

BOX 6

Liquid nitrogen tanks

The number and size of liquid nitrogen tanks needed for a gene bank will depend upon how much germplasm is expected to be stored. The capacity of tanks available for purchase will be specified in terms of numbers of straws, but it is also important to consider the quality of the tanks. Choosing to buy a given tank because the purchase price is low may not be the optimal financial strategy in the long term. Higher-quality (and thus more expensive) tanks will usually last longer and usually use less liquid nitrogen. They will also be less subject to leaks and other accidents that result in the loss of stored material. The value of the stored germplasm will usually far exceed the cost of the storage tank.

Any gene bank will need three types of tanks:

- long-term storage tanks;
- vapour shippers (for transporting germplasm); and
- storage tanks (for storing liquid nitrogen itself).

Long-term storage tanks are the most important part of a gene bank's equipment. Remember that the stored germplasm may be unique and irreplaceable. The tanks' liquid nitrogen consumption should be as low as possible, because liquid nitrogen is one of the major costs of maintaining a gene bank. In addition, it is recommended that individual tanks have electronic monitors that measure temperature and liquid nitrogen levels and sound an alarm if either parameter is outside set limitations. The size of tank required can vary greatly depending upon the size of the repository and how much germplasm is to be stored.

Vapour shippers are designed for the safe transportation of germplasm. They contain a hydrophobic absorbent that holds the liquid nitrogen, repelling moisture and humidity and thus maximizing the holding time (usually two to three weeks).

The third type of liquid nitrogen tank required are those used for storing surplus liquid nitrogen for future use in the other types of tank. Such tanks also serve as a buffer if regular supplies of liquid nitrogen are interrupted. Thus, the decision as to what size of storage tank is needed should take into account the level of security required given the frequency and reliability of liquid nitrogen deliveries, or alternatively the production schedule of the gene bank's own liquid nitrogen generation plant.

CENTRALIZATION AND ACCESSIBILITY

The development of the gene bank will be easier if it is located in an area that has sufficient infrastructure to ensure its smooth and continuous operation. For example, collection efforts will be facilitated if the gene bank is located near to major highways or airport hubs. Depending on the country, this may or may not be in or near the capital city. If the country has several well-connected cities, those found in the parts of the country that have the largest livestock populations will be the preferable options. If the country is large enough

TABLE 6
Estimated equipment costs by gene bank size

Item	Small		Medium	Large
	Necessary	Optional		
Long term liquid nitrogen tanks	$5 000 to $40 000		$40 000 to $120 000	> $120 000
Shipping tanks	$3 000		$3 000	$3 000
Equipment for straw filling	$500		$40 000	$40 000
Equipment (ranging from Styrofoam box to programmable freezer) for freezing samples	$200		$200 to $20 000	$200 to $20 000
Microscope	Compound $500		Phase contrast $5 000	Phase contrast with fluorescence $15 000
Centrifuge		$10 000	$10 000	$10 000
Spectrophotometer (fixed or portable)		$2 100	$2 100	$2 100
Makler counter chamber	$850		$850	$850
Haemocytometer	$200		$200	$200
pH meter	$1 200		$1 200	$1 200
Osmometer		$8 000	$8 000	$8 000
Water bath			$800	$800
Total	$11 450 to $46 450	$31 500 to $66 550	$111 350 to $211 150	$201 350 to $221 350

Note: costs are shown in United States dollars at 2012 prices.

to have notable regional differences in climate, situating the gene bank in a relatively colder region can help to minimize liquid nitrogen evaporation and thereby reduce the cost of maintaining the gene bank.

Other factors to consider in choosing the site for a gene bank include the extent to which public – and private – sector arrangements can be made for germplasm collection and processing and the location of important stakeholders. For example, AI centres may already be operating in the country, and the national germplasm programme may be able to develop arrangements with the AI companies to acquire germplasm from their facilities. By formulating such arrangements, the national programme can focus its own attention and resources on breeds and species for which there is a lack of commercial infrastructure for collection. As noted above, existing gene banks for plants or wild animals are also potential collaborators. In addition to aiding the collection effort, the presence of collaborating institutions may increase the accessibility of inputs such as liquid nitrogen.

HUMAN RESOURCES

The number and type of personnel needed to operate the gene bank will vary with the size of the facility. Common to all sizes, however, is the need for three different disciplines: genetics; reproductive physiology and cryobiology; and information systems and database development. Minimally, the gene bank will require:

- a curator (who will likely have a genetics background);
- technical support to cryopreserve germplasm; and
- technical support to develop a database and enter information into the database.

The curator has to develop targeted collection goals for each species and breed of interest. Obviously, this task is a significant responsibility for one person. Thus, in formulating plans for the development of the collection, the curator may need to seek input from a wide array of livestock expertise. Such consultations should involve not only the research community, but also the livestock industry, including groups involved in raising various livestock breeds.

As gene bank activities increase in size and scope, more staff will be needed. Much of this increase will be because of the increased flow of germplasm into the repository. However, as the size of a gene bank increases, additional technical needs will also arise. There may be a need to use a broader range of reproductive and cryopreservation technologies on a wider range of species. There may also be a need to quantify and understand the genetic diversity in the gene bank, which may require the use of molecular genetic techniques. With additional requirements comes the need for additional scientific support. Of course, many of these additions can be addressed by integrating conservation activities with those of pre-existing laboratories that have the necessary expertise (e.g. laboratories that specialize in molecular genetics). Nevertheless, the gene bank absolutely needs to have a reasonable level of competence with respect to these additional technologies if it is to fulfil its conservation mission.

Proper training is a critical factor in ensuring the smooth operation of gene banks. Regardless of his or her particular role, every gene bank employee should have an initial course of orientation and training at the beginning of their period of employment. Thereafter, training must be continual, with particular emphasis given to biosecurity.

CONTINUITY OF OPERATIONS

As with any important national resource, gene banks need to have procedures in place for handling the collection in the event of an emergency. Such emergencies might include the loss of electricity, flooding, earthquake, civil unrest, or a disease outbreak that affects the animal or the human population. If such an event take places, it is important to have a predetermined plan providing a set of guiding actions that can minimize the impact of the emergency on the maintenance of the collection.

To ensure the sustainability of the gene bank in the event of a disaster, the following points should be taken into account (some of these are mentioned above in the subsection on gene bank security, but their importance cannot be overstated):

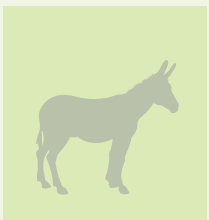
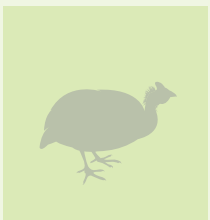
- Establish the gene bank in a location where hazards such as earthquakes, floods and tornados are minimized.

- Split the collection into two parts and store them in geographically separated facilities. This provides potential protection against earthquakes, floods, tornados, etc. The decision on whether or not to split the collection will depend on the finances available and the level of security afforded by the primary bank. In general, only the facilities for long-term storage (core and historical collections) will need to be replicated, so the costs of having two gene banks will be much less than double the cost of a single bank.
- Consider developing contingencies for moving the entire collection, or a predetermined subset of the collection, in the event of civil unrest.
- Establish minimum numbers of staff required to report to the gene bank in the event of an emergency.
- Develop plans for acquiring supplies (e.g. liquid nitrogen) from alternative sources.

Contingency plans should be subject to periodic review in order to ensure that they are still viable.

SECTION 6

Developing gene bank collections


Developing gene bank collections

Developing and updating gene bank collections is a long-term endeavour that involves several processes. Major steps include understanding the dynamics of the AnGR population of interest, determining the status of the population (e.g. whether numbers have dropped to a predetermined critically low level), establishing collection targets for germplasm (semen, embryos or oocytes) or tissue, and selecting the animals from which samples will be obtained for the collection.

As mentioned in Section 1, the choice of which breeds and animals to include in the gene bank will depend upon the country's objectives for AnGR management (preferably set out in a national strategy and action plan for AnGR) and the country's capacity to obtain and store the samples. In countries that do not have an existing gene bank, conservation needs for all the country's AnGR should be reviewed as a first step in determining whether establishing a gene bank would be an appropriate part of the country's conservation strategy. Factors to consider include both the accessibility of the animals for collection and the state of technical and financial capacities within the country for processing and storing the samples.

Primary considerations for all gene banks have to be the need to acquire sufficient quantities of germplasm to reconstitute the targeted populations and the need to ensure that the samples collected sufficiently represent the genetic diversity of these populations.

CHOICE OF POPULATIONS TO INCLUDE IN THE GENE BANK

Before initiating collection activities, gene bank managers will have to assess the various breeds and species in the country in order to determine where to start. More details on identifying breeds at risk and prioritizing AnGR for conservation will be presented in the forthcoming FAO guidelines on *in vivo* conservation of AnGR. Such decisions can be made on the basis of breeds' population size, potential genetic uniqueness, economic importance and/or cultural importance. In general, the choice of breeds for conservation should be a group decision undertaken by the National Advisory Committee on AnGR (FAO, 2011b) or similar committee, in consultation with the wider stakeholder community. Nevertheless, gene bank managers and curators should be allowed flexibility to be pre-emptive and initiate the collection of germplasm from breeds that are viewed to be in an extreme state of risk or critical to the country's livestock sector.

Various quantitative measures can be used for deciding which breeds should be prioritized for inclusion in collections. In general, two primary factors determine the conservation priority of a breed:

- level of risk of extinction; and
- conservation value.

Risk status

From a quantitative perspective, risk status can be thought of as expected future population size. The best measurable indicator of future population size is the current population size. Data on past population size can improve the prediction of future population size, as trends can be extrapolated into the future. Unfortunately, breed-level population data are often not available, especially where breed associations have not been established. Although many countries undertake periodic censuses of livestock, very few of these censuses record which breeds the animals belong to. As a result, it may be relatively easy to obtain a general overview of the national livestock industry, but gaining accurate information about the status of breeds within species is likely to be more difficult. The best way to overcome these difficulties is to initiate a national programme of surveying and monitoring for AnGR as an element of the country's national strategy and action plan for AnGR. As well as supporting decision-making at national level, this will enable regular reporting of population data to FAO via the Domestic Animal Diversity Information System (DAD-IS: http://www.fao.org/dad-is) and thereby contribute to reporting on global status and trends of AnGR. FAO has produced guidelines on *Surveying and monitoring of animal genetic resources* (FAO, 2011c) to assist countries in the task of obtaining data on their AnGR.

If the availability of data on national breed populations is insufficient, gene bank managers (in consultation with relevant national working groups on AnGR) may wish to initiate their own protocols for surveying and monitoring AnGR, provided the necessary financial and technical support is available. If new surveying initiatives are planned, the sampling procedure must be well designed. A statistician should be enlisted to design an appropriate sampling strategy. Further advice can be found in the above-mentioned guidelines (FAO, 2011c). If breed associations are present in the country, collaboration with them is recommended, as these organizations typically keep track of annual registrations and may be willing to share this information.

Factors other than population size can also influence risk status. Breeds that are distributed across more farms and/or across a wider geographic area tend to be at less risk than breeds with more concentrated distributions. When a breed is maintained by large numbers of livestock keepers, the action of a single individual will have less impact on the population size. Wider geographical distribution decreases the risk that the entire population may be wiped out by a single geographically concentrated catastrophic event, such as a disease outbreak. Increased cross-breeding and increased inbreeding also put AnGR at risk – even if the number of animals stays the same, the diversity may decrease.

Conservation value

As discussed in Section 1, conservation of AnGR may be undertaken to meet various objectives. These objectives and their relative importance are likely to vary from country to country. In turn, breeds will vary in terms of their relative contributions to meeting these conservation objectives. The following factors will influence the conservation value of a given breed (Ruane, 2000):

Genetic uniqueness. Genetic distinctiveness is an important criterion for establishing conservation priorities. Understanding the genetic history of particular breeds, or formally estimating genetic distances among breeds, will assist in determining breed uniqueness.

Genetic variation within a breed. Genetic variation gives an AnGR the capacity to adapt and allows for genetic response to selection. Conserving the most genetically diverse breeds is the most efficient way to conserve the diversity of a species.

Traits of economic importance. Breeds that are genetically superior for traits that are economically important (at present or in the foreseen future) should receive priority in conservation. Prioritization decisions require evaluation of both current and potential future importance of particular breed characteristics and performance.

Unique traits. Breeds with special behavioural, physiological or morphological traits should be given high priority for conservation.

Adaptation to a specific environment. The adaptation of breeds to specific environments is likely to be under some genetic control. Thus, the conservation of AnGR that thrive in specific environments (e.g. those that are in some way harsh) may be important. Breeds that perform valuable environmental services will also often be priorities for conservation.

Cultural or historical value. Breeds with special cultural or historic values merit consideration for conservation.

Species a breed belongs to. The above criteria are important in selecting breeds within species; some consideration should also be given to the species to which the breeds belong when considering which genetic resources to conserve and which approach should be applied. For example, in some countries, particular species may be especially important (e.g. alpacas in Peru). With regard to cryoconservation, the technology and procedures for cryopreservation are generally more advanced for cattle than for other species, meaning that costs are lower and success rates are higher. Considering species in conservation decisions therefore helps ensure that financial resources are fairly allocated to all important livestock species and accounts for the costs and likelihood of success of different conservation options.

Accounting for all of these factors can be quite difficult. Clearly, prioritization of breeds for collection and entry into the gene bank can be done in a number of different ways, and the approach chosen will depend on national circumstances. Advisory committees of experts, including stakeholders from industry and breeders' associations, can advise the gene bank about the risk status of various populations and their genetic, economic and cultural importance.

Molecular markers can be used for evaluating genetic distances and breed diversity (see FAO, 2011a). Various objective methods have been proposed for incorporating molecular measures of diversity into conservation decisions (see review by Boettcher *et al.,* 2010). These methods typically allow for simultaneous consideration of risk status and the various genetic and non-genetic factors listed above. Their use will be described in more detail in the forthcoming FAO guidelines on *in vivo* conservation of AnGR, because prioritization should be done as part of an overall conservation plan rather than just for cryoconservation.

Although formal prioritization methods may increase the efficiency of conservation decision-making, a willingness to be flexible in establishing collections is also needed so as not to miss unexpected needs and opportunities when they arise. When particular AnGR are at high risk of extinction, or if they can be collected a very low cost, collecting them for cryoconservation can be justified even if no formal prioritization analysis has been undertaken.

COLLECTION TARGETS FOR RECONSTITUTING POPULATIONS

Once the decision has been taken as to which breeds and populations should be collected for the gene bank and the type of germplasm to collect, the next step is to determine the amount of germplasm needed. The quantity will vary depending on the conservation goal, the type of germplasm and the species. In general, the goal of reconstituting extinct populations will require the greatest amount of germplasm.

Targets for cryoconserved semen

When semen is cryoconserved, the principal method for reconstituting a breed or population is through backcrossing (Figure 3). Starting with females of a common breed, four backcross generations allow the reconstitution of more than 90 percent (four generations ~94 percent; five generations ~97 percent)[4] of the nuclear genome of the conserved breed or population. The following subsections describe how semen can be used to reconstitute a population and the amount of germplasm needed to do this.

The length of reproductive cycles and conception rates influence the speed and efficiency with which populations can be reconstituted. To better establish collection goals, the manner in which the cryoconserved material will be used to reconstitute a breed needs to be quantified. Previous publications (FAO, 1998; Boettcher *et al.,* 2005) described a single backcrossing approach, such as that shown in Figure 4. In this approach, females are used as mothers only once in their lives. This allows the breed to be reconstructed with maximum speed and simplicity. All animals of the same age will be of the same generation and will have the same proportion of genetic material from the reconstituted breed. Because females are used only once, the amount of semen needed for reconstituting a population is quite large. This is primarily because of the 50:50 sex ratio that must be assumed, which in the early generations results in a large number of excess males.

Some livestock species, including cattle, buffalo, small ruminants and horses, have longer reproductive cycles and/or smaller family sizes than others such as pigs and poultry. Advanced strategic planning with regard to mating has the potential to drastically improve the efficiency of gene banking and breed reconstitution. The mating plan shown in Figure 5 uses a slightly different backcrossing scheme. The approach allows females in the first, second and third backcross generations to be mated up to three times. This gives these animals a much greater chance of producing a female offspring. Expanding the mating opportunities of the later generations means that less semen is used, and decreases the cost of maintaining the animals used in reconstituting the breed. The principal drawback of this approach is that it will take slightly (e.g. one to three years) longer to reconstitute the population.

[4] Proportion of target breed recovered = $1 - 0.5^b$, where b is the number of backcross generations.

FIGURE 3
Population reconstitution with cryoconserved semen

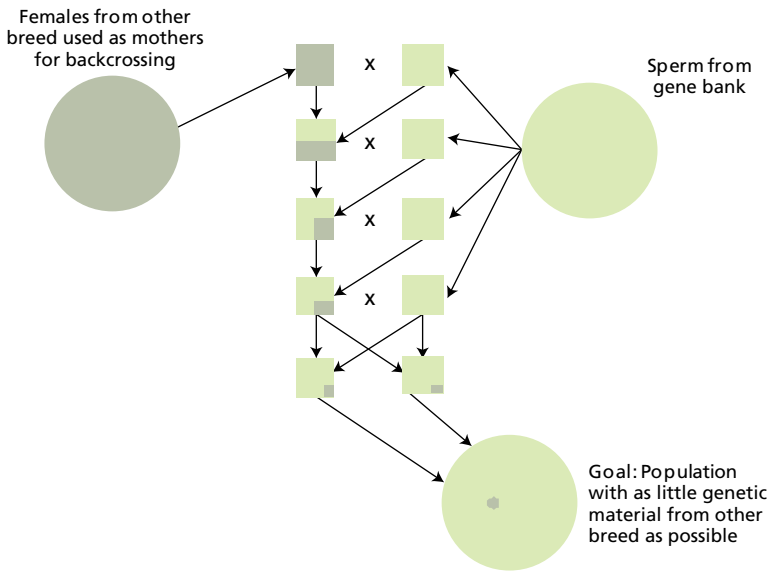
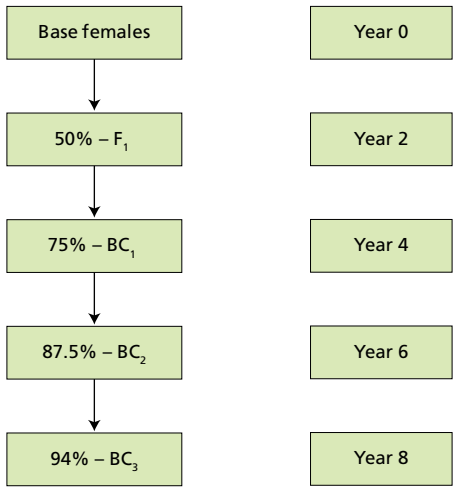
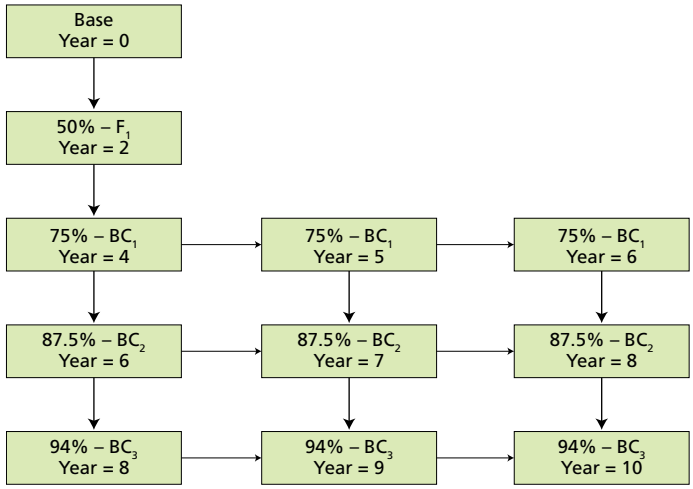


FIGURE 4
Standard backcrossing plan for breed reconstitution (using cattle as an example)



Note: BC = backcross (numbers indicate the first, second and third backcross generations).

FIGURE 5
Alternative efficient mating plan for breed reconstitution



Note: BC = backcross (numbers indicate the first, second and third backcross generations).

Ruminants and horses. Table 7 shows the quantities of semen needed for a "150 percent breed replacement plan" in which a breed is reconstituted using the breeding plan shown in Figure 5. A "150 percent breed replacement plan" means that the gene bank will save 1.5 times the amount of germplasm that is expected to be necessary to reconstitute a breed (i.e. to obtain 25 males and 25 females with 94 percent of the genetics of the conserved breed). Planning at the 150 percent level provides flexibility and additional germplasm that may be needed to compensate for lower than expected pregnancy or survival, excess animals of one sex, or other failures in the reconstitution process. The table shows targeted quantities of semen for various rates of pregnancy (which will vary according to circumstances). Another variable shown in Table 7 is the number of founder-breed females used to start the reconstitution process. The final number of animals with 93.75 percent of the targeted genome that will be generated (a critical factor in the success of the reconstitution process) is also shown (in parenthesis) in Table 7. Note that the reconstituted population sizes are also estimates of N_e. For several of the conception rates and initial numbers of animals bred shown in the table, the N_e is below 50, i.e. below the recommended threshold (see Section 3). However, this deficiency can be addressed by having semen form extra males in the repository, which can be used to broaden the genetic base of the newly regenerated breed.

Estimates of the quantities of semen required per male are presented in Table 8. For many mammalian species, the quantities shown in Table 8 are achievable in a single collection. Nevertheless, gene bank managers should follow a conservative approach and store samples from at least two collections (separated by at least two weeks) from each male.

With cattle, and potentially other species in the future, the utilization of sexed semen would dramatically change the projected amount of semen needed to reconstitute popula-

TABLE 7
Number of semen doses required to reconstitute a breed of cattle, small ruminant or horse

Founder females (N)	Pregnancy rate			
	0.4	0.5	0.6	0.7
75	...*	...	449 (26)	460 (37)
100	...	564 (22)	599 (35)	615 (49)
150	771 (17)	846 (33)	897 (53)	...
200	1 029 (23)	1 128 (44)	...	...
250	1 287 (29)	1 410 (55)	...	...
300	1 544 (34)	...	...	...
350	1 800 (40)	...	...	...
400	2 058 (46)	...	...	...

Note: The figures in parenthesis show the effective population size of the reconstituted population. The quantity of semen is calculated at the 150 percent level (see main text for explanation).
* Missing values indicate that results are not practical, resulting in either too few (upper-left corner) or too many (lower-right corner) animals or requiring large quantities of semen.

TABLE 8
Number of doses per male required to reconstitute a breed of cattle, small ruminant or horse

Founder females (N)	Pregnancy rate											
	0.4 Males (N)			0.5 Males (N)			0.6 Males (N)			0.7 Males (N)		
	25	50	100	25	50	100	25	50	100	25	50	100
75	...	...	...	...	...	...	18	9	5	19	10	5
100	...	...	...	23	12	6	24	12	6	25	13	7
150	31	16	8	34	17	9	36	18	9	...	...	...
200	42	21	11	46	23	12	...	...	...	...	...	...
250	52	26	13	57	29	15	...	...	...	...	...	...
300	62	31	16	...	...	...	...	...	...	...	...	...
350	72	36	18	...	...	...	...	...	...	...	...	...
400	83	42	21	...	...	...	...	...	...	...	...	...

Note: Quantity of semen is calculated at the 150 percent level (see main text for explanation).

tions. The figures in Tables 7 and 8 assume a sex ratio of 50:50. If sexed semen were used, the number of doses could be reduced by 30 to 45 percent.

Another option that countries may consider as a means of decreasing the number of doses of semen (and time for reconstitution) is to accept a larger proportion of the founder breed in the final "reconstituted" population. For example, if a ratio of

TABLE 9
Number of straws of semen required to reconstitute a breed of pig

Founder females (N)	Pregnancy rate		
	0.4	0.5	0.6
15	2 880 (56)	2 760 (66)	2 520 (72)
25	4 800 (96)	4 560 (108)	4 200 (126)
50	9 600 (192)	9 000 (225)	8 400 (252)

Note: The figures in parenthesis show the effective population size of the reconstituted population. The quantity of semen is calculated at the 150 percent level (see main text for explanation). Number of straws is based on an assumption of 20 0.5 ml straws per insemination, with a total of 1 billion cells per insemination.

seven to one (87.5 percent) of the reconstituted and founder breeds is acceptable, the number of backcross generations can be decreased to three and the amount of semen required can be decreased by 30 percent or more. This option may be particularly attractive in situations where reaching the goals shown in Table 7 would be difficult for practical reasons.

Pigs. The relatively high reproductive capacity and shorter gestation period of pigs enables quicker breed reconstitution than can be achieved in cattle and horses. Because of the prolificacy of the species, large numbers of animals are not needed in order to start the reconstitution process. However, because of dilution protocols, more straws of semen are required per insemination than are needed in other species. To a certain degree, the innate reproductive ability of the sow compensates for the occasionally high mortality of pig sperm cells during the cooling and cryopreservation process. In Table 9, the numbers of straws of semen needed for reconstitution are shown, as well as the numbers of offspring reconstituted at the 93.75 percent level. The data are based on the assumption that six piglets from each litter reach breeding age. This is a very conservative estimate given the results obtained by Spencer *et al.* (2010), who reported a 74 percent pregnancy rate and an average litter size of 11 when females were bred through AI. Quantities of semen needed per male are presented in Table 10. As with the other species, these quantities can potentially be obtained from a single collection, but a more conservative practice is to obtain two collections from each boar.

Chickens. In contrast to programmes for mammalian species, chicken conservation schemes may give less emphasis to breeds as categories. Although breeds are important, industrial populations consisting of distinct lines may (depending on the country) be of greater importance. In addition, poultry breeders and scientists have created numerous research populations, many of which can be categorized by Mendelian traits controlled by single genes or by quantitative traits.

Plans to reconstitute chicken populations need to account for the fact that cryopreservation has been problematic in recent years because of the contraceptive effects of glycerol on hen fertility. Multiple inseminations have been employed to overcome this problem and were a recommended in the previous guidelines on conservation (FAO, 1998). Other means

TABLE 10
Number of straws per boar required to reconstitute a breed of pig

Founder females (N)	Pregnancy rate								
	0.4 Donor males (N)			0.5 Donor males (N)			0.6 Donor males (N)		
	25	50	100	25	50	100	25	50	100
15	116	58	29	111	56	28	101	51	26
25	192	96	48	183	92	46	168	84	42
30	384	192	96	360	180	90	336	168	84

Note: The quantity of semen is calculated at the 150 percent level.

of solving this problem include using alternative media and performing intramagnal instead of intravaginal insemination (Purdy *et al.,* 2009). By using the intramagnal approach, multiple inseminations are not needed and the number of units of semen needed to reconstitute a population can be reduced significantly (Blackburn *et al.,* 2009).

Table 11 outlines the semen resources needed to create secure reserves of chicken genetic resources in gene banks. Various objectives for the eventual use of the stored materials are considered and the data assume the use of intramagnal insemination. Semen resources required for the reconstitution of a breed are presented in the first column of data. These figures were calculated based on a number of assumptions regarding the efficiency of reproduction and the survival of the resulting offspring. Specifically, the assumptions used in the calculations include:

- two fertile eggs produced per hen per insemination;
- 1.4 fertile eggs hatched per hen per insemination;
- 85 percent of hatched chicks become adults;
- two inseminations per 0.5 ml straw; and
- 50:50 sex ratio among surviving chicks.

Furthermore, the data assume an efficient mating system similar to that shown in Figure 5 for ruminants and equines. In the initial generations, females are allowed to produce only a single offspring, after which (the third generation for reconstitution of a breed) they are used to produce multiple offspring. This system decreases the number of excess males in the early generations.

Many research institutes around the world have developed specific within-breed lines that differ either for a single gene or for a single polygenic quantitative trait. Cryoconservation of germplasm from such lines may also be of interest, either to protect against loss or for economic efficiency (i.e. maintenance *in vitro* rather than *in vivo*). Reconstitution of such lines will require fewer resources, because a smaller amount of genetic variation needs to be captured in the donor population. The amounts of material that need to be banked in order to be able to reconstitute such lines are shown in the second and third columns of data in Table 11. Note that populations with single genes of interest can be reconstituted in one or two generations.

TABLE 11
Resources required for chicken gene banks to address various conservation objectives

	Objective for use of stored material		
	Reconstitution of a breed (five generation backcross)	**Reconstitution of a research line with a given quantitative trait (five generation backcross)**	**Single gene introgression**
Total straws used[a]	257	127	7
Initial number of hens	140	100	14
Inseminations for entire reconstitution process	513	254	14
Generation number to start multiple intramagnal inseminations per hen (inseminations/hen)[b]	3 (3)	4 (3)	0 (0)
Final number of birds in the reconstituted population (generation number)	62 (5)	44 (5)	16 (1)
Minimum number of straws for 150 percent reconstitution	386[c]	191[c]	11[c]

[a] Based upon a motile sperm concentration of 200 × 10^6 (Purdy *et al.,* 2009).
[b] Generation 3 and 4 hens will have 87.5 percent and 93.7 percent of the genome of interest.
[c] Assumes a 0.5 ml straw and two inseminations per straw.

Embryo use in breed reconstitution

As noted in Section 4, embryos have some advantages and disadvantages relative to semen with regard to the reconstitution of a population. Their principal advantage is the speed with which breeds can be reconstructed (less than five years). In addition, the use of embryos ensures the conservation of a breed's entire genome, whereas when only semen is used the mitochondrial genome is lost and a certain proportion of the founder breed used for backcrossing will be present in the reconstituted population. Embryos could also be particularly important for breeds with extremely unique characteristics that would be very difficult to re-establish by using semen in a backcrossing scheme. The Angora goat is such an example. Re-establishing the Angora's fibre quality through crossing with a founder population of goats that produce low-quality fibre would be very problematic from an economic standpoint, as the fibre from initial backcross generations would be of very little economic value.

However, embryos are significantly more expensive to collect and require greater technical capacity than semen (Gandini *et al.,* 2007). Moreover, embryo transfer (ET) is not possible in all species of livestock. Biologically, the embryo offers the complete genetic complement of the breed. However, the genetic combinations formed when making the embryos can become dated. Gene bank managers should be aware that this may affect the utility of embryos after long-term cryostorage.

Table 12 shows the number of embryos that need to be cryopreserved in order to obtain a reconstructed population of 25 breeding males and 25 females, depending on the survival of the embryo from thawing to birth and the subsequent survival of the animal

TABLE 12
Number of cryopreserved embryos required to reconstitute a breed population

Embryo survival (thawing to birth)	Offspring survival (birth to breeding age)			
	0.6	0.7	0.8	0.9
0.2	625	536	469	417
0.3	417	358	313	278
0.4	313	268	235	209
0.5	250	215	188	167
0.6	209	179	157	139
0.7	179	154	134	120

Note: The quantity of semen is calculated at the 150 percent level. Reconstituted population is assumed to have at least 25 males and 25 females. Numbers of embryos (n_{emb}) were obtained according to the formula $n_{emb} = 1.50 \times 25 (0.5 \times s_e \times s_o)$, where 1.50 is a multiplier used to obtain the 150 percent level, 0.5 is the sex ratio, and s_e and s_o are survival rates to birth and breeding age, respectively (Gandini and Oldenbroek, 2007).

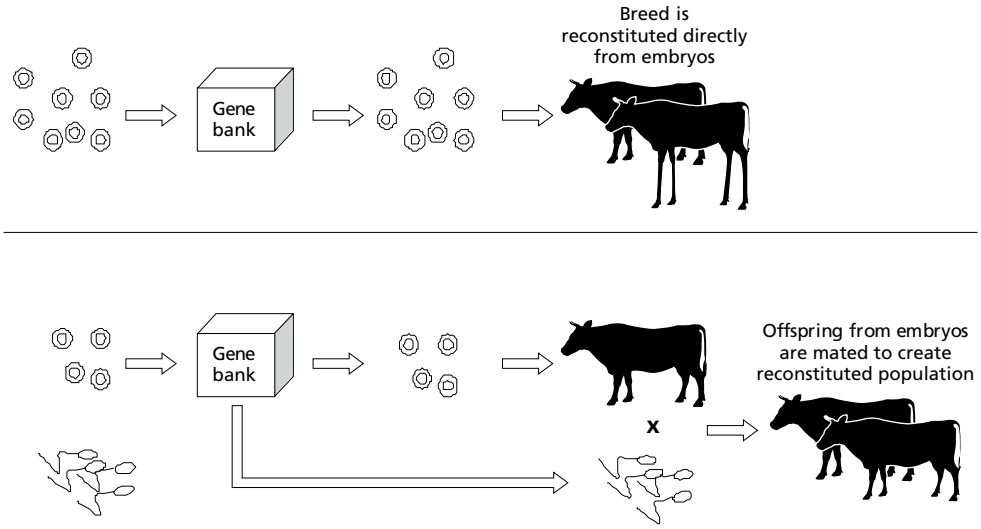
from birth to breeding age. Ideally, the numbers of embryos per donor will be nearly evenly distributed across at least 25 donor females, each mated to a different male (or multiple males), to capture the maximum amount of diversity possible from the existing population.

In cases where sampling 25 donors is not possible (or simply to economize), another option that may be considered is a hybrid scheme in which a combination of semen and embryos is stored (Boettcher *et al.,* 2005). In this strategy, fewer embryos are required than are listed in Table 12. Embryos are used to produce a population initially consisting of fewer than 25 breeding males and 25 breeding females, but these animals are subsequently mated with each other and with the stored semen in order eventually to obtain a population of the desired size after one or more years (Figure 6). This approach can reduce costs relative to storing only embryos while still allowing the maintenance of the mitochondrial genome and usually decreasing the time required for breed reconstitution relative to the backcrossing scheme utilized when only semen has been stored (Figures 3 to 5).

Nonetheless, a hybrid scheme requires some compromises. Although the reconstitution will usually be faster than that achievable with stored semen, multiple breeding cycles are nevertheless required to reach the final goal of 25 animals of each sex. Using embryos, this goal can be achieved in a single breeding cycle. Furthermore, genetic relationships in the reconstructed population increase (and N_e decreases) as the number of embryos decreases, as does the probability of failing to reconstitute the new population. Therefore, a hybrid scheme with fewer than 30 percent of the number of embryos required for an embryo-only reconstitution plan (Table 12) is not recommended. To increase N_e, the sires whose semen is stored in the bank should be different from the sires of the stored embryos.

A conservative approach to developing a germplasm collection would be to acquire semen samples as described in the above subsection on "targets for cryoconserved semen" and, where possible, to acquire embryos opportunistically and thereby enhance the quality

FIGURE 6
Reconstitution using only embryos versus a reduced number of embryos and semen



of the banked material. Collecting and maintaining sufficient embryos in the gene bank to generate 10 to 20 females will speed reconstitution and ensure that mitochondrial DNA is captured in the collection (Boettcher *et al.,* 2005).

Cryopreservation of embryos is most effective in ruminants. Pig embryos can be cryopreserved, but the success rates are lower. In poultry, embryo preservation is not yet possible. Therefore, to capture the genetic complement of a poultry population primordial germ cells have to be harvested and cryopreserved (see Section 4). Moore *et al.* (2006) demonstrated that primordial germ cells can be cryopreserved effectively. However, successfully inserting these cells into recipient eggs is problematic; the success rate is about 4 to 8 percent (Petitte *et al.,* 1990).

Before establishing a genetic reserve in an embryo bank, the number of available donor animals should be determined and a decision taken regarding whether these donors can produce a sufficient number of embryos to allow eventual reconstitution of the targeted breed(s). Ideally, embryos from at least 25 donor females mated to 25 different sires should be included in order to increase the genetic variability of the reconstituted population to the recommended level. The final decision will depend on the availability of donor females and the costs of the system used to access the germplasm. For example, if the gene bank has to buy the donor females from the breeders, the acquisition of exactly 25 donors may be financially optimal. However, if the breeders are paid for each collection (or if no payment for access is necessary), then the cost of sampling more than 25 donors may be similar to

the cost of sampling exactly 25 donors. In the case of at-risk breeds, gaining access to 25 donor females may be problematic.

Multiple embryo recoveries may be needed from each superovulated donor female. Embryo recovery following superovulation is notoriously variable, especially in cattle. The numbers of embryos per collection from a well-managed donor may range from zero to 40, with an average of five or six transferable embryos. In addition, females that do not respond well to superovulation during the first collection have a tendency not to respond well in subsequent attempts. Therefore, it is important that males used for matings are not associated with the same female throughout the collection period; otherwise, one or a few sires may dominate in the re-established breed. Repeated use of the same sire on a given donor female will also result in the banking of multiple full-sib embryos. There is an implicit penalty associated with storing full sibs: deleterious genes passed on by an individual of one sex penalize not only its own future contribution to the population but also that of its healthy mate. Avoiding full-sib embryos helps spread the risk (Woolliams, 1989).

Breed reconstitution with oocytes and semen

When oocytes and semen are stored in the gene bank, the number of oocytes required can be determined by using a slight modification of the formula used to obtain the figures presented in Table 11:

$$n_{ooc} = 1.5 \times 25\ (0.5 \times s_e \times s_o \times s_{ivf})$$

where n_{ooc} is the number of oocytes to be banked, 1.5 is a multiplier used to obtain the 150 percent level, 0.5 is the sex ratio, s_e and s_o are survival rates to birth and breeding age, and s_{ivf} is the success rate of the IVF procedure used to obtain an embryo from an oocyte.

The number of doses of semen stored should be sufficient to fertilize all the stored oocytes. A conservatively high estimate is one dose of semen per oocyte stored. However, a single dose can fertilize multiple oocytes. Ideally, the number of males from which semen is stored should be at least as large as the number of female donors of oocytes.

UTILIZATION OF GENE BANK MATERIAL IN LIVE CONSERVATION AND BREEDING

As noted in Section 3, in addition to reconstituting a breed after its extinction, gene bank material can be used for several other purposes, both in the short and the long term. First, the use of gene bank material for breeding animals in live populations can be helpful in controlling inbreeding rates or in revitalizing populations. Controlling the rate of inbreeding in a population is important for maintaining genetic variation. The N_e of a reconstituted population should be at least 50, which corresponds to a rate of inbreeding of 1 percent per generation. Gene bank collections can play an important role in maintaining the genetic variation of a breed and can actually increase the N_e of a breed if used properly. Box 7 presents an example of how this approach is being applied in the Netherlands. Obviously, semen taken from the bank for this purpose must not come from males that are part of the live breeding population. When managing a small population, three to ten males should be identified each year and their semen should be stored for future use. At least 20 doses of semen from each male should be conserved, with quantities increasing

BOX 7

Use of gene bank semen for revival and support of the breeding programme of the endangered Dutch Friesian Red and White cattle breed

In the 1800s, the cattle population in the province of Friesland in the Netherlands consisted mainly of Red Pied cattle. During the past century, however, preference for the Black and White phenotype, followed by sustained import and crossing with Holstein-Friesians, resulted in a sharp decline in the Red Pied population, so that only 21 Red and White individuals (4 males and 17 females) remained in 1993. At that point in time, a group of owners started the Foundation for Native Red and White Friesian Cattle. A breeding programme was developed in collaboration with the newly created national gene bank for farm animals. Semen from sires preserved in the gene bank in the 1970s and 1980s was used for breeding. Male progeny were raised by breeders, who were granted a subsidy from the gene bank. Semen from these males was collected, frozen and later used under new contracts. The breed increased in number, reaching 256 registered living females and 12 living males in 2004. In addition to the living populations, more than 10 000 doses of semen from 45 bulls are stored in the gene bank and kept available for AI. The combination of the living population and the gene bank stock results in a much larger N_e than is represented by the living population only.

depending on the reproductive capacity of the species (low capacity → more doses) and population size (larger population → more doses). At least 100 doses per sire should be conserved for species with low reproductive capacity such as cattle and horses. Because this practice will involve the use of "old" germplasm, it limits the amount of genetic progress that can be made for a given trait. However, the main objective is to maintain a high level of genetic diversity in the population.

Second, the material can be used in a cross-breeding system for introgressing specific characteristics into live populations. Breeders may want to introgress desirable characteristics of a cryoconserved breed into an existing breed. Introgression can be based on phenotypic information, and the desirable characteristics can be maintained in the subsequent generations by continuous selection. Alternatively, the genes underlying the desirable characteristics can be identified and molecular markers used to maintain the desirable genomic regions or traits. Introgression or crossing cryoconserved populations with live populations can be used to produce completely new breeds. Introgression generally involves only a single cycle of breeding to the conserved breed that provides the desired gene. In subsequent generations, the live animals are bred *inter se* or backcrossed to purebred animals of the live population. Therefore, the number of doses that need to be stored for this purpose will depend on the number of females in the live population that will be subject to crossing to initiate the introgression process. Box 8 describes the use of banked germplasm to introgress genes into a herd of pigs used for research.

BOX 8

Reconstituting a research pig line

Gene banks have an important role in backing up research populations. Purdue University in the United States of America had developed a line of pigs that were either homozygous or heterozygous for both the Napole and Halothane genes, which negatively affect pork quality in animals with the homozygous recessive genotype. In 2003, Purdue decided to discontinue this population and chose to have samples of semen from three carrier boars frozen and banked by the National Animal Germplasm Program. In August 2007, the University decided to re-establish a population in which the recessive homozygous condition was present so that it could be used to research meat quality. Samples of the semen stored with the National Animal Germplasm Program were therefore transferred back to Purdue and sows were inseminated. The results were a 100 percent pregnancy rate and an average litter size of 7.7 pigs. The resulting boars were genotyped, and 14 of 25 were found to be heterozygous for both genes. With the F_2 population, several boars were homozygous for both mutant genes. This case was the first in which a livestock research line was cryopreserved, discontinued, and re-established using germplasm frozen and stored by a gene bank.

For any breeding programme, regardless of the population size, periodic cryogenic storage of genetic material is recommended as a backup in case (genetic) problems occur. A cryo-aided live scheme can be very beneficial, especially for populations with a low N_e, mainly because it will result in prolonged generation intervals and therefore a larger N_e. Intensely selected breeds can have a small N_e even if the actual number of animals is very large (Bovine HapMap Consortium, 2009). It is important to collect new genetic material regularly (at least once each generation interval) in order to maximize genetic diversity.

The plan for collection and utilization of material from a gene bank must consider the characteristics of the breed in question, including the characteristics of its production system in addition to its genetic and phenotypic characteristics. This is especially important for breeds that differ greatly from the norm. Most scientific research on reproductive physiology, cryobiology, breeding and animal husbandry has been undertaken on international transboundary breeds kept under intensive management. To ensure optimal results when utilizing stored germplasm, complementary research to establish breed-specific protocols may be prudent. Box 9 presents an example from France in which the use of gene bank semen from two at-risk breeds did not yield the results expected because of differences between the characteristics of the breeds and those of the international transboundary breeds upon which protocols (and expectations) were based.

SELECTION OF INDIVIDUALS FOR CRYOPRESERVATION

As described in Section 3, the first target for germplasm collections is to enable the reconstitution of a population with an N_e of 50 animals. However, for the gene bank manager

BOX 9

Using frozen biological material from at-risk breeds: discrepancy between theory and practice

France has efficient conservation programmes for at-risk pig breeds, which include both *in situ* and *ex situ* conservation. Semen was collected from males from the unique bloodlines of all the at-risk breeds, mostly in the 1980s (semen pellets) and the 1990s (straws). The living populations of two local pig breeds (Bayeux and Blanc de l'Ouest) have suffered tremendous losses of genetic variability since the 1980s, prompting the respective breeding associations to contact the French National Cryobank and request permission to use pelleted semen from the collection (four males per breed). The quality of the semen in the pellets was evaluated as poor in Blanc de l'Ouest and good in Bayeux. This semen was used for mating four sows from each breed, with the primary goal of obtaining new breeding males. In the case of the Porc Blanc de l'Ouest breed, the four sows produced litters ranging in size from 6 to 13 live piglets, and semen from two of the males was collected for AI in 2007. However, in the case of the Bayeux breed, only two females were born from the four inseminated sows. The failure of the programme in the Bayeux breed was a result of several factors. The Bayeux is usually raised in an extensive production system and the sows behaved very aggressively when herded. Sanitary issues also played a role, as all the animals came from smallholdings where hygienic standards were lower than those in standard commercial operations. Last but not least, the physiology of the Bayeux sows was very different from that of sows from typical commercial lines. The protocols applied in this case, such as heat grouping, choice of insemination dates and so forth, were based on procedures that yielded optimal results for mainstream commercial breeds. This experiment yielded an important lesson. The French National Cryobank and breed associations have now agreed to develop special protocols tailored to each breed and to consider breed-specific measures in any future programmes for the use of stored genetic material.

the issue quickly turns to which animals within a breed should be sampled for the repository, and for which of the collection categories. Genetics are often among the primary considerations, but reproductive and sanitary aspects must also be accounted for.

Genetic aspects

Here the major consideration is to select animals that are as unrelated as possible to each other in genetic terms. This can be done in several ways. The appropriate option will depend upon the availability of information and resources.

- When pedigree information is available, simple procedures can be used to ensure that animals are not closely related, such as not selecting animals with common grandparents.
- More formal analyses can also be undertaken, such as the application of genetic contribution theory to select the least-related group of germplasm donors (Meuwissen, 2002).

Clustering approaches can be used to group animals that are closely related and identify clusters that are genetically distant from one another (see Box 10).

- Donors should be chosen from within lines if line breeding is practised.
- With or without pedigree information, various molecular DNA approaches can be used to determine the genetic uniqueness of animals or subpopulations within a breed. A principal obstacle to such approaches is that they require wide sampling of the animals within the breed, and many more animals will need to be genotyped than are actually needed for the gene bank. Alternatively, gene bank managers may consider collecting germplasm samples concurrently with taking blood or tissue samples and then utilizing the resulting genotypic data to enable more effective use of the stored material to decrease genetic relationships in the reconstituted population.
- Genetic markers can also be used to identify introgression from other breeds, the level of which may differ among subpopulations or geographical areas. This type of information is useful in determining how genetically pure the targeted animals are.
- If no reliable animal registration is available and resources are insufficient for the use of molecular genetics, donors should be carefully chosen based on their geographical location, phenotype and herd history.
- Particularly when pedigree information is not available, donor animals should be chosen from different areas and herds; genetic flows (i.e. exchange of animals) among herds and areas should be taken into account. One option is to collect along line transects drawn across maps of the regions of the country where the breed is located. Adequate geographic spacing of the collection sites should help ensure that the level of genetic relationships among collected animals is low.
- Even when animals are taken from geographically distant locations, owners should be interviewed to determine how unrelated their animals are from the immediate surrounding population and from other more distant flocks or herds.

Reproductive aspects

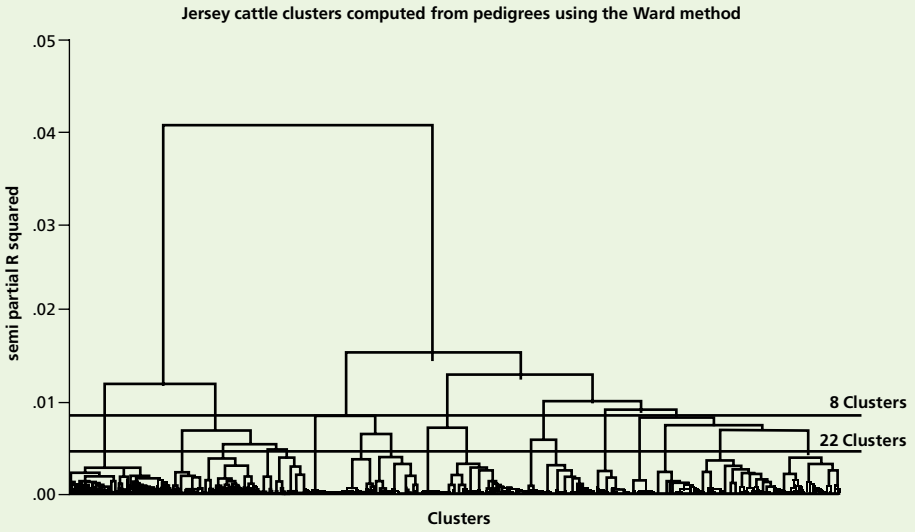
Only a small sample of animals can be represented in the gene bank, and with limited amounts of germplasm. Therefore, the gene bank manager must sample the animals that have the potential to yield the greatest number of offspring from the germplasm stored.

- Before the final decision about which animals to chose as donors, all candidates should be subject to a clinical and andrological or gynaecological evaluation. During this evaluation special attention should be given to animals that are selected as donors because of their genetic traits but which may be subfertile.
- When choosing female donors, animals with a good reproductive history should have a higher priority than animals that have a history of poor reproduction or have never given birth.
- When choosing male donors, priority should be given to animals that are known to produce semen that is of good quality after it is frozen and thawed.
- Both male and female donors must have morphological and behavioural characteristics that facilitate the collection of genetic material.

BOX 10

Selection of bulls for the National Animal Germplasm Program in the United States of America

Cluster analysis was used to evaluate the bulls of the Jersey breed stored in the United States of America's gene bank (Blackburn, 2009). Pedigrees of the 537 bulls in the gene bank were evaluated, along with pedigrees of the most popular bulls in 2004 and 2005. Genetic relationships among the bulls were used to assign them to clusters, which were visualized in a dendrogram or "tree" diagram (see the figure below).



(cont.)

Sanitary aspects

It is critically important that the conserved germplasm does not transmit pathogens into the future along with its genetic information. Thus, strict sanitary standards should be followed.

Donor animals should be clinically inspected to confirm that they are healthy and free from contagious and infectious diseases. They should fulfil all requirements established by OIE in terms of infectious and contagious diseases that may be transmitted through semen and/or embryos (see Section 9). To meet all the OIE standards, it may be necessary to quarantine animals to confirm their health status prior to collecting germplasm.

COLLECTION OF COMPLEMENTARY BIOLOGICAL MATERIAL

In addition to its role in promoting national food security, the gene bank has an opportunity and responsibility to collect germplasm, or tissue samples, for DNA analysis or other research purposes. It is advisable to undertake this type of activity at the same time as other germplasm collection activities. For example, it takes little additional effort to collect

The figure demonstrates how it is possible to define any given number of clusters (e.g. 8 or 22 clusters) by drawing a horizontal line across the dendrogram. The number of bulls in each cluster, the mean genetic relationship per cluster, and the number of bulls in the gene bank from each cluster were calculated for 22 clusters (see the table below). Several clusters were poorly represented in the repository (e.g. clusters 4, 15, 16 and 21) and efforts were therefore made to acquire samples to fill those gaps in the collection. A similar procedure could be followed in the initial selection of animals for a gene bank, by choosing similar numbers of animals from each cluster.

Cluster	n	Mean Rel.	No. in Rep.
1	350	.18	85
2	98	.20	30
3	451	.05	105
4	50	.23	2
5	115	.35	8
6	214	.21	38
7	193	.25	16
8	198	.11	13
9	342	.21	42
10	161	.20	37
11	116	.36	17
12	126	.22	36
13	142	.19	7
14	116	.33	15
15	62	.26	
16	104	.20	2
17	156	.26	35
18	105	.33	22
19	70	.30	18
20	86	.28	14
21	49	.22	2
22	46	.22	10

Note: n = number of bulls per cluster.
Mean Rel. = mean additive genetic relationship within the cluster.
No. in Rep. = number of bulls selected for the gene bank from each cluster.

blood samples for health tests and future DNA analysis at the time of germplasm collection. Such efforts will, in the long term, increase the utility of the collection maintained by the gene bank.