

Cryobanking Animal Genetic Resources **S. McClintock, E. Groeneveld, S. Hiemstra, and H. Blackburn**

Establishing national genebanks has been an under utilized avenue for conserving animal genetic resources. We will illustrate how national programs can utilize this conservation tool and show that it may be one of the most effective approaches for conserving animal genetic resources particularly when one considers cost and flexibility.

Background

To date the primary emphasis in conserving animal genetic resources has been on in-situ conservation, which has several positive attributes. However, given the global progress to date it would appear that actions have proceeded at a relatively conservative pace. This is not surprising considering the number of technical and socio-economic factors governing the maintenance of live animals. To date cryobanking of animal tissue and germplasm has been an under utilized tool in national conservation programs. The often heard comment is that the approach is too costly or that it requires greater technical expertise than is commonly available in developing countries. This is not the experience in the plant community that has successfully stored seed from over 300,000 species since the 1960's. Cryopreservation of animal germplasm is not new! Semen from cattle has been viably cryobanked and stored since the late 1950's. In the interim cryobanking techniques have been enhanced which has extended our capacity to store semen from other species and to store a variety of other tissues (e.g., embryos, blood cells, fibroblast cells, primordial germ cells) for farm animal species of interest.

Farm animal gene-banks are becoming more common in developed countries, but in developing countries, where much of the world's dwindling genetic variation currently resides, there are very few gene-banks.

Given the global contraction of animal genetic resources, the introduction of gene-banks is required urgently, particularly in developing countries, and in sub-Saharan Africa and parts of Asia.

Why Initiate Ex-situ / Cryobanking programs?

In practice, only a few breeds are being maintained as part of an in situ conservation program or actively further developed; the majority are still left exposed to the vagaries of genetic erosion.

Experience in developed and developing countries has shown that physically acquiring genetic material can be done relatively quickly and provides an important reserve of genetic resources that can be used for a wide variety of conservation, and research interests.

Cost has often been cited as a limitation of gene banking. However, in our evaluation these costs are relatively minor and depend upon the scale which the gene bank organizers chose. Our evaluation shows quite clearly that the recurrent storage costs for cryobanked samples are quite small and, in some countries, may be significantly less than in-situ conservation particularly when considering long-term storage (that is, for centuries).

Cryobanks: a Multifunctional Approach

Cryopreserved collections of genetic resources have several possible uses that include:

1. Reconstituting populations in the event of a national need;
2. Reintroduction of genetic variability into breeds whose genetic base needs to be broadened or to reintroduce genes that may have been lost or have suffered a reduction in frequency;
3. Development of new breeds or composite populations for either research or industry use and
4. A source of material for genomic studies. Major advances in genomics can be expected over the next century.

Available Technologies and their Costs

For all agricultural species of livestock there are cryobanking techniques available. These range from somatic cell tissues like blood to gametes or embryos and to primordial germ cells (precursors of ova and spermatozoa). In addition to a range of tissue types suitable for cryobanking, there is also a range of enabling technologies. At the top end, these include programmable straw fillers and freezers; at the low cost end, straws can be filled by hand and cryopreservation can be performed in a disposable Styrofoam box with a high degree of repeatability. Given this range of tissues and cryobanking techniques, policy makers and gene bank managers can decide which combination of

approaches will be appropriate and affordable. Some of these approaches for sample collection and cryobanking can be done in the field using mobile equipment.

It has often been stated that cost is a primary limitation in developing gene-banks for farm animals. We do not believe that this is correct in practice. There may be relatively high initial cost for facility establishment but if investments are amortized over 20 (or even 200) years, the cost is quite low. In addition there are wide ranges in the amount of space that such facilities require, in other words, one size does not fit all. As to actual collection costs these tend to vary by species, tissue type and local circumstances.

Another important cost is the long term storage costs. The recurrent costs for storing an entire breed are minimal. It is this extremely low annual cost for storage that makes cryobanking attractive relative to other conservation options. There will be some situations where liquid nitrogen may be more expensive; either the gene-bank can be relocated to a site where it is more affordable or a higher annual running cost is anticipated.

To sample a breed ideally one needs samples from about 100 unrelated animals. Typical scenario's might be collecting somatic cells or semen in the field or conserve abattoir derived semen or embryo's. In many cases it could cost less than €10,000 to conserve an entire breed.

Case studies from our experiences in both developing and developed countries when collecting and freezing tissues for conservation purposes are shown in Appendices 1, 2, 3 and 4.

How to Start

From our perspective and in canvassing a number of our colleagues in other countries there is little reason for not starting liquid nitrogen gene-banks for germplasm or tissues. This method of conservation provides a security backup for a country's genetic resources. The most important component of the annual running cost of a gene-bank is liquid nitrogen which is available in many countries. In areas where liquid nitrogen is unavailable, it can be produced on-site using small-scale, electrically powered equipment. Alternatively, a country may decide – at least in the short term - to collaborate with a neighboring country where these facilities are already available. In many countries there is both the expertise and the interest in starting a gene-bank. The issue is how to get started – we suggest the following steps.

1. A workshop for technical and livestock sector persons that are interested in gene-banking and may provide the needed leadership in the development of the program. The product of such a workshop would be a strategic plan that determines what resources are available for the gene-bank to use, what types of tissues are to be collected, and what will be the collection priority.
2. There is also a need, in the same or a separate workshop, to discuss operational issues of cryopreservation techniques, development of a database for monitoring the status and progress of the gene-bank, and to understand the breadth of genetic diversity captured or in need of collecting. There is also the opportunity to build some demonstration of techniques in such a workshop which can re-enforce training and build enthusiasm.
3. Because livestock are privately owned it is essential for gene-bank managers to establish a working relationship with breeders and the various segments of their livestock industry. It is advisable to have livestock breeders/producers on steering committees that provide input and support for the gene-bank's development and genetic resource acquisition process.
4. Consolidate the existing infrastructure so that the sampled genetic resources can be securely stored. This may include officially designating a preexisting facility (e.g., a national artificial insemination center) as the national livestock repository.
5. Start the process by capturing the "low hanging fruit". In many instances cryopreserved stores of animal genetic resources may already exist, portions of those samples can become components of the national collection.
6. National repositories are clearly the responsibility of national governments. They require and deserve appropriate levels of funding to make them operational. At the same time gene-bank managers must keep there collections relevant and explore new ways that the collections can be used to solve national problems or support national livestock industries.

Appendix 1.

CREATION OF A LOW COST GENE BANK FROM SOMATIC CELLS IN A DEVELOPING COUNTRY.

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Somatic cell cryo conservation is proposed as an emergency measure for the rapid setup of national genebanks. In a pilot study in Vietnam, the protocols developed were tested by sampling 6 breeds from swine, sheep and goats with 50 samples (from 25 males and 25 females) from each breed. Overall, it took two months to organize and execute the project. Initial investment for the tagging equipment, 2 cryo tanks for transportation and storage, a computer for the genebank database, digital camera and a GPS device was around €3300. The total variable cost for collecting 6 breed with 50 samples each including numbered vials, consumables and travel was around €4200. The procedure developed proved feasible and cost effective under rural Vietnamese conditions with less than €1000 per breed.

As indicated in table 1 the total investment for the creation of a somatic cell genebank is in the order of €3000. Not included are costs for buildings and other infrastructure that might be used.

Table 1. Fixed cost

Item	Amount/€
GPS device	100
Digital camera	260
Transportation Cryo tank	317
Main Cryo tank	900
Computer	1.630
Tagger	15
Scale	15
Syringe etc	15
Training of crew	50
Total costs	3.302

Table 2. Variable cost

Item	Amount/€
Ear tags / vials 400	850
Liquid Nitrogen	200
consumables	317
Transportation	900
Accommodation	900
Incentives for animal owners	380
Incentives for crew	700
Total costs for 300 samples	4.247

Variable costs are listed in Table 2, Here, we have the well defined costs for vials which are around €2 per sample. Other blocks of costs are travel of the sampling crew, and incentives for both the sampling crew and the animal owners. In the pilot it has proven very useful to pay the animal owners a small sum for their willingness to contribute samples. The variable costs for one breed amount to around €700. As a result, if countries want to create an emergency backup €10.000 will get them a long way.

Appendix 2.

The following are a set of expenses from field collections performed in the U.S. Travel costs are based upon having a person from the national program, located in Fort Collins, Colorado travel to the collection site. The cattle collection was a herd of Shorthorn cattle located in Broken Bow, Nebraska; the sheep collection was performed in western Oregon; and the rooster collections took place in Durham, New Hampshire and Tolland, Connecticut. The cattle and sheep collection were focusing upon minor or rare breeds, while the rooster collection was aimed at collecting research chicken lines at the University of New Hampshire and the University of Connecticut.

U.S. on location collection costs for cattle, sheep and chickens, in US dollars.

Item	Cattle	Sheep	Chicken
No. animals collected	15	37	49
Tissues collected	Blood & semen	Blood & semen	Semen
No. straws collected	2,266	1,697	223
Travel costs	\$ 131.24	\$3,384.99	\$2,138.17
Contract services cost	\$ 952.00	0.00	0.00
Health tests	\$ 146.25	\$ 100.00	0.00
Straw costs	\$ 929.06	\$ 695.77	\$ 91.43
Total cost	\$2,158.55	\$4,180.76	\$2,229.60
Cost per straw	\$ 0.95	\$ 2.46	\$ 10.00
Cost per animal	\$ 143.90	\$ 112.99	\$ 45.50

Appendix 3.

The following are three examples of germplasm collection activities from the Netherlands, governed by the Centre for Genetic Resources the Netherlands, including 1) embryo collection from cows of Deep Red cattle breed, 2) epididymal semen collection from rams of Dutch rare breeds after slaughter and 3) semen collection from roosters of rare poultry breeds at the experimental station in Lelystad. Please note that costs include costs of labor, lab and experimental station.

Item	Cattle	Sheep	Chicken
No. animals collected	2	8	60
Tissues collected	Embryos	Epididymal semen	Semen
No. straws collected	10 embryos	800	3,000
Travel costs		€ 84.00	€ 560.00
Labor costs	€ 250.00	€ 600.00	€ 59,024.00
Contract services cost	€ 750.00	€ 150.00	€ 1,500.00
Health tests	€ 0.00	€ 0.00	€ 250.00
Straw costs	-	€ 24.00	€ 90.00
Other materials/variable costs		€ 226.00	€ 5,734.00
Total cost	€1,000.00	€1,084.00	€67,158.00
Cost per straw	€ 100.00	€ 0.73	€ 22.00
Cost per animal	€ 500.00	€ 135.50	€ 1,119.30

Appendix 4.

The following relate to germplasm collections in Kenya. For the cattle this involved the abattoir collection of testes, followed by laboratory extraction of epididymal sperm. For the sheep, this involved on-farm collection of testes, followed by laboratory extraction of epididymal sperm. Embryos were derived from ovaries collected from an abattoir using in vitro fertilization and culture. The exact donor cow would normally not be identified except by batch (e.g. group of zebu cows slaughtered on a certain date. To identify embryos as coming from a specific donor would be more time consuming and might cost two or three times more per embryo. The marginal cost of Cryo-banking a breed (50 males and 50 females) would be about €2,500. An IVF lab setup cost for equipment such as incubator & microscopes might be in the order of €30,000.

Item	Cattle	Cattle	Sheep
No. animals collected	20	20	20
Tissues collected	Epididymal semen	Abattoir derived Ova followed by IVF	Epididymal semen
No. straws collected	2000	150	2000
Travel costs	€ 20.00	€ 20.00	€ 20.00
Labor costs	€ 80.00	€ 300.00	€ 80.00
Laboratory cost	€ 5.00	€ 400.00	€ 5.00
Health tests	€ 0.00	€ 0.00	€ 0.00
Straw costs	€ 600.00	€ 45.00	€ 600.00
Total cost	€ 705.00	€ 765.00	€ 705.00
Cost per straw	€ 0.35	€ 5.10	€ 0.35
Cost per animal	€ 35.25	€ 15.30	€ 35.25

Note that these costs do not include overheads or setup costs; they indicate the marginal costs of processing additional samples.