
IMAGE

Innovative Management of Animal Genetic Resources

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Abstract: Recommendations for guidelines for the development of livestock gene banks have been developed on the basis of IMAGE results and achievements. Recommendations are suggestions to improve or to update the FAO Guidelines “CRYOCONSERVATION OF ANIMAL GENETIC RESOURCES” (FAO 2012). This document presents the additions, modifications, suppressions that are proposed on the basis of the current structure of FAO guidelines.

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● Executive Summary

Background	Guidelines for the development of livestock gene banks are aimed at helping actors to develop their strategies and to organize their operations, taking advantage of state of the art knowledge and technologies. FAO has published the guidelines “cryoconservation of animal genetic resources” in 2012. IMAGE has resulted in new knowledge and insights which can be used for the further development of gene banks, and can be included in a next update of the FAO Guidelines. The FAO Commission on Genetic Resources for Food and Agriculture will decide on future updates of the FAO guidelines.
Objectives	To provide recommendations for a future update of the FAO guidelines based on the results of the various IMAGE Work Packages. IMAGE will make the recommendations publicly available, next to the current FAO guidelines.
Methods	<p>Each of the 12 sections of the current FAO guidelines (FAO 2012) was analysed by NordGen and the WP leaders as follows:</p> <ul style="list-style-type: none"> ● Is the content still relevant? ● Are there any gaps or new insights / knowledge that should be included? ● Which deliverables of IMAGE could help to fill these gaps and provide recommendations to update the current FAO guidelines? <p>Recommendations were drafted by NordGen with input from WP leaders and discussed with IMAGE partners at the final general assembly in Madrid.</p>
Results & implications	<p>The most relevant recommendations and proposed changes are:</p> <ul style="list-style-type: none"> - To develop a new Section 1 entitled ‘Building a gene banking strategy’, that could replace current section 1 and 3 of the FAO Guidelines. The new section should include the following key messages: <ul style="list-style-type: none"> ○ Stakeholder involvement and building a multi-actor governance ○ Envision scenarios of future use ○ Encourage cryopreservation for breeds before the status of endangerment is reached, regularly conserving a back-up of within breed genetic diversity ○ Collect samples for genomic studies at the same time for characterization purposes and for optimizing collections - Section 2 (Implementation and Organisation) should be focused on implementing the key principles of a quality management system, including a risk-based approach. - Section 3 (Choice of biological material to be preserved) is pointing out recent evolution in technologies, including advance in non-mammalian species and with alternative methods and materials.

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| | <ul style="list-style-type: none"> - Section 4 (Establish a gene bank -physical structure and costs) could be updated with economic optimization. - Section 5 (Developing and using gene bank collections) is highlighting the importance of a prior molecular characterization of collected gene bank material. - Section 6 (Basic principles of cryopreservation) is presenting improvements of the basic sampling methods of cryopreservation. - Section 7 (Collection of germ plasm and tissues) completes a list of methods, materials and species that were previously not possible to cryopreserve. - Section 8 (Sanitary recommendations) conveys a thorough up to date policy report on sanitary considerations. - Section 9 (Databases and documentation) represents improvements on exploitation of currently available data. - Section 10 (Legal issues - contracts and access) addresses major legal changes in gene banking. - Section 11 (Capacity building and training) focuses on training needs and innovative exploitation of gene bank collections. |
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1. Structure of the FAO guidelines

FAO Guidelines on Cryoconservation of animal genetic resources (2012):

1. Confirming the decision to cryopreserve
2. Implementation and organization
3. Objectives of cryoconservation programmes
4. Potential use of different types of germplasm and tissues
5. Establishing a gene bank - physical structure and costs
6. Developing gene bank collections
7. Basic principles of cryopreservation
8. Collection of germ plasm and tissues
9. Sanitary recommendations
10. Databases and documentation
11. Legal issues - contracts and access
12. Capacity building and training

2. Quality Management GAP analysis

The Quality Management gap analysis, based on a gene bank survey and diagnostic tool developed in T2.4, was the first step within IMAGE to identify key elements which should be addressed or should be more emphasized in the current FAO Guidelines. One important conclusion from IMAGE is that quality management principles should

be well reflected in the FAO Guidelines, and recommendations from the IMAGE Quality Management gap analysis should be taken into account when updating the FAO Guidelines.

Gene banks must ensure high quality by providing secure, long-term storage to guarantee future viability and biosecurity. Dedicated systems for quality management, be they formal or informal, are important tools that can assist managers of gene banks to achieve satisfactory results.

A Quality Management GAP analysis was performed with the following objectives: 1) identify key factors and processes associated with quality assurance in animal gene banking; 2) develop a tool for gene bank managers to use to self-assess their quality management; and 3) apply this tool to European gene banks to identify current areas of strengths and gaps in quality management.

The results of the GAP analysis for Quality Management Systems (QMS) have been reported in Deliverable 2.4, including the questionnaire survey lists. Below, a summary is given of factors identified in the GAP analysis in cases where $\geq 30\%$ of the respondents reported difficulties in meeting the requirements.

Topic	GAPs identified	Corresponding section in FAO guidelines
General gene bank management	<ul style="list-style-type: none"> • Lack of formally documented organizational and management structure • Lack of a stakeholder analysis • Lack of a communication strategy or plan 	Section 2
General gene bank management	<ul style="list-style-type: none"> • Lack of formal cryopreservation goals 	Section 3
General gene bank management	<ul style="list-style-type: none"> • Lack of mitigation plan for major risks • Lack of major risk analysis in long-term sustainability 	Section 5
General quality management	<ul style="list-style-type: none"> • Lack of formal quality policy • Lack of specific employees to serve as the Quality manager • Standard operating procedures not documented • The key processes not identified • No up-to-date system for management of quality management system documentation • Lack of formal certification and internal guidelines for QMS • Lack of a full-cost evaluation system 	Section 5

Gene bank equipment	<ul style="list-style-type: none"> No standard operating procedures for regular maintenance of critical equipment No system of record (SOR) when critical equipment undergoes controls, routine maintenance and/or calibration 	Section 5
Gene bank personnel	<ul style="list-style-type: none"> Lack of formal job descriptions for key persons Lack of bank manager Lack of training program for employees 	Section 5
Material collection	<ul style="list-style-type: none"> Lack of quality control system from the management viewpoint Unique labelling system missing (depending on the species) 	Section 5
Material storage	<ul style="list-style-type: none"> No recording system of persons visiting into storage area Storage area is not restricted Separate storage of different types of material missing 	Section 5
Material distribution	<ul style="list-style-type: none"> No policy for sample distribution and Lack of legal framework for material others than semen and embryos 	Section 5
Gene bank equipment	<ul style="list-style-type: none"> No identification of the critical equipment 	Section 8
Introduction of previously processed material	<ul style="list-style-type: none"> Lack of specific areas for incoming material from outside source Lack of formalized quality control of incoming material from outside before being stored Attention should be paid on existing quality control tests required for professional gene bank management 	Section 9
Information system	<ul style="list-style-type: none"> Lack of genomic information associated to germplasm collections Notable need for guidance for purchasing or developing a proper database system Lack of security and protection system for data storage Frequent lack of a backup-system 	Section 10
Genetic material acquisition	<ul style="list-style-type: none"> Lack of formal agreements/contracts 	Section 11
Material distribution	<ul style="list-style-type: none"> No formal procedures to grant access to stored material and for distribution of material to other persons or organizations 	Section 11

3. List of relevant IMAGE deliverables per section

Below is presented an overview of reference to IMAGE deliverables that provide new or updated knowledge to be included in the next revision of the FAO Guidelines on Cryoconservation of animal genetic resources (2012).

FAO Guidelines Section	IMAGE Deliverable
1. Confirming the decision to cryopreserve	D2.6: Rationalisation of gene bank strategies and of genetic collections D2.7: Identified gaps and priorities for further development of collections
3. Objectives of cryoconservation programmes	D1.5: Improved acceptance of technological innovation by the stakeholders up to the general public D9.3: Ethical advice for the choice of breeds to be conserved in gene banks
2. Implementation and organization	D2.4: Quality management gap analysis and framework for certification of individual gene banks
4. Potential use of different types of germplasm and tissues	D3.3 and D3.5, to be detailed in section 8
5. Establishing a gene bank - physical structure and costs	D2.5: Report on costs and potential values/benefits of genetic collections
6. Developing gene bank collections	D4.5: A standard multi-species chip for genomic assessment of collections D6.3 : Novel methods and software to optimise conservation and introgression schemes (MoBPS) D6.6: Simulation-based comparison of methods for introgression and conservation of genetic diversity in and from genetic collections and live populations
7. Basic principles of cryopreservation	D3.3: Validated PGC cryopreservation protocols

8. Collection of germ plasm and tissues	<p>D3.3: Validated PGC cryopreservation protocols</p> <p>D3.4: Validation after fertility studies of new protein, DNA and miRNA markers from sperms quality</p> <p>D3.5: Optimised protocols for gonad grafting, cryopreservation and transfer; pig embryo vitrification and semen cryopreservation</p>
9. Sanitary recommendations	D1.7: Policy report on disease control and sanitary regulations of gene banks
10. Databases and documentation	<p>D5.3: Web portal for Europe wide gene bank material with downloadable information on sample, genotype and annotations</p> <p>D5.4: Diversity browser in place and functional</p>
11. Legal issues - contracts and access	D1.6: Status, regulations and needs of ABS in genetic collections
12. Capacity building and training	<p>D7.7: Technical workshop in third countries no. 1</p> <p>D7.10: Technical workshop in third countries no. 2</p> <p>D7.13: Guidelines for management of gene banks</p>

4. Main recommendations

Overview of the main changes proposed for the FAO Guidelines for Cryoconservation of animal genetic resources, based on new or updated knowledge from the IMAGE project.

Current FAO title	New title	Recommendation
1. Confirming the decision to cryopreserve	1. Building a gene banking strategy	<p>Major revision - including:</p> <ul style="list-style-type: none"> • Stakeholders • Ethical considerations • Governance • Coupling with genomic collections • Scenarios of use • Rationalization (also relevant for other sections) • Defining priorities
3. Objectives of cryoconservation programmes		

2. Implementation and organization	2. Implementation and organization	Section 2 should follow merged old sections 1 and 3. <ul style="list-style-type: none"> Quality management (also relevant for other sections)
4. Potential use of different types of germplasm and tissues	3. Choice of biological material to be preserved	Technical update: <ul style="list-style-type: none"> Primordial germ cells Gonadic tissues
5. Establishing a gene bank - physical structure and costs	4. Establishing a gene bank - physical structure and costs	Technical update: <ul style="list-style-type: none"> Cost analysis
6. Developing gene bank collections	5. Developing and using gene bank collections	Technical update: <ul style="list-style-type: none"> Multispecies array MoBPS program
7. Basic principles of cryopreservation	6. Basic principles of cryopreservation	Technical update: <ul style="list-style-type: none"> Use of modelling to optimize embryo vitrification
8. Collection of germ plasm and tissues	7. Collection of germ plasm and tissues	Technical update: <ul style="list-style-type: none"> Cryopreservation of ram semen Vitrification of pig embryos Optimized protocols for chicken PGC and gonadic tissues
9. Sanitary recommendations	8. Sanitary recommendations	Major technical update: <ul style="list-style-type: none"> Animal health law principles
10. Databases and documentation	9. Databases and documentation	Major technical update: <ul style="list-style-type: none"> Concept of data integration
11. Legal issues - contracts and access	10. Legal issues: acquisition, storage and transfer of genebank material	Major technical update: <ul style="list-style-type: none"> Nagoya protocol and EU ABS legislation National ABS legislative, administrative and policy measures MAA and MTA guidelines

12. Capacity building and training	11. Capacity building and training	Technical update: <ul style="list-style-type: none"> • Training for gene bank staff • Training for users
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5. Proposed recommendations

WP7 Guidelines for the management of gene banks

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1. Building a biobanking strategy (new title)

Short introduction of the topic (from FAO manual):

Conservation of animal genetic resources for food and agriculture (AnGR) may be undertaken for different reasons. Conservation strategies can be categorized either as *in situ* conservation (in which animals are maintained within the environments or production systems in which they were developed) or as *ex situ* conservation (all other cases). The latter can be further divided into *ex situ – in vivo* conservation and cryoconservation.

Gene banks and their collections of germplasm and tissue can have multiple functions and objectives. While the primary function of gene banks is conservation of AnGR for use in the medium or long term, the material stored may also be used for other purposes. Collection goals may include breed reconstitution, support of *in vivo* conservation, back up in case of genetic problems such as inbreeding, development of new lines or breeds, and serving as source material for DNA research. Depending on the goals there may be a need to differentiate between different collection categories such as core collections (usually not accessed but updated regularly) and working collections (accessed regularly for e.g. research purposes or to support *in situ* breeding programs).

Short overview of activities related to this topic carried out in IMAGE:

Whereas IMAGE did not specifically target FAO Guidelines Section 1, its general objective of enhancing gene banking operations has led to results supporting the

definition of a strategy, particularly considering output of WP1 and WP2. The IMAGE survey T2.1 (D2.2) showed that most gene banks have multiple objectives and that objectives are different between gene banks. Sampling strategies for cryopreservation depend on available technologies, yet sampling requires choices to be made at the level of the species, of the breed, and of the animals within the breed.

To study the motivations and concerns of different actors for gene-banking and their expectations regarding the decision-making process, a questionnaire-based ethics survey was carried out among participants at various meetings and events concerning gene banks and breed conservation. The results of the *ethics survey* showed that stakeholders did not consider gene banks as competing with conservation programmes of live populations, that gene banks should be supported by governments and be managed by consensus among actors. All this supports the perception of genetic resources as a "club good", a subtype of public goods. Results also suggested that a risk/benefit approach should be considered for the use of invasive technologies or sensitive biotechnologies (cloning, transgenesis).

The detailed analysis of the survey revealed that different stakeholders had different perceptions and expectations, so that gene banks stand at a crossroads of many drivers: legal, economical, social and biological. This diverse landscape shows the importance of a multi-actor governance process, as well as the need for a tailored communication strategy.

References:

Deliverable 2.2, 2.6 and 2.7.

Recommendations (guidelines):

1. Introduction

Ex situ conservation and *in situ* conservation are complementary methods to preserve farm animal genetic diversity. *In situ* conservation is the preferred strategy, but a complementary backup strategy is needed to prevent loss of genetic diversity within and across breeds, due to genetic drift, demographic factors, sub-optimal breeding programs or other factors, including diseases and other disasters. Breeds should be sampled at regular intervals, before they reach the endangerment stage, so that the back-up would already be made at the time of a crisis.

2. Objectives

The aim of gene banks should be to effectively and efficiently conserve the existing diversity over time. The main criterion should be to maximize (potential) benefits at minimum costs for current and future stakeholders concerned.

3. Co-construction of the strategy

A gene bank should first define its strategy in cooperation with its stakeholders: policy makers, breed associations, breeding companies. The gene bank should set up its

governance respecting equity and ethical considerations, which generally ends up by creating a multi-actor board. Livestock genetic resources are generally considered as a “club good”, which is why a multi-actor board of different stakeholders should govern the establishment and management of livestock genetic collections. Germplasm collections can be public or private. In most cases, collections have both public and private characteristics and public-private partnerships are common. While private ownership of farm animal genetic resources is most common, long-term conservation of farm animal genetic diversity is generally seen as a government responsibility.

4. Prioritization

Key questions are: i) which species, ii) which breeds, iii) what type of material, iv) which donor animals, and v) which amount of material per breed/donor animal? Decisions about prioritization of species and breeds will be country specific and depends on the interests of stakeholders and society at large. The choice of type of material is species specific and is, first of all, a technical question, however ethical considerations and budget limitations also play a role. Current and potential future societal acceptability of reproductive technologies should be taken into account when determining the medium and long term strategy of a gene bank. The use of genomic and phenotypic characterisation data to compare collections with those already existing in other gene banks will help determine the uniqueness of material to be stored. Analysing collection gaps and redundancies at national and international level contributes to optimizing gene bank strategies and gene bank portfolios. Storing well documented material will also facilitate future use of collections.

5. Rationalization

Livestock gene banks need regular rationalization of their policies and implementation strategies, in order to optimize gene bank management and gene bank operations, and for further development of the gene bank strategy. A major question when developing or rationalizing a gene bank portfolio is what can be done technically, with which promise of success for the user. Rationalization exercises can be done *ex ante* and *ex post*. When rationalizing and developing a gene bank strategy many different aspects and considerations should be looked at, including policy, legal, economic, genetic and other technical aspects.

6. Projection towards the future

The strategy should anticipate future demand motivated by possible changes in climate, production systems, markets, consumer preferences and possible calamities (diseases, disasters, etc). Future use scenarios should be explored in order for the gene bank to prioritize its activities. Technology breakthroughs and innovations will likely influence future use. Innovative reproductive technologies could also change the value of different types of genetic material stored in gene banks. Future scenarios have a higher or lower uncertainty, while conservation decisions have to be made on the short term, taking into account budget constraints. Alternative strategies and scenarios should be compared before determining the optimum strategy. Different objectives can

also be competing in terms of budget allocation and prioritization. Developing future strategies could make use of a SWOT approach. The SWOT methodology could be used 1) for developing policy as well as institutional and legal framework, and 2) for implementing the strategy, deciding about priorities when building collections.

3. Implementation and organization (should become section 2)

Short introduction of the topic (from FAO manual):

Once the decision to establish a cryoconservation programme has been taken, a plan for implementation and organization should be developed. Key elements of effective implementation are a national policy framework and a national strategy or action plan, an organizational and institutional system that is optimal under the given circumstances, well-functioning quality management systems (QMS) and active involvement of relevant stakeholders. Especially stakeholder participation may prove a challenge in many countries, therefore sufficient time and energy should be devoted to involve relevant stakeholder groups.

Short overview of activities related to this topic carried out in IMAGE:

The general objective of IMAGE was to enhance the usefulness of gene banks for stakeholders. One of the specific objectives was to understand and formalize the expectations of stakeholders regarding gene banks and to identify the gaps or the needs that IMAGE could address with its work programme. This was achieved first by executing a GAP analysis for Quality Management System (QMS) for identifying key factors associated with quality assurance in gene banking and secondly by developing a self-assess tool for quality management.

The GAP analysis clearly showed, with respect to general gene bank management, a lack of formally documented organizational and management structure, lack of a stakeholder analysis and lack of a communication strategy or plan in many gene banks. Regarding other specific QMS topics the survey also revealed a lack of formal quality policy, absence of a formal Quality manager, lacking documentation for standard operating procedures (SOP) and other key processes, as well as a lack of a formal Quality Management System including certification and internal guidelines.

References:

- Deliverable 2.4
- Zomerdijk et al. (2020). Quality management practices of gene banks for livestock: A global review. Biopreservation and Biobanking (in press).

Recommendations (guidelines):

Gene bank operations will benefit from the implementation of a quality management system (QMS), in terms of setting objectives, risk analysis, quality checking and satisfaction of users. The ISO 9001-2015 standard provides a good reference to identify the steps to be taken in order to set up a QMS. The new ISO 20387 standard lists the activities which are specific to a biobank. Developing a QMS should follow some key steps:

1. Self-evaluation of the gene bank Quality Management System:

Measure QMS compliance through a GAP analysis for using the standard 10-point procedure from collection to utilization in the checklist and carrying out a voluntary self or peer based review using the procedure described in Deliverable 2.4. to identify an action plan to improve the weak points.

A well-described and well-functioning QMS should be the backbone of any gene bank. To assess this, the IMAGE survey was structured to cover the following aspects:

1. General gene bank management
2. General quality management
3. Gene bank equipment
4. Gene bank personnel
5. Genetic material database
6. Genetic material acquisition
7. Material collection
8. Introduction of previously processed material
9. Material storage
10. Material distribution

2. Securing stakeholder involvement:

- Multi-actor governance of genetic collections and gene banks should be secured through a board of stakeholders
- Essential agenda points for board meetings should be agreed (e.g. strategic and policy issues, sharing of information/data and material, sanitary issues, standardization, ethical issues) to ensure optimal governance
- Develop a communication plan for chosen targets

3. Organising activities:

- Processes and procedures supporting the activities should be identified and formalised
- Cooperation among gene banks, including sharing good practices, exchanging protocols and sharing data should be encouraged and facilitated
- Secure documentation: has to be justified by its usefulness, not only to show that documents are kept

4. Choice of biological material to be preserved (new title, new section 3)

Short introduction of the topic (from FAO manual):

Potential means of conserving genetic diversity have traditionally included storing semen, embryos, oocytes and somatic cells. However, in recent years technologies for cryopreservation of ovaries and other gonadal tissue, primordial germ cells (PGC) and spermatogonia have been developed and implemented to varying degrees. In addition, while most methods have been developed for and are widely used in cattle, several technologies e.g. PGC are now tested in other livestock species such as pigs and poultry.

Short overview of activities related to this topic carried out in IMAGE:

IMAGE has produced new knowledge about other types of germplasm (primordial germ cells, gonadic tissues) has become available and should be introduced in this section. Technical details about their use including activities in IMAGE are presented in Section 8.

Introduction of primordial germ cells (PGCs)

Primordial germ cells (PGCs) are embryonic diploid germ stem cells that are early precursor of gametes. In the chicken PGCs can be collected with the embryonic blood at the time of their migration into the developing gonads and propagated *in vitro* in order to increase their number for subsequent use in the reproductive biotechnologies. For a long time, frozen sperm was the only tool for the conservation of poultry genetic resources. However, it has some limitations: **i)** it does not allow the conservation of the genetic resources of female birds linked to the specific sex chromosome W and mtDNA; **ii)** restoring the genotype using frozen semen requires several backcrosses and take several years. In this context the cryopreservation of amplified *in vitro* PGCs is of great interest as a conservation strategy complementary to the sperm-based biotechnology. After thawing and re-amplification *in vitro* PGCs can be transplanted in a surrogate host embryo, where they can develop to functional gametes and give offspring (van de Lavoie et al., 2006, Whyte et al., 2015.). Using this process alone or in combination with the frozen semen would restore the male and female genotypes of interest in a single generation.

Introduction of gonads and gonadal tissues

Nowadays - regarding avian species – semen freezing is the only practically used preservation method. Cryopreservation of oocytes and embryos *per se* is impossible, because of their biophysical traits and, as the females are heterogametic and the males are homogametic, the female genome falls out of the long term maintenance of genetic

materials. It was proven that the ovary of newly hatched chicks can be successfully frozen in the first 24 hours after hatching, because its construction is different in this age from that of the adult ones, and grafting of these tissues is possible into recipient day-old chicks. These gonads can be cut into 2-4 pieces and from each tissue piece there will be a completely developed entire gonad which has the capacity for gametogenesis. Later if these adult chimera hens are artificially inseminated with the frozen / thawed semen of the donor breed, we can obtain donor derived progeny in the F1 generation. The advantages of this method are that we can regain the donor genotype in 100% within one generation and every chimera hen can produce several donor-derived eggs as well as it can be carried out under relatively simple circumstances.

Recommendations (guidelines):

- Paragraphs about potential use of semen, embryos, oocytes and somatic cells are outdated and should be updated (FAO)
- Current introduction should be revised to be applied also to non-mammalian species.
- Specific recommendations on potential utilization are provided in section 8.
- The gene bank should keep up to date with advances in cryopreservation and reproductive technologies and maintain a close connection to research, for instance through a scientific advisory board.

5. Establishing a gene bank - physical structure and costs **(new section 4)**

Short introduction of the topic (from FAO manual):

The size and capacity of the gene bank and the types and amount of equipment needed are dependent upon the quantities of germplasm to be placed in the gene bank, the objectives of the gene bank, the range of species and breeds to be conserved, and last but not least the financial resources available for the conservation programme.

The “FAO Guidelines for cryoconservation of animal genetic resources” provide detailed recommendations for the requirements of the physical plant, including equipment, safety protocols and human resources.

Another important aspect is the cost of cryoconservation, both in terms of the individual gene bank facility as well as, in case of multiple plants, on a national and even international level. There is general agreement that ex situ collections offer value of preserving a back- up collection of (threatened) breeds so that this genetic diversity might be available for use in the future. However, optimizing ex situ livestock collections has largely focused on optimizing genetic variability, i.e. which breeds to conserve. The logistical dimension of collections is an important but neglected limiting factor in this

context. Ex situ conservation implies cost and benefits that must be compared to identify optimal conservation decisions.

Short overview of activities related to this topic carried out in IMAGE:

As part of the IMAGE project a report on **costs and potential values/benefits** of genetic collections was prepared. The objectives of the report on costs and potential values/benefits of genetic collections were 1) to collect information on costs of genetic collections from gene banks in member countries and to evaluate costs and benefits of alternative gene bank objectives; and 2) an economic analysis of alternative conservation strategies and the potential returns to gene bank development and use.

A survey was conducted to collect cost data from a sample of European gene bank collections (Annex 2). These included costs for maintenance, semen collection and freezing, labour, documentation, average distance between banks to farm zones, costs of skilled labour, materials and equipment and collection failure rates. The survey covered information on germplasm in current collections from cattle, sheep, goats, horses, pigs and poultry across 11 European gene banks. In addition, an optimisation model was developed to estimate the economic advantages (cost savings) from alternative collaborative collection scenarios. Specifications of the model have been published by De Oliveira Silva et al. (2019).

The results showed that there is significant overlap in the current allocation across the 11 gene banks analysed, specifically cattle and sheep. Optimizing breed collections would reduce costs by around 25%, but this assumes existing breeds are native to the regions where they are currently collected/stored. Centralizing breed conservation would significantly increase *ex situ* collections costs. Costs per conserved breed varies depending on targeted diversity, i.e. higher diversity targets (in number of breeds) means higher costs per breed since this will require the use of less-efficient gene banks due to cryogenic tank capacity and more cross-regional collections.

In terms of preventing extinction of endangered breeds, a study in Spanish livestock breeds (De Oliveira Silva et al. 2020) predicts that if no ex situ programs are intensified, around 25% of breeds have a 50% probability of being extinct between 2040 and 2060. The study defines a metric for action (“acceptable level of risk”) that allows decision makers to specify tolerable levels of in situ breed endangerment when taking ex situ collection and storage decisions. The study also suggests that collection costs represent relatively small increases irrespective of population status scenario. This means that intensifying ex situ collection programs for breeds other than “requiring urgent action” would present only marginally additional costs.

References:

- De Oliveira Silva, Rafael De Oliveira Silva, Oscar Cortes Gardyn, Sipke-Joost Hiemstra, Joao G. Oliveira Marques, Michèle Tixier-Boichard, Dominic Moran. Rationalizing ex situ collection of endangered livestock breeds (manuscript).

- Rafael De Oliveira Silva; Bouda Vosough Ahmadi; Sipke Joost Hiemstra; Dominic Moran. Optimizing ex situ genetic resource collections for European livestock conservation. Journal of Animal Breeding and Genetics 2019. <https://doi.org/10.1111/jbg.12368>
- Deliverable 2.5

Recommendations (guidelines):

Based on the analyses presented above, the guidelines should now recommend to perform a full cost analysis of collection enrichment, maintenance and future regeneration step, as follows:

- Collect better cost estimates per gene bank/country where possible using the data collection list provided in Annex 2. This prevents collection of inconsistent cost data across gene banks as managers tend to consider different components when estimating costs, and some costs, labour, electricity, documentation are not exclusive for managing the collections.
- Use mathematical modelling to estimate costs in specific scenarios, but determine first whether modelling required/beneficial and for what purpose? Mathematical modelling offers a flexible tool for rationalizing *ex situ* collections avoiding redundancy, at the same time providing a systematic approach to cost data collection and in relation to formulating conservation objectives including acceptable in situ extinction risks.
- Requirements for modelling: consistent gene bank data, information on the quantity and nature of germplasm (e.g. number and volume of semen doses or goblets), cryotank capacity, census data (to link collection decisions with in situ populations and policy scenarios), available and/or projected conservation budget and conservation priorities for the formulation of conservation scenarios
- Estimation of benefits: Ex situ collections are generally costly and resources are limited. Rationalizing collections through cost-efficiency analysis can prevent suboptimal collection strategies.

Economic optimization of ex situ collections begs important questions about the specific conservation objectives, which in turn require more institutional coordination to define the mix of private and public good objectives and hence potential cost and benefit sharing. This implies clearer articulation of in situ risks including endangerment due to climate change and other pressures, expected economic returns and other attributes that determine stakeholders' conservation preferences.

6. Developing and using gene bank collections (new title)

Short introduction of the topic (from FAO manual):

As described in the “FAO Guidelines for cryoconservation of animal genetic resources”, developing and updating gene bank collections is a long-term endeavour that involves

several processes. Major steps are choice of populations to include in the gene bank, defining collection targets for reconstituting populations, and selecting the animals from which samples will be obtained for the collection. On the other side, gene bank material can be utilized in live conservation and breeding for purposes like controlling inbreeding or cross-breeding for introgression. The FAO guidelines provide detailed descriptions on how this may be achieved, including a series of examples.

With the advent of new molecular techniques for genetic analysis, however, there is a need for guidance on the collection of molecular data by gene banks. This includes use of the latest methods as well as processing the data originating from these analyses.

Short overview of activities related to this topic carried out in IMAGE:

The IMAGE project has carried out two major tasks related to the utilization of gene bank material:

1. The development and test of a standard multispecies array
2. The development of a tool box for the optimization of designs for introgression of cryopreserved genotypes into an existing population.

In addition, some IMAGE case studies have also shown that optimal contribution methods can combine high genetic value of current population and high genetic diversity brought by gene bank collections to reach a compromise between genetic trend and genetic diversity.

Standard multi-species array:

One of the activities in IMAGE was the aggregation of data sources in a global, integrated dataset of farm animal genetic diversity, at the same time providing standardised protocols to add data at later stages, such as information deriving from future characterisation of genetic collections by sequence- or genotype-based tools. In addition, sequencing and genotyping of close to 2.000 samples from cattle, pigs, horses, sheep, goats and chicken was performed. Finally, as the key deliverable a cost-efficient genotyping assay for large scale characterisation across breeds/ species was developed.

The use of the multi-species genotyping assay(s) is twofold. First, it provides a means to reduce data density for population managers, making it easier to work with relevant genetic information. Second, it provides a cost-effective tool that can be applied to a large number of individuals of different species by any gene bank, for the purpose of rationalising and evaluating in-situ and ex-situ collections.

Tool box for introgression analysis (MoBPS):

Using cryopreserved genomic repositories to improve current breeding populations requires some type of breeding scheme (Figure 3 in Section 6 of the FAO guidelines).

Such schemes can differ widely in general structure, number of animals used, or the applied methods. In the IMAGE project we developed a tool to optimize such schemes, both with respect to the best result, but also with respect to the used resources and costs: ‘Modular Breeding Program Simulator’ (MoBPS). MoBPS consists of an R-package, which simulates breeding programs under this concept and is publicly available at <https://github.com/tpook92/MoBPS>. This tool has been tested in case studies (D6.4), see Annex 1.

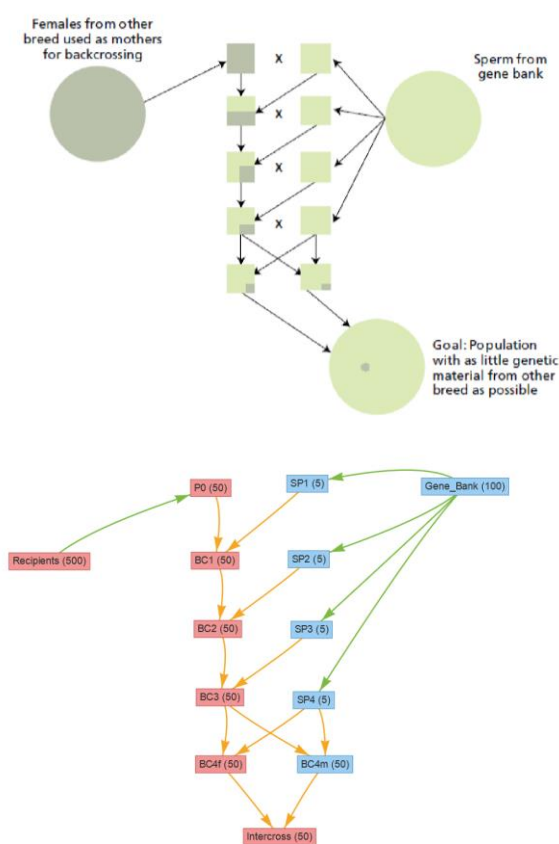


Figure 1: Left: An example backcrossing program taken from the FAO Guidelines “Cryoconservation of animal genetic resources” (FAO, 2012); Right: How the same program looks in the MoBPS editor (numbers in brackets are the assumed cohort sizes).

To make the package as user-friendly as possible, a web-based graphical user interface in JavaScript was developed, that allows to describe breeding programs in a very intuitive way, accessible through www.mobps.de.

A MoBPS analysis typically consists of four steps:

1. Define the breeding setup, i.e. the number of populations to work with, traits and their genetic characteristics, phenotyping and selection patterns, etc.
2. Draw the breeding program in a very intuitive ‘drag and drop’ type of approach (see Figure 1).

3. All information given in the first two steps is combined into a JSON file, being the input for the R-package, which then is executed in step 3 to simulate the breeding program.
4. In step 4 results are analyzed and all relevant variables can be plotted. MoBPS can work both with fully simulated data, but also can read in real data, e.g. genotypes or sequences of existing populations.

To illustrate its potential, MoBPS was used with an example backcrossing scheme taken from the “FAO guidelines on cryoconservation of animal genetic resources”, showing that MoBPS not only readily provides results for genetic progress, but also for the development of genetic diversity and inbreeding. Further information can be found in Deliverable 6.6, as well as two other case studies using MoBPS in Annex 1.

References:

Deliverable 6.6.

Recommendations (guidelines):

This section should be updated with two main objectives:

1. Emphasize the need for a complete molecular characterization of collections

The multispecies array:

- For every sample that is or will be stored for cryopreservation a blood sample (in EDTA) needs to be collected for DNA isolation. Extract DNA and genotype with the IMAGE multi-species arrays (as described in D4.5)
- Submit sample information to BioSamples (Inject tool, D5.2)
- Submit genotypes to BioSamples (D5.2)
- Compare and validate with reference data set per species (D5.4)

2. Diversify the use of gene bank collections

Tool box for introgression analysis:

- The software package MoBPS can be used for the restoration of populations from gene banks by simulations and for optimize breeding schemes
- The procedure is described in IMAGE Deliverable 6.6 and the MoBPS program can be accessed at <https://github.com/tpook92/MoBPS>.

7. Basic principles of cryopreservation

Short introduction of the topic (from FAO manual):

The most commonly used cryopreservation methods for animal germplasm are slow-freezing and vitrification. In both cases, understanding of relevant physio-chemical reactions occurring during freezing and thawing using different cryopreservation

protocols is important as well as the influence of cryoprotectants. Freeze drying is a very cost-effective method but may not be suitable for semen and somatic cells.

Short overview of activities related to this topic carried out in IMAGE:

The basic principles for cryopreservation have not changed a lot since the FAO Guidelines were published 2012. Some of the work carried out in the IMAGE project was done to optimize the existing methods for more specific and applied use, such as modelling the effect of vitrification on embryo quality in pigs and cryopreservation of ram semen. The results on ram semen cryopreservation will be presented in Section 8.

Identification of key transcripts affected by vitrification:

The purpose of this study was to identify key transcripts affected by vitrification - candidates for controlling quality in vitrification. Three different embryo treatment groups were established and compared at the RNA-level. RNA-sequencing analysis revealed different mRNA profiles for embryos subjected to different treatments. Results suggest that both vitrification and in vitro culture treatments have an important impact on the embryo. Our results reveal that an important part of the alterations on the embryonic transcriptome may be shared by both treatments, allowing to identify critical genes that can be used as biomarkers for improvement of IVC and vitrification treatments (D3.2).

References:

- Woelders H et al., 2018. Simulations of osmotic events in vitrification of equine oocytes and porcine embryos. Cryobiology 85, 154-155.
- Deliverable 3.2

Recommendations (guidelines):

- The osmotic effect of cryopreservation on vitrification of pig embryos should be taken into account in order to identify critical genes that can be used as biomarkers for improvement of IVC and vitrification treatments.

8. Collection of germplasm and tissues

Short introduction of the topic (from FAO manual):

As described in the “FAO Guidelines on cryoconservation of animal genetic resources” several factors determine whether germplasm is collected on farm or at a collection facility. Collection and processing procedures will differ depending on the type of germplasm being collected and the donor species.

Short overview of activities related to this topic carried out in IMAGE:

Upstream, the objectives of IMAGE were to develop and implement methods and tools to improve the cryoconservation and reproductive efficiency of biological samples; downstream the objective was to ensure the reliability of their use in breeding, e.g. breed reconstitution. The main impediments hindering collection and utilizing collections were prioritized, such as reproductive techniques in birds or pigs. The following activities were carried out:

- Chicken sperm proteins as candidate markers of fertility prediction (D3.2)
- The effects of in vitro culture on porcine embryonic transcriptome were compared to fresh embryos by RNA-sequencing (D3.2)
- Development and validation of a protocol for the cryopreservation of chicken PGCs (D3.3)
- Protocols for grafting of gonadic tissues (D3.5)
- Simulation of the osmotic effect of cryopreservation on vitrification of pig embryos (D3.5)
- Standardized semen cryopreservation process for rare chicken breeds (D3.5).
- Illustration of different freezing rates used for cryopreservation of ram semen (D3.5)

Chicken semen:

Several differentially expressed seminal proteins of poultry semen were found to be linked to male fertility. One of these proteins, SPINK2, expressed in the testis and epididymis epithelia, was chosen for closer examination with combined proteomic methods. The results indicated that SPINK2 was positively correlated with male fertility in several divergent chicken lines. Results indicated that new components in sperm quality and cryopreservation process were detected, and can be taken as a candidate fertility marker (D3.2).

The protocol for long-term storage of chicken semen was standardized and optimized for rare breeds (Thelie et al., 2019). The standardized semen freeze-thaw method included the use of the internal cryoprotectant glycerol (11%) that showed higher fertility results than Dimethyl formamide, Dimethylacetamide, and Ethylene Glycol cryoprotectants, when applied on the same rare breed. The method include the concomitant sampling and treatment of a low number of ejaculates (max 8), the immediate conditioning of semen with adapted dilutions and cooling, the freezing in straws at the rates 7°C/min from +4 to -35°C, and 60°C/min from -35°C to -140°C before storage in liquid nitrogen. Thawing was made at 4°C and glycerol was removed by successive dilutions (final: 1:19) before 15 min centrifugation at 500g and resuspension in insemination diluent. The effectiveness of the method was demonstrated on samples stored for 19 years (D3.2).

Efficient transmission of chicken PGCs:

For transplantation of chicken donor germplasm material into surrogate hosts the elimination of endogenous reproductive cells is required to increase the transmission frequency. For Gonadic tissues, and PGCs one means to achieve this is using sterile recipients obtained using genome editing or transgenesis. This however requires a specific implementation of national regulations in order to allow the use of genetically modified recipients in specific actions of conservation of genetic resources (D3.3).

Efficiency of chicken PGCs and their limits of long-term cultures - cryopreservation protocols.

To avoid contamination from animal products (serum) and to optimize growth, cryopreservation protocols using synthetic media were tested and optimized for chicken PGCs. Two serum-free cryopreservation media were compared to standard serum-containing cryopreservation media. The cryopreservation protocols with serum-free media serve as well as the standard serum-containing media for the freezing and restoration of PGC lines. Cryopreserved PGCs also recovered well after thawing and re-culturing (Woodcock et al., 2019) (D3.3).

The time spent by PGCs in culture and cryopreservation may affect these cells at different molecular levels with consequences for their reproductive capacities. Short term (1 month) cultures were compared with long term (7 month and 10 month) cultures, and fresh PGCs cultures were compared with cryopreserved, thawed and re-amplified *in vitro* PGCs cultures by RNAseq and RRBS approaches. Long term culture affected PGCs gene expression and DNA methylation in both sexes, but this effect was much more important on male PGCs. In germ line transmission experiences no progeny was obtained from 9 month male PGCs culture that indicate on the possible negative effect of long term culture on PGC reproductive potential. Almost no effect of cryopreservation, followed by thawing and reamplification *in vitro* was observed on PGCs gene expression, but it had an impact on DNA methylation. Short term male and female PGCs cultures demonstrated good germline transmission rate after freezing, thawing, reamplification *in vitro* and development in surrogate host, that provided additional evidence on the efficiency of PGCs reproductive biotechnology. In the context of this study it is not recommended to use long term cultures for restoration of genotypes.

Gonadic tissue: An efficient protocol for chicken semen suitable couples donors/recipients

Gonads from day-old donor chicks are vitrified using acupuncture vitrification. Day old recipient chicks were anesthetized, their gonads were ablated using an electrocautery, and they received allotransplants of vitrified-warmed donor gonad pieces. After surgery, dexamethasone was given intramuscularly, followed by oral administration daily of mycophenolate mofetil for 2 weeks and then once a week for an additional 6 week. For finding donor/recipient combinations we included information regarding

genetic differences between intensively selected lines for which there was successful donor-recipient pairing.

Applying this procedure the adhesion of the frozen / thawed gonads are similar to the native ones (70-80%) and capable for gametogenesis. We can obtain donor-derived progeny. It can be a suitable method in the *in vitro* poultry gene conservation.

It was proven that not all breeds are suitable recipients (Liptoi et al. 2013). Information obtained from evaluating genetic differences of intensively selected lines - in which there was successful pairing - was used in the indigenous breeds. According to the results donor/recipient combinations were created which could be effectively used for gonadal tissue transplantations. For the indigenous Hungarian breeds the decisions for pairing of different genotypes were based on a large-scale analysis of genetic diversity in chickens (Bodzsar et al., 2012; EC project GLOBALDIV). Based on the results of this study, and considering the theory of determining genetic difference between breeds to find successful pairings, other recipients were selected from the commercial lines (Liptoi et al. 2020). This method can be applied for creating successful donor / recipient combinations among other local chicken breeds as well (D3.5).

Cryopreservation of ram semen:

Three different protocols were studied for cryopreservation of ram semen. A protocol that mimics freezing in static nitrogen vapor was compared with two other protocols with different cooling rates. The optimal protocol [freezing rate: from +5°C to -10°C (5°C/min), and then from -10 °C to -130 °C (60°C/min)] provided the highest percentages of sperm motility, integrity of plasma, acrosome, and mitochondrial membranes, and the lowest percentage of fragmented DNA (Galarza et al., 2019). Thus, it is a highly recommendable method in the establishment of germplasm banks for threatened breeds as well as to support a greater use of artificial insemination with frozen sperm into sheep breeding programs (D3.5).

References:

- Galarza DA, López-Sebastián A, Woelders H, Blesbois E, Santiago-Moreno J. Two-step accelerating freezing protocol yields a better motility, membranes and DNA integrities of thawed ram sperm than three-steps freezing protocols. 2019. Cryobiology, 91, 84-89. <https://doi.org/10.1016/j.cryobiol.2019.10.007>.
- Liptoi K., Buda K., Rohn E., Drobnyak A., Meleg E., Palinkas-Bodzsar N., Vegi B. and J. Barna. Improvement of the application of gonadal tissue allotransplantation in the *in vitro* conservation of chicken genetic lines. Animal Reproduction Science Vol 213, 2020, 106280. (<https://doi.org/10.1016/j.anireprosci.2020.106280>.)
- Santiago-Moreno J., Bernal B., Perez-Cerezales S., Castaño C., Toledano-Diaz A., Estes MC., Gutierrez-Adan A., Lopez-Sebastia A., Gil MG., Woelders H. and E. Blesbois. Seminal plasma amino acid profile in different breeds of chicken: Role of seminal plasma on sperm cryoresistance. Plos One Jan 4 2019. <https://doi.org/10.1371/journal.pone.0209910>.

- Thélie A., Rehault- Godbert S., Poirier J-C., Govoroun M., Fouchécourt S. and Blesbois E. The seminal acrosin- inhibitor CIT11/SPINK2 is a fertility- associated marker in the chicken (<https://doi.org/10.1002/mrd.23153>). Molecular Reproduction and Development pp.762-775 Vol 86, issue 7, 2019.
- Woelders H et al., 2018. Simulations of osmotic events in vitrification of equine oocytes and porcine embryos. *Cryobiology* 85, 154-155.
- Woodcock ME, Gheyas AA, Mason AS, Nandi S, Taylor L, Sherman A, Smith J, Burt DW, Hawken R, McGrew MJ. Reviving rare chicken breeds using genetically engineered sterility in surrogate host birds. *Proc Natl Acad Sci U S A*. 2019 15;116(42):20930-20937. doi: 10.1073/pnas.1906316116. <https://www.ncbi.nlm.nih.gov/pubmed/31575742>.
- Whyte J, Glover JD, Woodcock M, Brzeszczynska J, Taylor L, Sherman A, Kaiser, P, McGrew MJ. FGF, Insulin, and SMAD Signaling Cooperate for Avian Primordial Germ Cell Self-Renewal. *Stem Cell Reports*. 2015, 8;5(6):1171-1182. doi: 10.1016/j.stemcr.2015.10.008. <https://www.ncbi.nlm.nih.gov/pubmed/26677769>
- Deliverables: 3.2, 3.3 and 3.5

Recommendations (guidelines):

1. Standardized method of semen cryopreservation in rare chicken breeds (Thelie et al., 2019).
2. In national-level legislation, consider specific actions to allow the use of gonadic transfer or of genetically modified recipients in specific actions of conservation of genetic resources.
1. An efficient protocol for creation suitable donor/recipient combinations for chicken gonadal tissue transfer (Liptoi et al. 2020).
2. The synthetic serum free media in routine use to avoid animal pathogens present in serum (Whyte et al. 2015, Woodcock et al. 2019).
3. Optimal freezing rate for use of artificial insemination with frozen sperm into sheep (Galarzo et al. 2019).
4. New protocols suggested for use in cryobanking (Table 8.1).

Table 8.1. New protocols suggested for use in cryobanking

Species	Type of cell/tissue	Method	Reference	Task
Chicken	Semen	Chicken semen cryopreservation protocol for local breeds	Thelie et al. (2019) Deliverables 3.2 and D3.5	T3.1
		Sperm DNA fragmentation	Santiago-Moreno et al. (2019)	T3.2

Ram	Semen	Ram semen cryopreservation	Galarza et al. (2019) Deliverable 3.5	T3.1
Chicken	Gonadic tissue	Gonadic cryopreservation and transfer	Liptoi et al. (2020) Deliverable 3.5	T3.3
Chicken	Primordial Germ Cells (PGCs)	PGCs cryopreservation	Whyte et al. (2015) Woodcock et al. (2019) Deliverable 3.3	T3.4
		PGCs mycoplasma test	Not published Deliverable 3.3	
		PGCs long term culture	Woodcock et al. (2019) Deliverable 3.3	T3.4
Pig	Embryos	Embryo vitrification	Woelders et al. (2018) Deliverable 3.2	T3.5

9. Sanitary recommendations

Recommendation to FAO: revise section 9 completely

Short introduction of the topic (from FAO manual):

Collection and banking of animal genetic resources presents several challenges regarding disease transmission and biosecurity since samples will originate from different animals, different farms and possibly different countries. As stated in the “FAO Guidelines on cryoconservation of animal genetic resources” each country will need to balance its breed conservation strategies with national and international health regulations. The primary issue for a gene bank collecting germplasm in the field is to minimize the risk of spreading diseases from farm to farm while collecting germplasm from animals belonging to different owners. Additionally, efforts must be made to reduce the risk of spreading diseases during the utilization of germplasm that the repository has collected and cryopreserved.

The Animal Health Law and the related Delegated Act ensure that germplasm traded and used in the Member Countries is free of diseases. However, germ plasm from rare breeds is not always able to comply with the regulations, which means that current regulations present a barrier for transboundary exchange and use of gene material.

Short overview of activities related to this topic carried out in IMAGE:

As part of the IMAGE project a policy report on sanitary recommendations to gene banks was prepared. The main objectives were 1) to establish a status quo of current sanitary policies of gene banks containing germplasm material; 2) to identify bottlenecks in acquisition, storage and use of germplasm caused by current sanitary policies; and 3) to provide recommendations for new sanitary policies complying with the Regulation (EU) 2016/429 with special regard to the needs of endangered transboundary breeds and of germplasm collections containing old material.

Results of the survey “Inventory and mapping of European animal genetic collections” (Passemar et al., 2018) showed that 21 of 51 answering gene banks encountered problems in acquisition and/or use of gene bank material because of sanitary regulations. A second questionnaire was sent to the 21 gene banks reporting bottlenecks in the survey and was answered by 11 gene banks situated in seven countries.

All respondents answered that they were familiar with the contents of the drafted Delegated Act “Germinal Products” to the Regulation (EU) 2016/429. The majority did not intend to change sanitary policies and did not expect the new regulations to make it easier to enlarge their collection, some expected to acquire more material of other sanitary status while all gene banks were planning to change or rearrange storage and consider bilateral agreements for use of gene bank material of regional transboundary breeds.

References:

Deliverable 1.7.

Recommendations (guidelines):

Based on the policy report on sanitary considerations the following recommendations can be made:

- Samples should be free from listed diseases in Regulation (EU) 2016/429 Annex II
- Tests for diseases should be carried out before and after collection of samples. Storage of blood/tissue samples as well as germplasm for further testing is important.
- National derogations should be used to facilitate collection and use of germplasm material of rare breeds on a national level.
- Samples should be collected at approved collection centres if possible. In case of field collections, the sanitary status of the farm/herd/donor should be documented as completely as possible. Backup samples of non-germplasm material (blood, tissue and other) should be stored

- Other sources of germplasm material should be considered for collection (e.g. slaughterhouse material)
- Germ plasm samples of one species with the same sanitary status may be stored in one tank
- Samples of sheep and goats with same sanitary status may be stored in one tank
- Samples with different sanitary status and/or of different species must be stored in separate tanks, but may be stored in the same room, provided the tanks are clearly marked and no cooling agent can pass from one tank to the other
- Comprehensive information on the sanitary status of the samples should be included not only in the documentation but also be available on the tank
- Material with same sanitary status of one species may be transported in one vessel
- Material of sheep and goats with same sanitary status may be transported in one vessel
- National use of gene bank material not complying with Animal Health Law on a national basis depends on national derogations, e.g. allowing use of such material of highly endangered breeds in case of loss of genetic diversity in situ.
- Transboundary exchange and use of gene bank material not complying with Animal Health Law should be regulated by the countries involved on a bilateral basis.
- Exceptions may be made for diseases listed in Regulation (EU) 2016/429, Annex II (European Union, 2016), if they are not transferable by frozen germplasm.

10. Databases and documentation

Recommendation to FAO: revise section 10 completely

Short introduction of the topic (from FAO manual):

It is essential to recognize the importance of data administration systems in day-to-day management of the gene bank collections, but also in allowing potential users to access up-to-date information on the material in the collection. Basic information about gene bank collections should be easily accessible without the need for any additional information from outside the database in order to promote awareness of the country's AnGR programme. The administrative systems of collected data varies from a basic information storage using a spreadsheet to more developed computer software systems specifically designed for database construction. In section 10, the essential components of a gene bank information system are introduced.

Short overview of activities related to this topic carried out in IMAGE:

A large amount of sequence and genotyping data have become available through public funded research projects and breeding programmes. In addition to the genomic information produced by modern genomic technologies, other types of information are

also available, such as existing gene bank information, GIS and phenotypic data. Although these resources are extremely valuable and large projects, which generate data and information on thousands of samples and individuals, need to properly organise the genotypic and phenotypic collection undertaken to facilitate the submission of data to these public archives. Information is often segmented and there is a lack of direct connection between all the different sources of information, which has hampered the full exploitation of the currently available genetic resource.

IMAGE has aimed to close this gap by creating ad hoc user-friendly solutions and interfaces to aggregate the information from different resources and allow both simple and complex queries. The objective was to create a European web portal that integrates data from gene banks and collections with genomics data, geographical information systems data, and other information generated by IMAGE. The following activities were carried out:

- A well-defined metadata rule set ensuring high quality and comparable data across the diverse collections originating in different storage formats and languages.
- Development of a single entry point 'Inject tool' helping gene bank managers to enhance, standardise, tag and submit their gene bank data to sustainable archive.
- The possibility to archive this data within the EBI BioSamples public archive.
- A common data pool that integrates the various informations (gene bank data, genomic and geographical information).
- A data portal that integrates gene bank metadata with generated 'omic datasets from within IMAGE and cross referencing to other gene bank and breeding database resources from across Europe such as those hosted by the Food and Agriculture Organisation (FAO).
- Within the data portal, a Geographic Information System tool is included to assist the user in identifying/storing the geographical origin of the samples as well as displaying individual/population genetic parameters and biological attributes through interactive maps (see Annex 1, Box 2. An example of Landscape analysis).
- Starting from data derived from the portal, computing tools and methods have been developed to browse the diversity of sample and/or genomic data. The Diversity Browser is a stand-alone tool that computes principal component analysis (PCA) of a reference dataset and a batch of samples of interest. An interactive web interface to guide the use of genetic material was created. It allows selective downloading of collection and genotype information to be leveraged using the linked MoBPS software package that provides a computationally efficient and flexible framework to simulate complex breeding programs and compare their economic and genetic impact.

References:

- Wilkinson, M.D., et al., The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data*, 2016. **3**: p. 160018.
- Salloum, S., et al., Big data analytics on Apache Spark. *International Journal of Data Science and Analytics*, 2016. **1** (3): p. 145-164.
- Zaharia, M., et al., Apache spark: a unified engine for big data processing. *Communications of the ACM*, 2016. **59** (11): p. 56-65.

Recommendations (guidelines):

Data management based on the IMAGE data framework:

- Samples submitted to the database should be assigned a unique identifier and a minimum set of descriptors matching the metadata rule set, as defined in IMAGE, ensuring high quality and comparable data across the diverse collections originating in different storage formats and languages.
- The Inject tool developed by IMAGE should be used to enhance, standardise, tag and submit the gene bank data to a Common Data Pool that integrates all gene bank records from across Europe.
- Data should be uploaded in the IMAGE data portal that integrates gene bank metadata with generated 'omic datasets from within IMAGE and cross referencing to other gene bank and breeding database resources from across Europe such as those hosted by the Food and Agriculture Organisation (FAO).
- The Geographic Information System tool should be used for identifying and storing geographical origin of the samples.
- IMAGE encourages collecting and storing data to BioSamples with a wide variety of characters, even not yet standardized, as free text, images or other attachments for virtual characterization, using diverse technologies (for example microscope images, scans etc).
- Rules for data collection (a general recommendation in the project). Any data collection should start with a definition of level of data privacy, property rights etc.
- Finally, the scientific community should devote further work to development/ definition of ontologies. An ontology is a controlled vocabulary that describes objects and the relations between them in a formal way. They allow sharing of information among the people and software agents. Trait ontology is necessary in forming a standard so that researchers and stakeholders may communicate with each other more consistently and effectively.

- Update the Tables 14 and 15 in the FAO Guidelines.

11. Legal issues - contracts and access

Recommendation to FAO: revise section 11 completely

Short introduction of the topic (from FAO manual):

In the development of country-based gene banks, there may be a need for various types of agreements covering the acquisition of any gene bank material and the dispersal of it when they are requested by potential users. As stated in the “FAO Guidelines on cryoconservation of animal genetic resources”, the agreements should delineate the rights and responsibilities of the gene bank, the users of the gene bank’s germplasm/tissue and (where relevant) the donors of the samples. Because of the potential legal ramifications, the gene bank must have clear policies and procedures for drawing up such agreements. Such policies may be established by the gene bank management or may be established at a higher level, such as through national legislation.

In developing policies and general agreements for acquiring and dispersing germplasm, a suggested guiding principle is that these instruments should facilitate the sustainable use, development and conservation of AnGR and the enhancement of the country’s livestock sector.

Short overview of activities related to this topic carried out in IMAGE:

Until now the gene banks were generally considered as backup solutions. A lot of samples are stored, but not used in any way. With rapid improvement in life science technologies has made it possible to extend the usability of existing genetic collections. However, challenges may arise from access and benefit sharing for exchange of genetic material according to the Nagoya protocol unless contracts and other legal agreements are not considered.

Two surveys were conducted for gathering information on implementation of access and benefit sharing regulation. The first survey gathered information on existing practices, internal gene banks’ procedures and protocols on acquisition, exchange and provision of biological material in EU countries. The second one focused on determining ABS provisions in the national legislation developed to implement the Nagoya Protocol and EU regulation 511/2014. It addressed both access requirements and potential benefit sharing provisions, especially in the context of utilisation of genetic resources from *ex situ* collections.

The FAO guidelines were developed almost 10 years ago. Over this period substantial developments of gene-banking operations were observed at the European and the global level. Moreover, significant changes have occurred in the legal environment affecting genebanks. In addition to the above mentioned facts, new knowledge gained from the surveys indicating gaps needed to address in revised FAO guidelines.

Identified gaps

- Lack of reference to the new legal agreements that were not in force while the FAO guidelines were prepared (Nagoya Protocol, the EU ABS legislation and national ABS legislative, administrative and policy measures).
- Lack of reference to the need to obtain and maintain a proper documentation on the genetic resources that have been acquired and stored in the genebank.
- While in the past documentation associated with the sample entering the gene bank and being transferred from the gene bank was gradually being developed and considered useful, at present getting, storing and transferring such information is a must.
- There was no strong recommendation on the need for internal MAA and MTA contracts/protocols developed and implemented by genebanks.
- There was not sufficient reference to veterinary requirements and need for phenotypic/genetic data on the donor of the sample.

References:

- The Nagoya Protocol
- 511/2014 EU ABS Regulation
- ERFP guidelines on MAA and MTA
- EU horizontal ABS guidance
- EU sectoral ABS guidance
- Deliverable 1.6
- E. Martyniuk, B. Berger, D. Bojkovski, D. Bouchel, S. J. Hiemstra, C. Marguerat, V. Matlova & N. Sæther. Possible consequences of the Nagoya Protocol for animal breeding and the worldwide exchange of animal genetic resources. *Acta Agriculturae Scandinavica, Section A — Animal Science* 2018.

Recommendations

Gene banks need to define access and use policy and associated access criteria and access procedures. The gene bank should put in place specific policies and necessary legal arrangements in order to provide clarity to all actors, short and long term, and build trust. Section 11 in the FAO guidelines requires substantial changes to accommodate recent developments and provide well informed recommendations.

Genebanks managers have to be aware of the procedures required by the implementation of ABS provisions

Proposed new section 11 in FAO guidelines

1. Introduction - A new legal ABS landscape: the Nagoya Protocol and national ABS legislation

- A short reference to the Nagoya Protocol and 511/2014 EU ABS Regulation
- National legislation: diverse ABS measures and regulatory frameworks, some examples

2. Gene banks: statutes, internal decision making structures and processes

- Various legal basis for establishment and functioning of genebanks
- Decision making bodies
- Decision making processes, the role of the owner of sampled animals

3. Acquiring samples for collection

- ABS documentation associated with the sample
- Veterinary requirements
- Documentation required by animal breeding law, if relevant
- Options of acquisition: How, what for and where from
 - ✓ Samples obtained on routine basis (AI station)/ national programme
 - ✓ specific actions/programmes to collect samples
 - ✓ taking over gene bank material from other entities
 - ✓ long-term storage / to be used for conservation/breeding/research
 - ✓ origin of the material (domestic /international)

4. Developing MAA (ERFP guidelines)

5. Access to the gene bank collections (EU guidelines)

- Who and what for would like to access samples
 - ✓ public institutions/private entities
 - ✓ conservation
 - ✓ breeding
 - ✓ research
 - ✓ research and development

6. Developing MTA (ERFP guidelines)

7. Transfer of germplasm

- ✓ ABS documentation associated with sample made available to the user
- ✓ Veterinary requirements
- ✓ Documentation required by animal breeding law, if relevant

8. Benefit sharing in the gene bank context

- When and how?

- ✓ User want to patent development based on the material
- ✓ BS guided by the national ABS law
- ✓ Public goods as a form of BS

9. ABS impact on operation of the genebanks

- Importance of documentation especially when material is acquired not only from domestic populations
 - ✓ for acquired material
 - ✓ while making material available for users
- Current impact of ABS measures

12. Capacity building and training

Short introduction of the topic (from FAO manual):

The development of sustainable conservation programmes is only possible if it is combined with the development of human resources, institutions and long-term organizational support. Well-trained researchers and decision-makers are critical for creating awareness of AnGR related problems and for implementing programmes to conserve and sustainably use AnGR.

The FAO Global Plan of Action for Animal Genetic Resources emphasizes the need for well-trained human resources, including researchers and decision-makers, to enhance programs aimed at the management, conservation and sustainable use of animal genetic resources (AnGR). Moreover, the Convention on Biological Diversity (CBD) calls for access to, and transfer of technology, exchange of information relevant to the conservation, management and use of biological diversity, including information on research, training, surveys and specialized knowledge, and technical and scientific cooperation through, where necessary, appropriate international institutions, with special attention to capacity building.

In compliance with FAO and CBD, relevant subjects related with conservation and sustainable use of AnGR have been incorporated into university curricula worldwide, aiming to promote awareness of the importance of AnGR, and disseminate scientific knowledge regarding their characterization, documentation, conservation and improvement. Recently, the European region, through its Regional Focal Point for Animal Genetic Resources, has established the European Genebank Network for AnGR (EUGENA). This network of Genebanks from European countries was set-up with the aim of supporting the *ex situ* conservation and sustainable use of AnGR and facilitate the implementation of the FAO's GPA and the Nagoya Protocol for Access and Benefit Sharing (ABS) in Europe.

Short overview of activities related to this topic carried out in IMAGE:

The “Inventory and mapping of European animal genetic collections”, an overview of how European collections are managed (including security backup, sanitary requirements, database set up and existence of a quality management system), as well as conditions of access to these resources (including questions about the implementation of the Nagoya protocol on ABS) clearly showed that there is ample room for improvement.

The results indicate an urgent need to disseminate and develop knowledge transfer actions within the AnGR community focusing on: a) better organization of AnGR gene banks, in addition to their *in situ* and long term conservation; b) the Nagoya protocol on ABS and its implications; c) documentation and databases; d) metadata for gene banks.

In addition to general dissemination activities such as newsletters and website, the following activities were carried out:

- Technical workshops for AnGR in third countries
- Guidelines for the management of gene banks

Recommendations (guidelines):

Adding to the recommendations outlined by the FAO regarding education in topics related to AnGR, based on experiences from the IMAGE project the following topics are proposed:

1. Innovative uses of Genebanks:

This topic should cover the possible use of gene bank collections for the reintroduction of diversity into standing populations, the characterization of diversity dynamics, contribution of gene banks to the development of new crosses and breed-types, and the contribution of cryobank to the management of the diversity of endangered breeds. As an output of IMAGE, MoBPS software was developed which allows to optimize the choice of cryobank resources to manage the diversity of a population in conservation or selection or to redirect its selection objectives.

2. Characterization of genetic diversity using genomic indicators

The availability of next generation sequencing and of high-throughput genotyping platforms allows to characterize the genetic diversity of collections, to unforeseen levels of complexity, which should allow a better understanding of the factors that affect genetic variation, at both the genome and at epigenome level. Enhancing education regarding how the data generated by these new technologies can and should be used for the management of genetic diversity is required.

3. Long-time maintenance of genetic diversity

New developments in reproductive technologies, genomic tools and in the theoretical framework underlying the management of small endangered populations provides novel opportunities for the establishment of in situ and ex situ conservation programs aimed at the long-time maintenance of genetic diversity. These new developments require up-to-date skills at various levels and should therefore be among the top priorities for training.

4. Characterization and documentation of collections and involvement in networks

In order to better use, manage and promote gene bank collections, the recording of associated metadata is required. This recording should be performed systematically and in a standardized manner, using available databases. Examples of these databases are Cryoweb as well as the newly developed web portal for gene banks developed through the IMAGE project. Training in the use of these databases and in the compiling of metadata information is required and of crucial importance.

5. Legal Issues related with access and exchange of germplasm

As pointed out in FAO guidelines, management of gene banks requires knowledge regarding national and international policies affecting the exchange of AnGR. Within IMAGE project activities it was revealed that there is insufficient knowledge regarding the implications of the Nagoya protocol on Access and Benefit Sharing, as well as regarding the implementation of MTA and of MAA procedures, which are essential for the establishment of clear rules in order to optimize the use and access to these collections.

6. Annexes

6.1. Three examples of novel methods to optimize conservation and introgression schemes

An example of introgression analysis using the MoBPS program:

Box 1: Breeding programs in the South American Creole cattle

The objective of this work was to enhance the breeding programs of the Creole cattle populations in two steps. The first step was a simulation of the breeding program and the possible increase in genetic gain, given the country specific characteristics. This was followed up with the second step for the optimization of the breeding program to constrain inbreeding levels.

For the simulation of the Creole cattle breeding program the MoBPS (Modular Breeding Program Simulator) software was used. The estimated breeding values for weaning weight were used to demonstrate the projected changes within the Colombian Blanco Orejinegro (BON) population, including the bulls from gene banks. The optimization of the breeding program was done utilizing the optimal contribution methodology, using the GENCONT software.

With the implementation of selection in the BON population we achieve the expected increase in production levels. In addition, average heterozygosity in the population also decreases, thus the inbreeding levels increase. Such one-sided selection might lead to unsustainable breeding practices. In a simulation we show that the inbreeding levels of the population could be improved by using the gene bank bulls from the conservation program (Figure 1).

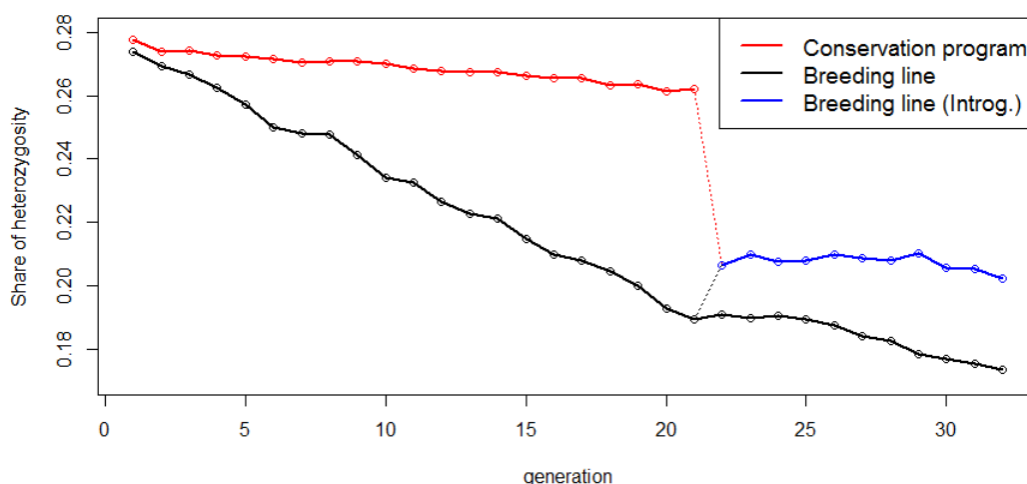


Figure 1: Development of the heterozygosity levels in a simulated conservation and breeding programs.

The follow up optimal contribution selection approach was implemented to suggest suitable mating plans for simultaneous increase of production level, while putting constraints on inbreeding levels. The use of gene bank bulls had a clear added value, and enabled an even better management of genetic diversity in the population.

Box 2. An example of Landscape analysis

The main objective of IMAGE is to fully exploit the potential present and future value of farm animal genetic resources biobanked material. The identification of genes having adaptive value contributes to reach this objective, considering the presently occurring rapid changes in climatic conditions. Climate change affects livestock in multiple ways, directly, in some cases decreasing animal welfare and productivity, and indirectly, changing pasture and both feed composition and availability, and parasite, vector and pathogen range. Once identified, adaptive genes can be used to characterize biobanked samples for their potential reserve of adaptive value, to optimize the choice of samples to be newly introduced in gene banks and to set up genotyping and breeding tools to improve livestock adaptation and welfare.

With this aims, IMAGE has set up two landscape genomics investigations to understand molecular mechanisms underlying livestock adaptation to environmental challenges. Two different models have been selected: a) a large set of sheep breeds reared in different climatic areas across Europe, South West Asia and North Africa; b) a cosmopolitan cattle breed (Holstein Friesian cattle) reared in different countries across Europe.

IMAGE has used SNP genotyping and whole genome sequences to associate genome variants of sheep and cattle to environmental variables using a landscape genomics approach. Genes that contain or are adjacent to significant SNPs are identified and analysed for function, involvement in metabolic pathways and association to traits in livestock and other species.

A total of 1156 geo-referenced sheep samples from 77 breeds from 12 countries distributed in eight environmental clusters and 32 subclusters have been characterized with the Illumina OvineHD SNPchip, containing more than 600,000 SNP markers. Landscape genomics analysis identified significant association between environmental variables and 483 candidate genes mostly associated to temperature or temperature associated environmental variables. These are involved in fundamental metabolic mechanisms for adaptation to different environmental challenges such as immune response, energy metabolism, morphology and behaviour. Comparison with results obtained with two independent selection signature methods identified 26 of these genes as strong candidates to have adaptive values. A total of 500 Holstein cows have been analysed by landscape genomics and environmental GWAS. Environmental GWAS identified 16 candidate genes associated with fat yield and isothermality. Significant SNPs have been included in the low-density SNP array developed in IMAGE and may be used for the characterization of sheep biobanked material, the choice of novel material to be stored and for breeding purposes.

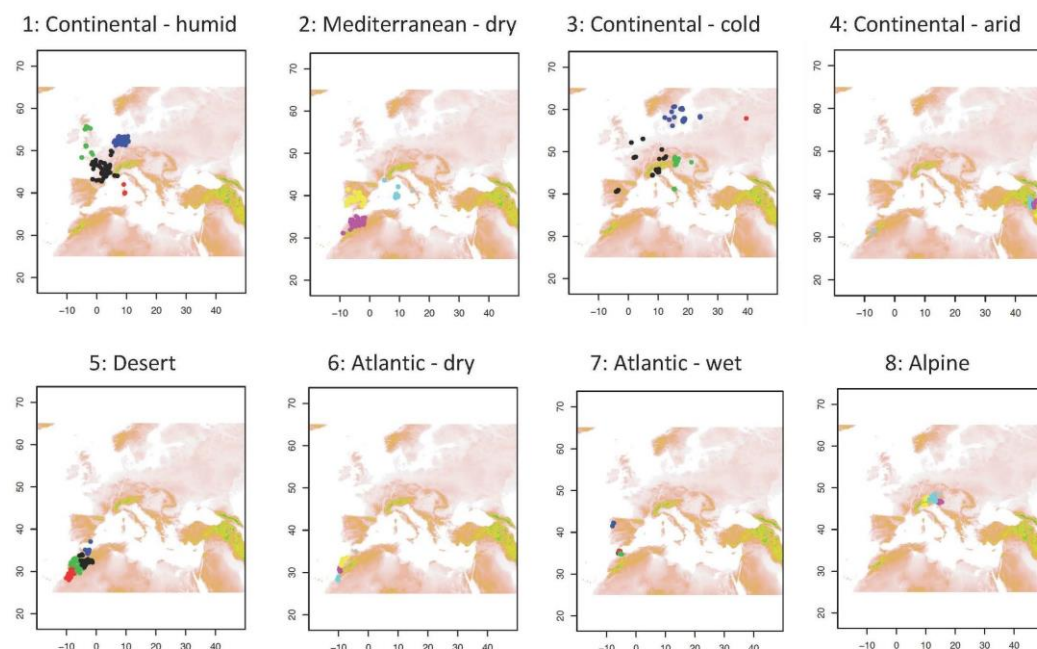


Figure 2. Distribution of the 1,156 samples (coloured dots) across the 8 environmental clusters (images 1-8) and 32 subclusters (colors within clusters).

Box 3: Blue Eggs.

One demonstration project in IMAGE aimed at introgressing the gene responsible for blue egg-shell colour from the Araucana breed into a high producing commercial white layer line. The objective was, to derive a line, which (a) is homozygous for the allele causing blue egg shell colour; (b) otherwise carries a maximum proportion of the white-layer genome, especially in the vicinity of the target locus for the blue egg shell gene on chromosome 1; and (c) shows as much genetic diversity as possible.

This goal was pursued with a backcrossing experiment: an F1 was followed by two backcross-generations and one intercross (see Figure 3). All selection and mating decisions were based on extensive simulation runs with MoBPS, using genome wide 54k SNP genotypes as well as genotypes for a specific set of markers up-and downstream of the target locus.

The overall strategy was to use for the backcross only those carriers of the blue egg shell colour allele with recombinations close to the target locus, and among those to maximize the genetic contribution of the white layers. By this, it could be achieved, that 91.2 % of the genome in the backcross 2 generation was of white layer origin, while the expected proportion would have been 87.5 % under random selection and mating. Hence, by using the simulation-based selection scheme the genetic contribution of the Araucana breed to the final product was reduced by about 30 per cent, leading to animals that produce blue eggs but with a similar performance profile as commercial layers both with respect to laying rate and egg quality.

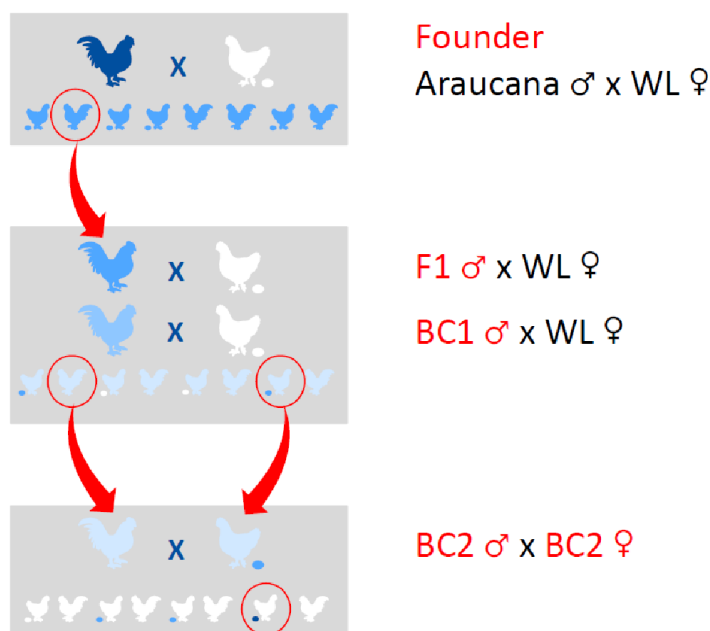


Figure 3: Breeding scheme to produce a highly productive layer line laying blue eggs.

References/acknowledgement:

Box 1: Breeding programs in the South American Creole cattle. Mészáros, G., Martínez, R., Lucero, C., Burgoz Paz, W.O., Naves, M., Doekes, H., Windig, J., Pook, T., Simianer, H.

Box 2. An example of Landscape analysis. Licia Colli, Oliver Selmoni, Mario Barbato, Marcello Del Corvo, Elia Vajana, Elisa Eufemi, Elisa Somenzi, Badr Benjelloun, Stéphane Joost, Paolo Ajmone-Marsan & the IMAGE consortium.

Box 3: Blue Eggs. N.T. Ha, A. Weigend, D. Caverio, M. Schmutz, B. Andersson, R. Preisinger, H. Simianer, S. Weigend.

6.2. Annex 2.

Online survey: Gene bank managers' views on cost of collecting and storing genetic material