

- **Historical overview of gene bank development in your country**

The gene bank development in the Walloon region of Belgium (there are two main regions in Belgium: Flanders and Wallonia) has been funded by the Public Service of Wallonia since 2005 via several research projects conducted by the Université catholique de Louvain (UCL). A first project aimed to list and describe the local ruminant breeds present in Wallonia and to study the feasibility of the development of a gene bank based on the experience of the European countries with national gene banks. A second project aimed to characterize the intra-breed genetic diversity of three Belgian endangered sheep breeds using a multi-dimensional approach combining results of molecular, genealogical and animal exchanges analyses for the choice of donors representative of the genetic diversity. Then the constitution of the collection of semen, DNA and serum has started for the three characterized breeds. In the future we expect to preserve material from local pig, cattle and horse breeds.

- **Objective(s) of national cryopreservation programme/policy**

- Collection goals

Multiple collection goals are considered in the gene bank. Material could be used:

- to reconstruct a breed in case of diminution of the amount of breeders and animals or in case of sanitary problems;
- to support *in situ* conservation;
- to have a back-up of the genetic diversity without interfering with progress of selection (for commercial breeds);
- for research purposes.

- Collection categories

Given the multiple purposes, several collection categories were considered in relation with the status of the breed. For local endangered breeds, the main collection is a Core Collection. The quantity of preserved material is evaluated to allow the reconstruction of a breed (to reach an effective population size of 50 individuals) but no update of the collection is planned. A part of the collection could be used as a Working Collection to support *in situ* conservation and as an Evaluation

Collection for example to verify the sanitary status of the donors or for genetic studies. For commercial breeds, Working and Evaluation collections will be the main types of collection and to a lesser extent a Historical collection could be constituted.

- Achievements until today

To date, semen, DNA and serum from 68 sheep of three local breeds (Entre-Sambre-et-Meuse, Ardennais Roux et Tacheté, Mouton Laitier Belge) were collected and preserved in the gene bank. The objective of preserving semen from 25 donors in each breed with 200 doses of semen per donor (FAO recommendations) will be reached at the end of this year.

- Future plans

During the three next years, we will collect the same material from two local pig breeds (Piétrain and Landrace Belge), two commercial cattle breeds (Blanc-Bleu belge and Rouge de Flandre) and two local horse breeds (Cheval de Trait Belge et Cheval de Trait Ardennais).

- **Participation of stakeholders: responsibilities/roles by stakeholder/actor**

- Laws, regulations or arrangements between stakeholders

A scientific and technical council was created in 2011 to make decisions about all the steps of the development and the management of the gene bank. It is composed of scientific experts (one expert of reproduction and cryopreservation and one expert of population genetics) from the three French-speaking Belgian university academies, from the Walloon Agricultural Research Centre and representatives of breeding associations and of the Public Service of Wallonia which funds the gene bank development. Within this council, conventions between stakeholders for the sampling, the conservation and the utilisation of biological material of the gene bank are established for each species. Concerning the sheep breeds, agreements are established between the council and the owner of the donor for all the steps realized by the managers of the gene bank: transport of rams from the herd to the collection centre, collection of biological samples for sanitary tests, conservation in the gene bank and use of the samples. Breeders are invited to provide information about the donors (pedigrees, sanitary data, etc.) to be stored in a database. Conventions will be made for other species taking into account specificities of each species concerning the process and the place of collect, sanitary requirements and the demands of the breeders in particular for the ownership and the use of the preserved material. Conventions are also written with breeding associations for the access and the conditions of use of pedigree data of the breeds. Sanitary protocols are set up for each species taking into account both national regulations and the risk of transmission of diseases through the use of the semen.

- Transboundary issues/arrangements

The development of the Walloon gene bank is based on FAO guidelines and the experiences of the other countries with gene banks for the sampling strategy, the protocol of collect, the sanitary requirements, the ownership issues, etc. At the Belgian level, contacts have been established with breeding associations of the Flemish region with which arrangements are made for access to information about the animals and the breeders. The gene bank includes material from donors of the two regions of Belgium.

It is planned to preserve material from the Blanc-Bleu mixte breed which is present in Belgium and also in France (called Bleue du Nord). Transboundary collaboration will be necessary to avoid to conserve material from the same populations in two countries. Other types of collaboration could be considered. For example, arrangements could be made to use material from a same breed preserved in another country if it can be beneficial to enhance the genetic variability of a local population. Secondary storage sites of the gene bank could be located in neighbouring regions or countries.

- **Decision making process**

- Type of material

Semen (ejaculated and epididymal sperm) was chosen as preserved material because this material is widely used in the insemination centres (easier to collect and to use). In addition, serum of each donor is preserved for sanitary tests and DNA for genetic analyses.

- Collection targets (populations, individuals)

It is planned to preserve local endangered breeds present in Wallonia and commercial breeds of Belgian origin for sheep, pig, cattle and horse species. For local breeds, the choice of donors is made to have a good representation of the genetic diversity of the population based on analyses of pedigree data, information about exchanges of animals between herds, molecular or phenotypical data. For sheep breeds, donors were chosen on the basis on the combined results of molecular (microsatellite markers) and animal exchanges analyses.

- **Storage and documentation**

- Storage facilities and rules

Semen doses are preserved in two storage sites (duplication of each sample): a primary site in the UCL university and a secondary site in a breeding association in Wallonia also. In each site, there are two storage rooms (one per sanitary status of doses, A or B based on the FAO guidelines) with one storage tank per species. Serum samples are located in the primary site and DNA samples in the laboratory that makes molecular analyses.

- Data management and documentation

A specific database with a Web interface was developed to save information about donors (pedigree, owner, phenotypic, genetic and sanitary data, etc.), the samplings (sampling dates, quality tests, processing information, etc.) and the collection (localisation of the samples in each tank of each location).

- Gene bank security

Low level nitrogen alarm devices were installed for each tank. The alarm will sound if the level falls below 25%. Alarm devices are connected to a central system sending messages to managers of the gene bank in case of alert. Access to the storage rooms is limited to the managers of the gene bank.

- **Sanitary arrangements/regulations**

Sanitary rules depend on both national health regulation and specific requirements decided by the scientific council. The national regulation depends on the species. For pigs and cattle derogations will be asked to collect samples in farms to avoid moving of animals, quarantine periods and associated costs. For each species, sanitary tests are realized for all the diseases with a transmission risk by semen. All samples from animals seropositive for any disease potentially transmitted by the semen are discarded. For specific diseases (for example for ovine catarrhal fever) tests on semen can also be performed to assess the quality of the semen in seropositive animals (for example after vaccination).

- **Legal issues (related to genetic material and data)**

- Ownership and IP

Ownership of preserved material is defined in conventions established by the scientific and technical council for each species. To date, such documents have been written for sheep breeds. The animal owner either renounces his ownership or maintains a veto right to the use of the material. In the two cases, utilisation of material is subjected to approval by the council. In the second case, the owner will be asked to contribute to the costs of collection and conservation. All the information about donors and preserved material are saved in the database. The access and the data utilisation are defined by the council.

- Collecting new material: Articles and conditions in Material Acquisition Agreements [translated agreement]

In addition to ownership issues, agreement between the owner of donor and the gene bank also includes in a single document:

- agreement on the transport and housing of the donors (if necessary);
- sanitary requirements: blood samples are tested for diseases with a transmission risk via semen and the owner has to mention the risk of diseases and gives the proof for vaccination(s) (ovine catarrhal fever for example);
- information about the donor to be saved in the database and access rights;
- agreement on insurance reimbursement in case of damage on animals during the transport, the housing and the collect;
- conditions of utilisation of the germplasm: the use of material is subjected to approval of the council.
- the list of the identification numbers of the animals concerned by the agreement.

Such agreements have been made only for sheep up to now and have to be adapted to the other species.

- Access to gene bank: Articles and conditions in Material Transfer Agreements [translated agreement]

No Material Transfer Agreement was established up to date.