

## Institutional and legal framework for ex-situ conservation in the Republic of Croatia

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### Historical overview

In January 2012 final version of Operational Programme for the establishment of the domestic animals gene bank in the Republic of Croatia was done. It was provided by the National Programme for the protection of autochthonous – native and protected breeds of domestic animals in the Republic of Croatia which was adopted earlier, at the beginning of 2010 year. The Operational Programme for the development of the Gene Bank is a well-designed, systematic, coherent and focused set of procedures and measures aimed at collecting, processing and storing tissue specimens of genetic material of interesting specimens with the function of their preservation.

### Objective(s) of national cryopreservation programme/policy

#### Collection goals

The main task of the Operational Programme for the establishment of the domestic animals gene bank in the Republic of Croatia is to point out and enumerate the main activities (measures) required for the establishment of the gene bank, which will be implemented from 2012 to 2016.

This multi-annual operational programme encompasses 26 autochthonous and protected breeds of domestic animals (9 sheep breeds, 4 horses breeds, 3 breeds of donkeys and cattle, 2 breeds of pigs, goats and poultry, and one breed of bees, Annex 1). Furthermore, the multi-annual operational programme predicts the collection and management of genetically interesting material of the conventional breeds of domestic animals.

Gene Bank is consist of systematically stored tissue and reproductive cells at selected locations and a traceable documentation and information system monitoring the storage of genetic material in Gene Bank Collection, its manipulation within the Collection and the accompanying operating procedures for specific situations.

#### Collection categories

The genetic material stored in the Gene Bank Collection is categorized according to:

- the level of storage (Core Collection, Working Collection),
- type of domestic animals (cattle, sheep, goats, pigs, horses, donkeys, poultry and other),
- type of tissue (semen/sperm, ova, embryos, tissue cells).

Whole collection of gene bank genetic material is divided in two categories:

1. Core collection of genetic material (which takes 65% of gene bank material)
  - genetic material permanently store in the Gene Bank. It is not generally used in the implementation of breeding programmes and may be used only in crisis situation (natural disasters, disease) which directly threatens the biological survival of the breed.
2. Working collection of genetic material (which takes 35 % of gene bank material)
  - material which serves as support to the *in situ* conservation programme of a breed, to research, needs of breeding organizations and use in genetic construction of other breeds.

#### Organizational structure of the Gene Bank of the Republic of Croatia

The institutional framework of the Gene Bank is consist of: state administration bodies and public institutions (Ministry; CAA and Croatian Horse Breeding Centre – Đakovo and Lipik National Stables), scientific and educational institutions; partner institutions with the required references in the area of agronomy and veterinarian medicine; breeding organizations (central breeders' unions,

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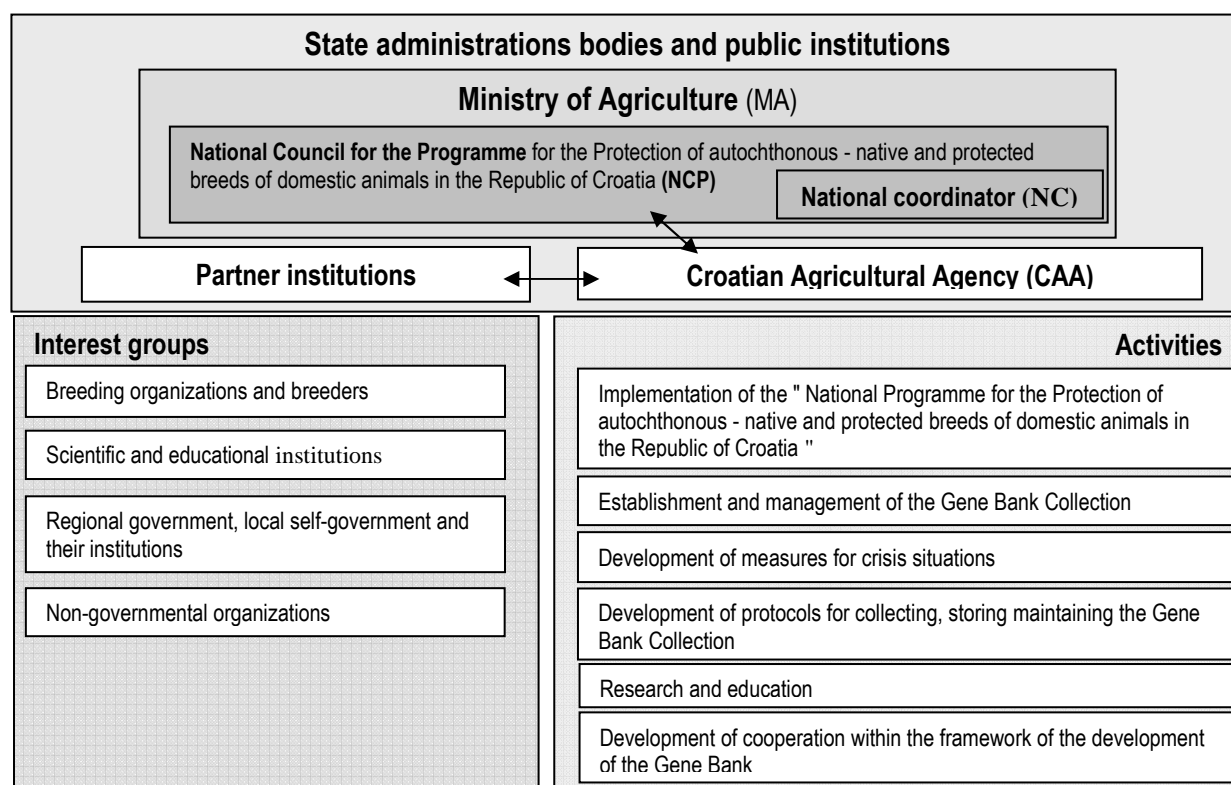
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breeders' unions, breeders' associations); non-governmental associations; companies from the related area (artificial insemination centres).

The genetic material stored in the gene bank is the property of the Republic of Croatia. Management of stored genetic material in the name of the Croatian Ministry of Agriculture is carried out by the Croatian Agricultural Agency (hereinafter: CAA), the central authority responsible for managing the Gene Bank Collection.

Although transboundary breeds exist, among Croatia and Slovenia, Austria, or other country contract or agreement on subject of exchange of genetic material is not yet established.

Scheme 1. Overview of the organizational structure of the Republic of Croatia's Gene Bank



### **Decision making process (selection and quantity of the genetic material)**

The genetic material stored in the Gene Bank Collection is chosen in a systematic and balanced way. The Gene Bank Collection primarily stores the genetic material of critically endangered breeds, followed by highly endangered breeds and potentially endangered breeds and, finally, non-endangered breeds (Annex 1). The type of genetic material collected and stored in the Gene Bank Collection is stored semen of male breeding animals (sperm), ova (oocytes), embryos, body tissues (somatic cells, blood, etc.), and isolated DNA. Two main principles on which the breeds and types of tissues stored in the Gene Bank Collection are selected are:

- the level of a breed's endangerment of extinction (i.e. the real and effective population size, proportion of population under the scope of breeding, degree of inbreeding, the level of risk exposure (disease, natural disasters, etc.), demographic structure of breeders of autochthonous species (i.e. age, motivation, education level).
- the conservation value of a breed (i.e. genetic uniqueness of the breed, genetic variability within the breed, possession of unique and economically important traits, genetic adaptability of the breed to a unique environment, cultural and historic value of the breed).

The necessary quantity of genetic material per breed is as follows:

- semen of male breeding animals (sperm): minimally 50 male breeding animals/breed; 300 doses/breeding animal; minimally 15 000 doses/breed

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- of this number, 200 doses per breeding animal (total 10 000 doses/breed) should be placed in permanent storage and named as Category I at two locations
  - the remaining 100 doses of each breeding animal (total 5 000 doses/breed) would be in the function of supporting the *in situ* programme (breeding programme), named Category R.
- b) ova (oocytes): minimally 50 female breeding animals/breed; 4 to 12 ova/female animal; 200 to 600 ova/breed
- c) embryos: minimally 100 embryos/breed
- d) body tissues (blood, somatic cells): minimally 200 animals/breed
- e) semen of allochthonous breeds interesting for breeding purposes (old bulls lines): 200 doses

For breeds with a small census number (autochthonous or rare breeds, critically endangered breeds,  $N_e < 50$ ), the genetic material of every available (and healthy) specimen is collected regardless of the degree of kinship. Selection of specimens within a breed should be conducted on the basis of pedigree (if any) in such a way that kinship between specimens is minimized, which contributes to the preservation of greatest genetic diversity.

Depending on the available source, genetic material is collected based on an annual plan made by the CAA together with its partner institutions, and which must be adopted by the NP Council. The genetic, reproductive and zoohygienic aspects are taken into account while drafting the annual action plan for storing genetic material in the Gene Bank Collection.

### **Storage and documentation of genetic material**

Storage of samples will be medium size with all necessary equipment and facilities. Measures of precautions should be always taken due to possibility of contamination of the genetic material stored in containers (like conservation of previously contaminated material, storing of samples of doubtful sanitary status, for example taken outside of planned collection location or animals not recoded by protocol, by persons unauthorized for collecting, etc.).

All the genetic material stored in the Gene Bank Collection must be properly labelled and labelling system depends on the type of genetic material that is stored. Packaging in which the genetic material stored is stamped with text (country's code, animal's unique number, tissue type code, and date of processing of the genetic material).

Additional data entered into the CRYO-IS HR database which accompanies the collection, processing, storage and activation of the stored genetic material. The information stored in CRYO-IS HR is selectively available to all stakeholders in accordance with their powers and rights.

### **Sanitary arrangements (measures) / regulations**

There are three important factors that shall be observed in order to reduce the risk of transferral of some diseases. They are: setting up quarantine, observing the procedures of collecting of genetic materials, and correct procedures with the genetic material after collection. Use of sanitary protocols and disabling of samples contamination will be consequent from personnel (use protective equipment defined by regulations: rubber boots or protective feet covers, protective aprons or overalls, rubber gloves etc.) and also applicable in using material like portable refrigerators, collectors, medical equipment, etc.

The genetic material of animals which are tested positive for diseases and are contagious, is not taken for storing. Since quarantined animals sometime don't show visible symptoms of diseases, sampling of blood or other tissue is recommended (for example nose wipes) for complete tests for presence of infectious agents.

### **Legal framework related to genetic material and data**

Existing legal frame (>21 laws, orders and ect.) and legal regulations that will be made shall govern the relationships between all the participants storing the genetic material into the gene bank,

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including the scope of genetic reserves, manner and collection procedures, storing and use and managing of records on biological material.

There are several approaches to the exchange of material between the gene bank and the animal owner, and the choice of most suitable one is on the team of professionals. Approaches are: the gene bank can buy an animal from the owner, the animal owner can donate the genetic material of the animal to the Gene Bank, providing a waiver of all the rights of the donated material, the owner of the donor animal can store the genetic material in the Gene Bank for certain time period (embargo) and after the embargo expires, the ownership over of the genetic material belongs to the Gene Bank. Documentation related to the ownership of genetic material is in phase of preparation.

Below stated elements should be taken into consideration: proprietary rights (proprietorship over cryoconserved genetic material should be clearly specified), costs of collecting, processing and freezing, rights of intellectual property (if the Gene Bank is founded as public property of a state, research results of use of materials in the Gene Bank should be publicly available, without the requirement for intellectual property), veterinary / sanitary issues (contract on ownership of the genetic material should contain the list of diseases the animal has been tested for), and data protection (the gene bank decides which information on stored genetic material shall be available to the public).

### ***International transfer of the genetic material stored in the Gene Bank Collection***

Secondary role of national Gene Bank is evident in the possibility of the international exchange of the genetic material where attention should be put to the regulations which govern the issue of health aspect. In this case main regulations are defined by the World Organisation for Animal Health (Office International des Epizooties, <http://www.oie.int/>). Protocols for transfer of genetic material between countries are used by the signatory countries of the Sanitary and Phytosanitary measures (SPS, [http://www.wto.org/english/tratop\\_e/sps\\_e/sps\\_e.htm](http://www.wto.org/english/tratop_e/sps_e/sps_e.htm)).

### ***Conclusions and future plans***

Founding, management and sustainable use of the Gene Bank is a complex project with interinstitutional cooperation as well as support of state institutions. Based on current knowledge, we present concise recommendations:

- project should be seen as the occurrence of permanent value which holds growth potential throughout the years,
- legislation should be regulated and harmonized with the relevant European legislation,
- proprietary rights should be regulated (samples),
- intellectual rights of property should be regulated,
- quality management system should be introduced,
- communication strategy and transparency should be defined.

## Annex 1. Overview of the status of endangerment of native and protected breeds in the Republic of Croatia

| Species      | Breed                      | Number of animals |      |        |        | Effective size of population (Ne) | Categorisation of breed endangerment status |
|--------------|----------------------------|-------------------|------|--------|--------|-----------------------------------|---|
|              |                            | Total (*estimate) | of   |        |        |                                   |   |
|              |                            |                   | Male | Female | Young  |                                   |   |
| Horses       | Lipicanac horse            | 1550              | 177  | 462    | 909    | 511.89                            | Potentially endangered                      |
|              | Croatian cold-blood horse  | 6300              | 265  | 2849   | 3181   | 969.79                            | Potentially endangered                      |
|              | Croatian Posavac horse     | 4900              | 124  | 2072   | 2713   | 467.99                            | Potentially endangered                      |
|              | Medimurje horse            | 57                | 5    | 21     | 31     | 16.15                             | Critically endangered                       |
| Donkeys      | Istrian donkey             | 220*              | 20*  | 150*   | 50*    | 70.58                             | Critically endangered                       |
|              | Littoral Dinaric donkey    | 2000*             | 200* | 1300*  | 500*   | 693.33                            | Highly endangered                           |
|              | North Adriatic donkey      | 150*              | 20*  | 100*   | 30*    | 66.66                             | Critically endangered                       |
| Cattle       | Buša                       | 563               | 33   | 308    | 222    | 119.23                            | Highly endangered                           |
|              | Istrian cattle             | 1041              | 36   | 627    | 378    | 136.18                            | Highly endangered                           |
|              | Dalmatian Gray             | 2000*             | 200* | 1300*  | 500*   | 693.33                            | Highly endangered                           |
|              | Slavonian Symirian podolac | 257               | 9    | 145    | 103    | 33.90                             | Critically endangered                       |
| Sheep        | Pag island sheep           | 30000*            | 218  | 4143   | 680    | 828.41                            | Not endangered                              |
|              | Krk island sheep           | 18000*            | 7    | 111    | 41     | 26.34                             | Potentially endangered                      |
|              | Lika sheep                 | 30000*            | 159  | 6553   | 1032   | 620.93                            | Potentially endangered                      |
|              | Ruda sheep                 | 712               | 38   | 564    | 110    | 142.41                            | Highly endangered                           |
|              | Rab island sheep           | 6500*             | 18   | 443    | 75     | 69.19                             | Potentially endangered                      |
|              | Dalmatian "pramenka        | 200000*           | 293  | 8184   | 838    | 1131.49                           | Not endangered                              |
|              | Istrian sheep              | 2314              | 96   | 1769   | 449    | 364.23                            | Potentially endangered                      |
|              | Cres island sheep          | 15000*            | 32   | 737    | 143    | 122.67                            | Potentially endangered                      |
| Tzigai sheep | 3000*                      | 26                | 1135 | 255    | 101.67 | Potentially endangered            |   |
| Goats        | Croatian white goat        | 5000*             | 4    | 58     | 13     | 14.97                             | Potentially endangered                      |
|              | Croatian spotted goat      | 35000*            | 30   | 394    | 97     | 111.51                            | Potentially endangered                      |
| Pigs         | Black Slavonian pig        | 5000*             | 109  | 896    | 297    | 388.71                            | Potentially endangered                      |
|              | Turopolje pig              | 400*              | 129  | 30     | 75     | 97.36                             | Critically endangered                       |
| Poultry      | Hen "Hrvatica"             | 1500*             | 103  | 949    |        | 371.66                            | Potentially end                             |
|              | Zagorje turkey             | 3000*             | 428  | 2255   |        | 1438.90                           | Not endangered                              |
| Bees         | Grey bee                   | 320000**          |      | 6500   |        |                                   | Not endangered                              |

\* estimate; source: annual report CAA, + bee hives