# Foreword

Livestock agriculture is in a period of tumultuous change and upheaval. General economic development, and population growth and mobility, have increased demand for livestock products, but have also placed pressures on the sustainability of rural environments and animal production systems. Livestock keepers will need to increase their efficiency to meet the rising demand while continually adapting their animal genetic resources to changing economic and environmental conditions. The genetic diversity necessary to allow this adaptation is in a state of decline, and the genetic resources that remain are not utilized in the most efficient way. *The State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007a) confirmed that a significant proportion of the world's 7 000+ livestock breeds are at risk of extinction and that many countries lack the technical capacity to ensure the proper management and sustainability of their animal genetic resources.

To address these problems, the Member Nations of FAO developed the *Global Plan of Action for Animal Genetic Resources* (FAO, 2007b) (*Global Plan of Action*), which was adopted at the first International Technical Conference on Animal Genetic Resources for Food and Agriculture in Interlaken, Switzerland, in September 2007. The *Global Plan of Action* contains four strategic priorities areas that provide a basis for enhancing the sustainable use, development and conservation of animal genetic resources throughout the world. It calls on FAO to continue to provide technical guidelines and assistance and to coordinate training programmes in order to support countries in their efforts to implement the *Global Plan of Action*.

Conservation of animal genetic resources is the third Strategic Priority Area of the *Global Plan of Action*. Conservation involves both *in vivo* maintenance and management of genetic diversity within livestock populations that are actively contributing to the livelihoods of their keepers or that are maintained in small numbers on research or demonstration farms and *in vitro* storage of genetic material that can be used at a later time to increase diversity in live populations or re-establish a population. A previous FAO publication on conservation – *Secondary guidelines: management of small populations at risk* (FAO, 1998) – covered both types of conservation. However, given the advances in technology and in the availability of information that have occurred during the past decade, the present guidelines will be complemented by a separate publication on *in vivo* conservation.

The development and operation of a gene bank for cryoconservation of animal genetic resources requires technical capacity in genetics, reproductive physiology, cryobiology and data management. Coordination among a wide group of stakeholders is also essential. These guidelines were developed to provide an overview of the fundamental issues involved in developing and operating gene banks as elements in comprehensive national strategies for the management of animal genetic resources.

# Acknowledgements

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The guidelines were prepared under the supervision of Paul Boettcher, with the full support of the Chief of FAO's Animal Genetic Resources Branch, Irene Hoffmann, and of officers of the Animal Genetic Resources Branch: Badi Besbes, Beate Scherf, Roswitha Baumung and Dafydd Pilling. Administrative and secretarial support was provided by Kafia Fassi-Fihri and Silvia Ripani.

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# The goal and structure of the guidelines

These guidelines are intended to serve as a decision aid with respect to the various cryoconservation options that are available, and to provide technical guidance on the design and establishment of animal gene banks. The guidelines are written under the assumption that a decision has already been taken that cryoconservation will make a valuable contribution to a programme for conserving the animal genetic resources of interest. The advice provided is intended to be relevant to all species of domestic livestock, but species-specific guidance is given where appropriate. Much of the information may also be relevant to cryoconservation of wild relatives of livestock and to other wildlife species. Thus, countries may consider developing joint gene banks for both domestic and wild animals.

The guidelines focus on cryoconservation of animal genetic resources. Matters related specifically to *in vivo* conservation, as well as general issues of conservation, are presented in a separate publication – In vivo *conservation of animal genetic resources* – forthcoming in this series.

The guidelines are intended to provide the technical background information needed by countries wishing to set up, implement and monitor gene banks. Although reading all sections is recommended, certain sections are aimed at specific stakeholders with specific technical interests and responsibilities.

The terms "cryoconservation" and "cryopreservation" are both used frequently throughout the guidelines. Although these words are in some cases interchangeable, an effort has been made to restrict the use of "cryopreservation" to the actual process of freezing biological material for long-term storage. "Cryoconservation" is used to refer to the conservation of animal genetic resources through the use of cryopreserved germplasm.

**Section 1** reviews reasons for conserving animal genetic resources and compares the various conservation options that are available. This is intended to help the reader confirm that cryoconservation will be a valuable component in a plan for conserving the animal genetic resource(s) under consideration (assuming a conservation programme of some kind is needed).

**Section 2** discusses what must be done before the freezing and storing of germplasm can start, i.e. the preparation, implementation and organization of gene banks.

**Section 3** discusses the objectives that can be addressed by gene banking programmes.

Section 4 describes the various types of germplasm and tissue that can be cryopreserved, as well as the uses to which they can be put. This is intended to provide a basis for informed choices regarding the type of material to store.

**Section 5** discusses requirements for, and costs of, establishing gene banks of various sizes and degrees of technological sophistication.

**Section 6** deals with the genetic issues that need to be considered when designing and implementing a cryoconservation programme, considering in particular the amounts

of various types of germplasm that need to be stored in order to capture desired amounts of genetic diversity. Biological material undergoes a number of (sometimes drastic) changes when subject to cryopreservation, and some of these will decrease the viability of the conserved germplasm.

**Section 7** describes the process of cryopreservation at cellular level, and the possible effects that the process may have on the stored material. This overview is intended to provide the basic information needed to diagnose and avoid damage to genetic material during the cryopreservation process.

**Section 8** describes methods for collecting and cryopreserving various types of genetic material from various species of livestock.

**Section 9** addresses the health and sanitary issues that must be considered when establishing and operating gene banks for animal genetic resources in order to help prevent the conservation of potentially dangerous pathogens along with the valuable genetic material.

**Section 10** describes documentation and database requirements for storing information on individual animals and on the samples of genetic material stored in the gene bank. To be of use, material stored in the gene bank must eventually be thawed and used to create new animals. Therefore, good organization and annotation of the stored material are essential.

**Section 11** addresses the legal issues associated with cryoconservation. Although animal genetic resources can be considered a public good, the animals from which germplasm is taken for cryoconservation are usually privately owned. Ownership may or may not change during the gene banking process, but the terms of agreement between gene banks and the breeders providing germplasm must be explicitly defined.

**Section 12** discusses priorities for capacity building and the need to train livestock keepers and extension workers. It also discusses the need for the inclusion of cryoconservation and related topics in higher education curricula.

The main sections are followed by a series of appendices, which provide step-by-step instructions on procedures for collection and cryopreservation of germplasm.

# Abbreviations and acronyms

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| **AI** | artificial insemination |
| **AnGR** | animal genetic resources (for food and agriculture) |
| **AV** | artificial vagina |
| **BSA** | bovine serum albumin |
| **CASA** | computer assisted sperm analysis |
| **DNA** | deoxyribonucleic acid |
| **ET** | embryo transfer |
| **FAO** | Food and Agriculture Organization of the United Nations |
| **FSH** | follicle stimulating hormone |
| **ICSI** | intracytoplasmic sperm injection |
| **IVF** | *in vitro* fertilization |
| **MTA** | material transfer agreement |
| **$N_e$** | effective population size |
| **OCM** | oocyte collection medium |
| **OIE** | World Organisation for Animal Health (Office International des Epizooties) |
| **OMM** | oocyte maturation medium |
| **PBS** | phosphate-buffered saline |
| **PHE** | penicillamine, hypotaurine and epinephrine |
| **SCNT** | somatic cell nuclear transfer |
| **TUGA** | transvaginal ultrasound-guided oocyte aspiration |