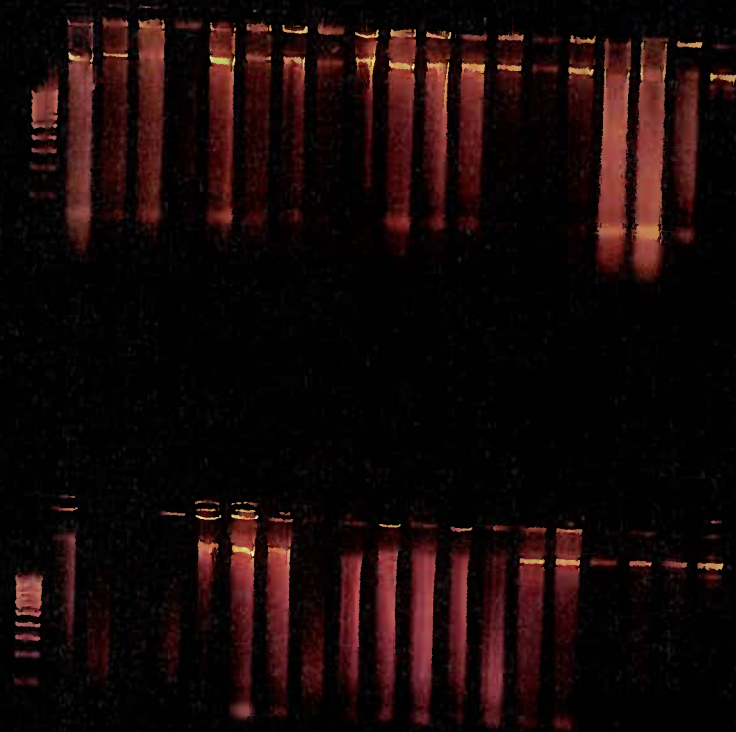


# DNA Banking for the 21st Century



Wendy L. Applequist and Lisa M. Campbell, Editors

# Preserving Biodiversity for Long-term Research: The Genetic Resources Repository of the Florida Museum of Natural History<sup>1</sup>

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## ABSTRACT

The Genetic Resources Repository (GRR) of the Florida Museum of Natural History (FLMNH) is a centralized facility that preserves DNA and other genetic resources of a wide array of organisms, mostly specimens derived from research projects of the divisions of the FLMNH. In a period of six years the collection had rapid growth and now encompasses 42,833 specimens with potential value for molecular studies that should prove instrumental to a broad array of research programs worldwide. The establishment and different facets of organization and administration of the GRR are here described, with the goal of contributing guidelines for the establishment of other genetic resources collections.

## RESUMEN

El Repositorio de Recursos Genéticos (GRR –siglas en inglés) del Museo de Historia Natural de Florida (FLMNH) es una colección centralizada de los recursos de genéticos los cuales son producto de los proyectos de investigación de las diferentes divisiones que conforman el Museo (FLMNH). En un período de seis años esta colección alberga a 42,833 especímenes con potencial para estudios moleculares, los cuales serán instrumentales para una amplia gama de programas de investigación a nivel mundial. Este artículo describe el establecimiento y varias facetas de la organización y administración del GRR que podrían contribuir como directrices para otras colecciones de recursos genéticos.

**KEY WORDS:** cryopreservation, natural history collections, DNA, tissues, liquid nitrogen

## HISTORICAL BACKGROUND OF CRYOGENIC PRESERVATION TECHNIQUES

Cryopreservation, a long-term method for the preservation of living cells at extremely low temperatures (ca.  $-190^{\circ}\text{C}$ ), has been used in biological sample storage since the 1960s, but the field has advanced greatly because liquid nitrogen is an alternative non-pollutant and high-efficiency method. This preservation

technique has been extensively applied in the medical field and has been influential in research, medical treatment, and preservation of cell cultures, blood, tissues, gametes, and embryos. Cryopreservation is also used at an industrial scale for food preservation and for the optimization of bio-reproductive technologies in aquaculture and agricultural industries (Bailey et al., 2008; Graham, 1996; Khadatkar et al., 2004). Biobanking and

<sup>1</sup> The establishment of the Genetic Resources Repository was funded in part by National Science Foundation Grant No. DBI-0545232. This paper was supported in part by iDigBio (NSF Cooperative Agreement EF-1115210).

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cryopreservation have played a major role in conservation projects devoted to the flora and fauna of entire regions, and for crops or livestock important for society (Berjak et al., 2011; Carolsfeld et al., 2003; Dixit et al., 2005; Fickel et al., 2007; Kaviani, 2011).

With the rapid advance of molecular and genetic research, and the fast decline of habitat and species diversity due to anthropogenic influences on ecosystems, collections that hold genetic resources are increasingly valuable for research. Natural history museums are increasingly considering the addition of cryogenetic collections to complement traditional specimen collections, but they typically face a number of impediments with regard to facilities, databasing, and operations. Here we present our experiences with establishing and operating the Genetic Resources Repository at the Florida Museum of Natural History at the University of Florida as a resource and incentive for other natural history collections to add similar facilities.

#### THE GENETIC RESOURCES REPOSITORY OF THE FLORIDA MUSEUM OF NATURAL HISTORY

The Genetic Resources Repository (GRR) was established as a collection of the Florida Museum of Natural History (FLMNH) in 2006, and its function is to centralize and preserve the genetic samples obtained through the seven divisions of the FLMNH (Birds, Fishes, Herpetology, Invertebrate Zoology, Plants, Lepidoptera, Mammals). The mission of this collection is to maximize the long-term value of the samples and to facilitate the use of these resources by the scientific community.

#### COLLECTION MANAGEMENT

The collection policy of the FLMNH (<http://www.flmnh.ufl.edu/collections/policy/>) regulates the operations of the GRR, but the

latter is an autonomous collection directed by a curator who supervises the management of the growing collection, the physical transfer of existing genetic resources to the GRR freezers, the development of appropriate data and loan management protocols, and loan authorizations. A collection manager is in charge of the maintenance of the facility, the preparation and storage of samples in the cryofreezer, the preparation and processing of loans, and the information management of the collection.

Due to the wide array of organisms deposited in this collection, and the different collecting and curatorial protocols used by the neontological collections where these samples originate, the challenges for the establishment of this collection were: 1) to consolidate the genetic samples under a common cryogenic storage facility, 2) to develop protocols for the preservation of the samples, 3) to capture and manage the information, and 4) to develop protocols for material loans. Herein, we describe the GRR collection, and the processes and protocols that were developed to achieve our mission.

#### NATURE OF THE GRR COLLECTION

As of 22 February 2014, the GRR served as a repository for 46,396 specimens with potential value for molecular studies. The collection represents a wide diversity of taxonomic groups and geographic regions (1289 families, 4410 genera, and 121 countries). More than half of the collection consists of specimens deposited in the GRR by the FLMNH Divisions of Invertebrate Zoology and Fishes (Fig. 1) based on surveys funded by the National Science Foundation. Tissues from the Birds, Fishes, and Mammal Divisions were prepared years prior to the establishment of the GRR, and although a subset of these specimens was obtained through research and exploration

projects, a significant proportion was acquired from the holdings of the FLMNH as the official repository for the State of Florida Natural History specimens (Florida Statute §1004.56). Therefore, the collection maintains important specimens from species of special interest in Florida (e.g. Florida panther, specimens donated by the Florida Fish and Wildlife Conservation Commission). Most recently, the GRR accepted genetic materials from the Florida panther conservation genetics program, formerly housed at the National Institutes of Health. The transfer of these specimens to the GRR reunites these genetic samples with the museum specimens from which they were derived.

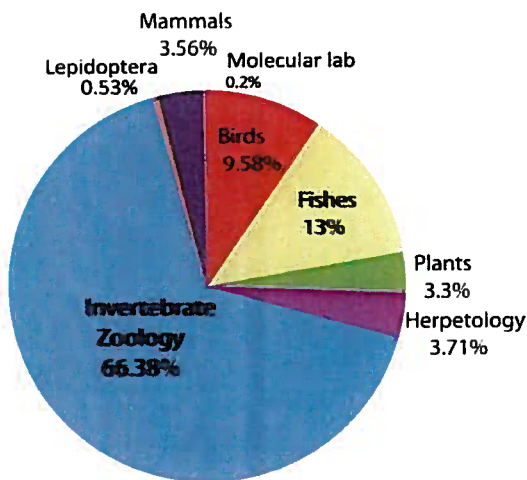


Figure 1. Summary of the collections deposited in the Genetic Resources Repository of the Florida Museum of Natural History.

#### SAMPLE TYPES

The GRR collection contains a variety of tissues and DNA extractions. Samples are stored in 1.8-mL vials (49 mm length, 12.5 mm diameter) that have a screw cap and a silicone gasket seal, specialized for vapor phase cryogenic storage. Tissues from the Fishes, Herpetology, Lepidoptera, and Invertebrate

Zoology collections are normally suspended in alcohol (variable concentration between 60 to 95%) and consist of a plug of tissue or a whole organism. Tissues obtained from the Mammals and Birds collections are frozen dry and are extracted from different organs of the specimens (e.g. liver, muscle, heart); in some cases the tissues have been previously treated with saturated salt (SED) buffer. Blood samples stored in FTA cards are mainly prepared for herpetological specimens and are stored in polycarbonate boxes suitable for cryogenic storage.

Plant accessions in the GRR consist of DNA samples, as well as leaf and flower tissues that have been previously dried in silica gel. Total DNA or DNA products are typically the result of research projects that have been concluded and are accepted in the GRR in quantities of 50 uL or more.

#### CRYOGENIC STORAGE FACILITY AND ITS ORGANIZATION

The GRR holdings are stored under cryogenic conditions (ca. -195°C/-319°F) in a liquid nitrogen vapor phase cryogenic freezer (Fig. 2A). The freezer contains a liquid reservoir below the sample platform that is the source of the cooling vapors that disperse along an internal jacket. The samples are therefore preserved under vapor storage and are never submerged in liquid nitrogen.

Currently the collection consists of one cryofreezer with a capacity for 70,200 vials. The system comes from the manufacturer (CHART MVE Biomedical, Ball Ground, GA) with an inbuilt rotating interior plate divided in six sections whose function is to provide easy access to the samples. Samples are accessed from the small offset neck door located on top of the cryofreezer (Fig. 2A - arrow).

Because of the default configuration of the cryofreezer, FLMNH ranges that contribute the largest number of samples have been assigned an entire section of the cryofreezer to accommodate their holdings and to allow growth (Fig. 2B). Each section holds nine racks, and each rack contains 13 boxes, with a capacity of 100 vials per box (Fig. 2C). Smaller collections share space within a section. An advantage of the spatial organization of the cryofreezer is that its clear delimitation (Fig. 2B) enables easy localization and also facilitates access to curators of the different ranges in the absence of the GRR collection manager or curator.

In order to facilitate recognition of the sections in the cryofreezer, and to locate racks, an aluminum colored tag has been placed on the racks handle (Fig. 2D), with the number of the rack and the letter of the section engraved on it (Fig. 2E). This feature is especially useful because nitrogen vapors obscure the reading of internal signs of the cryofreezer.

Boxes in each rack are identified with inert polyolefin white cryolabels that are top-coated and accept laser printing. Labels have an acrylic adhesive that easily adheres to dry or frozen surfaces, and they are placed on the upper left corner of the box. The box labels include the freezer number, the section, the rack number, and the box number (Fig. 2F). Boxes have 100 cells, each of which contains a vial (Fig. 2H) that is identified with a low-relief, self-laminated vinyl label with the following information: Vial number, Family, *Genus species*, Collector and number (if available), DNA number (if available), Vial number, and the identifier of the collection (GRR-FLMNH).

In order to store the information of the accessions deposited in the GRR collection, the spatial configuration of the cryofreezer dictated the requirements of the database. Currently a custom relational database is used

to manage the GRR collection; it is web-based, hosted on the FLMNH server, and integrated with all neontological databases to link genetic samples to their vouchers. The following section explains the fields and information captured for each accession.

#### INFORMATION STORAGE AND MANAGEMENT

Samples without adequate information diminish the scientific value of the specimen. Therefore, the GRR gathers complete information for each accession deposited in the cryofreezer. Few exceptions are made (e.g. fish or shark samples collected in markets), but, in general, collections without voucher information are avoided, and disassociation of specimens and their corresponding information is prevented. Table 1 lists and explains the fields that are stored in the GRR database. The information collected for each sample can be summarized in three types: (1) the exact placement of the sample in the freezer, (2) the collecting protocols and locality, supplied by the neontological collections, and (3) results and publications derived from the sample. The latter is stored in a different table of the database, and this information is a criterion for evaluating loan requests. For example, if tissues are requested for a project and the information is already available in GenBank, the requester is informed, and the loan request might be declined. Table 1 provides examples of the data stored in this field.

An important feature that was implemented for this collection is the field "Availability Status". The donors of the samples can choose among different categories to restrict or allow the use of the samples deposited in the GRR; these categories are: (1) Yes. No restrictions, (2) Yes. Contact donor to approve loan, (3) Yes. Notify donor of loan request, (4). No. Loans currently prohibited, (5) No. Sample

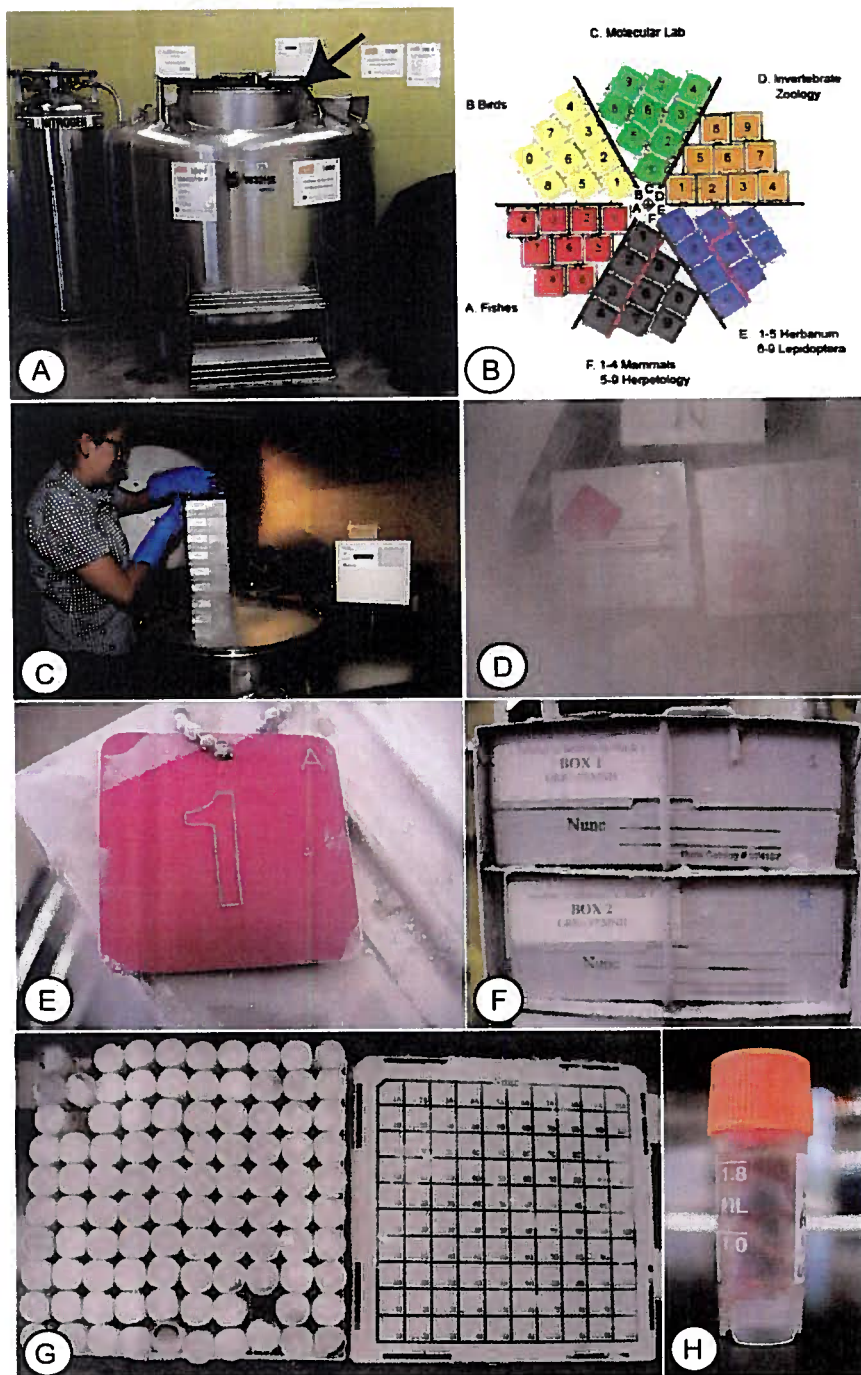


Figure 2. Organization of the Genetic Resources Repository. (A) Frontal view of the vapor phase cryofreezer of the GRR, arrow indicating offset neck door; (B) schema of the collection's arrangement; (C) rack retrieval with a detail of the offset neck door; (D) low visibility due to nitrogen vapors; (E) detail of aluminum colored tags used to identify the racks and sections of the cryofreezer; (F) boxes labeled to identify their corresponding section and rack; (G) internal organization of boxes and a detail of cells and vials; (H) cryovial used for specimen storage.

exhausted. The objective of this feature is to allow the donors to contact and collaborate with researchers requesting loans, to avoid replication of efforts, or to allow enough time for the publication of manuscripts. In the case that a donor chooses the category: "No. Loans currently prohibited", the policy of the GRR is to keep this category for a maximum of five years.

Another important field in the database is related to permitting. Specimens contributed by the neontological collections of the FLMNH have been legally incorporated into the collections, implying that the tissues donated to the GRR are within the legal protocols. After checking some permit documents, it has been found that in some cases permits explicitly prohibit genetic resources preparation or distribution to third parties without the authorization of the management authorities of the institution or country of origin. In the permit field, the latter information is recorded. This field is also intended to capture information of collecting and/or exporting permits (e.g. if a species is subject to restrictions under CITES or the Endangered Species Act). This is a precautionary measure that facilitates loans. When possible, digital files of the original permits corresponding to the samples are obtained, and although they are not currently in the database, it is expected that in the future permits will be linked to the GRR accessions in the central database.

#### LOANS AND TRANSACTIONS

There are two types of loans. Internal loans are requested by researchers within the FLMNH through a web-based application; external loans are conferred to users outside of the FLMNH. Information regarding the terms of loans and the application process are explained in detail at [\[ufl.edu/tissues/FLMNHGRR\\\_protocols.htm\]\(http://ufl.edu/tissues/FLMNHGRR\_protocols.htm\). Loan requests are received and evaluated by the GRR curator, who evaluates the merit of the project, checks the availability status of the samples, contacts the donor of the samples if necessary, and evaluates permit requirements. In general, preference is given to researchers who have demonstrated success in obtaining preliminary data that will guarantee the success of the project and have also collected materials independently and/or who contribute new holdings to biological research collections. Loans are not granted to research projects or individuals with commercial purposes. Loans are registered in a separate component of the database. A description of the data gathered is presented in Table 2.](http://www.flmnh.</a></p>
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#### COSTS

It is estimated that on a yearly basis, the average liquid nitrogen costs for operating the GRR approaches \$1,800 US. (corresponding to ca. 8,000 L of liquid nitrogen). To conserve resources and to maintain a stable temperature of the cryofreezer, the time that the system is opened for specimen deposit or retrieval is minimized (once per week). This policy may influence the processing time that for loan. To date, the cost per tube (including vials, labels, and the cell of the box where each tube is placed) is \$0.90 US.

#### FINAL REMARKS

The rapid growth of the GRR is largely related to the active research of the neontological collections of the FLMNH. The collaboration of researchers and collection managers who contributed samples and information associated with the collection in the correct formats greatly facilitated the establishment of the GRR. The diverse specimens are valuable resources for ichthyologists, herpetologists,

malacologists, ornithologists, mammalogists, and botanists, as well as for state and federal natural resource agencies. As of 19 July 2013, 2450 loans had been processed, demonstrating that in the short period since the GRR was established, it has been extremely active and is achieving its mission. This synopsis of the establishment and operation of the FLMNH's GRR will hopefully contribute to the founding of similar facilities at other institutions.

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