BEST PRACTICES FOR GENETIC RESOURCES ASSOCIATED WITH NATURAL HISTORY COLLECTIONS: RECOMMENDATIONS FOR PRACTICAL IMPLEMENTATION

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Abstract.—Researchers associated with natural history museums have made the collection of genetic resources a priority due to their importance in molecular studies, but often the long-term curation of these collections is difficult due to decentralized curation over multiple storage locations and lack of community best practice guidelines for their stewardship. Unlike traditional natural history specimens, the research utility of genetic samples increases with lower storage temperatures and fewer freeze–thaw events and, in addition, their use is consumptive. Collection managers must, therefore, maximize the research potential of each sample by carefully considering use on a case-by-case basis. This paper presents standardized guidelines accumulated for the management of genetic collections associated with natural history collections. These recommended practices are informed by general standards for biorepositories and augmented by information unique to natural history collections with the goal of providing a foundation for those curating genetic samples. Information pertains to all aspects of genetic sample curation and will assist those in making decisions regarding how to collect, store, track, process, and distribute genetic specimen samples. These guidelines also will allow users to make informed decisions regarding how to apply and improve the curation of their collection given their institution's goals and available resources.

Introduction

Genetic resources have become a fundamental and central part of natural history collections, expanding the value and usefulness of traditional scientific specimens to include genetic analyses. Although collections are generally focused on the preservation of traditional specimen preparations for the examination and comparison of morphology and anatomy, genetic and genomic collections encompass numerous sample types, including frozen tissues, chemically preserved tissues, and/or associated extracts. Genetic samples differ from typical museum specimens by the likelihood of their complete consumption and, unlike traditional specimen types, they generally must be collected, transported, maintained, and monitored in a low-temperature environment to retain a broad utility for molecular studies. These collections often require specific expertise to assemble, and the appropriate processing and storage is needed to maintain high-quality samples for long-term use. In addition, collection managers must comply with increasing regulations (e.g., Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity; CBD 2011) that govern complex issues regarding sample ownership, transport, storage, and use. For these and numerous additional reasons, this type of collection poses a number of unique curation challenges to the natural history community.

No current and comprehensive best-practice standards exist for the documentation, arrangement, and housing of genetic resources associated with natural history museums and other collections with similar educational missions, such as botanical gardens, colleges/universities, science museums/science centers, and zoos. Those working to establish genetic resource collections within natural history collections often must refer to more general guidelines for biorepositories or those published for other specific purposes, such as preservation of human samples (OECD 2007, NCI 2011, ISBER 2012). The

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curatorial problems unique to frozen tissue collections were first highlighted as a result of a workshop on frozen tissue collection management, outlining methods for collecting and preserving tissues for molecular studies, as well as presenting information regarding regulations governing acquisition and transport of tissue samples (Dessauer and Hafner 1984). Prendini et al. (2002) also presented practical guidelines for the collection and storage of zoological and botanical samples, but, with many advances in these methods over the past decade, this information and that presented by Dessauer and Hafner (1984) are now dated. Nagy (2010) outlines the tissue-preservation methods originally presented by Prendini et al. (2002), but this work does not address unique issues relating to the storage and use of genetic resources in natural history collections. A number of case studies related to nonhuman tissue and DNA banking have been published, but only present the policies and procedures used for a single collection or institution (Corthals and DeSalle 2005, Corthals 2006, Fay et al. 2006, Franco et al. 2006, Miller 2006, Campbell et al. 2012, Endara et al. 2014). A recently published survey of genetic collections associated with natural history museums revealed that curation practices involving sample collection, processing, storage, and distribution varied greatly (Zimkus and Ford 2014). In addition, more than one-half of the collections surveyed that had written departmental or institutional guidelines had not used any published resources in the development, curation, or management of their genetic samples.

''Best practices'' are defined as consensus-based practices that comprise both standards and guidelines (Cato 2001). The reasons that best practices should be set for the management and curation of genetic resources collections are similar to those for other natural history collections and include the following (based on Cundiff 2011):

- Standard procedures are methods that consistently work well for collections;
- Standardized terminology improves consistency in record keeping (Holm 1998);
- Standards outline the appropriate use of the collection (Stanley 2004);
- Standards can be used to justify the allocation of resources needed for curation; and
- Standards address the special needs of a collection and act as a resource for those curating the collection, especially if they do not have a background in the field or practical knowledge of the subject (Stanley 2004).

This paper presents the most current and effective practices for the curation of genetic and genomic collections associated with natural history collections, which can also be applied to other academic institutions and nonprofit organizations. These guidelines provide a decision-making method for preventative conservation by presenting data from both evidence-based methods and current practices used by natural history museums provided via community survey data (Zimkus and Ford 2014). It should be noted that although these guidelines can be applied globally, references to regulatory agencies and legislation are specific to collections within the USA because survey results had a 73% US and 27% non-US representation; collections outside the USA should research their relevant national and local regulations and laws. Most repositories associated with natural history museums maintained genetic samples for use in DNA analyses; research involving the extraction of highly ephemeral molecules (e.g., RNA) or whole-genome sequencing might require more detailed documentation, processing, and storage parameters. In addition, few natural history collections currently preserve living cells (e.g., cell lines, tissue cultures; Zimkus and Ford 2014); the cryopreservation of living cells requires additional measures (e.g., controlled-rate freezing, vitrification with the addition of cryoprotectant solutions) to prevent damage caused by formation of ice crystals within

the tissues (Karlsson and Toner 1996). Even though these studies require stricter standards for research success, the guidelines presented here are generally applicable. These recommendations are not designed to address the curation of medically-valuable and/or human samples, the latter of which are governed by additional regulations and require informed consent signed by the subject (Bell et al. 2010, Grizzle et al. 2011, NCI 2011).

The recent ratification of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity (CBD 2011) draws high-level attention to the access and use of genetic resources by calling on greater accountability and documentation. The CBD defines ''genetic resources'' as ''genetic material (any material of plant, animal, microbial or other origin containing functional units of heredity) of actual or potential value'' (Schei and Tvedt 2010). It should be noted that the term ''genetic resources'' is used both in this publication and the recent survey by Zimkus and Ford (2014); however, the use of this language does not imply or assign value to genetic material, including extracts and biproducts, because assigments of value to genetic resources must be made on a case-by-case basis by contracting Parties to the CBD. These recommendations do, however, provide best practice means to enable more detailed accountability for genetic resource collections.

Collection personnel must always balance institutional goals with the curation needs of their respective collections. Therefore, the recommendations provided can be broadly or narrowly applied, depending on the mission of the institution and other factors that influence the curation of genetic samples, including budget, staffing, and space. These guidelines provide an organized method to evaluate the relative costs and benefits of various strategies and to prioritize potential improvements and minimize risks to genetic collections, which is applicable to both enhancing a single existing collection, as well as those forming a centralized repository composed of samples from multiple independent collections.

Best Practice Recommendations

Policy Best Practice

Many natural history collections have institutional guidelines that outline how traditional specimens should be curated, but these guidelines generally are not specific enough to address the special curation needs of genetic samples. Therefore, all genetic repositories should develop dedicated written policies, including a Standard Operating Procedure (SOP) and a comprehensive guide to operations, to ensure that genetic samples are curated consistently for long-term preservation and use. Policy should govern all aspects of the use and storage of materials housed in the collection, including acquisitions, accessions, and cataloging procedures, as well as the active care and use (i.e., loan, gift, material transfer) of the genetic samples. In addition, the curation activities resulting from the near-exhaustive or exhaustive use of genetic samples (e.g., subsampling, deaccessioning) should be addressed in policy documents, because this situation is unique among traditional natural history collections. Policies and procedures should be written by those with experience in the management of the genetic collection and should comply with all governmental and regulatory requirements. All personnel should be trained using these written guidelines to ensure accuracy and consistency of procedures when working in the repository.

The SOP document should outline the tasks performed by the personnel of the repository, summarize the scope of activities performed, and address how the collection

satisfies the mission and goals of the institution. In addition, the SOP should summarize access to the collection, equipment and tools present, information technology needs, mandatory training for personnel, health and safety programs, personal protective equipment (PPE) required, equipment monitoring, maintenance activities, backup precautions, and emergency procedures. Collection managers should ensure that all genetic samples, even those without associated traditional voucher specimens, were acquired and transported legally, and policy documents should specify where and how copies of all relevant permits and acquisition documents are maintained. The SOP should also outline any specific requirements for acceptance of genetic samples, which can include provisions regarding acceptable sample types, preservation methods, storage containers, or sample organization (e.g., sample vials arranged numerically, data and vials submitted concurrently). Policy should also be clear regarding minimal requirements for the data associated with genetic samples (e.g., submission method, acceptable formats, Darwin Core standards; Wieczorek et al. 2012). All aspects of a collection's loan/gift policy should be clearly outlined, including the requirements for requesting genetic samples, internal procedures associated with the approval or authorization of loans/gifts, and an explanation of any fees associated with material transfers. The SOP should also include the contact order for personnel in case of operational questions or emergencies, and this information should be clearly posted in the collection. A more comprehensive guide to operations should detail the complete day-to-day collection procedures, including specimen processing (e.g., accessioning, cataloging, sample transfer, sample labeling, sample tracking), temporary and long-term sample storage criteria, database procedures, detailed use of equipment (e.g., operation, calibration, certification, maintenance, repair), subsampling procedures, shipment of loans/gifts, and disposal of waste, including biohazards.

Funding and Budget

The current and future potential of a collection for researchers conducting molecular studies is highly impacted by the efficient monitoring and storage of the genetic samples. A malfunction in simple equipment, such as a freezer, can render a genetic or genomic collection useless. To fulfill their research mission and ensure the long-term conservation of genetic collections, repositories must function efficiently, which requires dedicated and consistent funding. The successful operation of genetic resource collections is complex, and detailed budgets are needed to ensure that support from internal and external sources is sufficient to offset operational costs. Comprehensive budgets allow institutions to track spending associated with curating genetic resources so that they understand the costs of maintaining their collections and can make informed decisions. For those collections that are not centralized, the cost of curating genetic samples is often included in the overall budget for the institution or department, or funds come from personal research budgets (Zimkus and Ford 2014). Budget cost trends can also help these institutions estimate whether the costs of having separate departments curate genetic resources could be reduced if these collections were centralized.

Operational budgets should include labor (e.g., personnel salary, benefits), equipment, equipment maintenance, supplies (e.g., chemicals, consumables, equipment accessories, labware), shipping costs associated with loans/gifts (if not paid for by researchers), and facilities maintenance; plus, they should be reviewed regularly to account for any changes to procedure. The completion of an operational budget allows those managing

genetic and genomic collections to pinpoint specific areas where funding is deficient. Some collections have found that calculating the average curation cost per sample provides valuable information regarding the funding needed to maintain collections (Bradley et al. 2012, Baker et al. 2014). Repositories can then decide whether to apply for external funding to assist in immediate projects, such as the organization or labeling of genetic samples, or in long-term projects, such as building a centralized facility. The type of institution affiliated with the repository will affect the types of public or private funding available to the institution as a whole. In addition, detailed budgets allow those managing the genetic resource collections to determine if users must pay fees to offset processing or shipping costs for loans/gifts. Zimkus and Ford (2014) found that more than one-third of those collections surveyed requested that researchers offset costs of receiving loans/gifts of genetic samples, including paying shipping costs or a processing fee, the latter being assessed either on a per transaction or per sample basis. Thirty years ago, Baker and Hafner (1984) found that researchers paid for shipping costs in 43% of the tissue collections surveyed. With the increasing number of laws governing genetic samples (e.g., Convention on Biological Diversity), Applequist (2014) suggested that collections refer to fees associated with loans/gifts as ''shipping and handling charges,'' rather than ''per-sample fees,'' to clarify that repositories are not selling samples for profit.

Facility Management

Regardless of whether genetic collections are in separate locations in an institution or are housed together in a centralized repository, those curating these collections should clearly examine the storage conditions and address any issues related to the space(s). Addressing potential issues, such as restriction of access to collection rooms and/or cold-storage equipment, reorganization of equipment, improvements in lighting, upgrades to flooring, increased ventilation, and backup precautions, can ultimately improve the long-term preservation of genetic samples.

Access and security.—Genetic samples have significant inherent value to research because they are often collected from remote locations as a result of costly and time-consuming collection trips. Samples should, therefore, be stored in secure locations to minimize misplacement or inadvertent loss, and their access and use should be limited to those with the appropriate training. A risk assessment should be conducted to assess both building and collection security, including current procedures and protection devices. In addition to aspects of health and safety, training should encompass best practice techniques for handling genetic samples and the appropriate use of cold-storage equipment (see Operational Best Practice: *Training*). Many genetic collections currently store samples in a room or facility that remains unlocked during the day, which is likely because many research collections are stored within college and university laboratories allowing student access (Zimkus and Ford 2014). If access to rooms where genetic resources are stored must remain unimpeded, individual freezer units can be locked with key access restricted to approved and trained users. Policies should be developed in relation to visitors to ensure that they have the appropriate training before working with collections (see Operational Best Practice: *Training*). Records of training and activity in the collection areas should be maintained and archived for security, as well as health and safety requirements (see Operational Best Practice: *Records Management*).

Space planning.—Space planning encompasses the organization of equipment (e.g., storage units, tools), adequate workspaces, and storage for supplies, in addition to the

collections themselves. Although the layout of a functioning work area might not be greatly altered for an established collection, minor changes can be made to the placement of laboratory tables, desks, shelving, biosafety cabinets, or benchtop equipment (including centrifuges, electrophoresis equipment, incubators, rockers, scales, spectrophotometers, and tabletop autoclaves), that can increase research efficiency or improve functioning of equipment. Understanding every procedure that will be undertaken in the space will allow more accurate organization of the space, which is essential for those designing a centralized repository, as well as those working to improve the organization of a single laboratory. The inclusion of dedicated space for working with genetic samples or hazardous chemicals (e.g., biosafety cabinet, chemical hood, designated laboratory bench, or room) is recommended to protect both personnel and samples (Savolainen et al. 2006). An essential, efficiency-improving addition might be a dedicated cold-storage unit for specimens being actively processed, either prior to permanent storage, or while being subsampled or aliquoted for a loan/gift. Cold-storage units should be selected and organized based on their work purpose, because the type of storage might differ between those used for temporary storage (e.g., refrigerator units, freezer units) and those for long-term (e.g., mechanical freezer units, liquid nitrogen cryovats).

In a genetic resource collection, open spaces must be reviewed along with dedicated storage areas. Efforts should be made to ensure that mechanical refrigerator and freezer units are placed with sufficient space around them to allow for adequate airflow because compressors can overheat if units are placed too close to other units or walls. Moveable or mobile equipment, including laboratory carts or dewars (cylinders) of liquid nitrogen (LN_2), should have dedicated resting places so as to not obstruct exits, access, or equipment, including fire extinguishers, eyewashes, or safety showers. In addition, routes traversed for specimen transport, maintenance of equipment, and delivery of dewars of LN_2, should be reviewed to consider facility and/or safety issues, such as elevators (e.g., personnel may not transport pressurized dewars in elevators if ventilation does not accommodate oxygen deficiencies), steep inclines, floor surfaces, and movement through populated and public areas.

Lighting.—Lighting in the collection space should be sufficient to allow personnel to complete small-scale tasks, including examining vials for cracks, reading hand-written or typed labels on containers or subsampling specimens. If the light source is in close proximity to frozen samples (i.e., task lighting), a lighting unit that generates minimal heat (e.g., florescent fixtures, LEDs) should be used instead of incandescent or halogen lighting because these latter can cause samples to thaw. Emergency lighting that functions in the event of power loss should also be present to illuminate exit routes and, if needed, allow personnel to monitor cold-storage equipment (see FACILITY MANAGEMENT: *Backup Precautions*). Focused, portable light sources (e.g., flashlights, headlamps, battery-powered lanterns) should also be readily available within the collection in case of emergencies or if needed to examine equipment.

Flooring.—Flooring used in genetic repositories should be durable enough to accommodate all activities performed in the space. Flooring surfaces should be washable, able to support heavy cold-storage equipment without cracking, and level so as not to obstruct the movement of equipment, including laboratory carts and dewars (cylinders) of LN_2. If LN_2 is being used, the flooring should not crack if spills or leaks occur. Flooring surfaces, such as vinyl tile and linoleum, are not recommended because they can crack and lift, presenting a hazard. Sealed concrete is recommended over epoxy because if LN_2 is spilled on the latter, it can break the seal between the epoxy and concrete, causing cracking. If harder surfaces, such as concrete, are selected because of their durability,

antifatigue mats should be placed in areas where personnel stand for prolonged periods of time for potential ergonomic issues. Additionally, absorbing mats or floor grates might be needed in the front of refrigerator condensers or in the vicinity of freezers to protect personnel from slipping on occasional condensation or ice.

Ventilation.—Ventilation must be adequate in all collection spaces to prevent excess heat or humidity and, more importantly if using LN_2, it must consistently maintain sufficient oxygen levels. Excess heat and humidity within collections cause mechanical units to overheat and create condensation, which can damage equipment and result in an electrical shock hazard. Using liquid nitrogen for maintaining genetic collections requires constant monitoring of oxygen levels because nitrogen is a colorless, odorless and tasteless gas/vapor that is an asphyxiant, causing oxygen depletion. If LN_2 leaks into an enclosed space from a defective cryovat or supply dewar, oxygen levels might become depleted enough to be unsafe for occupants (see HEALTH AND SAFETY: *Liquid Nitrogen Safety*). To understand potential oxygen depletion due to LN_2 use in a genetic collection, the normal evaporative losses from storage vessels and the spillage of the entire contents of a vessel should be estimated for each collection room. Potential oxygen depletion can be determined by analyzing the size of the room, evaporation loss for each vessel (reported by the manufacturer as ''volume of LN_2 lost per day''), total spillage of each vessel, and the number of air changes in the room per hour. If neither normal evaporation losses nor spillage cause a significant reduction of oxygen content in a room, then general exhaust ventilation or use of natural ventilation (e.g., opening doors, windows) can be considered as an option to maintain safe oxygen levels. Natural ventilation is not always possible, however, because some laboratories might have institutional restrictions or special designations (e.g., Biosafety Level 2 or 3) requiring that windows are sealed and doors shut during operation. If risk assessment calculations of potential oxygen depletion indicate that evaporation losses and/or spillage would significantly deplete oxygen concentrations, forced-air ventilation must be installed. For accuracy in such high-risk matters, a professional engineer should be contacted and the health and safety group of the institution consulted.

Forced-air ventilation can include extractor fans that run continually, or fans that are either activated manually or automatically. An extractor fan that runs continually provides 100% exhaust and assures that none of the cycled air is returned back to the air handler. A noncontinuous extractor fan is a ventilation system activated in one of two ways: manually by the user when alerted that oxygen levels are low or, preferably, by an automatic trigger because this option removes the need for human intervention to rectify the issue. Automatic activation of an extractor fan can be linked to sensors that detect room occupancy and/or oxygen levels (see FACILITY MANAGEMENT: *Oxygen Monitors*). Using oxygen levels to activate an extractor using a predetermined set point has the advantage that it can remove built-up nitrogen that results from slow leakage when a room is not occupied. Having both occupancy sensors and oxygen levels activate an extractor fan serves as a safety redundancy should one sensor fail. Regardless of the type of ventilation system chosen, exhaust systems should include forced extraction ducts that are installed at low heights close to the floor because cold nitrogen vapors are denser than ambient air and settle at floor level. Exhaust ducts should also be in close proximity to storage units for the most efficient removal. With all ventilation systems, noise exposure from fans must fall within limits recommended by the Occupational Safety and Health Administration (OSHA), and collection managers should contact their environmental health and safety group to evaluate the noise levels and attenuate the fans if needed.

Oxygen monitors.—Ambient air normally contains 20.9% oxygen by volume, but nitrogen gas can quickly displace the air in the event of a spill or failure of storage equipment, reducing the percent of oxygen to unsafe levels. By OSHA standards, a ''hazardous atmosphere'' can include one that is oxygen-deficient, containing less than 19.5% oxygen by volume. This type of environment exposes people to the risk of incapacitation, impairment of ability to self-rescue (i.e., escape the area unaided), injury, acute illness, or possible death. Oxygen monitors should, therefore, be present in all rooms where LN_2 is stored or dispensed, and these monitors are essential in rooms where personnel are working with LN_2 storage equipment. Some ventilation systems using extractor fans can be automatically activated using oxygen levels; these systems can operate independently or be tied into the general room oxygen monitors (see FACILITY MANAGEMENT: *Ventilation*).

Oxygen monitors can be either portable or permanently installed within the collection space; their placement might be regulated by local authorities. Portable monitors are small, light-weight, and can be carried by individuals working in a room with LN_2 equipment. Permanent installations, which are either battery operated or electrical units mounted to the walls, are recommended over portable units to ensure constant and consistent surveillance of oxygen levels. Displays that continuously indicate the oxygen level and include alarms should be mounted approximately at eye level; however, sensors should be positioned closer to floor level but above the level of any exhaust-extraction fan located in close proximity. To assure that oxygen monitors comply with local regulations and function accurately, a professional engineer should be contacted and the health and safety group of the institution consulted. Regardless of whether these devices are fixed or mobile, oxygen monitors should include both audible and visual alarms. Additional alarms in adjacent rooms or hallways are recommended to prevent personnel from potentially entering a room with deficient oxygen. Alarm notification to a remote location is also a worthwhile precaution, because emergency personnel and those responsible for the collection can be alerted to problems. Unfortunately, some electrochemical sensors used within oxygen monitors are sensitive to the surrounding environment and are subject to drift with barometric changes or can even fail if placed in extremely cold temperatures. In addition, the sensors used within some oxygen monitors can become saturated over time, so they must be replaced at regular intervals. Dedicated maintenance and periodic calibration of the monitoring devices is therefore essential for all collections using LN_2. Both the SOP document and comprehensive guide to operation should contain the information needed to properly maintain and regularly test oxygen-monitoring equipment.

Backup precautions.—Collections that use cold-storage units to store genetic samples should include backup precautions to protect against power loss and ensure constant temperatures, thereby preventing sample loss (Hanner et al. 2005). Backup precautions are the necessary counterpart to a monitoring program, which protects samples from loss due to specific equipment failure (see OPERATIONAL BEST PRACTICE: *Manual Monitoring*). Fortunately, audible or visual alarms are included in many cold-storage units, but are merely the first step in protecting genetic samples. A risk assessment should be conducted to determine the appropriate backup system given the cold-storage type (e.g., refrigerator, mechanical freezer, LN_2 cryovats), institutional infrastructure (e.g., available backup power sources), regional factors (e.g., risk of severe weather, proximity to populated areas), and the running duration of each type of backup system. Automatic systems are recommended over those that must be activated manually to ensure that collection storage equipment remains functional at all times, even when personnel are not

readily available. It is also recommended that equipment alarms include both on-site and off-site notifications. Off-site alarms include 24-hour monitoring and ensure that the appropriate personnel can be contacted at any time in case of emergency. Contact information for multiple staff members trained to respond to emergencies should be included in the SOP document and posted within the collection to ensure that emergencies and alarms are addressed appropriately and immediately. Written emergency procedures should address how personnel should respond to various situations (see Operational Best Practice: *Emergency Procedures*).

As part of a risk assessment, collection managers should identify what equipment is essential and requires backup power in the event of power loss. Risk assessments should be conducted regularly because contents of individual cold-storage units can change, which might alter priorities for backup power. Mechanical freezers should always be connected to backup power. Managers of collections using LN_2 storage units should consider having backup power for the LN_2 cryovat controllers to ensure that cryovat units continue to receive LN_2 supply from dewars when the commercial power is not present; however, the addition of LN_2 can be done manually if needed (see Genetic and Genomic Collection Storage: *Liquid Nitrogen Cryovats*). Environmental monitoring systems and safety equipment, such as oxygen sensors, specialized ventilation, and emergency lighting, should also be protected by backup measures.

If regular power is lost, an auxiliary or emergency-power system generally uses an automatic transfer switch to connect to emergency power. Most emergency-power systems use diesel, natural gas, or liquid propane gas-driven generators to power the identified essential equipment in a facility. Multiple generators might be necessary to support large collections if many mechanical freezers are present. Enough fuel should be present to sustain power continuously for at least 48 to 72 hours, but there also should be an established plan for how fuel supplies can be replenished if needed (ISBER 2012). An uninterruptible power supply (also called uninterruptible power source, UPS) or battery/flywheel backup can also provide short-term emergency power if commercial utility power fails. UPS units are designed as a steady, continuous power source during interim breaks in power and generally only function for relatively short periods of time. In an emergency situation, these units can provide personnel with essential time to connect to other secondary power sources, such as backup generators. In addition, some mechanical freezers require users to manually restart the unit after a power failure to protect the compressors, and an UPS would make this restart unnecessary. Power systems should be stored in an easily accessible and dedicated storage space so collection personnel can access them when needed. All backup power systems should be tested on a regular basis as part of the SOP to ensure that they function and can sustain the electrical load in an emergency (see Policy Best Practice).

Genetic and Genomic Collections Storage

The type of storage used in a genetic or genomic collection ultimately influences the potential future uses of samples in molecular research because colder temperatures reduce cellular degradation. Documentation of the number of freeze–thaw events and any chemical preservatives or buffers should also be considered because genetic sample integrity might be reduced, depending on the preparation, as well as the duration and number of freeze–thaw cycles. Collection managers must, therefore, track all storage methods utilized and usage events for their genetic samples from the time of initial collecting until their use for research.

Temperature considerations.—Collections might include genetic samples preserved in a number of ways, depending on the size of the collection (including estimated future growth), personnel, funding, dedicated space for equipment, and length of time the samples will be stored. Cryopreservation at very cold temperatures is considered the most effective technique for the long-term stabilization of genetic samples because it inhibits enzymatic and chemical activity that leads to sample degradation (Engstrom et al. 1990, Karlsson and Toner 1996, Kilpatrick 2002, Mutter et al. 2004, Corthals and DeSalle 2005). When genetic sample temperatures are reduced below −137°C (the vitrification point of water), biological activity substantially slows and, at −196°C (the boiling point of liquid nitrogen), DNA degradation virtually stops because there is insufficient thermal energy for chemical reactions. Thus, the type of cold storage chosen for samples affects the long-term viability of those samples, with the most reliable form of cryogenic preservation being the storage of samples at temperatures below −137°C and improving as storage temperatures decrease below this threshold.

In addition to long-term storage in a collection, there must be considerations for short-term storage situations in relation to when samples are collected and transferred, such as for loans/gifts. Various methods, including freeze-drying samples or preparing them using buffers, chemical preservatives, or desiccants, are currently used for the initial preservation of genetic samples (Gemeinholzer et al. 2010, Nagy 2010, Buś and Allen 2014). These methods, which generally allow genetic samples to be stored at room temperature, are most appropriate for short-term storage or transport and not for long-term preservation (see Genetic Sample Processing: *Initial Preservation Procedures*). Long-term viability can be increased for genetic samples initially preserved using these methods if they are ultimately stored in a low-temperature environment. In general, genetic samples should be stored at the lowest temperature possible at any available time to reduce degradation and maximize future use.

Liquid nitrogen cryovats.—Liquid nitrogen cryovats are cryogenic freezers that are cooled in various ways using a supply of nitrogen in the liquid phase. All methods of cryopreservation that use LN_2 are considered to be the most effective because samples are stored at temperatures below the glass-liquid transition temperature of water (−137°C). There are currently four different storage methods that use LN_2: 1) vapor-phase cryovats that include a standing level of LN_2 present below a rotating carousel that houses sample racks (≤−150°C), which was the most commonly used method by collections surveyed by Zimkus and Ford (2014); 2) liquid-phase cryovats that allow submersion of samples in nitrogen (−196°C); 3) isothermal cryovats with a specialized jacket surrounding the interior chamber and allowing nitrogen vapor to enter the freezer space via directional vents (approximately −190°C); and 4) MVE Variō^{TM} cryovats (−50°C to −150°C) that allow LN_2 to flow through a heat exchange system located in the top head of the freezer, using vaporization energy of the LN_2 to cool the unit. All four types of cryovats are available in various sizes with vial capacities that range from approximately 20,000 to almost 100,000. Use of liquid-phase cryovats are not recommended because nitrogen in a liquid state can penetrate all commercial cryogenic vials if not placed in secondary containment (e.g., polyethylene tubing), making them at risk of exploding when they expand upon warming (see Genetic Sample Processing: *Storage Containers*). In addition, cross-contamination can occur when immersing samples directly in LN_2 without securing the cryovial in secondary containment (Clark 1999). The circulation of vapor within isothermal cryovats allows for improved visibility due to less clouding at the top of the chamber, but these cold-storage units have increased consumption of LN_2

when compared to traditional vapor-phase cryovats. MVE Variō^{TM} cryovats are the newest freezer systems, having the benefit of a dry storage area, but this technology is not yet used or tested by natural history collections (Zimkus and Ford 2014).

Most liquid nitrogen cryovats are equipped with electronic controllers that automatically monitor and regulate the supply of LN_2 to the unit. Liquid levels, temperature readings, and alarms are generally displayed on a controller unit panel. Cryovats with electronic controllers generally use a two-sensor system to detect LN_2 at user-defined levels. When LN_2 levels are below the low-level sensor, a solenoid valve is opened, allowing LN_2 to enter the cryovat from a nearby source (e.g., small-volume dewar, large volume bulk tank) connected via a cryogenic hose and/or vacuum-jacketed piping (see Genetic and Genomic Collection Storage: *Liquid Nitrogen Supply*). When LN_2 levels reach the high-level sensor, the solenoid valve is closed, stopping the flow of LN_2 into the cryovat.

Liquid nitrogen cryovats (i.e., liquid-phase, vapor-phase, isothermal) can maintain ultracold temperatures for periods of time if they are not opened but, ultimately, they must have LN_2 to maintain their cold temperatures. In addition to both on-site and remote alarms, it is paramount that cryovats are checked at regular intervals and levels of LN_2 are recorded (see Operational Best Practice: *Manual Monitoring*). When using LN_2 cryovats, backup power for the electronic controller functions to keep a continuous supply of LN_2, although the unit itself stays cold without power (see Facility Management: *Backup Precautions*). If a cryovat controller unit that automatically dispenses LN_2 is not connected to a backup system, readings of LN_2 levels and the addition of more product (i.e., pouring LN_2 into the cryovat) need to be done manually. For those cryovats that store samples in the vapor phase (i.e., vapor-phase, isothermal), LN_2 would likely need to be supplied at regular intervals to maintain ultracold temperatures if power remains off for an extended period of time and there is no backup connection. In the event of a power failure, collection procedures should detail if and when cryovat lids are opened to minimize use of LN_2 (see Operational Best Practice: *Emergency Procedures*).

Liquid nitrogen supply.—Liquid nitrogen to supply cryovats can be obtained and stored in three ways: 1) delivery in small-volume dewars (cylinders); 2) delivery to large-volume bulk tanks; and 3) self-production using an on-site LN_2 plant. If a commercial vendor is present in the area, LN_2 can be delivered as needed on a regularly scheduled basis. For most small collections, mobile dewars, generally up to 240 liters, are sufficient to supply cryovats. These dewars are generally placed in close proximity to the cryovats and connected with cryogenic transfer hoses, unless permanent vacuum-jacketed piping designed to transfer cryogenic liquids is installed within the repository. The dewars themselves can be purchased and refilled by a delivery vehicle, or these storage vessels can be leased so that an empty dewar is exchanged for a full one. In addition, Department of Transportation (DOT) codes regulate the specifications of liquid nitrogen delivery vessels in the USA, so collection managers should be aware if these regulations affect their delivery. LN_2 deliveries should be scheduled to minimize the amount of time that a dewar is not connected to a cryovat to ensure that a cryovat has a steady supply of LN_2. Collection managers should be aware that LN_2 evaporates at a constant rate, which can be affected by the atmospheric conditions, vessel integrity, and manufacturing tolerances.

For large collections with many cryovats, a pressurized bulk or microbulk tank should be considered. Depending on the size of the bulk vessel, the tank might need to be located outside of the building where the collection is housed. If bulk tanks are placed indoors, it

might be possible for external wall boxes to be installed to allow bulk tanks to be filled without having to enter the building. Costly vacuum-jacketed cryogenic pipes are needed to deliver the LN_2 from the tank to the point of use, so for cost efficiency, bulk tanks should be located as close to the cryovats as possible. In many areas, bulk tank storage is regulated by city or state ordinances; oxygen monitors are also essential if these storage units are placed indoors (see FACILITY MANAGEMENT: *Oxygen Monitors*). In smaller collections that are located in remote locations where LN_2 cannot be easily delivered, collection managers might consider the use of LN_2 plants, which generate nitrogen by separating it out from the ambient air. The plant-generated LN_2 is stored in a tank that then can be moved and connected to a cryovat. Regardless of how nitrogen is obtained, personnel must balance multiple competing factors, including product stock versus available space and over-supply stock versus both cost and leakage, to ensure cryovats have a constant supply. To manage this balance, collection managers should track the amount of LN_2 used to estimate an efficient timeline for when more supply is needed (see OPERATIONAL BEST PRACTICE: *Manual Monitoring*).

Mechanical freezers and refrigerators.—Mechanical freezers and refrigerators offer a large range of storage temperatures, including ultracold freezers that can sustain temperatures between -50° and -86°C, general-purpose or laboratory freezers that normally operate between -12° and -30°C for manual defrost models (automatic defrost models allow temperatures to fluctuate more broadly), and general-purpose refrigerators that maintain temperatures between 1° and 12°C. As previously discussed, cryogenic storage at extremely low temperatures is considered the most effective method for the long-term stabilization of genetic samples. However, refrigeration is sometimes considered for short- or long-term storage if certain buffers or chemicals were used in sample preservation, and these additives only require that genetic samples be kept below ambient temperature. Collection managers must record all chemical additions and preservatives used with their genetic samples to ensure that the samples are stored at the appropriate temperature recommended by the manufacturer. If multiple cold-storage methods are used within a collection, the SOP should clearly outline the criteria for storing samples at the different temperatures.

Freezers and refrigerators are available in a variety of sizes, styles, and voltages, including upright or chest configurations. Many models are available with locks for additional security (see FACILITY MANAGEMENT: *Access and Security*). Collection managers should ensure that each electrical unit has a dedicated circuit so power is not overloaded, and that units are positioned to allow for sufficient airflow around them (see FACILITY MANAGEMENT: *Space Planning*). Heat released from cold-storage equipment should be monitored and counter-balanced by an HVAC or cooling system when needed. Those collection managers purchasing new equipment should consider freezer units that have increased energy efficiency, which generally ensures that less heat is emitted to the surrounding air. Compressors can also be placed outside of the building for some freezer units, removing the need for HVAC or cooling in the collection space where the mechanical freezer is located. Fortunately, compressor systems on newer cold-storage units include those being cooled by water, whereby valves connected to local water sources automatically control the flow needed to maintain cooler compressor temperatures, which enhance the stability and reliability of the unit. Because cold-storage units are integral to the functioning of a genetic or genomic collection, the SOP should outline specific protocols for regular preventative maintenance and emergency

repair of all mechanical units (see Operational Best Practice: *Equipment Preventative Maintenance, Repair, and Replacement*).

Mechanical freezers and refrigerators require less dedicated time in their day-to-day operation when compared to LN_2 cryovats, but collections have an increased risk of loss in the event of mechanical breakdown or power disruption. Even a short power outage can be detrimental to samples being stored in a mechanical cold-storage unit. Battery backup systems or an UPS are needed to maintain units in the event of a short power outage or until power can be switched over to a robust backup system for longer term emergencies (see Facility Management: *Backup Precautions*). Internal and, if possible, remote monitoring should be present to prevent sample loss due to mechanical failure of units (see Operational Best Practice: *Manual Monitoring*). To safeguard against all possible risks, including power outage and mechanical failure, both monitoring procedures and backup precautions should be clearly outlined in the collection SOP.

Operational Best Practice

Genetic and genomic collections are more equipment- and personnel-dependent than traditional natural history specimens, with more catastrophic consequences if storage equipment fails or samples are handled improperly. In addition, genetic samples are consumable resources, so they become more limited with each use for research.

Facility functions and services provided.—Genetic resource collections can provide many key services to fulfill institutional missions and assist internal and external researchers in achieving project goals. How broadly and consistently these key services can be addressed is related to how genetic collections are set up at an institution (i.e., separate locations or centralized facility). The main functions of most centralized genetic resource facilities associated with natural history museums include sample storage, sample tracking, and tissue subsampling and/or processing of loans/gifts (Zimkus and Ford 2014). A small percentage of repositories provide additional genetic laboratory functions, including DNA/RNA extraction, polymerase chain reaction (PCR), and sequencing. Although most institutions are moving toward the centralization of genetic resources, institutions that have maintained genetic samples within multiple locations have prioritized accessibility to collections for internal researchers; collections are generally stored within the laboratories of the principle investigators conducting research using those particular genetic samples, and it is unclear how accessible these samples are to the scientific community (Zimkus and Ford 2014).

Besides genetic sample maintenance, additional research services could potentially be provided by genetic resource collections, although this would be more easily achieved in a centralized facility, including assistance with project planning or proposal submissions, sample collecting, assistance with sample transportation from the field, sample quality assessment using spectrophotometry or other methods, and student or intern training (Hanner et al. 2005). Collections could also provide or lend supply materials for those researchers who will be depositing samples in the biorepository, including sample vials, sample boxes, reagents, LN_2 dewars, dry shippers, LN_2, or dry ice. To ensure that sufficient equipment, supplies, trained personnel, and funding to maintain high-quality specimens are available, and to potentially offer additional services, collection managers should clearly outline priorities, as well as the molecular laboratory tasks performed by collection staff (e.g., DNA/RNA extractions, fluorometric/spectrophotometric quantification of DNA, DNA barcoding to identify or confirm identifications), in the SOP document. Policy should include standards for the receipt of genetic material to the

collection, defining how samples and metadata must be stored (i.e., acceptable sample storage vessel sizes and types), labeled, and organized, so personnel can easily incorporate new samples into the collection.

Personnel.—Genetic and genomic resource collections must be managed properly and continuously to ensure that samples are properly stored and maintained, and remain available for use in research. Depending on the size of the collection and institution type, personnel might include a single manager or numerous employees. A recent survey of genetic resource collections associated with natural history museums found that the vast majority had curators/professors with higher academic degrees or collection managers with higher degrees maintaining the collections (Zimkus and Ford 2014). Other personnel working with genetic resources included collection managers without higher degrees, staff or technical assistants, paid and unpaid student assistants, and volunteers. At minimum, collections staff should include a dedicated manager who is trained to manage the day-to-day activities within the collection and can ensure that all procedures are completed as instructed in SOP documents. A collection manager also prevents unauthorized access to storage areas, thus protecting samples from misplacement, accidental thawing, or contamination (Savolainen et al. 2006). Duties and reporting relationships for each staff member should be clearly outlined in a written job description. Collection managers should ensure that all personnel working in the collection have the appropriate training to undertake the duties assigned in their particular job description, especially with regard to health and safety issues. Although it is ideal to hire personnel that have previous experience, it is understandable that many collections must function with the assistance of students, interns, or volunteers owing to limited budgets or their nature as educational institutions. Therefore, adequate training and oversight by managers is crucial to guarantee that all policies are implemented and the genetic material is protected for long-term availability. Proper maintenance of genetic resource also requires assistance from additional departments outside of those working directly with the collection, including building maintenance and operations, environmental health and safety, information technology, and security.

Training.—All genetic collection staff must receive adequate training to ensure that they can perform all aspects of their job description and follow all policies and regulations, including those mandated by international, national, regional, and local statutes. All new personnel, including staff, students, interns, and volunteers, should be trained by authorized staff to understand the details and justification for the SOP documents and comprehensive protocols. Policies should also be developed in relation to both short-term and long-term visiting researchers to ensure that they have the appropriate training before working with collections. Training should outline best practice for working with genetic resources because improper handling can reduce the quality and long-term integrity of a sample, which can occur if it thaws or is contaminated by instruments or contacted surfaces (see Genetic Sample Processing: *Sample Transfer*; Use of Collections: *Aliquoting Samples*). In addition, personnel should be trained in database usage and the proper storage of samples in accordance with corresponding database records (see Inventory Control and Data Management: *Databasing for Genetic Samples*). Training should also include all aspects of safety and security, including the appropriate use of tools and equipment, proper personal protective equipment, and risks of working with chemicals and biospecimens (see Health and Safety). Internal records should be kept of all approved personnel and dates of their various training events, especially because some training requires mandatory

updates at regular intervals. All training programs should be regularly assessed to make sure that information is up to date, and it is recommended that training be done in collaboration with the health and safety group at the institution.

Manual monitoring.—The viability of genetic samples is dependent on storage equipment operating at optimum efficiency. Diligent and consistent monitoring by staff ensures that the collection is maintained and all equipment operates properly. Cold-storage equipment requires regular review to ensure that samples are being kept consistently at the appropriate temperature. Automatic alarms are generally present in most units, but personnel should also visually inspect and record the temperature of the equipment on a regular basis (e.g., daily, multiple times per week). The levels in liquid nitrogen cryovats should also be measured manually on a regular basis to confirm that the measured levels match the control displays to ensure the proper functioning of the equipment. In addition, managers should evaluate the temperature and nitrogen-level data on a weekly basis to identify any problematic trends. All transportable pressurized vessels, including dewars (cylinders) of LN_2, should be examined upon receipt from outside vendors to check that the pressurization is compatible with the cold-storage equipment and that there is no visible damage or leakage. When in use, these vessels should be closely monitored to estimate rate of use and ensure that valves are functioning properly. Collection procedures should clearly outline how to monitor and check collection equipment, how often it should be done, and how to maintain documentation.

Equipment preventative maintenance, repair, and replacement.—Proper functioning of cold-storage equipment is essential for the long-term stability of genetic material. All equipment should have regular routine maintenance in accordance with the recommendations of the manufacturer. Preventative maintenance of mechanical freezers should include comparing the temperature set-point versus the actual temperature (see Operational Best Practice: *Manual Monitoring*), testing backup batteries, ensuring that gaskets and seals are intact without tears, and routine cleaning of freezer coils and condenser filters. Mechanical freezers also might need to be defrosted regularly. Another benefit of preventative maintenance, in addition to increased dependability, is that significant energy savings are associated with regular maintenance of cold-storage equipment, such as mechanical freezers. Liquid nitrogen cryovats should also have routine maintenance to ensure that internal temperature sensors function properly, LN_2 readings are accurate, fill valves operate normally, and alarms activate under the defined conditions. Maintenance of cryovats by collection personnel should also include regular checks of the insulating materials (e.g., polystyrene foam) fitted underneath the lid to identify cracking, which reduces the insulative properties, or built-up frost, which prevents the lid from closing properly. In addition, ice and frost build up in a sensor tube results in false readings, whereas build up in vacuum-jacketed cryogenic pipes can block the flow of liquid nitrogen into the cryovat during the fill cycle.

Biosafety cabinets and chemical fume hoods should be tested and certified routinely to ensure that they are functioning appropriately and in compliance with both local regulations and any requirements associated with biosafety level status. Oxygen monitors also need regular maintenance and/or calibration as life safety equipment (see Facility Management: *Oxygen Monitors*). All safety equipment, including eyewashes, safety showers, fire detection units, and fire suppression systems, should be tested regularly to ensure reliability, which is generally best accomplished in collaboration with the health and safety group of the institution. Managers should keep records of all routine and

special maintenance, including dates, description of the issues (e.g., alarms, incident reports), tests performed, and actions taken to resolve any problems. The SOP should provide plans, including instructions and schedules for preventative maintenance, calibration, repair, and replacement of equipment.

Regular assessments of equipment should be made by collection managers to help establish a timeline for replacement. ISBER (2012) reports that generally, mechanical freezers last from 5 to 15+ years with manufacturers reporting a range of 8 to 12 years, whereas liquid nitrogen cryovats generally last longer, functioning for 10 to 35 years. Collection managers should track the ages of all equipment and know the manufacturer's expected lifespan for each. Based on the age and performance of the equipment, long-range plans for replacement can be anticipated and replacement funding can be ensured. If a cold-storage unit should fail, written procedures should be in place to identify how to transfer the samples from the failed unit to their emergency storage location (see Operational Best Practice: *Emergency Procedures*).

Records management.—Collection managers should maintain records that detail the collection, processing, storage, and use of each genetic sample. Copies of all relevant permits and acquisition documents must be maintained by the institution, collection, or laboratory, which detail the legal attainment of all genetic samples either alone or associated with traditional voucher specimens (see Policy Best Practice). Besides being a legal obligation for the institution, this information allows both collection managers and end-users to assess the sample history and quality. Records should also be maintained for all curation activity in a genetic collection because of the specialized training and equipment required (see Operational Best Practice: *Training*). Up-to-date records should be available that document personnel training, equipment activity (e.g., monitoring, preventative maintenance, repairs), safety inspections, and legal compliance (e.g., active permits) as outlined in the SOP.

Genetic sample data can be recorded as part of an institutional database, which potentially tracks both the original voucher specimen and any subsequent associated genetic or genomic derivatives, or a stand-alone tracking system that is used only for genetic samples (see Inventory Control and Data Management: *Databasing for Genetic Samples*). Documentation of genetic sample locations in cold-storage units should be as precise as possible so that samples can be located quickly (see Inventory Control and Data Management: *Sample Labeling and Tracking*). Dates can be easily confused because their format differs throughout the world; thus, recorded dates should be formatted consistently and in an unambiguous manner. Security for electronic records should include password protection and automatic time-outs on computers and online databases, as well as scheduled backups for all data. Large collections should have electronic records backed up daily on a network or remote secure server, whereas small collections might consider doing local backups on a weekly basis.

Emergency procedures.—Collections should have emergency procedures, including evacuation procedures and detailed disaster plans, outlined in their SOP to address how personnel should respond to various situations, ranging from laboratory incidents to natural disasters (ICOM 1993, Dorge and Jones 1999). Generally, emergency procedures and disaster preparedness should be developed to address incidents at various levels of involvement, including issues confined to the genetic collection (e.g., clean-up of chemical spills, transfer of genetic samples from a failed cold-storage unit), building-wide or institution-wide events (e.g., flood or water damage as a result of plumbing issue or roofing leak), local issues (e.g., city-wide power outage), or regional events (e.g.,

earthquake, hurricane, tornado, tsunami). Because emergencies with genetic resources can become disasters if immediate action is not taken, it is recommended that a risk assessment is conducted and personnel have regular reviews and drills to ensure their familiarity with emergency procedures and backup plans for every conceivable situation (see FACILITY MANAGEMENT: *Backup Precautions*). It is also essential that personnel are familiar with the locations of all equipment and tools needed to quickly and efficiently perform their responsibilities during each type of emergency, ranging from how and where to transfer samples from a failed unit to how to turn on backup power during a power outage. Contact information for personnel responsible in case of emergency in the order that they should be contacted, facilities managers, and outside contacts for emergencies (e.g., power companies, fuel supply companies, important contractors) should be included in the SOP document, provided to those responding to off-site alarms, and clearly posted in the collection. Contact information should be regularly reviewed to ensure that it remains current.

Genetic Sample Processing

Genetic samples require specialized storage to maintain their viability, but the manner by which they are initially preserved when collected and any subsequent changes made prior to their deposition in a collection also greatly affects their utility in research. In addition, procedures used by collection personnel to prepare the samples for deposition in long-term storage can have detrimental consequences if not completed properly.

Initial preservation procedures.—The methods currently used to preserve genetic material during initial sampling vary widely owing to the sampling location, tissue type, and intended research use (Dessauer and Hafner 1984, Prendini et al. 2002, Buś and Allen 2014). A comprehensive treatise of methods to preserve genetic samples is discussed in Nagy (2010). Of all the methods examined, flash-freezing using liquid nitrogen or a mixture of dry ice and ethanol is considered to be the one of the best ways to preserve samples because the speed by which the sample is frozen prevents large ice crystals from forming and no preservative is needed, which maximizes the research potential of the sample. Some chemical agents or buffers (e.g., glycerol, dimethyl sulfoxide [DMSO]) are used to protect macromolecules and/or tissue integrity from damage caused by ice formation during cryopreservation techniques, such as freeze–thaw procedures or vitrification (Withers 1980, Karlsson and Toner 1996). Buffers, chemical preservatives, and alcohols, especially ethanol, do not require immediate refrigeration and are frequently used for the initial preservation of samples because it circumvents the complicated logistics involved with flash-freezing samples in the field or keeping them cold during transport. All preservation techniques have caveats to their use, however, and genetic resource collections must be fully aware of both the benefits and limitations to make informed decisions.

In a recent survey of genetic resource collections associated with natural history museums, samples were found to be initially preserved in a variety of different ways (Zimkus and Ford 2014). The majority of collections surveyed included samples that were flash-frozen or preserved with $\geq$ 95% ethanol. Ethanol at concentrations of 95% to 99% is commonly used by researchers in the collection of zoological samples but is generally not used for the preservation of plant material. Ethanol can be considered expensive when compared to alternative preservatives and can be difficult to transport, especially at high concentrations, because of its flammable classification (Williams 2007). A number of buffers and chemical preservatives are currently being used by natural history researchers,

including DMSO, RNA*later*® (Ambion, Austin, TX), and various lysis buffers. DMSO is used as a preservative in many different aqueous solutions. One of the most commonly used formulations is a salt-saturated solution of DMSO (20% DMSO, 0.25 M ethylenediaminetetraacetic acid [EDTA], sodium chloride [NaCl] saturated, pH 7.5; Seutin et al. 1991, Nagy 2010), which is a cost-effective method for preserving and transporting genetic samples from remote locations, but the long-term effects on sample quality are still unknown (Williams 2007). RNA*later*® solution and a similar product, Allprotect® Tissue Reagent (Qiagen, Valencia, CA), are used in sample collecting for the stabilization of DNA, RNA, and protein in tissues. Although these solutions minimize the need to immediately process and/or freeze tissue samples, material preserved in RNA*later*® and Allprotect® buffers cannot be stored at room temperature indefinitely if genetic samples are to remain viable. Procedures using lysis buffers to preserve samples are relatively inexpensive and can be used to yield high-molecular-weight DNA. Queen's lysis buffer is most often used for the preservation of DNA in nonmammalian vertebrates that possess nucleated red blood cells (Seutin et al. 1991, Nagy 2010). Although lysis buffers might not require immediate refrigeration, this type of preservation limits the ultimate use of the sample because proteins are denatured and RNA is not preserved (Longmire et al. 1997, Nagy 2010).

Other sample preservation techniques that do not require the immediate cold storage of samples are available but are used less frequently among natural history museums (Zimkus and Ford 2014). These techniques are not recommended for the preservation of samples used in genome-level sequencing (Wong et al. 2012). Buccal swabs are used to collect cells from the inside of the mouth and, more recently, skin swabs are being used to nondestructively sample amphibians to test for the presence of *Batrachochytrium dendrobatidis*, a disease-causing fungus commonly known as chytrid (Boyle et al. 2004). Swabs can generally be kept at room temperature (if not exposed to extreme temperatures or direct sunlight) for approximately 1 week before they need to be placed in a freezer for long-term storage. Whatman FTA® (GE Healthcare Bio-Sciences, Pittsburgh, PA) is filter paper impregnated with a proprietary mix of chemicals, which serve to lyse cells, prevent growth of bacteria, and protect the DNA in the sample. This product allows for the collection and storage of blood, cells from buccal swabs, and other tissues, such as blood, tissue, or saliva, by direct application of the biological sample onto the paper (Smith and Burgoyne 2004). DNA samples collected using FTA paper do not require immediate refrigeration because samples purportedly remain viable for at least 4 years at room temperature. Manufacturers of this technology also report that it allows for the collection of biological samples needed for RNA analyses, but samples for this type of analysis are more sensitive and thus must be immediately placed in cold storage for long-term preservation. This technique is convenient for the collection of DNA samples because of the small size of the collecting product and no need for immediate cold storage, allowing ease of transport by a carrier or in personal baggage.

Desiccation methods, commonly used to preserve botanical samples, use either physical processes or chemical agents to dehydrate samples. These methods can be very effective in preserving samples if they are maintained in a humidity-controlled environment after the desiccation process. Physical processes that lead to desiccation include sun-drying, air-drying, flash-drying, oven-drying, vacuum-drying, and freeze-drying, whereas substances that induce natural desiccation include rice, sodium chloride (NaCl), calcium sulphate ($CaSO_4$, commonly known as drierite; Liston et al. 1990, Nagy 2010), and sodium silicate (obtainable in powder, crystal or gel form; Chase and Hills

1991). Freeze-drying (lyophilization or cryodesiccation) is the controllable dehydration of samples by vacuum desiccation. This method involves the conversion of water within the sample into ice, crystallization of the sample, sublimation of ice under a vacuum, and subsequent evaporation of remaining water from the crystallized sample (Adams 2007). Silica gel beads are commonly used by botanical collectors as a desiccant with the advantage that whole samples can be stored at room temperature as long as the material is stored in tightly sealed containers and are regularly checked for dryness (Chase and Hills 1991); recent studies demonstrate that samples stored in containers with poor seals yield lower-quality DNA (Neubig et al. 2014). In addition, comparisons of frozen DNA extracts stored for 7 to 12 years and new extracts of the same original silica-dried tissues indicate slightly less degradation is present in the frozen DNA extracts. Chemical desiccation, which involves adding a chemical to extract moisture from samples, can be completed using a number of different substances, including amyl acetate, hexamethyldisilazane, xylene (which is the chemical Dimethylbenzene), methyl cellosolve/cellusolve (also known as ethylene glycol monomethyl ether), calcium oxide (CaO, commonly called lime), and sulphuric acid (H_2SO_4). Each of these chemical methods has certain considerations for the samples and personnel that should be closely examined before working with them (Nagy 2010). Regardless of the method of desiccation, moisture content must be continuously controlled or samples quickly degrade. In addition, samples preserved in this manner are more sensitive to thermal decay at higher temperatures due to their reduced water content and should be stored in a low-temperature environment as soon as possible to ensure that samples remain viable.

Collection staff often does not have control of how genetic samples are originally preserved, and most genetic resource collections associated with natural history museums accept samples that are preserved using numerous different methods (Zimkus and Ford 2014). As more information is discovered about preservation methods themselves, it is becoming more important to know how samples were preserved and handled. Collection managers should make every effort to record the initial preservation methods used and any subsequent changes to storage for their samples, including media or containers. Both media and temperature can affect the ways that samples can be used for future molecular analysis; thus, all curation data for genetic samples should be made available to researchers before their selection and use. Research advancements are constant in molecular studies, and future technological developments might allow for the stabilization and storage of biological samples at room temperature, which could eventually eliminate the need for low-temperature storage environments for newly collected samples. To determine if these new emerging technologies will be effective for existing genetic collections, the detailed curation history regarding how each individual sample was preserved, stored, and utilized will become increasingly important.

Storage containers.—Genetic resources can be stored in a variety of ways, depending on the tissue type, sample size, and cold-storage methods used in a collection. Primary storage containers of the samples themselves can include plastic bags, paper envelopes, reaction plates, or vials. When primary storage containers are accessioned into a collection, they must be examined to determine if they are appropriate for the type of cold storage being used in the collection. The SOP should clearly define acceptable storage containers and encourage their in-house researchers to use them because any variation could involve material and staff costs to rectify. For all types of cold storage, the most commonly used sizes of vials are between 1.2 and 2.0 ml, which is the size and configuration that maximizes storage capacity while retaining ease of handling (Zimkus

and Ford 2014). Some additional features that might be useful to those working with sample vials include self-standing vials (flat-bottom or skirted) and those that allow for locking into a rack for one-handed operation.

One area of immense variability is with the number of vial types used in natural history collections (Zimkus and Ford 2014). Potential vial issues are the same for both mechanical freezers and cryovats, although more extreme for cryovats because of their colder temperatures and quicker temperature change. Samples stored using LN_2 must have vials that are rated for use with cryogenic temperatures, preferably made of polypropylene with screw-top caps. Glass vials are problematic when frozen because of their fragility when handling and vulnerability to cracking when stressed, and their use with LN_2 is unacceptable because leakage into the vials can lead to the vessels exploding. In addition, pop-off lids are not recommended because this vial type can easily open on its own. Vials can have threads located internally or externally on the vial opening; both vial types exhibit advantages and disadvantages. Externally-threaded closures promote more sterile conditions because internal threading can allow contaminants to enter if the cap is placed on an unclean surface when removed, but these vials can be susceptible to cracks and loss of air-tightness, which can lead to sample dessication or oxidation (Corthals and DeSalle 2005, Corthals 2006). Internally-threaded vials might allow increased storage capacity, depending on the vial selected, but users also suggest that material can be trapped within the threads of this vial type (Johnson 1999). Some manufacturers suggest that vial caps that incorporate a silicone gasket or O-ring (internally or externally threaded) are ideal for vapor-phase LN_2 freezing because the seal is enhanced, but care must be taken if the vial cap is over-tightened because the gasket can become distended. In addition, the presence of gaskets or O-ring might improve the initial performance of seals but can be problematic when used with some alcohols (e.g., ethanol) because some gasket material, such as silicone, is vapor permeable. When working with liquid-phase nitrogen cold storage, extra caution must be taken because the accidental entrapment of liquefied nitrogen inside the vial leads to pressure build up and, upon removal, rapid vaporization of the liquid can result in leakage or even explosion (see HEALTH AND SAFETY: *Liquid Nitrogen Safety*). As a precaution, vial manufacturers recommend that samples not be immersed in LN_2 unless they have secondary containment. Heat-sealing vials into flexible polyethylene tubing is recommended for safe storage in the liquid-phase environment.

Depending on the size of the samples and their primary storage method in the collection, various secondary and tertiary storage containers (e.g., boxes, cassettes, sample storage canes for immersion in LN_2, racks) can be used to organize samples and maximize space. The majority of natural history museums recently surveyed organizes their collections with a vial box and rack system (Zimkus and Ford 2014). The box-and-rack system is a simple but effective way to maximize space within a cold-storage unit and reduce the amount of time needed to search for a specific box. The overall cost of box-and-rack systems depends on the style and number of both the boxes and racks purchased. The use of racks also decreases potential risks to the collection when personnel are retrieving samples. Collections without racks often stack boxes on top of one another within cold-storage units, forcing personnel to remove or displace boxes to access those underneath or behind. In addition, the movement of individual boxes to retrieve samples requires more time, increases the possibility that samples will thaw, and decreases the chance that boxes will be put back in their original location. Lastly, racks allow samples to be quickly moved to other freezers in the event of a freezer failure and

decrease the possibility that samples are misplaced while in a temporary storage location.

Racks systems made from aluminum or stainless steel can house most standard-sized boxes and can be oriented horizontally or vertically to fit in mechanical upright or chest freezers, as well as LN_2 cryovats. Aluminum might be chosen for racks in collections that need precise and controlled freezing of samples because this metal is a better energy conductor than stainless steel. Stainless steel racks are more durable and, because the steel does not oxidize, they remain clean. Racks most often come with a locking rod that runs through the front of the shelves, ensuring that boxes are held in place when the rack is moved. Racks also can have spring clips instead of locking rods, which allow quicker access to boxes, but can be problematic if the racks are bumped or dropped. Identifiers can be added by riveting or etching onto racks, or by labels (e.g., unique locating identifiers, barcodes) that can be affixed to the tops of racks and rack shelves (see Inventory Control and Data Management: *Sample Labeling and Tracking*).

A number of different types of vial storage boxes can be used, such as cardboard, chipboard (paperboard), fiberboard, metal, polycarbonate, or polypropylene. It is recommended that moisture-proof boxes be used even though they are more costly, because water repellency increases their long-term durability. When purchasing new boxes, care should be taken to ensure that they are compatible with both the existing rack system and the cold-storage equipment. Collection managers should be aware that polypropylene boxes with hinged lids, which are often used in the field because they are shatter-resistant and have attached lids, might not fit in standard-sized racks. In addition, personnel in collections using either the liquid or vapor phase of nitrogen should use boxes with holes or slots present on the underside of the box to allow the LN_2 to drain. Collections using cryovats should confirm that particles of cardboard, chipboard (paperboard), or fiberboard will not prevent equipment from functioning properly if boxes begin to degrade; otherwise, a more durable type of box should be used. Aspects of ordinary use are another important consideration for storage boxes in a collection. Collection managers should be aware that boxes that have numbered, gridded inserts inside might be difficult to read after being removed from cold temperatures because of frost. In contrast, boxes with grid numbering present on the lid, rather than the box itself, can have frost easily wiped off; however, finding the correct vial may be difficult once the lid is removed because care must be taken to keep the lid numbering aligned with the internal grid.

Sample transfer.—When samples are received by collection staff, they might need to be transferred into new primary storage containers, especially if they are stored in a manner that is incompatible with the cold-storage system or if initially preserved in suboptimal conditions for long-term storage (e.g., preservatives need to be removed; Williams 2007). Collection personnel should ensure that contact between different specimens is avoided and all instruments used in the tissue transfer process are handled in such a way that ensures that biological contaminants are destroyed. Disposable equipment (e.g., single-use blades, disposable forceps) replaced after one use greatly reduces the chances of sample contamination, but this practice can become too costly for many collections. Several methods (e.g., heat/flame, chemical agents, steam sterilization in an autoclave) can be used to prevent contamination between genetic samples, but collection managers must be sure that their particular procedures denature or destroy all contaminating genetic material. Some practices, such as cleaning instruments using detergent and water, render instruments and surfaces safe to touch, but these methods do

not protect samples from cross-contamination. Disinfection of work surfaces, decontamination of equipment, and sterilization procedures are all needed to ensure safe operations for both staff and genetic samples, and these techniques can vary depending on the task at hand.

Comprehensive procedures should clearly outline which handling methods are appropriate for the various tasks when working with samples. Some techniques, such as autoclaving and use of UV light, destroy all biological matter but require a longer time to accomplish, and thus are better utilized before and after processing a batch of samples. Alternatively, other methods, such as heat/flame or hydrogen peroxide, could be used to immediately decontaminate subsampling tools and ensure no cross-contamination while working through a batch of samples, because ease and speed of use is important. If a chemical agent such as hydrogen peroxide or bleach is used to clean equipment or surfaces, procedures should ensure that those agents do not contaminate samples because these techniques destroy biological matter (e.g., dry instruments thoroughly before manipulating samples). Personnel should also be careful not to inadvertently contaminate the samples by touching their own body or clothes, or allowing hair to come in contact with samples. In addition, they should always wear the proper PPE to protect genetic samples and for their own personal safety (see HEALTH AND SAFETY: *Personal Protective Equipment*).

Inventory Control and Data Management

The ability to find a specimen is essential for the curation of any natural history collection. The manner by which the physical locations of genetic samples are tracked is particularly important in their curation because it is virtually impossible for personnel to search the entire collection if a particular sample is missing. In addition, proper inventory and data management allows collection managers to maximize the capacity of their cold-storage units while reducing the amount of time needed to locate samples, which is crucial given that the quality of some genetic material can be reduced with each freeze–thaw event; more research is needed to understand these effects on various sample types over both short-term and long-term storage (Shikama 1965, Ross et al. 1990, Davis et al. 2000).

Sample labeling and tracking.—Proper sample tracking is key to ensuring that cold-storage equipment does not have to remain open for long periods, or samples are not repeatedly exposed to temperatures that initiate thawing. An appropriate tracking system could include sample labeling, multiple levels of container labeling, and the use of a database to record sample location data. Using a convention for numbering that assigns unique locating identifiers, such as barcodes, to all levels of sample storage (including primary, secondary, and tertiary containers) allows for quick location and retrieval from a complex cold-storage system (see GENETIC SAMPLE PROCESSING: *Storage Containers*). It is recommended that for maximum efficiency, sample vials stored within LN_2 cryovats or mechanical freezers should have unique locating identifiers assigned to the cryogenic vials, as well as unique locating identifiers assigned to the associated boxes, shelves within racks, racks in their entirety, and individual cold-storage units. Each sample vial can then be located in the collection by a unique location number combination. Any labels placed on vials or storage containers should be typewritten or computer-generated; hand-written identifiers might be difficult to read due to the handwriting or decomposition of the ink as a result of cold temperatures or mechanical friction. Upon arrival to a collection, samples might need further curation to meet collection standards, including the addition

of typewritten or computer-generated labels to vials or the transfer of samples into the appropriate type of vials. Extreme care must be taken because most genetic samples are initially labeled using reference numbers written on the vial by the researcher. All label placements should be tested under projected environmental conditions to ensure that ink, adhesive, and the label itself withstands the cold-storage temperatures. On secondary containers, such as boxes or racks, labels should be placed in areas where friction is minimal to reduce the risk that the label face is worn down by repeated scratching or rubbing.

Barcodes labels rated for cryogenic conditions or vials manufactured with barcodes are commonly used by genetic resource collections to facilitate sample tracking, including for primary and other hierarchies of containers (Zimkus and Ford 2014). Preprinted or self-printed barcode labels that are mostly transparent and wrap completely around vials, allow information hand-written on the vials to be viewed when positioned over the writing. Vials manufactured with barcodes on the side or bottom are less commonly used, most likely because these vials rarely have an area where researchers can include hand-written information. Barcodes inserted into vial caps do facilitate the barcoding process because they require less handling time when compared to vial-wrapping labels, but there are added risks that inserts could fall out or a cap could become disassociated from the vial itself. Radio frequency identification (RFID) tags transmit unique locating identifiers associated with tagged objects and, unlike traditional barcodes, these tags do not need to be within the line of sight of the reader. RFID technology is currently being used only to track secondary and tertiary containers (e.g., boxes, racks) within the natural history community, but this method could potentially be used to track primary containers when the tag size and associated costs decrease (Zimkus and Ford 2014).

Databasing for genetic samples.—By their nature, genetic resource collections generate and track a large amount of data associated with their samples. A computer-based inventory system, such as a stand-alone database, internal spreadsheet, or networked database, is essential so that all sample metadata, including location data and loan/gift sampling history, can be tracked (Cable and Fulcher 2006). The system should have the capacity to assign a unique identifier to each genetic sample entered in the database and associate all derived genetic samplings and derivatives with the original specimen, which might be the original genetic sample itself or a traditional voucher specimen. It is essential that a tissue sample or genetic extract can be readily linked to the traditional voucher specimen. Changes/updates to data, including taxonomic identification, should be made to all preparation types, even if data are tracked separately for the traditional voucher specimen and genetic sample, The chosen database should accommodate for museum-wide variables given that traditional voucher specimens are likely housed apart from their associated genetic samples due to their different storage and conservation needs and, in addition, personnel curating these two collections are likely different. Regardless of the chosen database, data standards are also critical to ensure that the format of the information, syntax, and punctuation are consistent (Cable and Fulcher 2006, Miller 2014). Data consistency allows databases to function as research and curation tools when used in basic queries, enhances the exchange of data among research and informatics partners, and facilitates the utilization of data with data aggregators (e.g., Global Biodiversity Information Facility [GBIF], iDigBio, VertNet).

Those curating genetic resource collections associated with natural history museums are currently using a number of different platforms to track data, including free-access database application systems developed for museums (e.g., Arctos, Specify), commercial database application systems (e.g., FileMaker Pro, Microsoft Access, FreezerPro®), web delivery through data aggregators (e.g., GBIF, HerpNET, ORNIS), and internally written applications (Zimkus and Ford 2014). All of the systems currently being used have benefits and drawbacks; before selecting a system, collection managers should evaluate the size of their genetic resource collection and internal computing resources, and determine how to integrate or link data associated with genetic samples and traditional voucher specimens. Free database application systems developed for natural history collections can easily associate specimen parts, but these systems might need to be customized to include data fields specific to genetic samples, such as changes to preservation, freeze–thaw events, and remaining volume. Some commercial systems (e.g., KE EMu) can accommodate genetic sample data, but some collection managers might find annual license fees too costly to maintain. Also, some specialized systems (e.g., FreezerPro®) can prioritize sample location data, which is important for a larger genetic collection, but disassociates traditional voucher specimen data from genetic samples. The use of a web-based data aggregator (e.g., Global Genome Biodiversity Network [GGBN; Coddington et al. 2014], GBIF) can allow external users to access data, but an internal platform is still necessary to allow collection personnel to track genetic sample metadata. Internally written applications might seem ideal because they can be tailored, but they require personnel dedicated to their development and maintenance, and who have an understanding of how to build the system with the flexibility and scalability needed for data and metadata growth.

Use of Collections

Most natural history museums have active loan/gift programs, but the consumptive nature of genetic resources requires collections to develop specific policies to address the demand on this unique type of collection. Genetic collections must also ensure that the manner by which genetic samples are processed and transported preserves the integrity of the original sample and all subsequent subsamples.

Loan policies.—In the context of natural history museums, a loan is a temporary transfer of a single specimen or lot of specimens, generally for research, for a specified period of time. Most loan policies for traditional natural history specimens are applicable to genetic samples but, owing to the consumptive nature of this resource and unique issues related to custodianship, clear policies should be developed in relation to the distribution of genetic samples. Baker and Hafner (1984) suggested that the term ''loan'' be replaced with ''gift'' or ''donation'' when discussing transfer of genetic samples between collections and researchers because the specimens are not returned; however, Zimkus and Ford (2014) found that some genetic collections do request unused samples to be sent back. Loan/gift policies should be explicit how custodianship issues, as well as permissions to approve the transfer of genetic material, are made, especially for institutions with a centralized repository. Unlike traditional natural history specimens within an institution, genetic samples might be part of a voucher specimen that was accessioned by another collection before the sample itself was deposited in the genetic collection.

Because genetic collections are consumptive, loan/gift policies should clearly outline the required information that should be submitted in sample requests to justify that a

material transfer is warranted. The following information is recommended for inclusion in loan/gift requests:

- Objectives of the proposed research and its scientific merit;
- Taxa and total number of genetic samples requested;
- Experimental protocol to be employed;
- Amount of genetic material requested per sample;
- Desired method of transport (e.g., room temperature in preservative, frozen on dry ice); and
- Plans for the dissemination of knowledge gained from the proposed research with the originating institution and scientific community, including publications resulting from use of the samples and online publication of sequence data.

Loan/gift policies should also clearly state the allowable use of genetic samples and associated metadata. Policies should stipulate that loans/gifts may not be transferred from one institution to another without the written permission of the originating institution, ensuring that material is used only for approved purposes. This also ensures that those receiving samples do not patent or otherwise profit from the use of specimens (as outlined in the Convention on Biological Diversity), which could compromise any original collecting agreement with the country where the specimens originated or any other entity that might have donated material to the collection. Researchers might also be obliged to acknowledge the institution in all publications resulting from use of their loan/gift material and make available citations or reprints of such publications.

Loan/gift policies for genetic resources differ from those of other types of natural history specimens because genetic sequence data is generated from their consumptive use. The majority of genetic resource collections surveyed by Zimkus and Ford (2014) request that researchers submit genetic sequence data to a public genetic sequence database, such as NCBI GenBank (http://www.ncbi.nlm.nih.gov/). This requirement is important for consumable collections because it ensures that sequence data is available to other researchers, unnecessary work is not duplicated, and originating institutions comply with granting agreements to make data accessible. Whenever possible, collections should link the end products of research (e.g., genetic sequences) captured in communal databases to the original genetic samples and/or voucher specimen records found in the originating institution's database. To ensure compliance, it is recommended that collection managers should follow up with researchers annually and consider loans/gifts "closed" only when all policy requirements are met.

If the institution provides the appropriate amount of material, the genetic samples loaned/gifted should be completely consumed by the researcher (see Use of Collections: *Aliquoting Samples*). If a portion of a genetic sample remains unused after the project, however, researchers might be unclear about what to do with the remaining samples. In a recent collection survey, institutions requested that researchers address leftover samples in various ways, including destroying the remaining samples, returning samples to the originating institution for destruction or reuse, or accessioning samples into the researcher's personal or institutional collection (Zimkus and Ford 2014). The loan/gift policy should clearly outline the requirements of researchers in regards to any unused genetic material. It is recommended that subsamples that are returned to loaning collections for potential future reuse be maintained separately from the original samples in case the returning sample's integrity was compromised (e.g., contaminated, mislabeled, improperly stored) while on loan. The use history of each sample is important to track,

especially for returned material, because this allows personnel to inventory and use only returned samples if all other portions have been consumed (see INVENTORY CONTROL AND DATA MANAGEMENT: *Databasing for Genetic Samples*). When returned samples are reused, tracking also allows collection managers to inform researchers of the chain of custody of the returned sample, so that future researchers can evaluate the risks of use of such samples.

Aliquoting samples.—To maximize their use and research potential, genetic resources should not be sent in their entirety; rather genetic samples should be aliquoted for internal or external use (see USE OF COLLECTIONS: *Loan Policies*). Regardless of the end user, subsampling methods should be employed that maximize collection utility by minimally handling samples, effectively removing the smallest sampling amount, and reducing freeze–thaw events while manipulating samples. Implementing efficient organizational methods, from storage containers to database management systems, minimizes the amount of time required to locate samples (see GENETIC SAMPLE PROCESSING: *Storage Containers*; INVENTORY CONTROL AND DATA MANAGEMENT: *Sample Labeling and Tracking*). At any one time, only a manageable number of samples should be retrieved, so that cold-storage units do not remain open and the time that storage containers are kept outside of these units is minimized. During the subsampling process, samples should be kept as close to their normal storage temperature as possible. Depending on the temperature from which the samples were taken, liquid nitrogen, dry ice, or wet ice can be used to help keep samples cold during processing.

The amount of a genetic sample provided to a researcher should be scaled to their individual request to ensure long-term availability of the original sample for other researchers. The SOP should provide baseline standards to guide the amount of genetic material sent as a loan/gift, including considerations for the type of preparation requested (e.g., tissue subsample, aliquot of extraction, PCR product) and the experimental protocol to be implemented. In a recent collection survey, collections were found to set these sampling criteria in various ways (Zimkus and Ford 2014). Over one-third of the surveyed collections provided the approximate amount of tissue needed for two to three DNA extractions; fewer collections quantified the amount of a sample sent (i.e., ranging from 1 mm^2 to 6 mm^2 in size, weighing from 10 mg to 2 g), likely because measuring or weighing individual samples is a time-consuming process that most collections cannot afford. Collection managers should also consider whether to extract DNA, rather than send tissue samples, especially when rare samples or those with low remaining volumes are requested. Regardless of the general criteria, personnel working with genetic samples should be knowledgeable of the sample amounts needed for methods commonly conducted (e.g., DNA extraction, RNA extraction, PCR, cloning). It is recommended that if researchers require more than the standard amount needed, justification should be provided in their sample request (see USE OF COLLECTIONS: *Loan Policies*).

Packaging and shipping loans/gifts.—As with traditional natural history specimens, loan/gift shipments of genetic samples should include loan paperwork on institutional letterhead that documents the contents, handling requirements, and policies of use (see USE OF COLLECTIONS: *Loan Policies*). Before any loans/gifts are processed, collection personnel must determine all the national or international laws and regulations pertaining to the genetic samples and the shipment, especially if a short shipping time is critical. Copies of all permits and certificates should be included with the shipment and placed in a location that is easily accessible on the outside of the package. Confirmation of the receipt of the samples should be documented for all loans/gifts and can be

accomplished in the same manner as traditional natural history specimens; namely, a loan invoice form is sent with the shipment to be signed and returned by the researcher. In addition to the regular import/export permits required for international loans, special permits or requirements might be necessary for genetic material, depending on the countries involved with the shipment (Renner et al. 2012). In addition, specific taxa might be regulated, including those monitored by the US Department of Agriculture (USDA) and/or those protected under the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA; Moore and Tymowski 2005), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; Davis et al. 2006, Donaldson 2006, Applequist 2014), and various US domestic laws, including the Endangered Species Act (ESA; Applequist 2014), the Lacey Act (Applequist 2014), and the Migratory Bird Treaty Act (MBTA). As the legal landscape evolves, collection personnel must receive the appropriate training, with applicable updates, in both national and international regulations, including the shipment of biological samples and dangerous goods (e.g., DOT, International Air Transport Association [IATA] Special Provision A180), and the recent ratification of the Convention on Biological Diversity Nagoya Protocol on Access and Benefit-Sharing.

Loans/gifts of genetic samples can be shipped using various methods and, depending on the sample type and ultimate use, material transfers might require special means of shipping to preserve specimen integrity and quality. Cold or frozen material should be shipped in a manner that will maintain the appropriate temperature for the duration of the shipment with allowance for delay in arrival time. ISBER (2012) suggested that refrigerant should last an additional 24 to 72 hours, depending on whether the shipment is domestic or international, especially because customs will be involved for the latter. It is advised that refrigerated or frozen shipments are not sent on days that require transit over a weekend or holiday period, so as to avoid delays and ensure that recipients are available for immediate receipt of the samples. The coordination of shipping and receiving samples is important for all parties, so to avoid mishaps, both the shipper and recipient should track the package while in transit. Collection personnel should contact the recipient before sending the shipment to confirm that they are present to receive the package as scheduled and, again, contact the researcher to notify them when the shipment is specifically scheduled to arrive.

When packing, genetic samples should be positioned in the package so that they are surrounded by refrigerant on all sides, rather than having samples placed on top of or underneath refrigerant. Any empty space between samples and refrigerant should be filled with dunnage material (e.g., loosefill nonbiodegradable peanuts, padding material). Specimens that are sensitive to humidity (e.g., botanical samples) should be shipped in sealed bags with desiccant to ensure that humidity levels are controlled in transit. The following are typical temperature conditions required for shipment of genetic samples, including the insulation and/or refrigerant that can be used to maintain the desired temperature (modified from ISBER 2012).

1. Ambient (20° to 30°C): insulated packaging with a minimum of 4-cm-thick walls to protect from fluctuations in ambient temperature; chemical preservative might or might not be necessary, depending on sample type.
2. Refrigerated (2° to 8°C): insulated packaging with a minimum of 4-cm-thick walls with wet ice or gel packs (conditioned at −15°C); chemical preservative might or might not be necessary, depending on sample type.

3. Frozen (−20°C): insulated packaging with a minimum of 4-cm-thick walls with gel packs designed for frozen temperatures (conditioned at or below −20°C).
4. Frozen (−70°C): insulated packaging with a minimum of 4-cm-thick walls with dry ice pellets, sheets, or blocks. Note that dry ice (solid CO_2) is considered a hazardous material and appropriate labeling should be included in accordance with DOT and IATA regulations.
5. Frozen (at or below −150°C): LN_2 dry shipper. Dry nitrogen shippers are insulated containers that contain LN_2 that is fully absorbed in a porous material within the walls and, therefore, considered a nondangerous product by DOT and IATA regulations.

Recently surveyed genetic resource collections were found to most frequently ship samples frozen using dry ice, or at ambient temperature using ethanol or DMSO as a preservative (Zimkus and Ford 2014). Shipping methods among collections likely differed due to overall cost, shipping restrictions, ease of shipment, and the intended use of the samples. With increasing regulations on shipments and shipping training for personnel, expenses have become prohibitive for many institutions and, as a result, many collections are either requesting or requiring that researchers offset the associated costs (see FUNDING AND BUDGET). Even 30 years ago, it was recommended that researchers planning to obtain samples from genetic resource collections include these costs in grant budgets (Baker and Hafner 1984).

Health and Safety

Health and safety policies are essential because they protect personnel who are working with genetic resource collections, but these policies also ultimately protect the collection itself because they promote proper and efficient practices. All health and safety policies should be reviewed with the appropriate health and safety group at the institution and fully outlined in the SOP of the genetic collection.

General laboratory practices.—Good laboratory practices should be implemented in genetic resource collections to protect staff from exposure to various hazards (e.g., potential pathogens, chemicals, sharps). For staff safety, certain activities should be prohibited in the laboratory or collection space, including eating, drinking, storing food, smoking, applying cosmetics, or handling contact lenses. Mechanical pipetting devices should be used in the laboratory; for safety concerns, mouth pipetting must be prohibited. For collections harvesting genetic samples from traditional voucher specimens, collection managers must ensure that any biosafety waste is processed in accordance with institutional regulations and any pertinent local, state, or national guidelines. Policies should be instituted for the safe handling and disposal of sharps (e.g., needles, razor blades, scalpels). Lastly, good laboratory practice includes washing hands at the end of preparation work, after gloves are removed, and prior to leaving the laboratory.

Personal protective equipment.—All personnel, including visitors, should wear appropriate and approved clothing (e.g., buttoned lab coats, long pants, shoes that completely cover the feet) when working in a laboratory environment, such as a genetic collection (Kapinos and Graham 2006). Shorts, skirts and open-toe shoes are not appropriate, especially in labs that require a Biosafety Level 2 designation. In addition, long hair and dangling jewelry should be secured. Depending on the laboratory application, personnel should wear the appropriate gloves; this includes latex or nitrile gloves when handling chemicals or biological samples, cryogenic gloves to protect against

frostbite when working with ultracold equipment or samples, and heat-resistant gloves when using autoclaves or other equipment that reach high temperatures. Eyes should always be protected from exposure to chemicals and biohazards by using safety glasses, goggles or face shields; eye and face protection devices should comply with American National Standards Institute recommendations (i.e., ANSI Z87.1) when used with chemicals.

Liquid nitrogen safety.—Those collections that use LN_2 storage equipment require additional safety precautions and equipment specifically rated for cryogenic conditions. Specialized ventilation and oxygen monitors might be needed to protect personnel from the risk of oxygen depletion and possible asphyxiation (see Facility Management: *Ventilation*; Facility Management: *Oxygen Monitors*). When working with LN_2, appropriate PPE is recommended to protect the eyes and skin from cryogenic burns. Cryogenic gloves, which are water-resistant insulated gloves rated for cryogenic temperatures, should be used at all times to protect the hands when working with LN_2 equipment (e.g., cryovats, dewars, hoses), storage equipment (e.g., racks, boxes), and samples. Cryogenic gloves will not protect the skin when immersed in the nitrogen liquid itself, so additional precautions must be taken to insure that gloves are only exposed to vapor. Cryogenic aprons are essential to protect the body when using the liquid phase of nitrogen but are also a good precaution when working with the vapor phase of LN_2. Eye protection should be worn when working with samples that have been stored in the liquid and vapor phase of nitrogen, but there is an added danger with the liquid phase because sample vials can burst without warning if LN_2 enters them through minute cracks while in storage and then rapidly expands when thawing. When dispensing LN_2, goggles and/or a face shield, rather than simple safety glasses, are recommended to additionally protect the face and eyes from possible splashing. Closed-toe shoes that cover the entire foot are required in genetic labs but, when working with LN_2, it is additionally recommended that footwear can be quickly removed (e.g., few laces, buckles, zippers). Therefore, if a spill occurs and liquefied gas enters the shoes, footwear can be easily removed, preventing severe burns that result when LN_2 surrounds the foot with nitrogen and is held in the shoe material. Canvas shoes are not recommended because LN_2 can easily permeate the material. With regard to clothing, lab coats are essential, and it is recommended that pants not have cuffs nor be tucked into shoes or boots because LN_2 can become trapped if spilled.

If nitrogen in the liquid form contacts the skin or eyes, the tissue should be immediately flooded or soaked with tepid or warm water (105°F to 115°F [41°C to 46°C]). Hot water (above 46°C) should never be used because this can cause additional damage to the skin. In addition, the skin must not be rubbed because this can damage the tissue; thus, any contact should involve only gentle patting or dabbing. If LN_2 comes in contact with the eyes, contact lenses should be removed immediately and the eyes flushed with tepid or warm water for at least 15 minutes, and the upper and lower eyelids should be lifted occasionally during the flushing process. If any injury occurs from a cryogenic burn, medical attention should be sought as soon as possible.

Cryogenic fluids must be handled and stored only in containers specifically designed for these products in accordance with SOP. Unapproved materials can become brittle and shatter or become over-pressurized, causing risks of explosion. All cryogenic vessels must be equipped with pressure-relief devices (e.g., relief valve, venting lid/stopper) to prevent excessive pressure build-up. A tremendous amount of force can be generated if liquid nitrogen rapidly vaporizes, so pressure-relief devices on LN_2 equipment should be

monitored to ensure that they are functioning properly. Transfer operations involving open cryogenic containers, such as dewars, should be conducted slowly to minimize boiling, splashing, and thermal shock to the receiving vessel. A phase separator (i.e., fitting that attaches to end of a transfer hose that separates gas from liquid) can also be used to control the vapor path while dispensing. When dispensing or pouring LN_2, receiving vessels should be placed as close as possible to the source; a table or other sturdy surface can be used to position the vessel in the proper location. Funnels should not be used to channel LN_2 because they can freeze, creating a splash hazard, or be propelled upwards by the LN_2 if the container is overfilled.

Dry ice safety.—Use of dry ice (i.e., solid phase of CO_2) is regulated and requires specialized training for use in shipments. When dry ice is used, collection managers should ensure that there is adequate ventilation because CO_2 can displace oxygen, causing loss of consciousness or even asphyxiation. All confined areas should be closely monitored when dry ice is in use. Walk-in freezers should be kept free of dry ice because carbon dioxide can rapidly build up without regular air exchange; owing to this inherent danger, proper signage should be posted outside walk-in freezers to prevent staff from placing dry ice into walk-in freezers.

Biosafety.—Collection managers should consult all federal biosafety and biocontainment regulations relevant to the activities conducted that involve potentially hazardous biological materials within the collection. Policies should incorporate all regulations that outline standard and special practices, safety equipment, and facility requirements to minimize potential hazards to laboratory personnel and the environment. USDA permits could be needed for work with some specific taxa, such as birds, some mammals, and plants. Depending on the biosafety level required for the collection as determined by the USDA, certain requirements might need to be met, including training in handling pathogenic agents, restrictions to collection access when work is being conducted, and conduction of particular work in a biological safety cabinet. A biosafety cabinet (BSC), also known as a biological or microbiological safety cabinet, is an enclosed, ventilated workspace for handling materials potentially contaminated with pathogens (e.g., Exotic Newcastle Disease, Foot-and-Mouth Disease, Hantavirus, Highly Pathogenic Avian Influenza). BSCs differ from fume hoods because BSC exhaust air is HEPA-filtered as it exits. BSCs are classified as one of three classes by their level of protection provided to personnel and the environment (i.e., Class I, II, III). Class II Type A2 BSC, which includes a minimum inflow velocity of 100 ft/min (30.5 m/min), is the most commonly used BSC providing protection for personnel, the environment, and thesamples. Collection managers should be aware that gas lines should not be installed into BSCs because air is contained within, rather than being exhausted from the cabinet, leading to the potential build up of flammable materials. Additionally, open flames, such as those generated by Bunsen burners, can detrimentally affect airflow in a BSC, disrupting the pattern of HEPA-filtered air supplied to the work surface. To provide adequate protection, BSCs require proper use, monitoring, and regular maintenance. SOP documents must outline these approved activities, including appropriate height of the sash opening to minimize airflow, methods used to decontaminate surfaces, as well as testing and certification protocols for the unit itself (see Operational Best Practice: *Equipment Preventative Maintenance, Repair, and Replacement*).

Chemical safety.—Genetic resource collections should maintain an inventory of all chemicals used and access to Safety Data Sheets (SDS) for every chemical used within the collection. A chemical hygiene plan should also be included in SOP documents, which

outlines the appropriate use of chemicals in the collection, as well as their containment and procedures for clean-up if spills occur. Personnel should have the appropriate training for all procedures related to chemical safety, which frequently involves training by institutional groups, such as health and safety and the fire group (see Operational Best Practice: *Training*).

Fire safety.—Collection personnel must comply with all local fire and building codes. Automatic fire detection units and suppression systems, including appropriate hand-held fire extinguishers and sprinkler systems, are recommended. The SOP should include information regarding major fire hazards and potential ignition sources, as well as what working materials are flammable hazards (e.g., ethanol). Personnel should be trained in fire prevention and emergency procedures relating to evacuation if a fire occurs (see Operational Best Practice: *Training*). All fire detection systems and suppression systems should be tested on a regular basis and frequently involve health and safety, fire, and building facility personnel at the institution (see Operational Best Practice: *Equipment Preventative Maintenance, Repair, and Replacement*).

Ethical and Legal Best Practice

The accumulation and use of genetic samples involve numerous ethical and legal issues. Most natural history museums have institutional policies that address legal compliance and ethical standards, but many times, genetic samples are present in individual laboratories or stand-alone collections whose activities might not be specifically addressed in the broader institutional policies. In these instances, dedicated policies might not be in place to govern broad aspects of legal and ethical issues associated with the initial collection, transport, and use of genetic samples. Genetic resource collections are subject to the same regulations, laws, and consequences as traditional natural history collections. As such, they must have accessioning practices that ensure and document that all samples are legally collected, transported, and acquired by the collection. Copies of all necessary permits, including collecting permits, relevant Material Transfer Agreements, export permits from the country of origin, and import permits into the country of the genetic collection, must be on file within the institution or genetic resource collection (see Policy Best Practice). The accession process should also ensure and document that all animals collected were done so in a manner consistent with pertinent guidelines, such as Institutional Animal Care and Use Committee (IACUC) regulations.

Unlike most traditional natural history collections, genetic resource collections make samples available to researchers for molecular analyses with the understanding that derivatives and even possible genetic modifications of the original sample will be produced. It is a legal responsibility that collections personnel, therefore, act as custodians to ensure that genetic samples are used in accordance with all laws and governing policies, including the original collecting or acquisition agreements and pertinent international conventions (e.g., Convention on Biological Diversity Nagoya Protocol on Access and Benefit-Sharing; CBD 2011, Applequist 2014). Collection managers should be aware that countries or other governing entities might have restrictions in relation to the commercial use of genetic samples, whereas educational or research use is permitted. In addition, entities that retain ownership (e.g., US National Park Service) can have specific regulations in relation to exportation, importation, or use of samples.

To document compliance and provide responsible stewardship for consumptive genetic resources, collection personnel should track the use history of all genetic samples and

their derivatives, and link this information to the available metadata (e.g., NCBI GenBank; see INVENTORY CONTROL AND DATA MANAGEMENT: *Databasing for Genetic Samples*). Loan/gift policy must, therefore, be explicit in the approved uses of genetic samples and associated metadata, as well as expectations for what the researcher should do with any samples remaining after the completion of the research (see USE OF COLLECTIONS: *Loan Policies*). Policy should also clearly outline requirements resulting from use of the genetic material, which can include the acknowledgement of the collection in publications, notification of any resulting publications to the originating collection, and submission of genetic sequence data to a public genetic sequence database. Research users and collection managers should both make a dedicated effort to ensure that collections are formally acknowledged for use of their samples and collections are notified of published studies that result from the use of their material. Knowledge of sample use is vital to genetic resource collections, because they must justify their significance in research to both their institution and outside funding bodies. Lastly, copyright and intellectual property rights in relation to genetic sample metadata provided to researchers or presented on public websites should be accompanied by policy that outlines the terms and conditions of its use, including the requirements to share results from the research.

Genetic and genomic resource collections, by their nature, are very process and equipment-dependent, requiring dedicated space, personnel, and funding to function properly. Operating at suboptimal levels can have deleterious and catastrophic effects on both the genetic samples and collection personnel. Institutions and/or laboratories that form genetic and genomic resource collections must be aware of the commitments associated with operating, monitoring, and maintaining these collections, including legal obligations and safety requirements. It is the responsibility of those overseeing genetic resource collections to properly assess and control risk hazards ensuring that personnel can work safely and samples are proficiently curated.

DISCUSSION

The main goal of this paper is to present standards to address the best practice curation of genetic resource collections associated with natural history institutions. Compared with traditional natural history specimens, genetic samples generally have a narrower window of tolerance in relation to storage and handling, and these samples must be monitored from the time of initial preservation throughout their research lifespan to maintain the quality needed for molecular analyses. In addition, institutions must develop policies that address the ownership, documentation, and research use of these collections to ensure that samples are collected, transported, and utilized in accordance with all relevant local, state, national and international regulations. These guidelines provide a foundation for the stewardship of these specialized collections both for museums creating centralized genetic resource collections and laboratories working to organize and house existing research collections. In addition, this information will help existing collections in prioritizing potential upgrades to equipment and strengthen current practices.

Genetic resources have become an integral part of natural history collections, but these collections are often not curated for maximum use, longevity, and potential when housed within departments curating traditional voucher specimens or stored within individual research laboratories. Many institutions have realized the intrinsic value of these consumptive resources because field expeditions are costly to execute and many species can no longer be sampled due to restrictions on collecting or, worse, extinction. Proper

curation of genetic collections ensures that genetic samples are readily available and of research quality for broad molecular analyses. In recognition of their growing importance, many institutions have moved samples into centralized repositories, dedicating space, funding, and personnel to their maintenance, and establishing policy that governs their curation.

Collection managers and independent researchers that incorporate aspects of best practice standards into their internal policy will improve the organization of their samples and efficiency of their personnel. Ultimately, improvements in the management of these collections will ensure high quality samples and minimize potential risks to both the samples and personnel. It is the authors' hope that these guidelines can be used as a point of reference for those curating genetic samples, because the main objective of this paper is to focus on the aspects of curation that are most important to the long-term preservation of these resources. As with all standards in a community, these will evolve as more collections prioritize the conservation of genetic resources and the technology related to sample preservation improves. We look forward to continual discussion and subsequent advancements for genetic resource collections within the natural history community.

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