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Now what? Pre- and post-analysis preservation of ancient animal and human molecular products

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Abstract

With the advent of the "genomic revolution" and the rapid refinement of techniques of molecular biology, academic institutions, such as museums and university collections, are at the forefront of specimen analysis. However, the lack and/or poor condition of preservation of molecular voucher generated from often fragile and rare specimens is a problem rarely addressed. To remedy this problem, the AMNH launched a frozen tissue collection, the Ambrose Monell Cryo-collection (AM-CC) in May 2001. The AM-CC maintains specimens below -150 o Celsius and supports ongoing genetic research across taxa, from ancient to modern samples, by insuring that all research materials are vouchered (i.e. they point back to a specimen in a curated collection), a much-needed service that the Museum extends to the entire scientific community. Scientists using the Monell Collection have access to legally collected, authoritatively identified and properly documented specimens for use in their research, complete with Museum catalog numbers to reference in their scholarly publications. Researcher are also offered the possibility of vouchering their research by depositing the DNA or tissue samples gathered for their studies.

The issues

The majority of talks are about collection-based, biological samples-based studies. However, the Manchester Mummy Tissue Bank (Under the initiative of Dr. Rosalie David, http://www.dnafoundation.com/cpope/mirror05.htm) remains to this day the only systematic effort to maintain ancient tissues according to up-to-date best practice of preservation and databasing.

80% of molecular samples used in all published studies have no proper post-study storage or very loose archiving protocols (either discarded or stored haphazardly) and suffer from an eclectic history of acquisition. This means that 80% of all studies performed currently have no voucher and are not reproducible. And without reproducibility there is no science. On a more pragmatic level, the problem of not vouchering specimens has add a tremendous impact on the literature and DNA sequence databases such as GenBank (EMBL) by creating "false" published sequences (20%).

Furthermore, museums should be at the forefront of preserving the molecular information contained in fragile specimens, which keep on deteriorating in inappropriate storage condition for retrieval of ancient molecules.

In many cases, the reason for inaction in vouchering DNA or other molecules on the part of traditional collection managers and curators, as well as researchers, is the hesitation on whether DNA can be considered a voucher. The answer is: "ABSOLUTELY YES". Ancient DNA (aDNA) extractions are always difficult and frustrating, and often stem from precious and rare aliquots, hard to re-sample, if need be. Furthermore, the repetition of an extraction from an additional sample might not yield at all the same quality or validity of results. If a vouchered sample of the original DNA is kept, not only is it a voucher for the scientific community at large, allowing reproducibility of results, but it is also a back-up samples in case of assay failures, or contamination problems.

This articles means to share with the scientific community, and in particular my colleagues in all fields of Biological Anthropology methods of best practices of preservation of post-analysis molecular samples in place at the AMCC.

Who we are and our mission

The AMCC's mission is to primarily create a genetic library of biodiversity for studies in genomics, evolutionary biology, phylogenetics and to voucher molecular products stemming from these studies. This has entailed

accessioning, and in many case extracting DNA from, tissue samples from the museum's legacy collections using techniques of aDNA. Our ancient specimens are varied and include, but are not restricted to, tissues and DNA from: amber preserved insects, human mummies DNA, "museum prepared" skins and skeletons, ancient parasites preserved in naturally mummified cadavers etc.
The broad scope of the AMCC addresses an aspect of cryogenic bio-repositories not yet approached by attempting to catalog all biodiversity at the molecular genetic level. The AMCC is further distinguished from other repositories because of its location at the American Museum of Natural History (AMNH), where tissue samples can be referenced with documented collecting events involving traditional voucher specimens and associated data, linking tissues to bibliographic citations, geospatial referencing information, genetic data, digital images and photographs as well as external relevant databases (see below).
The AMCC supports a broad range of comparative genetic and genomic research initiatives. We provide our researchers with collecting kits to readily sample and ship genetic material of high quality, enhancing the genetic information content of each specimen. The AMCC supports ongoing genetic research by insuring that all research materials are vouchered (i.e. they point back to a specimen in a curated collection, see below). We extend this service to the entire scientific community, under the guidance of our institutional policy.
(See the AMCC website @ http://researc.amnh.org/amcc/coll_pol.html).
Scientists using the AMCC have access to legally collected, authoritatively identified and properly documented specimens for use in their research, complete with Museum catalog numbers to reference in their scholarly publications. In each case, we record as much information as possible to document the existence of the sample, where and how it was collected and by whom as well as what research has previously been conducted on the specimen.

Our system and equipment

The AMCC maintains specimens in an array of liquid nitrogen cooled vats, at temperatures below -150° Celsius (Fig. 1).

Fig. 1 - Cryo-Storage room.

Frozen specimens held at -20° are subject to protein and lipid changes and damage from the growth of micro-organisms, while specimens held at -80° are also subject to protein and lipid changes, with extensive desiccation of specimens and some molecular damage (Frank, 1985; Florian, 1990). Thus, we advocate a "colder is better" position on archiving tissues, especially when the long-term use of the resource is undefined.
The AMCC lab facilities (BSL II; fig. 2) include a dry lab, complete with 2 bio-safety cabinets (where the accessioning of the samples is mostly done), a wet lab (fully equipped for all kinds of genetic assays), and a cryo-storage room (in which the physical collection resides).
Fig. 2: Floor plan of the AMCC
Each sample is archived in a vial on which a cryo-resistant label is applied containing the AMCC unique barcoded and human readable number.
Additionally, the AMCC has a wide array of equipment for field collection trips, such as our cryogenic dry shippers, which facilitate freezing samples collected in the field and transporting them back to the museum without ever thawing the specimens.

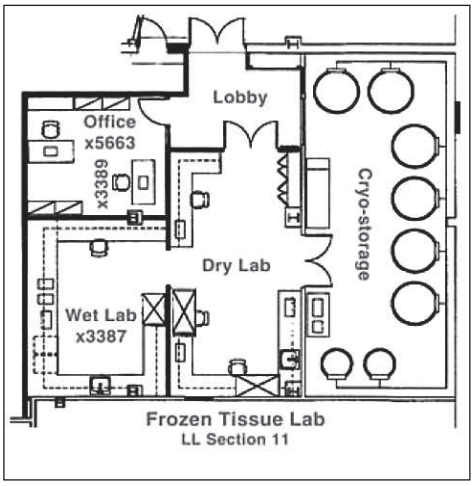

Fig. 2 - Floor plan of the AMCC.

The problem of non-centralized repositories

We advocate strongly the need for a centralized repository as a way to solve problems of security, safety and quality control. Museums and universities around the world face three main problem grandly jeopardizing their collections:

1) *Inadequate or non-standardized containers and labeling:* A variety of containers and labeling techniques were used for making tissue collections. Many, but not all the containers are proving inadequate for long-term archival. Moreover, many of the techniques for labeling are proving to be inadequate: numbers written on pieces of tape that were applied to collection vessels do not adhere properly under long-term storage conditions at low temperatures, in other cases the writing itself is disintegrating with changes in temperature associated with specimen sorting and retrieval, and/or buffer leakage.
2) *Uncontrolled storage conditions:* Many tissue collections

are located in mechanical freezers without sufficient backup freezer space to handle meltdown of a malfunctioning freezer, or backup power in the case of extended service interruptions. Past power failures, such as the blackout of August 2003, have revealed the urgency of accessioning samples in reliable, non-mechanical, non-power based freezers, such as the cryo-vats of the AMCC. Furthermore, by having an unrestricted and unguarded access to the freezers, tissue collections run a very high risk of disappearance, accidental thawing (sample left out by mistake), contamination and misplacements of specimens. In most cases, specimens to be used for research or sent out on loans are sub-sampled within laboratories, which house numerous PCR machines and no bio-safety cabinets. This increases severely the risk of contamination of the samples (through aerosol DNA), as shown by Scherczinger et al. (1999).

3) *Addition to data capture:* The electronic database for many tissue collections lack location reference in the -80^0 freezers. For most collections, the retrieval of samples relies critically on the memory of the collection manager or worse, the researcher using the samples, and at best, a single Excel spreadsheet to locate specimens.

As a bio-safety II, restricted centralized repository, the AMCC has answered these many problems both through the design of the facilities, as well as through bio-informatics solutions. Thus, we have answered the problem of mechanical freezers by maintaining samples into stable, liquid Nitrogen charged cryogenic freezers. The AMCC also has ample back-up freezer space in case of emergency. Furthermore, not only has the AMCC a restricted access and gate keepers of the collection, but contamination is also avoided by the use of two bio-safety cabinets in which all transfers are performed, and by the absence of PCR machines in the facilities. All instruments are also rigorously disinfected between the transfer of each specimen and the bio-safety cabinets are sterilized at the end of each day.

Databasing and inventory

In its daily operations, the AMCC tissue samples are indexed using Freezerworks", a relational database application program well suited to the task of freezer inventory management (Ioannou, 2000). The program creates a record for each specimen giving it a unique barcode ID. This technology is now being utilized in both museums (Monk, 1998) and other biodiversity assessment programs (Oliver, et al. 2000). Data entry in the database is made easy and reliable with the «import» feature, allowing donors to generate their own data spreadsheet and have it imported into the AMCC database without ever having a third party (lab tech) modify anything manually. Each record contains data ranging from the collecting event (who collected it, when, where, how and even why), to each the position of each vial in the collection's many freezers. Following data entry, the program generates a printed cryo-resistant label, which includes the unique ID number both as a barcode as well as a human-readable numbers. This feature allows for lab technicians to retrieve any vial from the freezers quickly and reliably by scanning the label to retrieve its associated data and thus confirm the identity of the specimen they are attempting to retrieve. The computer database tracks each bar-coded vial, noting the specimen's taxonomic identity, where the specimen was collected, by whom and tracks how many times the sample has been thawed and refrozen.

The AMCC online database

Because our relational database, FreezerworksUnlimited, has not yet been made compatible with the web, a "shadow" database had to be built in order to render the holdings of our collection accessible to the scientific community worldwide. In order to host the fully searchable database, the AMCC launched its own website in 2002.

The data available on the AMCC website constitute a subset of data from the facilities' database Freezerworks Unlimited. This allows the AMCC to have better control of the amount of data published on the web. The online database of the AMCC runs on the MySQL relational database management system. This database allows specimen records in the collection to be located by taxon name or browsed by taxonomic hierarchy. The web database front-end is written in PHP and is served on the back-end by an Apache web server running on Solaris. The PHP code is in a development stage, but is being steered towards an object-oriented design that should make it fairly portable to other collections.

The AMCC Online Database and GenBank/NCBI

The database is designed to integrate with the National Center for Biotechnology Information's Entrez indexing and retrieval engine. This allows for AMCC records with nucleotide sequence accession numbers to link out to corresponding pages on the NCBI Genbank and Taxonomy databases and inversely for GenBank sequences to link out back to the AMCC.

The core tables of the database are the specimen and taxonomy tables, and are joined by an associative table that allows a many-to-many relationship between specimens and taxonomic names. In addition, both the specimen and taxonomy tables form a many-to-one relationship with a table for "foreign resources," which flexibly store information about external URLs, files, or database resources. At the moment, only URLs to websites are stored in these tables, but the design, in theory, allows any type of resource-location information to be stored that pertains to an address that can be associated with an institution. In the future, the taxonomic names will be joined many-to-many with a common names table.

E-Vouchers in the AMCC

The term «E-Voucher» has been defined by Monk and Baker (2001) as follows: «An e-voucher is a digital representation of a specimen. An e-voucher may be

ancillary to a classical voucher specimen or it may be the only representative of the specimen in the collection.» The importance of e-vouchers has emerged not only with the advent of better digital imaging technology, but also with new approaches to specimen collection and the redefinition of «museum vouchers». The AMCC has linked, whenever possible, the specimen records to digital images (hosted by the museum's Digital Library server), making for a complete connection between sequence data and the visual identity of the specimen examined.

Future considerations and recommendations

Institutions and researchers around the world must assess the preservation state of their current specimens collections and their role in modern genetic and genomic studies. It is recommended that the salvage of ancient specimens should be of high priority in thinking about genetic resources at academic institutions. But what's more important is the vouchering of the molecular products of studies on ancient specimens, often too fragile for re-sampling. Vouchering post extraction DNA offers a tremendous service not only to science but to the individual researcher as well. Science is not science without reproducibility of results.

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