

Botany Best Practices (US National Herbarium): A product of GGI-Gardens

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I. About this Training Module

The Smithsonian Institution's Department of Botany (including the US National herbarium; US) has recently embarked on a new project to voucher and collect genome quality tissue samples from the plants growing in mid-Atlantic Botanical Gardens, Greenhouses, and Arboreta. This effort, funded by the Global Genome Initiative (GGI; <https://ggi.si.edu/>), is part of the GGBN (Global Genome Biodiversity Network; http://www.ggbn.org/ggbn_portal/). The recent increase in collecting for genomic purposes has led to the development of some 'best practices' and we have attempted to outline those here. Although these are, in some areas, specific to US we hope they will be useful to a broader audience as a starting point for other 'Best Practices' documents.

This training module is the beginning of our effort to establish field collection standards for botanical collections in the Department of Botany (Smithsonian Institution) and associated tissue samples that are intended for incorporation into the NMNH Biorepository. In this document you will find information on collecting voucher specimens, how to manage these specimens, and the incorporation of their data into EMu and the Biorepository. This document is intended as a supplement to other training modules, specifically the General Training Module and the Liquid Nitrogen Training Module from the Global Genome Initiative (GGI). There is an abundance of books and manuals on herbaria and how to collect herbarium specimens (Applequist & Campbell 2014; Davis AP 2011; Davis PH 1961; Fosberg & Sachet 1965; Hyland 1972; Jain & Mudgal 1999; Lot & Chiang 1986; Miller & Nyberg 1995; Mitchell 1982; Robertson 1980; Smith 1971; Steenis van 1977; Womersley 1981) so we will not cover collecting methods in this manual (see Appendix A for references for collecting).

II. Voucher Specimen Collection

(A) What is a Vouchers?

If possible, a voucher should be all or a portion of a plant that is collected, preserved, and maintained as reference material in an herbarium (Fig. 1). Since species identification in the field is often impractical and cultivated material is commonly misidentified, without a voucher it is often impossible to verify the correct name of a collection. A voucher specimen is typically used for taxonomic verification and morphological representation but can be used to infer a number of biological, ecological, and evolutionary characteristics about how and where the plant lived. The terms used to describe a voucher depend on the type of voucher and the relationship of the voucher to the genetic sample. The US National Herbarium (US) prefers pressed, dried voucher specimens with reproductive material (Fig. 1), however, the type of voucher used depends on the materials at hand, and/or institutional protocol. An example of materials that should be brought into the field is provided (Fig. 2) and a typical greenhouse collecting event is shown in Figure 3.

(i) Types of Vouchers

(1) Herbarium voucher: a *traditional museum specimen* (Fig. 1). This is a preserved, intact plant or part of a plant that has been pressed, dried, and mounted on archival paper with a label that provides information on where it was collected. Such vouchers are stored in an herbarium, preferably one that is listed in *Index Herbariorum* (a free online listing of all herbaria; Theirs, continuously updated). If a specimen is a voucher for a genetic sample, then the genetic sample consists of material that was taken from that exact individual that is represented on the herbarium sheet. Such pressed, dried herbarium voucher specimens represent the most common type of preserved plant collection (see Appendix A for literature on collecting). A dried voucher specimen can also be an exemplar (see below and Fig. 4).

(2) Exemplar voucher: a *phenotypic voucher*, an *allovoucher*, or a *representative voucher*. This is also a preserved, intact, specimen mounted on an archival, acid-free sheet and stored in an herbarium. If a specimen is an exemplar of a genetic sample, then the genetic sample is material taken from a *different* individual plant collected at the *same time* in the *same place* by the *same person* and deemed by the collector to be of *the same species* (Fig. 4).

For example, if there are 20 ferns collected in an area and they are determined to be the same species, then one fern can be taken in its entirety to become a dried, mounted specimen and the fronds from the other 19 ferns can be taken as genetic samples; the dried, mounted specimen is an exemplar of those 19 genetic samples.

(3) Other material as vouchers: There are other parts of the plant (e.g., seeds, fruits, wood, etc.) that can be mounted on an herbarium sheet, stored as bulky material or placed in a special collection (e.g., wood), and used as vouchers as long as the material is sufficient for identification.

(4) Photo voucher: a photograph of a plant with no physical specimen. This occurs when a physical genetic sample (leaf/petal/seed/root/etc.) is collected, but it is not possible to collect the plant or even a portion of the plant for an herbarium voucher and the photograph is the only evidence of the whole plant. This is not recommended. Because of the importance of an herbarium voucher (e.g., misidentifications) one should, when possible, make repeated trips to try and obtain an herbarium voucher (especially when the plants are in gardens or greenhouses). If a photo voucher is used, photographs should clearly indicate reproductive structures as well as habit and any characters that are useful for unambiguous species identification.

(5) Living voucher: There has been some discussion about ‘living vouchers’, which occur when a plant is left alive in a greenhouse, arboretum, forest plot, etc. and referenced with location and/or accession information. Such vouchers should not be used and are not

accepted in the NMNH database. One should always make a photo voucher if the plant cannot be collected.

(B) What information should be collected in your field notes as a part of the voucher?

Appropriate label information is as essential as the material collected for the voucher (see label in Fig.1). For the labels, collectors should include details that are not intrinsic to the specimen itself. This information must include **biographic information** (collector, collecting number and any collecting team members), **biological data** (taxon name and/or family, character notes such as flower color, plant height, etc.), and **geographic data** (location, latitude x longitude). In addition, one should try to include **ecological data** (e.g., soil type, landscape), and an indication that genetic samples were taken from the specimen. Genetic sample *unique identifying numbers* should be indicated in the field notebook and, if possible, on the label. It is also advisable to have a header on the label that indicates the collecting project (e.g., “Plants of the District of Columbia”) and a footer that lists the herbarium sponsoring the collecting trip (e.g. “Sponsored by the US National Herbarium & GGI-Gardens”). The format for entering these data may vary from collector to collector, but all collectors should have a clear, pre-determined organizational method for documenting this information, preferably in an archival field notebook that can be deposited in the herbarium library or other archival venue. In some instances the collector may prefer to fill out individual sheets for each collection and GGI-Gardens has sample collection sheets (Appendices B–C). Plant collections from Gardens may have information indicating where the plant was obtained and these data should be requested from the Garden, GGI-Gardens has a data sheet for this type of information (Appendix D) and when possible these data should be included on the label of the voucher specimen or on a separate label mounted on the voucher.

(i) Biographic collector information

All voucher specimens must include a clear record of the full name and affiliation (possibly in the footer of the label) of the primary collector as well as the collecting number. There should be a reference to all additional collectors. The collecting number is furnished by the primary collector. Collecting numbers should be sequential from the beginning of the collector’s career until the end. It is recommended that collectors avoid using collecting numbers with dates or letters and especially avoid the practice of starting the numbering sequence over every year (using the year as the first part of the collecting number, e.g., 2016-101 which indicates the 101st collection of 2016). These habits can create confusion in many databases

(ii) Geographic locality

The original location of the plant that has been made into an herbarium voucher is an important part of documenting biodiversity. All collectors must record detailed information regarding the locality that would allow subsequent researchers to revisit the exact location of the collection. Geographic locality information includes: Country, 1st political division (e.g., state); 2nd political division (e.g., county); nearest population center (e.g. town, village) and directions and distance to the collection site from that town; GPS coordinates; any physical landmarks or landscape features to help locate the specimen (e.g., adjacent to a river or stream, along a slope, next to a road, etc.); elevation; and any information on the substrate.

(iii) Biological and ecological character notes

Inevitably, a pressed, dried specimen will include only a fraction of biological and ecological information that the entire, living plant represents. Therefore, knowledge about this collection depends upon detailed notes regarding the state of biological and ecological characteristics related to this collection. Important information includes: taxonomic identification (if known), observations about the size of the specimen (height, width, etc.), habit, life history stage, features that are too large or difficult to collect, information about the color of physical characteristics (since this information can be lost during the preservation process), other organisms that are observed interacting with the collected specimen voucher (e.g., pollinators, herbivores), other organisms growing on or nearby the collected specimen (e.g., ecosystem type). Clearly not all of this information will be available or necessary but the notes you make should be as detailed as possible, so as to place the specimen within the context of its living state.

(iv) Genetic sample tissue information

Genetic sampling is detailed in section III, however, the information that a specimen is a genetic voucher should be included in the field notes and included on the label of the specimen voucher. The field notebook should include the unique NMNH Biorepository number (a 7 character alphanumeric number) for each genetic sample, the preservation method of the sample (liquid nitrogen, silica, etc.), and the relation of the sample to the specimen (voucher or exemplar). All genetic tissue samples should be labeled with the primary collector's last name and collecting number.

(v) Preparing Dried Voucher Specimens in the Field

Vouchers are collected in the field, pressed, dried in a plant drier (or air dried in parts of the world where the ambient temperature is high and the humidity low), frozen for 4–5 days at –20F° to eliminate any pests, and finally affixed to an 11.5" x 16.5" archival herbarium sheet. Skill in

the pressing, drying and mounting processes will have a great impact on the final quality of the specimen.

During the collection event, best practices for museum quality vouchers should be followed: make sure to include reproductive parts or other diagnostic features, use the entire plant with cleaned roots if it will fit on a standard herbarium sheet (11.5" x 16.5"), and the specimen should be arranged in an aesthetic manner with both surfaces of planar structures apparent (e.g., both sides of leaves visible). There are publications that detail collecting methods including many special techniques for some groups of plants such as bulky, large, succulent, or aquatic species (*aquatic plants*, Haynes 1984; *Araceae*, Nicolson 1965; *bamboo*, Soderstrom & Young 1985; *Cyclanthaceae*, Hammel BD 1987; *ferns*, Croft 1999; *Lecythidaceae*, Mori & Prance 1987; *lianas*, Gerwing et al. 2006; *palms*, Dransfield 1986; *succulents*, Baker et al. 1985; *Zingiberaceae*, Burtt & Smith 1976; see Appendix A for a more inclusive list). Specimens are placed in newsprint in a ‘sandwich’ of blotters and corrugates then compressed between boards with straps (Fig 3). The corrugates, or ventilators, can be cardboard or aluminum and must have openings to allow airflow between each specimen. Air-drying within a day or two of the collecting event is the preferable method of preserving a voucher and vouchers preserved in this manner may retain high quality DNA, which may be extracted in the future if the need arises. Plant driers ideally have forced air and gentle heat (45–50° C). High temperatures can give specimens a brown, scorched appearance and degrade their DNA. A simple, portable electric dryer, can be constructed fairly easily (Appendix E; Blanco et al. 2006).

Extended storage of unprocessed specimens in newsprint should be avoided. Recently Neubig et al. (2014) stated “...newspaper is highly acidic and probably contributes to DNA degradation, especially in specimens stored for long periods.” Neubig et al. (2014) provided some evidence that this may be true, however, there are other contributing factors such as humidity and temperature that could be responsible. To be safe, if material is intended for DNA use at some point in the future it should be subsampled and the tissue put into silica gel even if it is a few months old. Until it is subsampled it should be stored at a low humidity and temperature and in acid free paper. The specimens should be mounted and intercalated into the ordered collection as soon as possible.

(vi) Ethanol Preserved Voucher Specimens

An alternative method of specimen preservation, especially in wet tropical environments where drying facilities are often unavailable, is to fix them with ethanol prior to drying. This method prevents the decay of specimens for several months until they can be dried. Long-term storage in ethanol should be avoided. Ethanol preserved vouchers usually turn brown and any DNA extracted from them is often highly fragmented. To use ethanol preservation, voucher specimens are field pressed in newsprint, bundled, placed in a plastic bag and then saturated with ethanol (or other alcohol such as isopropanol, if ethanol is not available; the alcohol should be >50%). Bundles should be stored in a dark place until they are delivered to a drying facility and dried in a conventional manner. So far there have been no problems in bringing such bundles back on

airplanes as checked baggage, however any excess alcohol should be drained off and you should consider changing the papers). Bundles that have reduced alcohol content post transport can be frozen for temporary storage until they are dried without obvious affects on specimen quality.

III. Genetic Sample Collection

(A) Tissue Collections preserved in Silica Gel

(i) How to Choose and Work with Silica Gel

Silica gel is used as a desiccant to rapidly dry recently collected plant tissues destined for DNA extraction. For this reason, always store silica in a reclosable air-tight container (Appendix G) away from moisture (Appendix G; Fig. 3A). A variety of silica gel types, as well as other kinds of desiccants (see below), have been successfully used by researchers. While finer grades (i.e. sand-like 28-200 mesh size) will dry tissues more quickly due to a greater surface area relative to volume, they are more difficult to work with and larger (less expensive) grades will typically dry adequately to get genomic quality DNA (Fig. 3A). When working with all grades of silica gel, be aware of the hazards of accidental inhalation, which can cause respiratory problems. Finer grades of gel can be more easily inhaled and use of a facemask is recommended.

To assess the saturation of silica gel (to know when to change it out for fresh silica gel), indicating gel is used which undergoes a color change when saturated. Indicators on the market come in 3 versions: **orange>>green** (based on methyl violet), **orange>>clear/white** (based on iron III/II salts), **blue>>pink** (based on cobalt (II) chloride). We recommend the **orange>>clear/white**. Avoid using blue indicating gel as cobalt (II) chloride is an irritant, a carcinogen, and can cause environmental damage and the orange/green is not totally innocuous as the methyl violet is toxic and mutagenic. Orange/green is potentially safer but not totally innocuous as the methyl violet is toxic and mutagenic. To monitor moisture levels during storage, small amounts of indicator silica can be used or one can opt for *relative humidity cards* which may be a better option than indicator gel (they use inert salts instead of organic dyes). For storage, a relative humidity of 30% is a realistic target.

Once silica gel is saturated, it should be exchanged for smaller amounts of fresh silica gel (Fig. 3A; see next section for more information). To save money the saturated silica gel may be reconditioned (reactivated) to drive off water so that it is absorbent once more and can be reused (<http://www.agmcontainer.com/desiccant-reactivator-unit.html>).

Other substances that seem to work well as desiccants include salt (Carrió & Rosselló 2014; P. Acevedo pers. com.), silica-gel cat litter (B. Brooks & A. Egan pers. com.), regular clay cat litter (A. Egan pers. com.) and even a food dehydrator (Tai & Tanksley 1990). Reports from the US National Herbarium Botany staff indicate that all of these substances work to dry leaf material. The clay based cat litter seems to be the least favorite because it takes longer to dry plant parts than the others. In the end, when available, most collectors prefer to dry their leaf material in silica.

(ii) How to Preserve Tissue Samples in Silica Gel

When preserving plant material with silica gel, the goal is to gently dry samples as quickly as possible. The faster samples are dried, the higher the quality of DNA that can be extracted from them. Samples should be completely dried within 12–48 hours of collection, and should be dried in a cool, ambient-temperature environment.

When taking plant tissue for genetic samples, it is best to choose young (but not the very youngest) undamaged leaves. Young leaves have a higher density of cells per volume and should yield more DNA. However, the very youngest leaves are fragile and prone to damage before drying and the results are better with slightly older leaves. In general, older leaves are also more likely to have contaminants such as fungi or epiphylls. In wet tropical environments fungal endophytes rapidly colonize new leaves, especially from spores landing on those leaves (A. Herre, pers. comm.).

Collect an amount of tissue that is approximately 10–25 cm². Avoid stacking leaves in many layers because the inner most leaves will dry more slowly than the outer leaves. Leaves that are particularly thick, tough, or waxy should be torn (or cut) into small pieces to increase the amount of exposed edge to draw water through. Be careful of cross contamination on cutting implements or fingers due to sap or leaking cell contents. If tissues are dirty or contain epiphylls, wash in high quality water and pat dry. Other sources of DNA include flowers (corollas); which can be low in secondary compounds, and seeds. If preservation is delayed the tissues should be kept cool and moist to prevent wilting until they can be processed. Cold shock and deterioration (i.e., watery breakdown of tissues similar to freeze damage) can occur during refrigerator storage of sensitive species and may affect DNA quality.

At the present time, we recommend collecting genetic samples directly paper coin envelopes (2½ x 3½ inches) such as ULINE S-11485, or small reclosable polyethylene bags (2½ x 3½ inches) such as ULINE® or Ziploc® (Appendix G). The coin envelopes are porous, easy to label, allow for rapid drying of tissue samples, and are sized to fit into permanent storage boxes (such as Lock n Lock®) in the NMNH Biorepository, so little post-collecting processing work will need to be done. The coin envelopes are bleached, white paper envelopes but not necessarily archival quality. We are currently looking for a cost effective, archival storage solution that is acid free and unbuffered. Do not use brown or glassine envelopes that are acidic and less porous (respectively). Before the envelopes are placed in the freezer they can be closed with stainless steel paper clips (Appendix G): while this is not necessary it can help keep the envelopes from overlapping with one another.

Envelopes should be placed in an airtight container or Ziploc® style bag and surrounded by silica gel to maximize surface area contact for drying (Chase & Hillis 1991; Fig. 3A). Different sources estimate the correct amount of silica gel as anywhere from 9–15 times as much silica gel as plant matter and this partly depends on the water content of the tissues. The silica gel should be gently mixed around the envelopes to prevent local saturation of the silica and examined several times a day to make sure the silica gel is not changing color. The benefit to storing tissue in separate envelopes is that you can place many samples together in one container (Fig. 2A). Replacing hydrated silica gel in a shared container is less time consuming and the

silica gel can be reused. If we are working out of an herbarium with a plant dryer we put the used silica into a large cotton bag (e.g., pillow case) and flatten it on the wire rack or shelf of the plant dryer and turn the temperature on high. In more remote areas it can be cooked in a large frying pan over a fire. When the indicator has returned to its original color it is ready to be used again (<http://www.agmcontainer.com/desiccant-reactivator-unit.html>).

There are alternative collection methods. For instance, instead of collecting into coin envelops, some researchers collect into teabags or coffee filters that are very porous and allow samples to dry quickly. The drawbacks of teabags are that they are not as structured, so dried samples may be crushed more easily, and they are harder to label. For storage in the Biorepository, the collector may have to fold teabags and put them into coin envelopes or the samples may need to be transferred, requiring the collector to spend extra time processing them. Another method is to collect into small reclosable polyethylene bags containing individual quantities of silica gel. The benefit is that plastic bags are easy to label, tissues easy to see, and because tissues are in direct contact with silica gel they will dry more quickly. When using polyethylene bags it is advisable to remove the silica on a rolling basis as samples dry. Dry samples can then be grouped in larger 1 gal reclosable bags. There are several drawbacks to the use of polyethylene bags. First, the dried leaves may be pulverized by the abrasive action of silica gel as samples are transported (making them harder to prepare in the lab). Second, it is extremely labor intensive when one needs to change silica gel from each small bag. Third, the collector may need to empty the polyethylene bags and transfer the samples to coin envelopes for storage in the Biorepository, and finally, the silica cannot be used again so this method is more expensive.

(B) Liquid Nitrogen Tissue Collection

See the Liquid Nitrogen (LN₂) Training Module for additional details on handling liquid nitrogen and other items. For collection into liquid nitrogen, plant tissue samples are collected and placed into 8 ml, externally threaded cryovials with o-ring seals (Fig. 2F). Tubes are labeled with a Biorepository barcode sticker (Fig. 2D), wrapped in a ca. 4x8 cm piece aluminum foil (Fig. 2F), and placed into a cryogenic storage Dewar filled with liquid nitrogen (Fig. 3). Samples begin to degrade immediately after collection, so it is important to work quickly. The foil is critical as the jostling of the tubes may cause the barcode stickers to dislodge or the caps to come off when the tubes are first put into the Dewar. Because the barcode sticker is the link between the collecting information and the sample it is critical that it be protected. Writing the collection number on the tube is a way to create a secondary identifier.

If LN₂ is not available in the field, but LN₂ collections are desired, one possibility is to bring seeds back from the field and grow them locally. Genetic tissue samples could be cut from growing plants and deposited directly into LN₂, and new voucher specimens can be collected directly from growing plants. Although it is labor-intensive, it provides excellent material and is a viable alternative to working with LN₂ at a remote collecting site.

(C) Ethanol Tissue Collection

Historically, ethanol has been used to preserve animal tissues for DNA studies but not plant tissues. There has been some investigation recently in the use of ethanol for plant tissue preservation. Preliminary results suggest that, for some species, ethanol preservation may result in a higher yield of DNA but the quality of the recovered DNA is lower when compared to that recovered from the silica-dried method. The mechanism of preservation for alcohol is presumably dehydration, coupled with useful leaching of secondary compounds. As in animal tissues, higher alcohol concentrations (95–100%) appear to promote better preservation; changing the alcohol after an initial fixation can help reduce the total water for high water content plant tissues. The long-term viability of the DNA stored in ethanol has not been determined, however, preliminary tests show that while the quality of the immediately extracted DNA is excellent, the leaf material left in the alcohol for over a month it is no longer superior to silica collected material (J. Wen, pers. comm.). For the storing of samples for genomic use we recommend silica or liquid nitrogen.

(D) RNA Collection and Preservation

(i) General precautions.

Tissues collected for RNA extraction must be handled with additional care in order to prevent the activity of RNases, both endogenous and those introduced by environmental contamination. This is not trivial as RNases are nearly ubiquitous, sturdy enzymes that are hard to “destroy”. When working with these tissues, wear gloves and use forceps. If the goal is to preserve the transcriptome, it is important to process the sample as quickly as possible to prevent changes due to altered physiological state after collecting. While plants are slow to die after being picked, changes in gene expression can occur much faster and it is best to get tissues into the preservation medium quickly after harvesting (within seconds to a few minutes). If field decontamination methods are needed working surfaces can be cleaned by flaming with a micro-torch, dipping/wiping in chloroform (evaporates without residue but is carcinogenic), or a commercial decontamination solution (RNaseZap, RNase Away; but needs an RNase-free water wash afterwards). RNA can be preserved by flash-freezing or special preservative solutions such as RNAlater®.

RNAlater® is specified in its patent (US 6528641) as a solution “composed of 25 mM sodium citrate, 10 mM EDTA, 70 g ammonium sulfate/100 ml solution, pH 5.2”, although subsequent patents have suggested improvements. Pre-prepared tubes of RNAlater® are the easiest method of preserving RNA. Leaf tissues should be cut into small pieces ($\leq 0.5 \text{ cm}^2$ in any single dimension) and 5–10 volumes of RNAlater® to allow complete penetration. One problem with plant tissues is that they typically wet poorly in RNAlater® and float. To mitigate this issue use small vials that can be filled completely so that tissue can stay submerged, or macerate submerged tissue with a scalpel. Once tissues are in RNAlater® they should sit at room temperature to 4°C for 12–24 hours, then frozen (-20°C or -80°C). While manufacturer

guidelines indicate room temperature storage for up to a week will not affect quality (or up to a month at 4°C), in general, colder storage sooner is better. Long-term storage at -80°C is recommended. RNAlater® will freeze into an opaque white block at -80°C and must be thawed to remove tissues; preferably most RNAlater® is poured off prior to -80°C storage, which will allow selection of samples without thawing.

Standard LN₂ (see above) is another effective preservation method for RNA but keep in mind the comments above concerning the time between the removal of the tissue from the plant and its preservation.

IV. Samples in the NMNH Biorepository (Appendices D & E)

Below, we discuss standard Biorepository specimen deposition methods. These methods are the most efficient and productive for long-term storage, databasing, sampling (genomic DNA and RNA). As always, specimen collection handling procedures should be performed with utmost care to ensure that the Biorepository collections are preserved according to the indefinite access and preservation goal of the Biorepository.

(A) Storage of silica preserved collections in the Biorepository

Silica dried samples are stored in -80°C freezers in the Biorepository. The current setup (still under refinement) is storage of databased, tissue-filled coin envelopes in Lock & Lock brand plastic boxes (HPL836), which are airtight with a clamping lid and gasket. Each box has a custom plastic grid inside, producing nine compartments where envelopes are stored upright. The grids are sized to fit the standard coin envelopes that have been in use in Botany (Appendix G). Within each box will be a packet of desiccant to keep samples as dry as possible and absorb moisture when boxes are opened.

(B) Storage of liquid nitrogen preserved collections in the Biorepository

Samples selected for LN₂ preservation must be collected according to the procedure outlined in Liquid Nitrogen Training Module, using 8 ml cryovials. These cryovials will be stored in 6x6" cardboard boxes in vertical metal racks in the Biorepository nitrogen freezers. When returning from field collecting, be sure to take all liquid nitrogen samples to the Museum Support Center (MSC) to be deposited in the Biorepository as soon as possible.

Please try to follow the recommended collection methods (collection into coin envelopes and 8 ml cryovials). By setting forth these specific collection methods, we are minimizing the amount of processing and rehousing work that will need to be done to incorporate samples into the Biorepository. This would be most efficient (rehousing is a very time consuming process) and would protect the integrity of the samples the best (less risk of contamination, less physical handling of delicate dried samples, etc.).

V. Preparing for a Field Collecting Trip (Appendices D & E)

(A) Collecting Event Approval/ Permits

It is strongly encouraged that all collecting permits be acquired prior to departure. In some countries it is not possible to obtain a collecting permit before you arrive but the application process for a permit should be well underway before the collector departs. In addition, if you intend to bring or ship plants into the USA you need an APHIS permit. If the trip is expected to produce a substantial number of specimens the Collection and IT Managers should be alerted so they can plan for the arrival of specimens, etc.

(i) Obtain Acquisition Number for Collecting Event

Once the collecting event been organized, contact the Acquisition Manager (currently Erika Gardner) to receive an **EMu Acquisition Number** (previously referred to as the Accession number or OR number) for the event.

(ii) NMNH Field Information Management System (FIMS) database

If you chose to use the NMNH FIMS to generate a FIMS spreadsheet for recording specimen data you should consult NMNH Informatics for assistance in selecting the appropriate fields for your collection trip. If you use FIMS it will insure that collection events are linked with genetic samples and photos. If you chose to use a standard Excel spread sheet, make sure the fields match those required in the FIMS. The Botany Acquisition Manager or Botany IT office can supply you with such a spreadsheet. The Botany IT office has templates tailored for field collections (including tissue samples) which are designed for both vouchered genetic samples, and for genetic samples when the voucher is deposited elsewhere. Alternatively you may choose to enter your data directly into EMu upon your return to NMNH. This is especially useful if you are collecting locally. All alternatives to the NMNH FIMS database should be approved by the relevant departmental Information Technology staff (currently Chris Tuccinardi).

(iii) NMNH Biorepository Labels

The Biorepository will assign a range of Biorepository numbers and adhesive labels to label tissue samples in cryovials and envelopes. Please request labels from the Biorepository Manager (currently Chris Huddleston) at least two weeks prior to departure.

(iv) In the Field

When entering information in the FIMS or to your spreadsheet, you must enter a separate record (a separate row on the spreadsheet) for every voucher specimen and genetic sample that you collect. This includes pressed voucher specimens, leaves/tissue collected in coin envelopes, leaves/tissues collected in 8-ml cryovials, and any material that is collected into a Matrix plate (uncommon for Botany collections). For example, Funk-13245-1 might be the voucher, Funk-13245-2 might be the cryovial, Funk-13245-3 might be a silica sample, Funk-13245-4 might be a photo, etc.

When you are collecting specimen vouchers to ultimately deposit in the Biorepository, you must remember to add barcodes to Biorepository items. A unique barcode label will be added for both the LN₂ cryovial *and* the silica gel coin envelope. The corresponding circular stickers can be placed in the field notebook or the original collection sheet (Fig. 2D; Appendix A or B). Barcodes for silica envelopes can be affixed prior to departure, in the field, or upon return, as long as they are recorded along with the appropriate specimen data.

(v) After collection of vouchers and tissues

- 1) Plant Presses should be placed in an appropriate plant dryer to dry the specimens (either in the field or at NMNH). Presses should be checked daily, turned and tightened. Most specimens will dry in 2-4 days. Do not leave presses in a dryer longer than 2 days without checking them and removing the dry specimens.
- 2) Once the dried specimens arrive at NMNH, they must be frozen as part of Botany's Integrated Pest Management protocol before entering offices or areas of the permanent collection (see below)
- 3) When the specimens are available the Acquisition Record (formerly the Accession number or OR number) in EMu needs to be updated. The collector should work with Acquisition Manager to complete the Acquisition information. The collector will also need to provide a copy of all collecting and export permits (if applicable), an SI travel authorization (if you traveled on one), and the collector and collection number range to the Acquisition Manager. The Acquisition Record number is needed for the drop tag that accompanies all bundles of plants when they move around the herbarium and go to plant mounting.
- 4) All collection data must be entered into EMu. As mentioned above, there are three options for entering the data:
 - a) the label information may be entered into the NMNH-FIMS usually in the field;
 - b) the data can be recorded in a customized Excel spreadsheet, either in the field or when you return, or
 - c) the data can be typed directly into EMu,

If the collector has not used any of these methods before, the Botany IT Office will provide training. The person entering the data must be sure that all data, Acquisition numbers, etc. are entered in the correct tab. The entry of these data into EMu is the

responsibility of the collector unless prior arrangements have been made. When the voucher data are added to EMu an **Institutional Record Number (IRN)** will be generated for each specimen. If the collector uses the FIMS spreadsheet, or an approved excel spreadsheet, with all the field data, preliminary identifications, and Acquisition number on each record it can be submitted to the Botany-IT staff for uploading into EMu.

- 5) Voucher labels can be printed from EMu. The Botany IT office has designed a number of “reports” in EMu that allow the collector to print labels and new “reports” can be developed in cooperation with the IT Office.
- 6) The “**Primary Collector + Primary Collector’s Number**” together form the unique identifier for the voucher when it is collected in the field. Later, once the specimen is mounted, the **US Herbarium catalog number** becomes the primary identifying number. The Primary Collector + number (e.g., Funk 12345) is used to link all associated genetic samples (tissues, DNA extracts) as well as photos, and other collected items (e.g., wood samples). It is recommended that all tissue samples be assigned a unique Sample ID (or Tissue ID) Number derived from the Primary Collector + Primary Collector Number. The suggested standard is to take the Primary Collector name + number, and append an additional number for every related tissue. For example, if five separate samples are collected from Funk 12345, then those can be assigned the sample ID numbers (e.g., Funk-12345.1, Funk-12345.2, Funk-12345.3).
- 7) Samples preserved in LN² and silica should be transported to the Biorepository at the Museum Support Center (MSC) as soon as possible. This provides them with safe storage and handling even before the spreadsheets and data are entered into EMu. In addition, each tissue sample is given a Biorepository number (a seven-character alphanumeric number; e.g., AB3DE67). These numbers are printed on Biorepository labels and the labels should be attached to each tissue sample (cryovial, coin envelope, etc.) and the corresponding circular 2D barcode label should be affixed to the field notes close to the entry for the voucher specimen from which the sample was taken. Silica gel envelopes and liquid nitrogen tubes will each be given a unique barcode.

An example (very abbreviated) dataset might look like this:

Funk 12345:

Dionaea muscipula, collected at the US Botanic Garden on 19 June 2016.

Funk-12345.1: Leaf, silica dried. (AB3DE67)

Funk-12345.2: Leaf, liquid nitrogen preserved. (AB3DE68)

Funk 12346:

Carnegiea gigantea, collected at the US Botanic Garden on 19 June 2016.

Funk-12346.1: Leaf, silica dried. (AB3DE69)

Funk-12346.2: Flower, silica dried. (AB3DE70)

Funk-12346.3: Leaf, liquid nitrogen preserved. (AB3DE71)

Funk-12346.4: Flower, liquid nitrogen preserved. (AB3DE72)

- 8) After the collections are deposited in the Biorepository:

- a) The Biorepository will provide the collector with a spreadsheet that has biorepository barcode and where the collector adds the appropriate IRN, the barcode number, **the preparers unique IRN**, and the **Sample Field ID**. Remember for Botany, the Voucher & samples Field ID is the Primary Collector's Name + Number followed by a number suffix. The Biorepository will link the **Primary Collector Number**, the **Institutional Record Number**, all **Biorepository barcode numbers**, and **genetic sample numbers** in EMu.
 - b) If the collector or designate is entering the data directly into EMu, permission must be granted by Botany IT.
- 9) Photographs are treated the same way as genomic samples. Photos can be uploaded directly into the EMu record by the collector or designate or the information can be added to a spreadsheet and submitted to be batch uploaded. The fields are the Primary Collector + number, the IRN, the image name, and a title (ie: habit, flower, fruit). If there is more than one image, each should have Primary Collector + number and IRN repeated for each image for the same voucher.
- 10) Labels are the responsibility of the collector unless prior arrangements have been made with the IT Staff. Once voucher information is entered into EMu, labels can be generated directly from the database or from the collectors own label program.
- 11) Labels should be added to the vouchers and tied into ca. 25 sheet bundles with appropriate drop tag and Acquisition number and submitted for mounting.

Anyone wishing to contribute collection data should check with the Botany IT office to make sure they have the appropriate spreadsheet or have completed Botany FIMS or EMu training. When using EMu one indicates that a specimen is the voucher of a genetic sample, by entering the Primary Collector's Name and Number in the **Voucher Field ID** field of the genetic sample row. In addition, specify the type of voucher in the **Voucher Type** field. Be sure to include the sample preservation method. See Figure 4 for a graphic depiction of voucher, exemplar, parent, and child relationships.

Remember all DNA/voucher collections need to be data based in one of three ways: a) through the NMNH FIMS database, b) using the specially designed excel spread sheet obtained from the Botany department IT office, or c) entered directly into EMu.

A flow chart that summarizes the steps involved in vouchering specimens and making genomic quality tissue samples can be found in Appendix F and a list of vendors for some of the supplies in Appendix G.

(B) For GGI partners

All GGI collections need to be data based, available on line, and deposited in a GGBN facility. If you are collecting as a GGI partner and your home institution has its own database and/or collections database procedure, please refer to your institutional protocol.

VII. References

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Figure 1. Example specimen voucher from the US National Herbarium showing a dried, pressed herbarium voucher specimen with Catalog Number and specimen label.



Figure 2. Example of a typical set of field equipment required for voucher specimen collection (for plant press and LN₂ Dewar see Fig. 3). A. Bag with silica and envelopes, A1. Sample envelope, B. Camera, C. writing implements, D. Genomic sample barcode labels, E. clippers, F & F1. cryovial and aluminum foil, G & H. Data sheets.

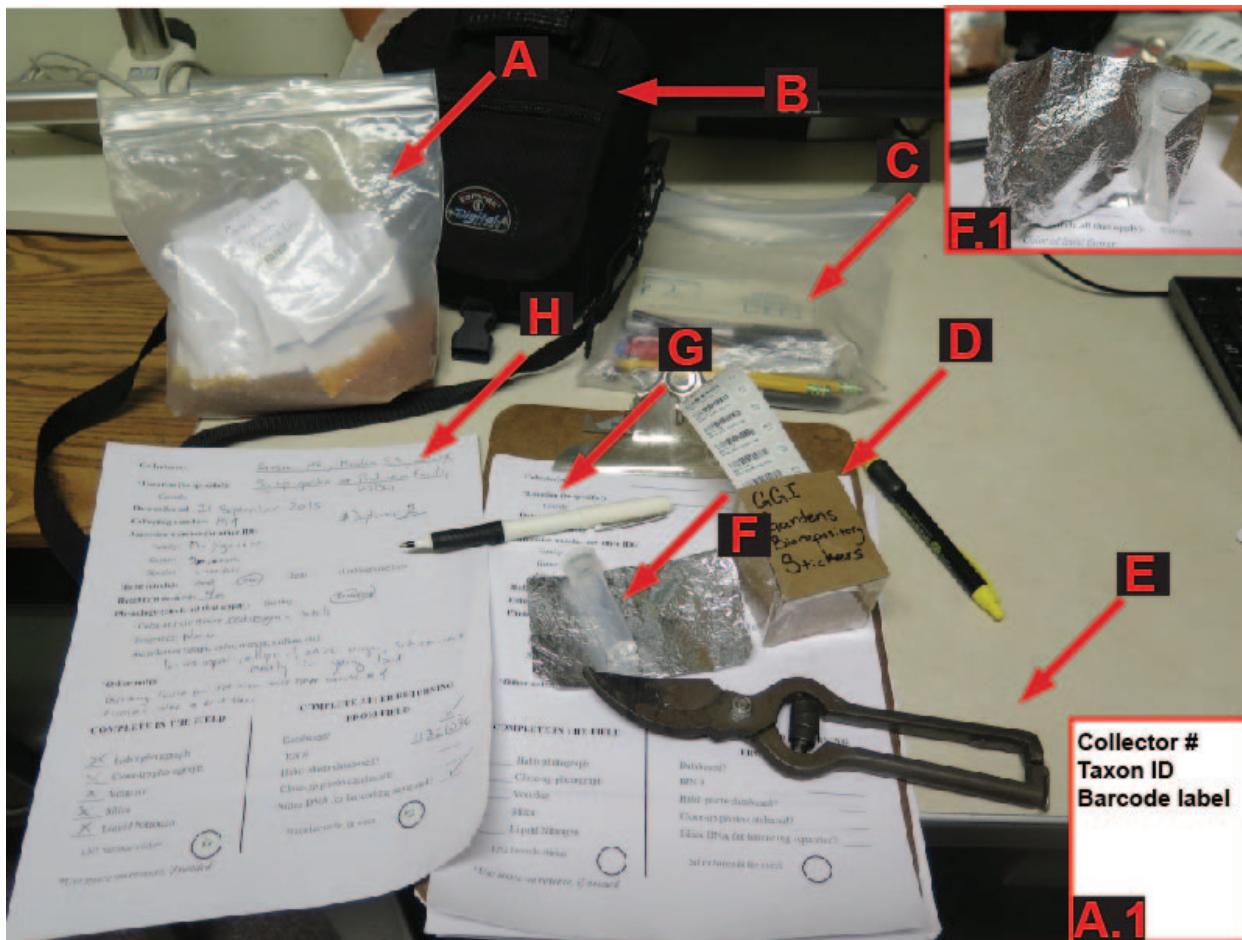
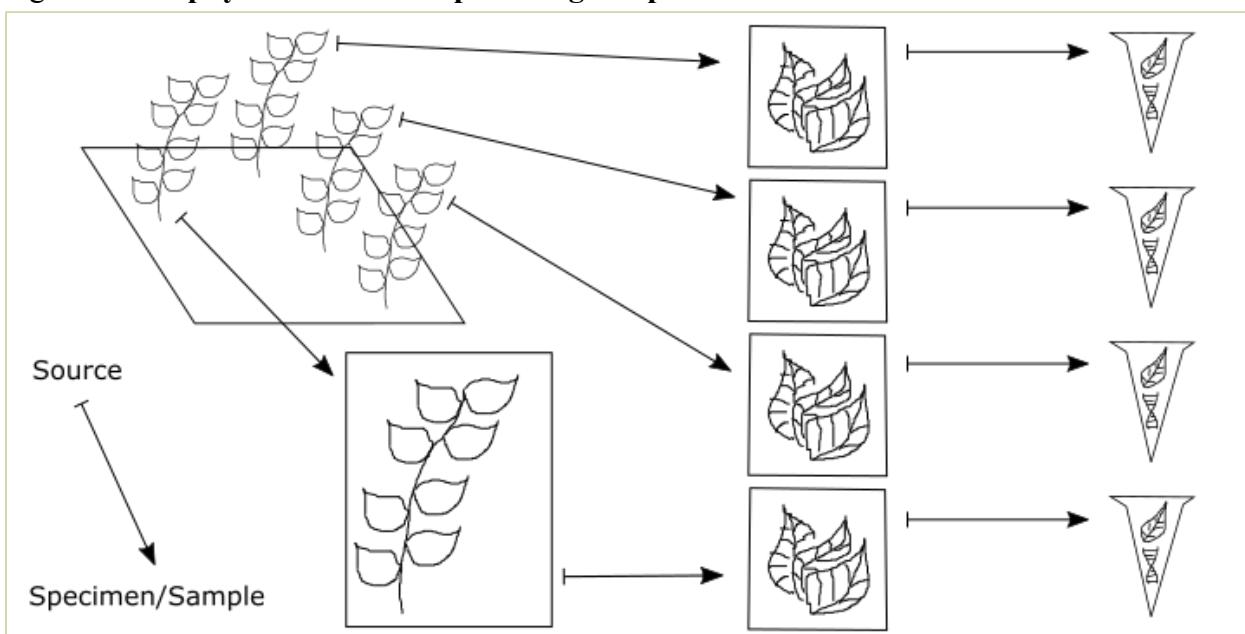


Figure 3. Example voucher specimen collection event with all necessary materials (from GGI–Gardens).

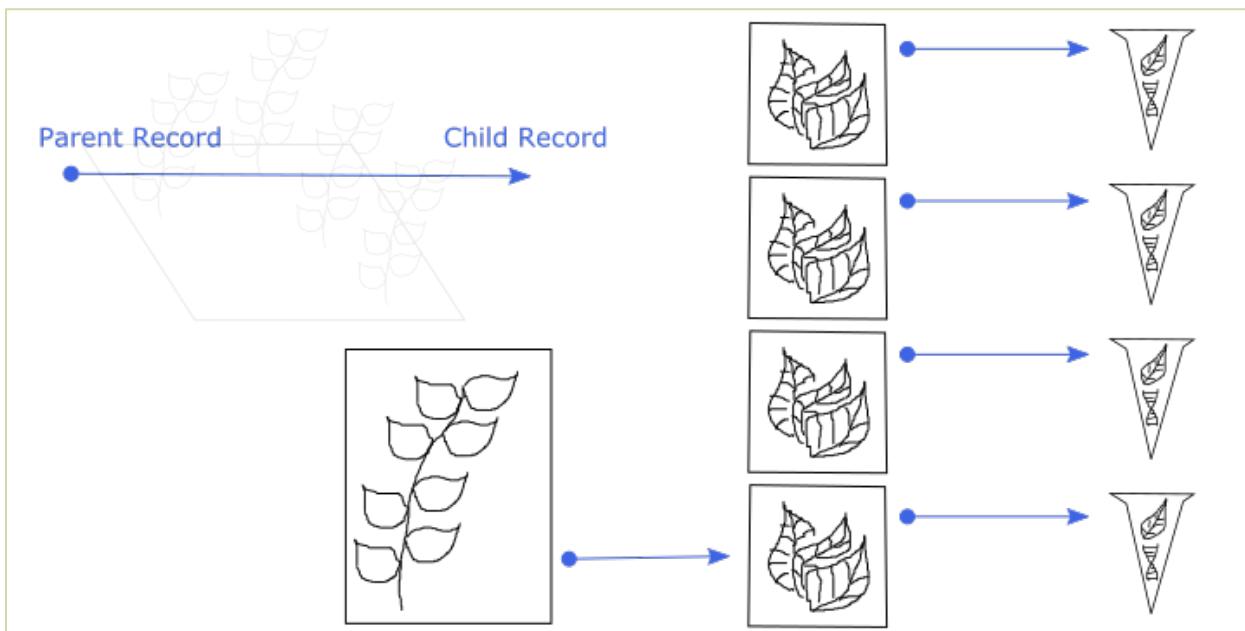


From left to right: Sarah Gabler (summer undergraduate intern) is taking notes for the field book, V. Funk (Research Scientist, SI) is pressing the voucher specimen, Asia Hill (YES High School Intern) is preparing the tube for the liquid nitrogen sample, Kristen Van Neste (summer undergraduate intern) is waiting for leaf material to put into the coin envelope that will go into the orange silica gel in the reclosable polyethylene bag. [Photo courtesy of the Smithsonian Institution and GGI]

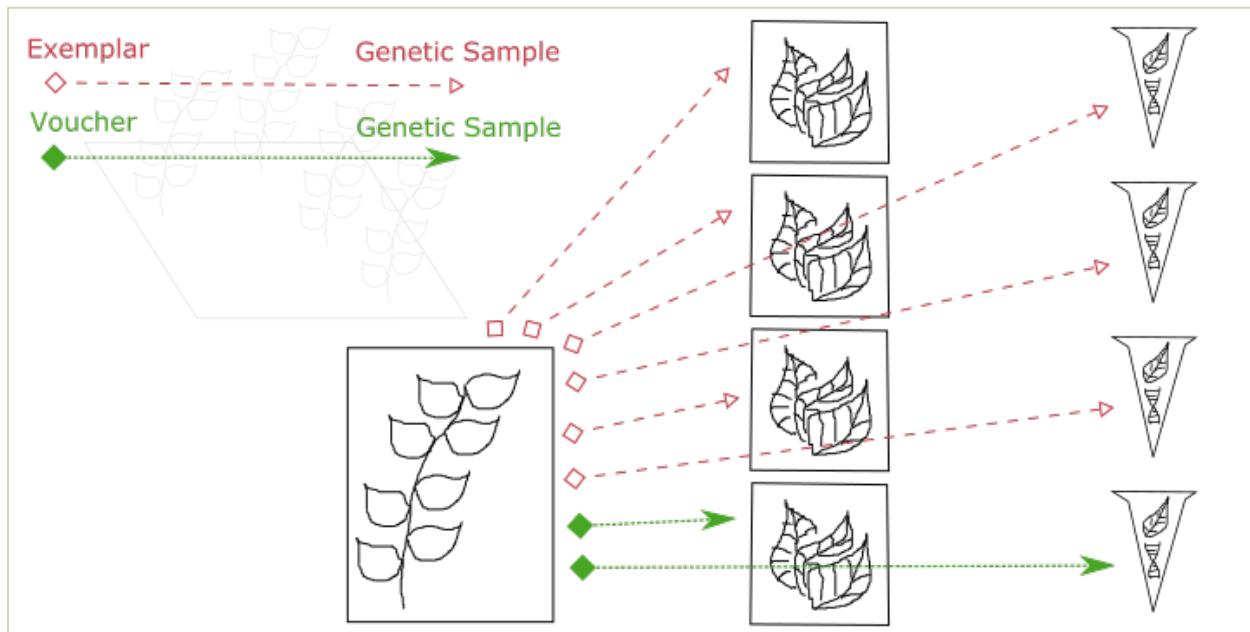
Figure 4. The physical relationships among samples



A. The physical relationships between samples. One voucher specimen and three genetic (tissue) samples are taken from an individual in the field. In addition, one genetic (tissue) sample is taken from the voucher specimen. Genetic (DNA) samples are extracted from each of the genetic (tissue) samples.



B. Every sample that is derived from another specimen/sample is a child of that specimen/sample. The original individual in the field is not cataloged, so it is not a parent. The bottommost DNA extract is a child of the bottommost tissue sample, which is a child of the voucher specimen.



C. The Voucher and Exemplar designations. When genetic samples are taken directly from the pressed, dried specimen, that specimen is the voucher of those genetic samples. When genetic samples are taken from individuals that are different than the pressed, dried specimen, but are thought to be the same species, then the pressed, dried specimen is the exemplar of those genetic samples.

Appendix A: Two lists of articles discussing various collecting techniques.

List Organized by Taxon or Group

- Algae: **González-González J & E Novelo-Maldonado** (1986) Algas. Pages 47–54, in A Lot & F Chiang (eds) Manual de Herbaria: Administracion y Manejo de Collectiones Tecnicas de Recoleccion y Preparacion de Ejemplares Botanicos. National Council of the Flora of Mexico: Mexico.
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- Aquatics: **Fosberg FR & M-H Sachet** (1965) Instructions for collecting macroscopic aquatic plants. Pages 107–109, in Fosberg & Sachet, Manual for Tropical Herbaria. Regnum Vegetabile 39, International Bureau for Plant Taxonomy and Nomenclature, Utrecht. [Republished in JS Womersley (1981) Plant collecting and herbarium development: a manual. Pp 98–99. Food and Agriculture Organization of the United Nations: Rome]
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- Araceae: **Croat, TB** (1985) Collecting and preparing specimens of Araceae. Annals of the Missouri Botanical Garden 72(2): 252–258.
- Balsaminaceae, *Impatiens*: **Grey-Wilson C** (1980) Notes on collecting Impatiens. Flora Malesiana Bulletin 33: 3435–3436.
- Begoniaceae, *Begonia*: **Logan J** (1986) A pre-pressing treatment for Begonia species and succulents. Taxon 35: 671.
- Bromeliaceae: **Jorgensen V** (1972) The preparing, pressing and mounting of bromeliads. Journal of the Bromeliad Society 23:211-214.
- Bryophytes: **Delgadillo C** (1986) Briófitas. Pages 77–82 In: A Lot & F Chiang (eds) Manual de Herbaria: Administracion y Manejo de Collectiones Tecnicas de Recoleccion y Preparacion de Ejemplares Botanicos. National Council of the Flora of Mexico: Mexico.
- Cyclanthaceae: **Hammel BE** (1987) The origami of Botany: A guide to collecting and mounting specimens of Cyclanthaceae. *Annals of the Missouri Botanical Garden* 70(4): 897–902.
- Epiphytes: **Aguirre-León E** (1986) Epiphytes [including Orchidaceae]. Pages 113–119 Lot A & F Chiang (1986) Manual de Herbario: Administracion y manejo de colecciones, tecnicas de recoleccion y preparacion de ejemplares botanicos. Mexico: National Council of the Flora of Mexico. [Orchidaceae, Bromeliaceae]
- Ferns: **Arreguín-Sánchez M de la L** (1986) Pteridófitas. Pages 83–86, in A Lot & F Chiang (eds) Manual de Herbaria: Administracion y Manejo de Collectiones Tecnicas de

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Lot A & F Chiang (1986) Manual de Herbario: Administracion y manejo de colecciones, tecnicas de recoleccion y preparacion de ejemplares botanicos. Mexico: National Council of the Flora of Mexico. 142 pages [algae, fungi, lichens, mosses, ferns, aquatic vascular plants, grasses, succulents, epiphytes, palms, trees]

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Robertson KR (1980) *Observing, Photographing and Collecting Plants*. Illinois Natural History Survey Circular 55, pages 1-72 + Cover page and Table of Contents.
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Steenis CGGJ van (1977) Three pleas to collectors - improve your field data. Flora Malesiana Bulletin 30: 2843-2844.

Womersley JS (1981) Plant collecting and herbarium development: a manual. Food and Agriculture Organization of the United Nations: Rome. Pp 137 [pandans; aquatics; banana; palms; bamboos, Araceae; wood]

Appendix B: Example collecting sheet (from the GGI–Gardens project) showing preferred data entry fields included as part of a voucher specimen collection event.

Collector(s): _____

***Location (be specific!):** _____

Coords:

Date collected:

Collecting number: _____ **Number of Duplicates:** _____

Accession number (or other ID):

Family:

Genus:

Species:

Habit (circle): shrub tree herb climbing/vine/liana

Height (in meters): _____

Phenology (circle all that apply): fruiting flowering

Color of fruit/ flower: _____

Fragrance:

Stem/leaves (shape, color, margin, surface, etc):

***Other notes:**

COMPLETE IN THE FIELD

- Habit photograph
- Close-up photograph
- Voucher
- Silica
- Liquid Nitrogen

LN2 barcode sticker



*Use space on reverse, if needed

**COMPLETE AFTER RETURNING
FROM FIELD**

- Databased? _____
- IRN # _____
- Habit photo databased? _____
- Close-up photo databased? _____
- Silica DNA for barcoding separated? _____

Silica barcode for excel



Appendix C: Alternative collecting sheet (from the GGI–Gardens project) showing data entry fields for Genetic Sample biorepository barcode number data; other field data are entered using a smart phone.

GGI-Gardens collection

Date:

Appendix D: Provenance for Garden Plants: Original Collection Information found on voucher label or from Garden database

Collector of the original material:

Collecting Number:

Family:

Subfamily:

Tribe:

Genus:

Species:

Authority:

Subspecific category:

Authority:

Original Collection date:

Country of Origin:

1st political division:

Location:

Lat Long:

Elevation:

Habitat:

Plant description:

Notes:

Appendix E: Making a Plant Specimen Dryer by Carol Kelloff

<http://botany.si.edu/bdg/plantdry/index.htm>

Materials needed:

Zinc-plated galvanized slotted angle can be purchased in any hardware store. You will need at least 4 pieces of angle 1-1/2" x 72".

16 - 1" bolts

16 - wing nuts

8" x 24" board

2 - 150 watt flood lamps

2 - lamp sockets (rated for the selected bulbs and with a wide base (e.g., a porcelain ceiling socket)

4 - wood screws

3 yards of canvas ca. 24" width or 1.5 yards of canvas if 48" in width

8 - 1" brass grommets

10 feet of 16/3 power cord with plug



Plant specimen dryer channels:

Cut the zinc into 4 pieces 18-3/4" in length and 8 pieces 24" in length. Remember to cut the zinc so that the holes line up.



Plant specimen dryer lights: Cut one piece 16/3 power cable 10 inches long. Strip off the ends exposing the wires. Connect the two lamp sockets (in series). Strip the bare end of the 16/3 power cord with plug to expose the wires and connect to one lamp socket. Screw the lamp sockets to 8" x 24" board approx. 6 - 8 inches apart.



Plant specimen dryer bolts: These are the 16 1" bolts and 16 wing nuts. Pick a diameter (probably 3/8") that is right for the holes in your Zinc frame.



Plant specimen dryer with lights: The bottom will be used to rest the lamp board and the end zinc angles can be moved to adjust the height of the lamps.



Plant dryer frame: Assemble the front and back framework of the dryer using four (4) 24" zinc per side. Fasten the zinc at the corners using the bolts with the wing nuts facing out. Place the angle of the zinc down, ie:

L. This will provide a "shelf" for the lamp board and plant press to rest. For the sides use the 18-3/4" pieces of zinc.

Plant specimen dryer finishing:

Cut the canvas 24" wide and approx. 88" in length. Fasten a brass grommet in the upper left corner of canvas and attach using wing nut and bolt of frame. Stretch canvas to next corner, place grommet appropriately and attach to frame. Keep going around frame until canvas is attached to frame. You may want to add a grommet to the bottom corners of canvas where the two ends meet. This will hold the canvas tight to the frame on the bottom.

A standard plant press will sit on the lower angle of the zinc at the top of the dryer. Close off any space on either side of a thin plant press by using a board or cardboard. Tighten and flip the press daily or as needed. Drying time varies according to several factors including specimen characteristics, humidity, and air circulation. Recheck specimens after they have been off of the dryer for a day or two to see if more drying is actually required.



CAUTION: FIRE HAZARD Check your plant drier frequently. Remember that you are dealing with paper and heat. Keep the heat source at a reasonable distance from the press. Make sure the press straps are not hanging on or near the heat source.

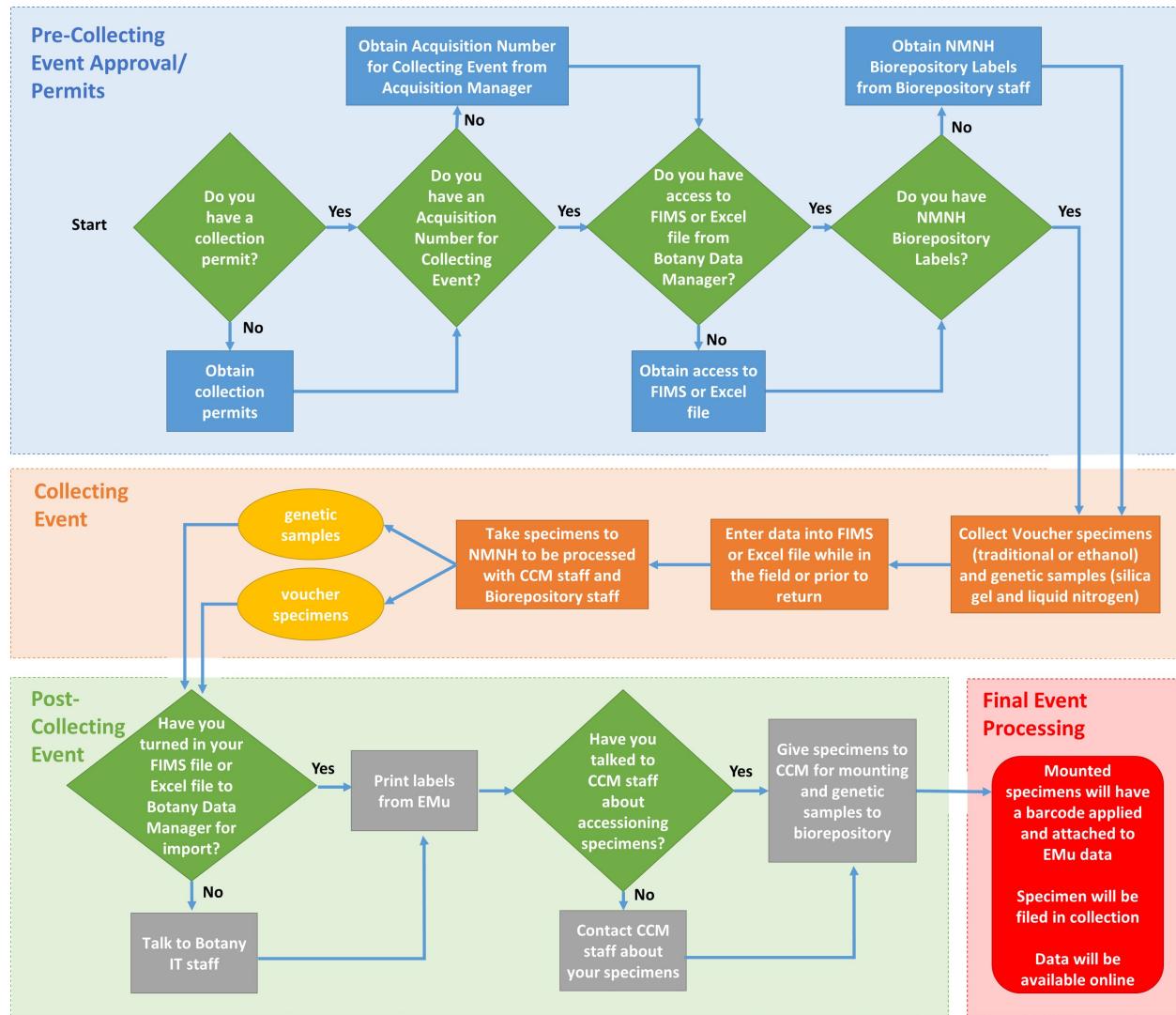
Any heat source, incandescent or involving combustion, can be **dangerous** and neither the author nor the Smithsonian take any responsibility for losses or injuries.

Use common sense!

Appendix F: Flow Chart for GGI-Gardens sampling (by Melinda Peters & Amanda Divine)

Incorporating Genetic Sampling into a Traditional Botanical Voucher Workflow.

By Melinda Peters and Amanda M. Devine (US)



Appendix G: Sources for commonly used resources.

CRYOTUBES (8 ml)

Sarstedt: <https://www.sarstedt.com/en/home/>

57x16.5 mm, polypropylene with EPDM o-ring, 8 ml sample volume; 60.542.024 (assembled and sterile); 60.542.007 (separate tubes and caps)

Paper coin ENVELOPS

ULINE S-11485

<http://www.uline.com/Product/Detail/S-11485/Paper-Office-Envelopes/2-1-4-x-3-1-2-White-Coin-Envelopes>

Stainless Steel PAPER CLIPS (1.25 in, 3.2 cm)

LINECO 497-2001

http://www.lineco.com/cart.php?m=product_list&c=536&s=1026

Reclosable POLYETHYLENE BAGS

http://www.uline.com/Grp_5/Poly-Bags-Reclosable

or Ziploc® from any grocery store

Bulk SILICA gel [option 1]

Sorbent Systems

<http://www.sorbentsystems.com/bulksorbents.html>

640SGO55, Orange indicating silica gel, 2-4mm bead, 55 lb drum

639AG55BG, White Silica Gel, 2-4mm bead, 55 lb drum

Bulk SILICA gel [option 2]

AGM Container Controls, Inc

<http://www.agmcontainer.com/products/desiccants/bulk-desiccant.html>

920010, Flower Dry Silica Gel, 0.08-0.6 mm bead (fine granular grade, blue indicator), 55 lb bag

920004, Silica gel mix, 90% white, 10% blue indicating, 2-5 mm bead, 55 lb bag

920013, Orange Indicating Silica Gel, 2-5 mm bead, 55 lb bag

Plant PRESS and other collecting gear

Herbarium Supply Company

P.O. Box 10966

Bozeman, MT 59719 U.S.A.

<http://herbariumsupply.com/product-category/pressing/presses/>

Plastic STORAGE BOXES used in the Biorepository

Lock & Lock USA <http://shop.locknlock-usa.com/> (sometimes out of stock)

HPL836, 1.45 Gal container

Also from:

http://www.qvc.com/kitchen-&-food/lock-&-lock/_N-lglvZ1z141jy/c.html?pageSize=ALL