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

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Table of Contents

I. About this Training Module

The purpose of this training module is 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 samples, how to manage these samples, and the incorporation of their data into 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).

II. Voucher Specimen Collection

(A) What is a Vouchers?

A voucher can be all, or a portion, of a plant that is collected, preserved, and maintained as reference material in an herbarium. 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, 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 in Figure 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: http://sweetgum.nybg.org/science/ih/). 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. 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 a voucher 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 leaves 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 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 example of a label, Fig. 1 and data sheets in Appendices A & B). Collectors should include details that are not intrinsic to the specimen itself. This information includes **biographic** (collectors and collecting number), **geographic** (e.g., locality, soil type, landscape), **biological** and **ecological** (character notes such as flower color, plant height, other plants found in the area), and should indicate that genetic samples were taken from the specimen. 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. GGI-Gardens has a sample collection sheet (See Appendix A). 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 (not shown).

(i) Biographic collector information

All voucher specimens must include a clear record of the name(s) and affiliation(s) of all collectors present, and a collecting number furnished by the lead 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 location of the plant specimen (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: 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, etc.). 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 during collection.

(iv) Genetic sample tissue information

Genetic sampling is detailed in section III, below, but should also be included with field notes and included on the label with the specimen voucher. This information should include the biorepository genetic sample record number (a unique barcode tag) for each genetic sample (e.g., different ones for liquid nitrogen, silica, etc).

(v) Preparing Dried Voucher Specimens in the Field

Vouchers are collected in the field, pressed, dried in a plant drier, frozen for several days 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*, Ceska & Ceska 1986; *Araceae*, Niciolson 1965; *bamboo*, Soderstrom & Young 1985; *Ethnobotany*, Jain & Mudgal 1999; *ferns*, Brownsey 1985, Croft 1999, Stolze 1973, Holttum 1957; *lianas*, Gerwing et al. 2006; *palms*, Dransfield 1986; *vascular plants* in general, Miller & Nyberg 1995, Robertson 1980; *succulents*, Baker et al. 1985). 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 C; 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, store plant collections at a low humidity and temperature and in acid free paper and have them 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 resealable air-tight container away from moisture. A variety of silica gel types, as well as other kinds of desiccants, have been successfully used by researchers. A variety of silica gel types, as well as other kinds of desiccants, 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. 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. 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). However, to prevent cross contamination new leaf material should be stored in coin envelopes or polyethylene bags (Fig. 2).

(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 cm^2. Avoid stacking leaves in many layers as the inner most leaves will not dry at the same rate as 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 (petals), 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 into paper coin envelopes (ULINE S-11485) or small Ziploc® style bags. The coin envelopes are easy to label, allow for rapid drying of tissue samples, and are sized to fit into permanent storage boxes in the NMNH Biorepository, so little post-collecting processing work will need to be done. These 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. Envelopes should be submerged in silica gel, to maximize surface area contact for drying in an airtight container or large Ziploc® style bag (Fig. 2). Different sources estimate the correct amount of silica gel as anywhere from 9-15x as much silica gel as plant matter and this partly depends on the water content of the tissues. The envelopes can be stirred to prevent local saturation of the silica gel.

There are alternative collection methods. 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 Ziploc® style polyethylene bags containing individual quantities of silica gel. The benefit is that plastic bags are easy to label, tissues easy to see, and tissues in direct contact with silica gel will dry most quickly. The drawbacks of polyethylene bags are that dried leaves may be pulverized by the abrasive action of silica gel as samples are transported (making them harder to prepare in the lab), and it is extremely labor intensive to change silica gel from individual bag versus a bulk volume of silica gel. The collector may need to empty the polyethylene bags and transfer the samples to coin envelopes for storage in the Biorepository. When using polyethylene bags it is advisable to remove the silica on a rolling basis as samples dry and group samples in larger 1 gal Ziploc® style bags. This saves weight and bulk during transport, and reduces tissue pulverization.

(B) Liquid Nitrogen Tissue Collection

See Liquid Nitrogen (LN_2) 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. 2). Tubes are labeled with a Biorepository barcode sticker, wrapped in a ca. 4x8 cm piece aluminum foil and placed into liquid nitrogen that is stored in a Dewar (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 can

cause the barcode stickers to pop off when the tubes are first put into the Dewar. Writing the collection number on the tube is a way to create a secondary identifier.

If LN_2 is not available in the field, but LN_2 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 LN_2, 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 LN_2 at a remote collecting site.

(C) Ethanol Tissue Collection

Historically, ethanol has been used to preserve animal tissues for DNA but not plant tissues. There has been some investigation recently in the use of ethanol and preliminary results suggest that ethanol preservation may result in a higher yield of DNA from plant tissue of some species, but a lower quality of the recovered DNA versus a silica-dried method. The mechanism of preservation 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 tissues. The long-term viability of the DNA stored in ethanol has not been determined however, preliminary tests shows that when immediately extracted quality of DNA is excellent but if the leaf material is left in the alcohol for 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 drying or liquid nitrogen instead.

(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 by flaming with a micro-torch, dipping/wiping in chloroform (evaporates without reside 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 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 LN_2 (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 will be 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 ULINE envelopes that have been in use in Botany. Within each box will be a packet of molecular sieve 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 collected for LN_2 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 MSC to deposit 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 this is not possible but the application for a collecting permit should be submitted before the collector leaves. 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 Melinda Peters) to receive an **EMu Acquisition 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. This allows collection events to be 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) are designed for both vouchered genetic samples, and for genetic samples when the voucher is deposited elsewhere. You may also 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 pre-assign and print stick-on labels for cryovials and envelopes. These labels have a special adhesive designed for cryovials. Please request labels from the Biorepository Manager 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 might be the cryovial, Funk-13245-3 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 LN_2 cryovial *and* the silica gel coin envelope. The corresponding circular stickers can be placed in the field notebook or the original collection sheet (Appendix A or B). Barcodes for silica envelopes can be added either in the field or some feel that it is less confusing to add them after they return.

(v) After collection of specimen vouchers

(1) Once dried vouchers arrive at NMNH, they must be frozen as part of Botany's Integrated Pest Management protocol before entering areas of the permanent collection. (2) When the specimens are available the collector should work with core collection staff to complete the Acquisition information. Labels are the responsibility of the collector unless prior arrangements have been made. However, some guidance will be given on label preparation if needed. 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. (3) Copies of collecting and export permits (if needed) must be supplied to obtain a number from the Office of the Registrar (OR number); often called an Accession number in Botany. Specimens cannot be mounted without this number. (4) Ultimately, all voucher data must be in the NMNH EMu database. (5) Submit vouchers for mounting and provide funding information if applicable for collecting event.

Every specimen and genetic sample should be assigned a unique identification number. For Botany voucher specimens, this number is the **Primary Collector's Name and Number** which together can be used as the **Sample Field ID** (e.g., Funk-13245.1 Funk-13245.2, etc.). The EMu database will generate an **Internal Record Number (IRN)** that is associated with a collection and this IRN will be used to link all of the parts related to this collection. EMu will make and maintain the links among the **Primary Collector's Name & Number**, the **IRN**, all **Biorepository barcode numbers**, and **genetic sample numbers**. The Biorepository will link the FreezerPro record to EMu so that the EMu records can supply all the taxon information, locality information, etc. to the Biorepository. [FreezerPro is the freezer management tool used by the Biorepository]

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 GGI collections need to be databased in one of three ways: 1) through the NMNH FIMS database, 2) using the specially designed excel spread sheet obtained from the Botany department IT office, or 3) entered directly into EMu.

(B) For GGI partners

All GGI collections need to be data based and 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.

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Stolze, R.G. 1973. Inadequacies in herbarium specimens of large ferns. American Fern Journal 63: 25–27.

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 (sans plant press and LN_2 Dewar) required for voucher specimen collection. A. Bag with silica and envelopes, A1. Sample envelop, B. Camera, C. writing implements, D. DNA barcode labels, E. clippers, F & F1. cryovial and aluminum foil, G & H. Data sheets.

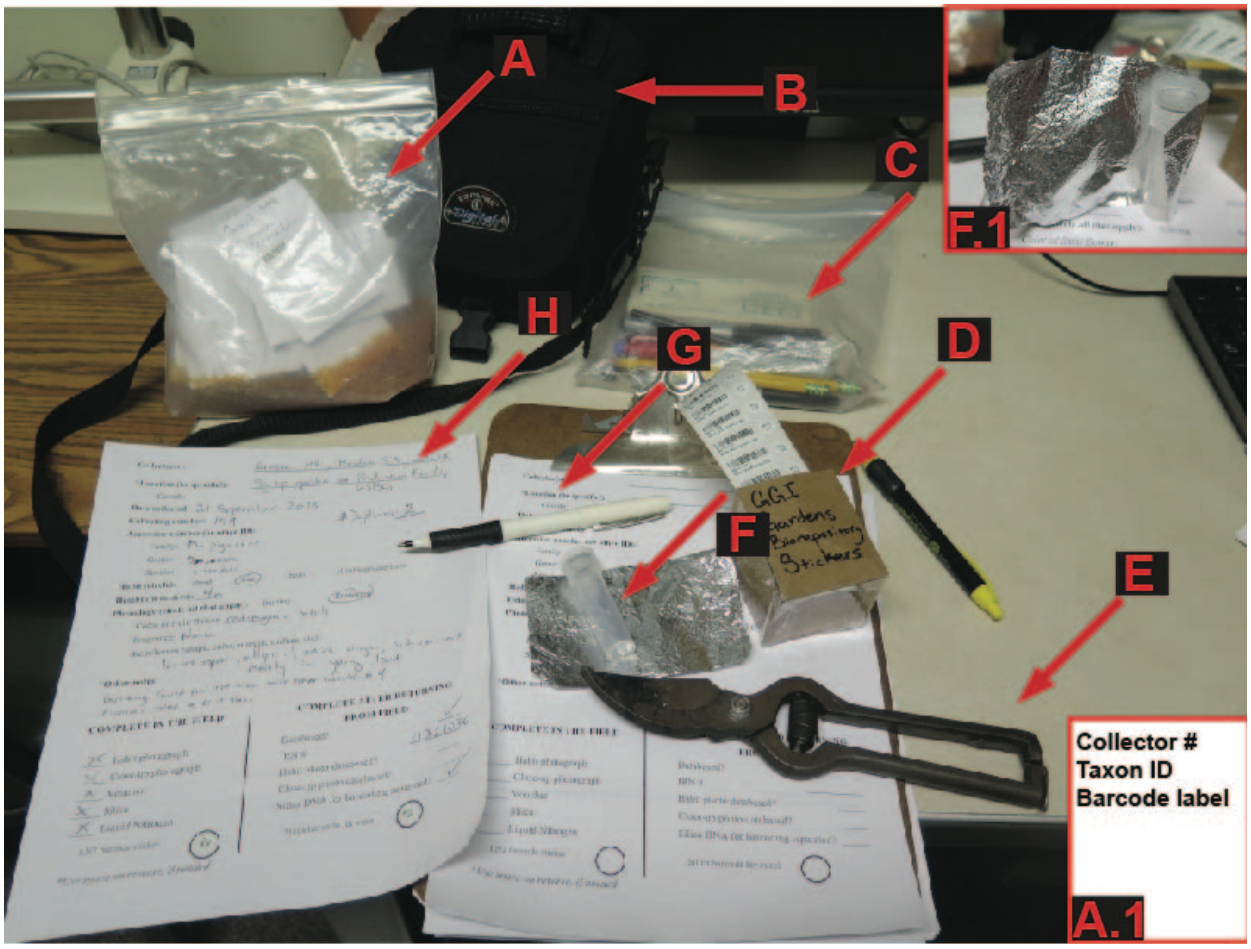
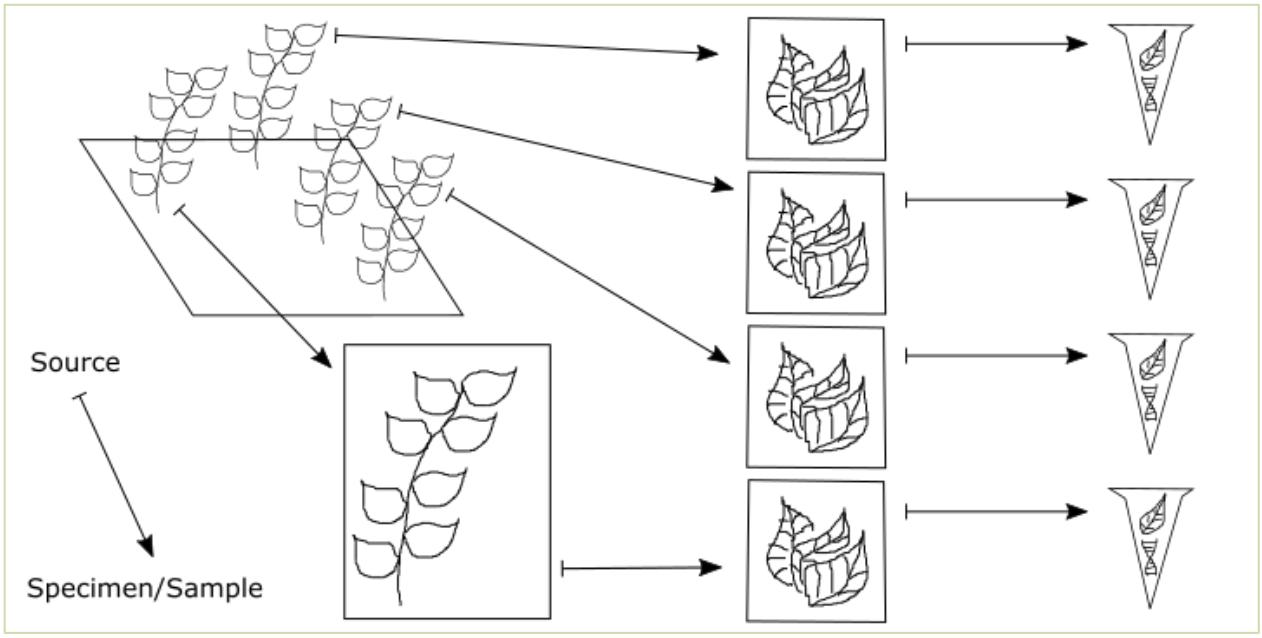


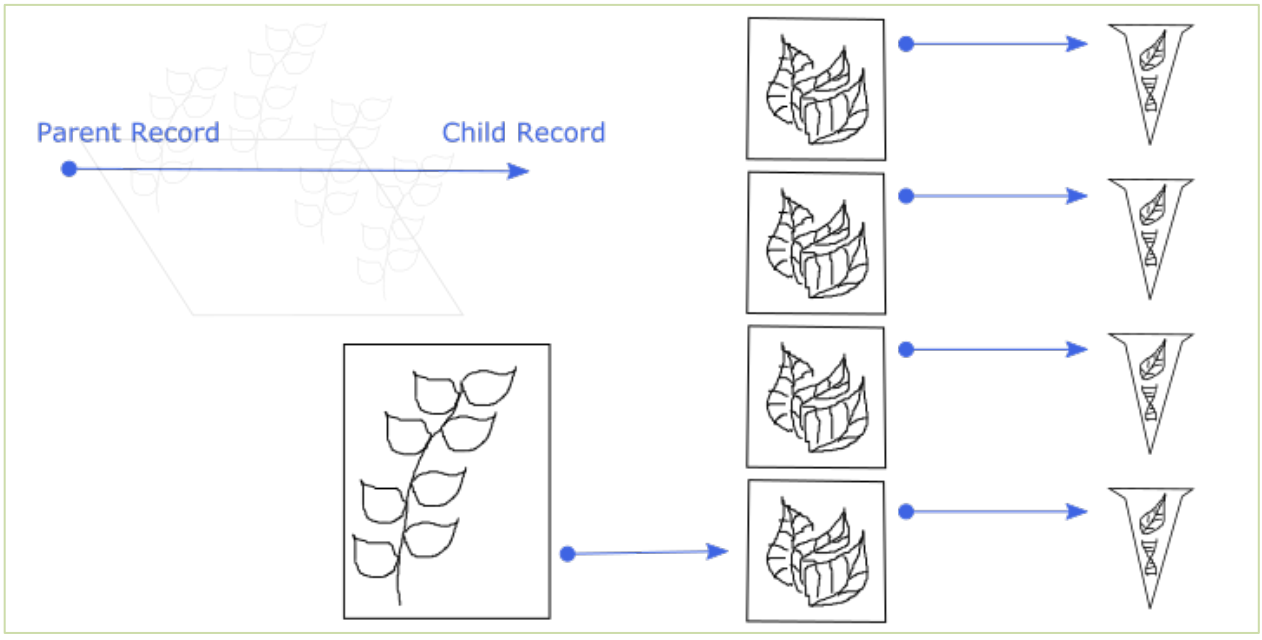
Figure 3. Example voucher specimen collection event with all necessary materials (from GGI–Gardens).

[Photo from left to right: S. Gabler, V. Funk, A. Hill, K. Van Neste]

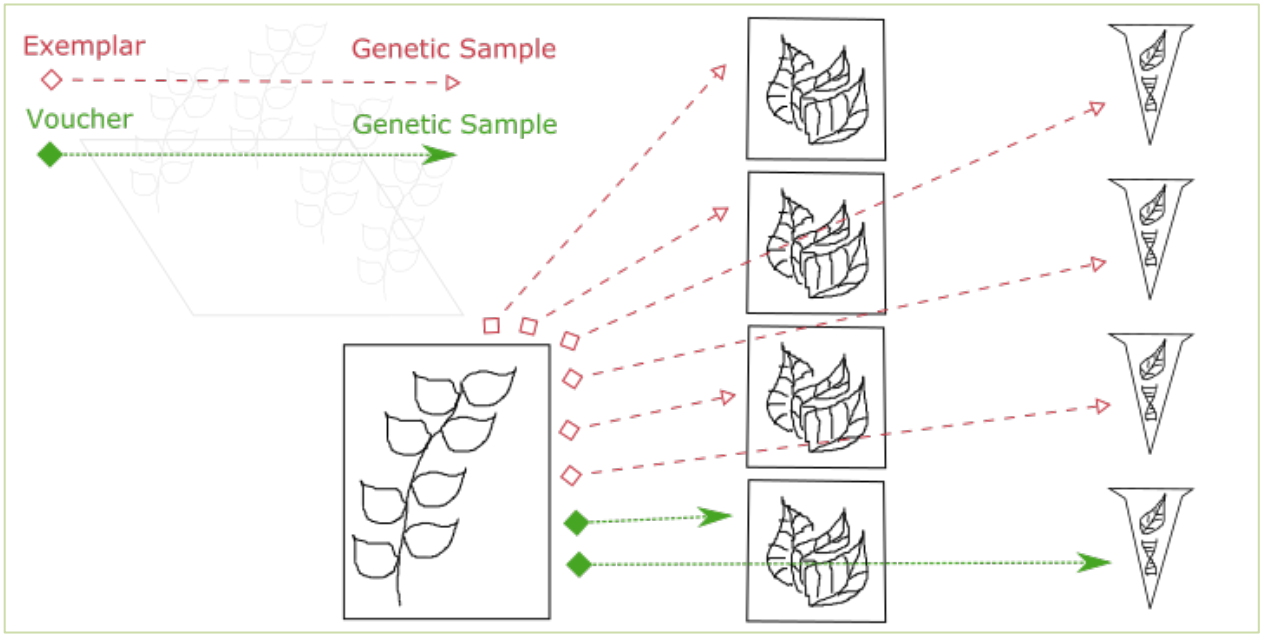
Figure 4. The physical relationships among samples



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: 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

COMPLETE AFTER RETURNING FROM FIELD

Databased? _____

IRN # _____

Habit photo databased? _____

Close-up photo databased? _____

Silica DNA for barcoding separated? _____

Silica barcode for excel

**Use space on reverse, if needed*

Appendix B: Alternative collecting sheet (from the GGI–Gardens project) showing data entry fields for Genetic Sample biorepository barcode number data.

GGI–Gardens collection **Date:**

Collector	Collector Number	LN2 Sticker	Silica Sticker

Collector	Collector Number	LN2 Sticker	Silica Sticker

Appendix C: 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.

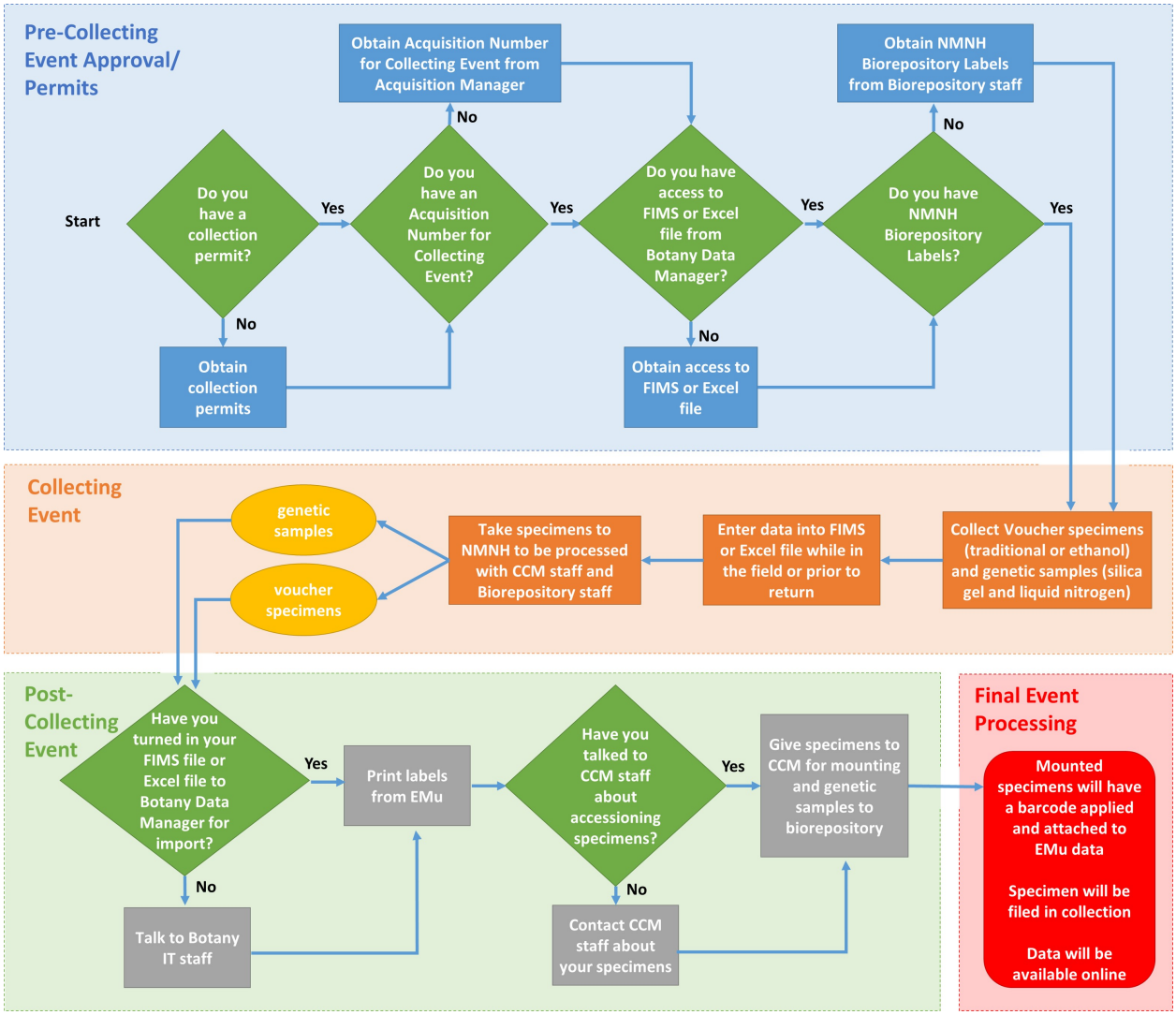
Plant specimen dryer finishing:

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 D: Flow Chart for GGI-Gardens sampling (from a poster at GGBN Berlin meeting)

Incorporating Genetic Sampling into a Traditional Botanical Voucher Workflow. Melinda Peters and Amanda M. Devine (US)



Appendix E: Links to commonly used resources.

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

Bulk silica gel
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

AGM Container Controls, Inc: http://www.agmcontainer.com/products/desiccants/bulk-desiccant.html
920010, Flower Dry Silica Gel, 0.08-0.6 mm bead (a fine granular grade with blue indicator preferred by Jun Wen), 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

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)

Plastic storage boxes used in the Biorepository
Lock & Lock USA: http://shop.locknlock-usa.com/
HPL836, 1.45 Gal container