To address specific questions about the information contained in this document or to request additional in-person training please contact the GGI program team at GGI@si.edu
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I. Introduction

A. Purpose

The Smithsonian organized Global Genome Initiative (GGI) is a collaboration to preserve and understand the genomic diversity of life on Earth. A primary goal of this program is to collect samples of genomic diversity from across the major branches of life and make them accessible to scientists and other stakeholders. GGI seeks “genome quality” samples. Our working definition of genome quality is at least 50% or more of the extracted DNA above 9 kb in size, and vouchered by classical museum specimens.

The purpose of this document is to provide a guide for field researchers who collect, preserve and make accessible genomic samples as part of a GGI/NMNH supported collecting expedition. This includes the collection of samples in the field, and the transfer of samples (into the Biorepository) from field expeditions. Please see the GGI website resource page (http://ggi.si.edu/resources) for protocols specific to departments and shipping. See Figure 1 for an overview of GGI’s workflow.

Figure 1. The GGI workflow, from the assessment of biological coverage in databases and sequence libraries to strategic collecting, preservation, and accessibility of genomic samples for research.
Below are general guidelines for collecting genomic material, with specific sections for taxonomic groups with particular requirements outlined in Appendix I. Department Specific Protocols.

B. **GGI Collections**

GGI aims to obtain tissues from which genome-quality DNA can be extracted. In order to preserve genome-quality DNA, it is important to collect material as quickly as possible after death of the organism, and to keep the tissue at the lowest temperature possible. Please keep this in mind when deciding which collection method to use.

Best practice recommendations for collection and long-term storage of tissue samples for genomic analyses are currently under development and review. Preliminary results for vertebrate tissue suggest that submerging tissue in an EDTA/DMSO (mixture) or AutoGen GENEprep Tissue DNA Extraction Kit M2 solution prior to flash freezing produces higher quality DNA than EtOH, DNAzol, or RNAlater. To ensure the best possible tissue preservation, we strongly encourage the use of liquid nitrogen (LN2) to flash freeze tissues after death as soon as possible, whenever feasible.

It is impossible to obtain LN2 in some countries, and, even if available, infeasible to transport LN2 to some field sites. Therefore, preservatives such as 95% ethanol, RNAlater, EDTA/DMSO, or AutoGen GENEprep Tissue DNA Extraction Kit M2 solution may be used for animal tissues, and silica gel may be used for plant tissues. Please refer to department specific protocols (Appendix I. Department Specific Protocols) for further information. Dry ice can be a useful alternative. Please refer to the list of Biorepository-approved containers, or consult Biorepository staff on whether containers used are appropriate for storage in the Biorepository.

C. **Roles and Responsibilities**

Collectors should follow standard NMNH, SI practices, including animal care protocols (e.g. Institutional Animal Care and Use Committee, ACUC) and obtain proper collecting and export permits. It is recommended that permit applications are submitted approximately two months prior to departing on collecting trips. Please send documentation of this process to GGI@si.edu for award tracking purposes. Upon return, specimens should be declared at customs (botanical collections require USDA approval at point of entry), a USFWS Form 3-177 should be filed and an approved copy should be returned to the appropriate NMNH department collection data manager prior to cataloging. It is also the responsibility of the collector to contact appropriate curators and collection data managers prior to their trip in order to obtain an Acquisition Number, which is generated by creating an appropriate EMu Transaction record and required to receive Biorepository approved containers for collection of genomic samples. Collectors should also ensure they are following the protocols of the department or division in which their specimens will be deposited (Appendix I. Department Specific Protocols).

II. **Pre-fieldwork considerations**

A. **Departmental considerations**

Staff from each Department or Division that will be receiving the voucher specimens from the expedition should be contacted as soon as the collector has firm plans to ensure that the department or division will accept the collections. Collectors should work out the following details with the Department or Division accepting the voucher specimens:
1. **Permits.**

Collectors should obtain appropriate collecting and export permits (if from a foreign country) -- **APHIS/USDA permits**, **ESA/USFW permits** and file a **USFW 3-177 Import form**. Please check with the department’s collections managers and curators for a list of required permits. The collector should follow all Animal Care Use Committee (**ACUC**) guidelines.

2. **Voucher Specimens accession, cataloging, and curation.**

Prior to departure, the collector should make sure the time and resources required to process voucher specimens upon return from the collecting trip are available. This should be negotiated directly with Department or Division staff accepting the voucher specimens or GGI.

3. **Pre-Registration and Data Templates.**

The collector should contact the Department or Division’s data manager or transactions staff to obtain an Acquisition Number that results from creation of an initial EMu Transaction record for the expedition. This effectively prepares the Office of the Registrar (OR) for specimens and samples that are expected. Data managers should also be consulted on the Department or Division’s preferred formats and standards for field data entry. Please refer to Pre-Registration and Data Templates, section II.C below for more information on registration and data entry responsibilities.

**B. Biorepository considerations**

In order to provide sufficient time for preparation of collection supplies please notify the biorepository manager, Chris Huddleston at **huddlestonc@si.edu**, about your collecting trip as soon as plans for the expedition are firm.

1. **Safety.**

Working with dry ice, liquid nitrogen and other chemicals can be hazardous. Do not allow dry ice or liquid nitrogen to contact your skin or eyes directly; please wear appropriate protective equipment (e.g. face shield, cryoapron, cryogloves, closed-toed nonabsorbent shoes). If traveling or working with liquid nitrogen or dry ice in an enclosed area (e.g. you are driving a personal vehicle with the chemical in the cabin), please leave windows partially open to ensure proper ventilation. For more information on safely handling LN and other chemicals, please send a request to **ggi@si.edu**.

2. **Equipment and Service Requests.**

Prior to going into the field, PIs should fill out, return and review the **GGI Project Checklist** with GGI staff. The checklist document is located under the RESOURCES FOR SMITHSONIAN AND
AFFILIATED RESEARCHERS section of GGI’s Resources webpage. See Table 1 for recommended timelines to consider when ordering items or services prior to departure.

Table 1. Recommended timelines to consider for ordering items or services prior to departure.

<table>
<thead>
<tr>
<th>Item or Service</th>
<th>One Month Prior to Departure</th>
<th>Two Weeks Prior to Departure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generate Biorepository Labels</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Request Preservative Solutions</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>(95% ethanol, EDTA/DMSO, Lysis buffer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Request Dewar or Dry Shipper</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Request Shipping Service</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>(Cryoport or FedEx)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Biorepository numbers and labels.

The collector should request Biorepository numbers and labels from the Biorepository staff at least two weeks prior to the collecting trip. The Biorepository staff will issue each collector a series of Biorepository number (seven character alphanumeric codes). The collector or department data manager is responsible for assigning each tissue sample one of these Biorepository numbers. Collectors collecting into cryovials or coin envelopes will be issued a roll of Biorepository labels, to be affixed to the side (Figure 2a) and optionally the top (Figure 2b) of cryovials or envelopes. Collectors collecting in 2D barcode cryovials will not receive physical labels – instead, Biorepository numbers will be associated with scanned 2D barcode numbers. See Instructions for Biorepository Numbers and Labels. We strongly recommend that collectors who anticipate collecting more than 200 samples apply their labels to their cryovials/envelopes prior to departure – this reduces processing time in the field. Along these lines, if foil wrapping will be necessary, it is helpful to cut pieces of foil prior to departure.
Figure 2a. Biorepository labels should be fixed to the side of cryovials, as shown here, and optionally the top of cryovials, as shown below in Figure 2b.
b) Equipment and supplies

Below is a recommended list of equipment and supplies for collection of tissue samples. Please consult department specific guidelines for additional recommendations specific to taxonomy and vouchers (Appendix I, Department Specific Protocols).

(1) **Alphanumeric plates.** Recommended for samples intended for DNA barcoding.

(2) **Aluminum foil.** Samples should be wrapped in foil before dropping in a dewar/dry shipper as a safeguard against loose labels, caps and specimens.

(3) **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.

(4) **Coin Envelopes.** Recommended for silica dried plant samples.

(5) **Cryogloves.** Recommended for work with liquid nitrogen.

(6) **Standard Cryovials.** A 2ml, self-standing, externally threaded polypropylene cryovial with a silicone or Teflon seal is generally used in the Biorepository. The current recommendation is a Simport T310-2A, available from cryostuff.com, but similar cryovials are also acceptable. Please consult with the Biorepository Manager before purchasing other cryovial types and sizes to make certain those cryovials will fit in existing storage configurations.

(7) **Kill Chambers.** If necessary to slow down organisms, or to anesthetize/kill them prior to processing, GGI has designed LN2-proof kill chambers suitable for submerging organisms in LN2 and retrieving them for processing. Detaching legs or other parts for barcoding is frequently easier if the organism is frozen rather than room temperature. If a large sample requires several cryovials, subdivision may be easier and less prone to contamination if frozen first.

(8) **Disposable Gloves (Optional).** Recommended for work with RNA samples for transcriptome sequencing.

(9) **Field sheets, notebooks, laptops, etc.** Use whatever is standard for your unit, but good data capture is imperative. We encourage
digital data capture through departmentally approved data templates or NMNH FIMS spreadsheets whenever feasible (See Pre-registration and Data Templates). Keep multiple backup copies of data whenever possible, both hand written and digitized, on thumb drives or on the FIMS server.

(10) **Forceps, disposable scalpels and other tools for sample manipulation.**

(11) **Hybrid LN2 Dewar/Dry Shipper with case.** The Biorepository has eleven MVE Doble QWick 10/660 for your use. These should be reserved one month in advance when feasible. Each dry shipper can hold up to 450 2ml, foil-wrapped cryovials. The dry shipper can be pre-charged the day before you go to the airport to speed up filling at the destination. A room-temperature dry shipper requires a 2 hour charge time. It maintains internal temperature for up to 30 days when filled with LN2 and for up to 10 days when charged and dry.

(12) **Internal labels (optional).** Some units use an internal label with a field or field series number on it. Anecdotal evidence indicates that Resistal and other types of plastic material and resins may damage tissues, especially in the presence of a solvent like ethanol. Instead print labels on thick 100% cotton rag paper with non-aniline ink. Or write in pencil on suitable paper.

(13) **Knockout blocks.** GGi has a few “knockout blocks,” which are plastic boxes designed for use on a microscope stage, with the surface made from porous foam. CO\(_2\) or N\(_2\) can flow continuously into the block and through the foam to anesthetize small organisms for manipulation, sorting, etc. Tanks with CO\(_2\) and regulators are required.

(14) **2-D Barcode Cryovials.** Recommended for long term storage of small tissue samples.

(15) **Number two soft lead wooden pencils.** Recommended for back-up labeling on cryovials. A pencil works well when writing on cryovials with a white writing surface. Graphite is resistant to ethanol, fat and other solvents. Wood is easy to sharpen and won’t dry up, but is NOT smudge resistant. A pin, thumbtack stylus or other sharp object can also be used to engrave labels into the cryovials.

(16) **PDF Datalogger (optional).** The Biorepository has four data loggers with a remote temperature probe to monitor the dry shipper temperature in transit. It is similar to a ShockWatch indicator, not an
alarm device. See Appendix I for instructions on how to use a PDF Datalogger.

(17) Liquid Nitrogen (LN2). Obtained from industrial gas suppliers, universities, welding stores, hospitals, military bases, or companies that ship bull semen. Locate an in-country supplier before you depart. LN2 is prohibited on aircraft.

(18) Preservative solutions. Recommended for the preservation of vertebrate, invertebrate, and entomology tissue samples. Please contact Chris Huddleston huddleston@si.edu to request preservative solutions (95% ethanol, EDTA/DMSO, Lysis buffer). Indicate whether collecting cryovials need to be pre-filled prior to departure.

(19) RNAs-Away Wipes (optional). Recommended for keeping RNA intact for transcriptome sequencing.

(20) Sterilizers. Bleach, hydrogen peroxide, or high ammonia-content soaps can be used to sterilize utensils (forceps, scalpels, etc.) between tissue samples. Note: 95% EtOH does not sanitize nor remove DNA, but preserves DNA. An open flame may also be used to sterilize utensils and EtOH can be used to insure suitable flame coverage.

c) Biorepository-approved containers

Use of appropriate archival sample containers is important. Do not transfer material to different containers after collecting, if possible, as it is both costly in time and materials, as well as potentially damaging to frozen samples.

Collectors should select containers based on downstream plans for tissue samples. Approved containers are listed below:

(1) Alphanumeric plates. Recommended for samples intended for DNA barcoding upon return from the field. This storage container does not fit inside a dewar/dry shipper. Collectors are advised to store the bulk of collected tissue in a standard or 2-D barcode cryovial (Figure 4a), and a very small sub-sample (that will be extracted for DNA barcoding) into an alphanumeric cryovial (Figure 3a). When using alphanumeric plates, collectors should carefully record the location in the plate, keeping in mind these cryovials have the alphanumeric location on the bottom of the cryovial, and this information is on the plate itself, columns 1–12 across the top and A–H along the left-hand side (Figure 3b).
(2) **Coin Envelopes.** Recommended for silica-dried plant samples. Samples should be collected directly into coin envelopes, and envelopes should be dried in silica gel then stored at -20°C in a large double-bagged Ziploc bag or in a Lock & Lock plastic box. For more information on coin envelopes, see: ULINE Coin Envelopes – White, 2 ¾” x 3 ½” (S-11485)

(3) **Standard Cryovials:** Recommended for the majority of tissue collection for long-term storage. These storage containers fit inside a dewar/dry shipper. A 2ml, self-standing, externally threaded polypropylene cryovial with a silicone or Teflon seal is generally preferred for the Biorepository. Our current recommendation is a Simport T310-2A, which is available from cryostuff.com, but similar cryovials are also acceptable. Please consult with the Biorepository Manager before purchasing other cryovial types to make certain those cryovials will fit in existing storage configurations.

(4) **2-D Barcode Cryovials (0.5 ml or 1.4 ml).** Recommended for the collection of small individual organisms for long-term storage of smaller tissue samples and where collecting equipment space is an issue (Figure 4a). 2-D barcode cryovials are stored in 2-D barcode plates (Figure 4b). This storage container does not fit inside a
dewar/dry shipper. Individual 2-D barcode cryovials do not need to be labeled with Biorepository labels; rather, prior to departure you should scan your 2-D barcode plate(s) and record the ten-digit number associated with each cryovial on your spreadsheets, taking special care to preserve any leading zeroes in the barcode number (Figure 5). Do not put any labels on the left side of the box – this space is needed for later Biorepository use (Figure 6 a–b). Also, be sure to keep the notch in the front right side of the plate clear from tape or any other obstructions. For more information on 2-D barcode cryovials and plates, see http://www.matrixtechcorp.com/storage-systems/tubes.aspx?id=63.

Figure 4a. 2-D Barcode Cryovial.

Figure 4b. 2-D Barcode Plate.
Figure 5. If using the 2-D barcode tissue plates you should scan the 2-D barcodes and include the ten-digit number associated with each cryovial on your spreadsheets.

Figure 6a. Please do not cover up the notch on the front bottom right of the box.
C. Pre-Registration and Data Templates

Well in advance of departure, the collector should contact and work with their Departmental or Division Data Management and Transaction Management staff. Pre-registration with the Office of the Registrar (OR) by recording upcoming collecting activities in EMu is a prerequisite for collecting. Doing this will ensure that the project will have an EMu acquisition transaction record in place for referencing and appending afterwards. Using approved data structures and entering standardized values where needed can help to ensure smooth data flows to EMu, the LAB, and the Biorepository.

Pre-registration involves creation of a new “In Process” Acquisition transaction record in EMu by your Transaction Managers. The Subtype of that acquisition should be “Collected for Museum”, and the field work’s primary sponsor or collector should be recorded as the Primary Transactor. Other collectors or collaborating institutions can be included as Secondary Transactors. General information about what will be collected and where should be included in the Material Description field of the transaction. Any permits or agreements received prior to the trip should be scanned and referenced via Rights records and linked to the Transaction. Item level information will be added after return from the collecting trip.

Data templates for recording field data should be acquired from, or approved by, Departmental/Division Data Management staff. Collectors should understand the Departmental/Division data entry recommendations to avoid problems after the data are submitted. It is recommended that critical values such as Biorepository 2-D Barcodes be entered by barcode scanner or by copy and paste whenever possible. Collectors should review and submit data to Departmental Data Managers as soon as practical after completion of fieldwork. All finalized data files should also be loaded as EMu multimedia assets and associated with the fieldwork’s primary transaction.
The NMNH Field Information Management System (FIMS) is a software tool in development at SI. It is available as an online data template generator and data validator, to be used at the discretion of the Collector’s Department/Division. The NMNH FIMS allows collectors to generate customized Excel spreadsheets and then during or following fieldwork use an online data validation system to ensure that data entered are free of formatting errors. Versioned copies of validated dataset are stored on the NMNH FIMS server upon validation and available to designated Data Managers. For additional questions about the NMNH FIMS or entering data in a FIMS-generated spreadsheet, please contact departmental data managers, the NMNH Informatics Office, or GGI.

III. In-Field Considerations

A. General Considerations

1. Cryovial Preparation. Label the cryovials with scanned Biorepository numbers. Hand writing or manually typing Biorepository numbers on labels is discouraged, as it results in frequent mistakes due to human error. It is recommended that collectors scan or copy and paste the Biorepository numbers from their spreadsheet. Affix the Biorepository number along the length of the cryovial. Do not cover other label areas. Make certain that the label is attached firmly with no air gaps or wrinkles. Cryovials can be pre-filled with aliquots of preservatives. When manually adding non-Biorepository numbers to cryovials for back-up purposes, numbers should be written or scratched onto the cryovial with a sharp object, such as a thumbtack, diamond stylus, or pin, prior to departure or in the field.

2. Sampling tissues. When sampling tissues from large specimens take as much tissue as possible without damaging the specimen. Tissues should be collected with clean scalpels, forceps, scissors, etc. and muscle or leaf tissue is recommended. For tissues that will be frozen in liquid nitrogen (LN2) without any additional preservative, fill the cryovial no more than ¾ full with tissue (to allow room for expansion during freezing). For tissues that are collected into a preservative (DMSO-EDTA, Ethanol, etc.), maintain a ratio of 1 part tissue to 6 parts preservative in the cryovial; this will ensure an adequate amount of preservative to permeate the tissue completely. If you are collecting into a preservative and also storing the sample in LN2, allow the tissue to soak in the preservative at ambient temperature for one hour before submerging in LN2 to allow full penetration of the tissue. If the specimen is uncommon and sufficiently large, you should consider taking multiple tissue samples; please label each of these samples with a unique Sample ID. (For further clarification on recording and labeling vouchers and tissue samples, please see Appendix III: Vouchering Genomic Samples.) Please consult your department-specific Appendix for considerations unique to your target organisms (e.g. shelled invertebrates, tiny insects, plants, etc., see Appendix I, Department Specific Protocols). For each tissue sample you collect, please record the
Sample ID and/or Biorepository number, preservative, type of tissue (e.g. whole organism, leg, leaf, muscle, kidney, etc.), Collector Number of the specimen that is the voucher/exemplar of the tissue sample, and Alphanumeric/2-D Barcode plate name and position if applicable.

3. **Adding tissue samples to the container.** Add the tissue sample to the cryovial and turn the cap tightly. The internal gasket should be compressed to keep LN from entering the vial and to keep the cap on the vial. Record pertinent information about the specimen and be certain to record the tissue type (i.e. “Genetic Sample Type Secondary” on NMNH data sheets; e.g., Muscle, Liver, Whole organism, etc.). For tissue that will be frozen in LN without additional preservative, fill cryovials only to ~3/4 volume; tissue is mostly water that expands when it freezes (Figure 9).

![Figure 9. Fill cryovials ~3/4 volume; tissue is mostly water and expands when it freezes.](image)

If using EtOH, EDTA/DMSO mix, or any other liquid preservative, monitor volume displacement when adding tissue. Allow expansion room for the freezing process. Make sure that enough preservative is available in the cryovial to sufficiently saturate the sample. Topping off a tissue-packed cryovial with a little EtOH will not sufficiently preserve the tissue. Instead use multiple cryovials. If using EtOH as a preservative, check tissues every few days and change out yellow, discolored liquid for fresh 95% EtOH.

For a single specimen stored in multiple cryovials, it is important to number each cryovial individually so that multiple cryovials from the same specimen can be distinguished from one another (see specifics for each department/division in Appendix I, Department Specific Protocols). Cryovials that are pre-labeled with Biorepository numbers or 2D barcodes meet these criteria. Cryovials without Biorepository numbers or 2D barcodes should be labeled with numbers that are specific to each cryovial.

4. **Transitioning the Dewar to a Dry Shipper.** When ready to transport via air or FedEx, convert the dewar (Figure 10) to a dry shipper (Figure 11). Dump the nitrogen out into gravel or bare dirt. **DO NOT DUMP LIQUID NITROGEN DOWN A DRAIN!** A sieve will catch cryovials that fall out.
Figure 10. Liquid Nitrogen Dewar.

Figure 11. Dry Shipper.
B. Sample Preservation Methods.

Information on sample preservation methods can be found in the department specific protocols.

C. Shipping

1. Shipping Samples to the NMNH Biorepository. Ship samples directly to the NMNH Biorepository. The full address is:

   Attn: Biorepository Manager
   MSC-Suitland
   4210 Silver Hill Rd.
   Suitland MD 20746
   USA

2. Shipping Options.

a) Cryoport Service. Consult with the Biorepository Manager in advance. Service is available in many countries, but is considerably more expensive when LN2 is difficult to obtain. Fill-out the Cryoport order form as completely as possible and return it to the Biorepository Manager along with a fund number from your unit (copy your funds manager on the message). The Biorepository Manager will get a price quote on the Cryoport service. The quote does not include freight or customs charges. Other documentation may be required depending upon the country of origin and items being shipped.

b) FedEx. Samples quotes via FedEx Priority Overnight as of August 31, 2017 for sending to/returning from:

   (i) Phoenix: $42.39/$42.39
   (ii) Honolulu: $63.39/$63.39
   (iii) Anchorage: $63.39/$63.39
   (iv) Littlerock: $43.39/$43.39
   (v) Mexico City: $108.51/$123.00
   (vi) Cape Town: $748.90/$304.10
   (vii) San Jose (CR): $409.82/$176.74
   (viii) Liberia (CR): $421.58/$188.50
3. **Shipping Protocols.** Refer to the GGI Resources webpage ([https://ggi.si.edu/resources](https://ggi.si.edu/resources)) for detailed directions on the steps to follow for shipping packages and samples via FedEx Domestic, FedEx International, and USPS “Official Business” Priority Express.

IV. **Post-Field Considerations**

A. **Returning to NMNH**

Ship or transport the genetic samples directly to the Biorepository. Transfer the vouchers to the relevant department or division. Provide department data managers with carefully reviewed or validated data sheets and all permits. Coordinate with department staff on preparation and filing of the USFW 3-177 Import permit.

The Biorepository/GGI staff will count and scan the samples immediately upon receipt, and email the collector with the list of samples received. **The collector should review the list to ensure that all samples arrived safely, and to flag any inconsistencies.**
V. Appendix I. Department Specific Protocols

A. Botany Protocols

1. Botany Best Practices: see GGI Resources webpage for protocol [Published 30 January 2017]

2. GGI Gardens-Protocols: see GGI Resources webpage for protocol (Published 4 September 2016)

3. GGI-Gardens-Samples Request Policy and Form: see GGI Resources webpage for request policy and form

B. Entomology Protocols

1. Entomology Best Practices: see GGI Resources webpage for protocol (Modified August 2017)

C. Invertebrate Zoology Protocols

1. IZ Genetic Workflow Procedures: see GGI Resources webpage for workflow (Modified 24 April 2017)

2. IZ Genetic Workflow and Collection Accessioning Procedures Workflow: see GGI Resources webpage for workflow (Modified 24 April 2017)

D. Vertebrate Zoology Protocols


VI. Appendix II. Frequently Asked Questions (FAQ): see GGI Resources webpage for FAQ document (Modified 28 September 2017)