Genetic Workflow Procedures

Applicable to Invertebrate Collections Transferred to the Smithsonian Institution's National Museum of Natural History

Version one

April 2017

Last updated 4.24.2017

Contents

I. INTRODUCTION

This document is intended as a general reference for those who are conducting fieldwork and collecting genetic samples to be deposited in the National Museum of Natural History (NMNH)'s Department of Invertebrate Zoology (IZ), specifically for the Global Genome Initiative (GGI), MarineGEO, and Autonomous Reef Monitoring Structures (ARMS) supported projects. This document is not intended as a comprehensive guide to museum collection management practices, but it does provide guidelines that will help to make the field processing and eventual transfer and incorporation of IZ vouchers and genetic samples into the National Collections as efficient as possible.

If you have collection-related questions that are not answered by the included information please do not hesitate to contact any of the following individuals in the NMNH Department of Invertebrate Zoology and Biorepository:

a. Bill Moser, Acting Collection Manager – Invertebrate Zoology

301-238-1761 or moserw@si.edu

b. Lisa Comer, Invertebrate Zoology Technician – GGI, MarineGEO, ARMS, Invertebrate Zoology

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c. Katie Ahlfeld, Museum Specialist and Tissue Manager – Invertebrate Zoology

301-238-1754 or ahlfeldk@si.edu

d. Geoff Keel, Museum Specialist and Curation Supplies – Invertebrate Zoology

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e. Courtney Wickel, Museum and Shipping Specialist – Invertebrate Zoology

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f. Chris Huddleston, Biorepository Manager – Biorepository

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II. PRE-TRIP ACTIONS

a. Field Work Alert

i. Researcher must inform the Invertebrate Zoology Collection Committee of proposed collection activity.
- IZ Collection Management Staff can provisionally approve the activity, but the final decision is ultimately made by the IZ Collection Committee.

ii. Researcher provides IZ Collection Management Staff and relevant technical staff (e.g. GGI) with required permits, documents, estimated date of field trip, and shipment plan. See details below.

1. Permits
- It is important to make sure you have the proper approval and permits for the specimens you intend to collect.

- Permit(s) should cover collecting and export permissions, ownership/rights, and approval for the phyla you intend to work on.
- If you are planning to ship back to the museum any species that are currently on any CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) list or that require an APHIS (Animal and Plant Health Inspection Service) permit, then those plans will need to be discussed well in advance of your trip.
  - **http://checklist.cites.org/#/en**
  - **https://www.aphis.usda.gov/aphis/home/**
- If you are collecting parasites and any host tissue, then both will have to be covered in the permit and the host tissue will have to be cleared through a US Fish & Wildlife 3-177 eDec form. Host tissue phyla may require a CITES or APHIS permit even if the parasite does not.
- Research protocols for vertebrates must be approved by the appropriate Institutional Animal Care and Use Committees (IACUC).
- Confirm with the permitting agencies if any MOU (Memorandum of Understanding) or MTA (Material Transfer Agreement) may be required by the government of the country of origin to legally export and obtain biological material.
- It is the researcher's responsibility to obtain all proper documentation.
- If the country or administrative zone does not have any structure in place for oversight, then this must also be documented by an administrative official. This can be high level government offices, local fish and wildlife agencies or even associate institutes who issue them in lieu of an actual government entity.
- Applications for U.S. Fish and Wildlife Permits can be found on the U.S Fish and Wildlife Service website: **https://www.fws.gov/permits/ApplicationMain.html**

2. Documents

- GGI Pre-Trip Collecting Questionnaire
  - To be populated during a meeting with GGI technical staff and GGI management.
- Project synopsis and scope of taxa you intend to collect
  - All documents pertaining to travel, permits, and plans for export/import of the specimens

3. Shipment Plan (Export/Import)

- Inform IZ Collection Management Staff how material will be transported to the United States (shipped via a courier or hand carried). Hand carries should only occur if absolutely necessary.
- Please provide IZ Collection Management Staff with a complete list of taxa down to the lowest level of identification possible.
- A U.S. Fish and Wildlife Services Inspector may not approve material with too general of an identification. A family level ID is highly preferred.

- Ship specimens in accordance with all US Department of Transportation (DOT) Guidelines, International Air Transportation Association (IATA) Special Provision A180 (ethanol/alcohol) and A152 (for liquid nitrogen dry shippers), and FedEx Hazardous Materials Guidelines.
  - US DOT: https://www.transportation.gov/
  - IATA Special Provisions: http://www.iata.org/Pages/default.aspx
  - FedEx : http://www.fedex.com/us/hazardous-materials/
- All relevant documentation must be included in the waybill pouch for the customs clearance, including 3 commercial invoices with original signatures (do not send copies).
- Please note that if there is any material which falls under CITES, then the entire 3-177 eDec form will need to be pre-filed with the port through which the material is expected to clear customs.

4. Shipment (Hand Carry)

You should hand carry specimens only when absolutely necessary. Shipping material via a courier service is recommended whenever possible.

- If the intent is to hand carry the material, then IZ collections staff need to know your travel plans as a 3-177 permit must be pre-filed with the entry port USFWS office up to 72 hours in advance. Your boarding pass and/or flight information will be required once you have returned.
- If the hand carry involves any chemicals, then you may be required to inform the pilot, who then may refuse your request for transport.
- You must go through a valid USFWS port and a USFWS Customs line, and present your material along with your permits to an inspector.
- For a list of designated Fish and Wildlife Service ports, please reference the U.S. Fish and Wildlife Services "Designated Ports" page: **http://tinyurl.com/lbv3fyt**

b. Trip Approved

i. IZ Collection Management staff provide researcher with custom FIMS.

- IZ Collection Management Staff will provide the researcher with an Excel spreadsheet designed for accurate and consistent data capture.

ii. Biorepository Manager provides researcher with Biorepository ID Numbers.

- The researcher should request Biorepository ID numbers and labels from the Biorepository Manager at least two weeks prior to the collecting trip. It is acceptable if the exact number of tissues to be collected is unknown. An estimate of intended samples is fine.
- Researchers collecting into cryovials or coin envelopes will be issued a roll of Biorepository labels to be affixed to the sides of the cryovials or envelopes.
- We strongly recommend that researchers apply their labels to their cryovials/envelopes prior to departure – this reduces processing time in the field.
- Researcher collecting in 2D barcode cryovials will not receive physical labels, as the Biorepository ID numbers will be associated with scanned 2D barcode numbers.

- Please refer to the Biorepository's Standards and Services, located on Darwin: **http://tinyurl.com/h4svs9q** Please refer to the Biorepository's Workflow for New Collections, located on Darwin: **https://tinyurl.com/j9cgclu**

iii. **LAB provides researcher with 0.75mL Alphanumeric Matrix tubes.**

- Please refer to LAB's Spreadsheets and Protocols for requesting plates, capturing data, and sampling tissues, located on Darwin: **https://tinyurl.com/htvj2rq**

III. COLLECTING TRIP

a. Collect Voucher Specimen:

i. Researchers collecting in the field must make sure that all preserved specimens are accompanied by the proper location and collection information, and any corresponding notes. IZ Collection Management Staff will provide you with an Excel template (i.e. FIMS spreadsheet) for collecting specimen information.

- Specimens lacking this information will be of less value to other researchers and the collection as a whole.

ii. For fluid preservation, collectors may immerse invertebrates in formaldehyde, alcohol, or other solutions. These techniques either kill the specimen or work as fixatives. The type of preservation fluid used depends on the sampling method and the species being collected.

iii. To be safe, the rate of preservation to specimen/tissue in the vial or jar should be at least 3 parts preservation fluid to 1 part tissue.

- For most small specimens, 4 or 6 dram glass shell vials with straight sides (no shoulders) filled with preservation fluid are suitable. The vial(s) should be plugged with clean cotton in such a manner that no air bubbles are trapped inside and the vial should be placed inverted into a 4oz or 8oz container, or an appropriately sized glass jar with a proper lid and gasket.
- Extremely small specimens may be double vialed, first in a ½ or 1 dram vial and then placed into a 4 dram vial, in order to ensure the specimen is not damaged by labels.
  - Medium sized specimens can go directly into appropriately sized glass jars with proper lids and gaskets. The jars should be topped off with preservation fluid to prevent air bubbles being trapped inside.
  - Oversized Invertebrate specimens can be stored in polycarbonate pails with screw top lids. These pails should also be topped off with preservation fluid.
  - Collecting supplies must be included in the researcher's budget.
- Voucher collecting supplies can be provided by the IZ collection staff.

iv. All specimens must be identified and sorted by taxon as narrowly as possible, preferably to the species level.

v. Jars should be kept in the dark and not exposed to artificial light or sunlight because the specimens will fade.

vi. Jars should be checked on a regular schedule to ensure that the specimens remain below the level of the fluid.

vii. Labels for Wet/Dry Specimens should be of cotton/rag bond.

viii. Labels should be completed using a #2 pencil or a pen having indelible ink.

- Laser printed or photocopied labels are not acceptable, as they can fade and bleed. Ballpoint pens and fugitive pencils (such as red pencils) will fade and therefore are also not acceptable for writing on labels.

Specimens should never be jammed or forced into the vials or jars. An appropriate container would allow an organism to fit comfortably through the opening of the jar and be completely submersible in preservation fluid.

b. Tissue Sample for Biorepository:

i. **Scan (or copy/paste from provided spreadsheet) Biorepository ID numbers (BR Numbers) into the appropriate columns within the Biorepository FIMS prior to collecting event.**
   - Biorepository Staff will provide researcher with Biorepository ID Number (BR) labels and Biorepository FIMS.

ii. **Prepare all vials prior to the collecting event.**
   - BR labels are applied by hand to vial exterior.
   - Corresponding collector specimen labels are placed inside the vial, and the vials left dry or filled with preservation fluid.

iii. **A tissue sample is promptly subsampled in accordance with the taxa-specific tissue sampling.**
   - The more tissue subsampled the better.
   - If a specimen is too small to subsample a tissue sample, the entire specimen is placed in the cryovial, and an identical specimen is kept as the morphological voucher.

iv. **The tissue sample is promptly placed within a new labeled vial and left dry or topped off with preservation fluid up to the 1.8 ml fill line.**
   - Use 2ml cryovials for tissue samples. Cryovials will be provided to the researcher by the biorepository upon request.
   - There is limited space in the biorepository for 8ml tubes. Only request if absolutely necessary.
   - Make sure caps have gaskets. Gaskets prevent liquids (i.e. alcohol, DMSO, etc.) from leaking out. Gaskets will be provided to the researcher along with requested cryovials.

v. **Wrap individual tubes in tinfoil so labels do not fall off while submerged in liquid nitrogen.**
   - Cutting tinfoil prior to collecting trip reduces processing time in the field.

vi. **The tissue sample is promptly moved to liquid nitrogen-cooled storage or a freezer. Please contact the Biorepository staff for available liquid nitrogen-storage containers.**

vii. **The voucher specimen is placed in a jar featuring the complementary ID label and topped off with the appropriate preservation fluid.**

viii. **Record accurate and consistent data in FIMS.**
   - Station data and other necessary information is recorded in the appropriate columns alongside corresponding BR numbers within FIMS.
   - Avoid blank cells within the spreadsheet if at all possible.

ix. **Please see GGI's Vouchering Genomic Samples document, located on Darwin under Protocols and Training: https://tinyurl.com/hwe8jyk**

Care must be taken to ensure that the specimen label matches its associated tissue sample label. Mix-ups will result in the tissue being attributed to the wrong specimen, and the tissue and associated DNA – as well as all the time and money put into obtaining and processing it – will be worthless.

c. Tissue Sample for LAB Extraction:

i. **Label extraction plate with a unique and explanatory code (e.g., Hawaii_Bioblitz_2017_plate1).**
   - LAB will provide the researcher with extraction plates**.**

- Remove G12 and H12 from the box, or leave empty. These are left blank for the positive and negative controls.

ii. **Use an empty box while sampling.**
- This will serve as a "sink" box – put tissue in the empty tube in the "source" box and shift the filled tube to the "sink" box. This goes a long way towards helping you keep your place in the box, and in the spreadsheet.

iii. **Record if sample is taken from voucher or from tissue sample taken for the Biorepository.**

iv. **Fill tubes with 150uL buffer (50uL 95% ethanol can be used instead).**
- This is an EXACT amount, as LAB will add the additional components when they receive the samples, and the total volume has to be exactly right.

v. **Subsample a small amount of tissue.**
- A dull pencil lead worth of tissue is generally sufficient.

vi. **When the specimen is added, you can plug the tube.**
- It's easiest to pull the tube out of the plate and press it against the sheet of plugs.
- The plug should come off the plastic sheet/card readily and you may need to push on it to get the plug completely seated.
- You will be able to see when a plug is all the way in. Once all plugs are used, discard the plastic sheet.

vii. **Keep filled plates in the fridge, if possible.**

viii. **Please refer to LAB's Protocols for Sampling Tissues, located on Darwin: https://tinyurl.com/htvj2rq**

ix. **Record accurate and consistent data in FIMS.**
- Avoid blank cells within the spreadsheet if at all possible.

d. Blended Fractions and Blended Environmental Samples:

i. **For proper procedures on collecting, stabilizing, and recording blended fractions and blended environmental samples, please contact the IZ Collection Manager directly.**

e. Observational Data:

i. **For proper procedures on recording and standardizing observational data, please contact the IZ Collection Manager directly.**

IV. POST-TRIP ACTIONS

a. Acquisition and Release for Work:

i. **Provide IZ Collection Management Staff with all relevant documentation from trip.**
- Required Import/Export permits and documentation
  - It is up to the researcher to do the background work to insure that the material can be legally acquired. Examples: Fish and Wildlife eDEC 3-177, Material Transfer Agreement (MTA), Memorandum of Understanding (MOU), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits, Animal and Plant Health Inspection Service (APHIS) permits, etc.
  - If an import is illegal, IZ Collection Management Staff will not attempt to fix the issue.

        - All legal material will be obtained and any illegal material will be disposed of or returned to the country of origin.
    - Travel Vouchers
        - This includes boarding pass and/or flight information.
    - Completed FIMS spreadsheet
        - Please complete the FIMS spreadsheet to the best of your abilities.
        - Avoid blank cells and gaps in information if at all possible.

ii. **Material must either be in the possession of IZ Collection Management Staff, the Biorepository, or LAB as they have the required storage to preserve the material during this process.**

iii. **No material can be worked on until the import has cleared and the acquisition has closed.**

b. **Specimen Vouchers:**

i. **Provide IZ Collection Management Staff with all voucher specimens and completed FIMS Spreadsheet.**
    - At this point, the vouchers and corresponding data will be assessed.

ii. **EMu catalog records.**
    - Catalog records for vouchers will be created in EMu by IZ Collection Management Staff.

iii. **Labeling and curation of voucher specimens.**
    - Once catalog records have been created in EMu, IZ Collection Management Staff will print the necessary labels and curate the vouchers into archival containers, as needed.

iv. **Vouchers incorporated into general collection.**

c. **Biorepository Tissues:**

i. **Provide Biorepository Staff with samples.**

ii. **Biorepository Staff will process tubes and assess samples.**

iii. **EMu catalog records.**
    - Catalog records for vouchers will be created in EMu by the IZ collections staff.

iv. **EMu catalog records and FreezerPro locations now linked.**
    - The Biorepository ID number provides the link.

v. **Data ready for upload to GGBN.**

vi. **Tissues put away in the Biorepository by the Biorepository staff.**

d. **Extraction Plate Tissues:**

i. **Provide extraction plates with tissues to LAB.**
    - Please refer to LAB's Protocols and Lab Procedures, located on Darwin: **https://tinyurl.com/htvj2rq**

ii. **LAB performs molecular work on plate.**

iii. **LAB provides IZ Collection Management Staff with completed plate and extraction data.**

iv. **IZ Collection Management Staff enter completed plate and extraction data into EMu.**
    - Catalog records and Biorepository ID numbers will be created in EMu by IZ Collection Management Staff.

v. **Completed plates delivered to Biorepository and scanned into FreezerPro by Biorepository Staff.**

- Scanning enters sample's plate and well location into FreezerPro, the Biorepository's database for extraction storage.

vi. **EMu catalog records and FreezerPro locations now linked.**
   - The Biorepository ID number provides the link.

vii. **Data ready for upload to GGBN.**

viii. **Plate data uploaded to BOLD/GenBank by researcher.**

ix. **Matrix plates put away in the Biorepository by Biorepository Staff.**

e. Field Photographs:

i. **Save/Convert all images to JPEG.**

ii. **Multimedia records can be created for all photographs and attached to corresponding catalog records by IZ Collection Management Staff.**

iii. **Provide IZ Collection Management Staff with high resolution images (the higher the better).**

f. Blended Fractions and Blended Environmental Samples:

i. **For proper procedures on archiving blended fractions and blended environmental samples, please contact the IZ Collection Manager directly.**

g. Observational Data:

i. **For proper procedures on archiving observational data, please contact the IZ Collection Manager directly.**

V. QUESTIONS, CONCERNS AND SUPPORT

a. IZ Collection Management Staff have extensive training in processing transactions, international shipping and permits. If your question falls outside of our expertise, then we have resources within the Smithsonian as well as outside that can quickly provide answers.

b. Our staff can also provide you with a tutorial on shipping protocols and procedures. If you are expecting to do a great deal of international shipping then it may be appropriate to point you in the direction of an official IATA/DOT Hazardous Materials course.

c. Shipments, even when prepared properly, can run into problems. Our staff is committed to putting into play whatever resources are needed to ensure that your specimens make it through US Customs. We have an excellent track record with US Fish & Wildlife and know many of their inspectors.

d. For all questions and concerns pertaining to the Biorepository, please refer the Biorepository's homepage located on Darwin, which will provide you with links to standards and protocols, workflows, and research support: **http://tinyurl.com/jo6qhmt**

e. For all questions and concerns pertaining to LAB, please refer to LAB's DNA Barcoding Resources page located on Darwin, which will provide you with contact information and links to protocols, spreadsheets, and lab procedures: **http://tinyurl.com/htvj2rq.** If you are performing sequencing work for a GGI, TMON or ARMS funded projects, please inquire with relevant technical staff to ensure proper sequencing pipelines are being followed.

VI. ADDENDUM

a. Field Practices and Protocols for the Florida Museum of Natural History

The following is a descriptive overview of GGI/TMON partner field practices and protocols from the Florida Museum of Natural History (FLMNH) by Dr. Gustav Pauley. This workflow is often used during collecting events where a subset of samples, including vouchers and tissues, collected are housed at FLMNH and a subset, specifically the voucher duplicates, tissues and DNAs, are housed at NMNH. Gustav Pauley is the lead collector during such collecting events.

b. <u>Pretrip</u>

i. **Permits organized by group.** For this Bioblitz, no specific actions are required by FLMNH.
ii. **Organize and ship supplies.**
iii. **Preprint field sheets and labels.** These are simplified equivalents of FIMS.

c. <u>Collecting Trip</u>

i. **Collection stations tracked on white board, and captured in FIMS each day.**
ii. **Specimens tracked by each worker in field sheets.** These are backed up photographically daily, and entered into electronic FIMS post trip.
iii. **Specimens are morphosorted.** ID'd to best practicable level.
iv. **Representatives of each ID are photo documented.**
v. **Specimens are subsampled for tissue, relaxed and fixed.**
    1. If specimen is too tiny to subsample, then the entire organism will be used.
    2. Tissues are fixed in ethanol.
    3. Tissues will be parsed out to GGI and barcoding (in M2) post-trip.
        a. Plating tissue in the field is too complex and often leads to poor outcomes.

d. <u>Post-Trip Actions</u>

i. **Specimen Vouchers**
    1. **Collection data entered into electronic FIMS in appropriate format for ingestion into FLMNH collection database.**
    2. **Vouchers rehoused and parsed among collections.** Duplicate vouchers will be sent to the IZ Department at NMNH.
    3. **FLMNH vouchers are curated and cataloged.** Data made available through FLMNH collection database and IPT.
ii. **Tissues**
    1. **Tissues parsed among collections, plated for archival storage with GGI and separately plated for barcoding at LAB**
iii. **Field Photographs**
    1. **Photos cleaned, cataloged, and matched to specimens.** Made available through FLMNH collection database.

e. <u>Data</u>

i. **All digitized and electronic data made available to partners at end of project.** Updates in identification from FLMNH's collection database will be available through IPT.
ii. **Identification carried further iteratively.**