ATLASea Standard Operating Procedures

General recommendations for sampling

Introduction

The Standard Operating Procedures (SOPs) contain general guidance on processing specimen within the scope of the ATLASea project. The guidance specifically refers to the tissue samples destined for DNA extraction, and to specimen vouchering. Beyond the present general recommendations, taxon-specific SOPs are also available, and describe more specific procedures for each taxon (see list below). Other taxonomic groups may be added in the future.

1- Crustacea
2- Bryozoa
3- Tunicata
4- Anthozoa
5- Medusozoa and Ctenophora
6- Echinodermata
7- Mollusca
8- Porifera
9- Annelida
10- Platyhelminthes – arriving soon
11- Macro-algae – arriving soon
12- Phytoplancton cultures – arriving soon
13- Zooplankton – arriving soon
14- Fish – arriving soon

Future plans for this SOP

This SOP will be reviewed on a regular basis by ATLASea team members to incorporate feedback from the community. If you have any advice, comments, techniques, or lessons learned that you would like to contribute, we would greatly appreciate it (please contact: ambassadors@atlasea.fr).

ATLASea sampling selection

ATLASea collects marine organisms of ecological, endangered, and economical interest observed in the French EEZ. The GoaT database (Genomes on a Tree) must be consulted before a species enters the ATLASea program, to avoid overlaps with other genomic projects. Only species that have not yet been sampled or sequenced by other programs will enter the ATLASea program. For any question or clarifications please write to ambassadors@atlasea.fr
****Please ensure that you hold sampling permits if these are required, please see the ATLASea Statement**

General procedure

Sampling and conditioning a specimen to be sequenced by ATLASea will generally involve the following 4 steps:

1. Taxonomic identification and status verification in the GoaT database (section A)
2. Photographing the specimen live with labels provided by ATLASea (section B)

3. Flash freezing tissues for sequencing (section C), and preserving a sample for a voucher (section D).
4. Shipping the samples to Genoscope (section E)

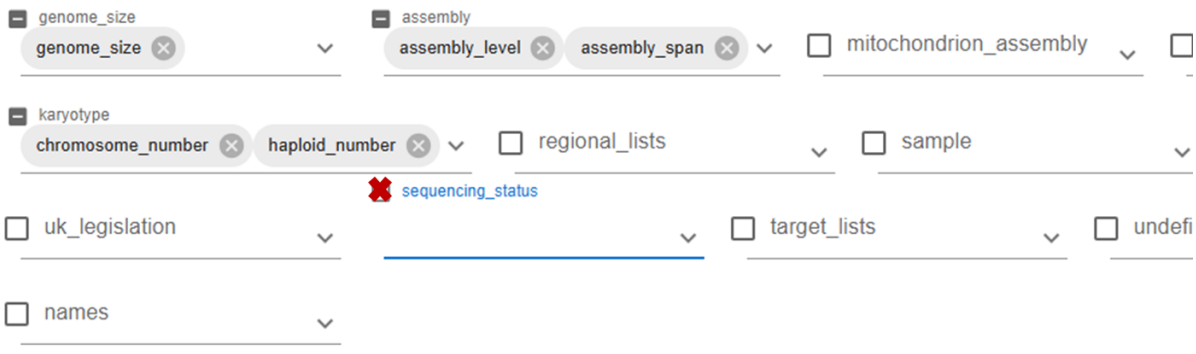
Before starting the specimen sampling, please ensure that you have the 2 types of labels (ATLASea and MNHN) and the screw cap cryotubes in sufficient quantity. Both can be sent to you by ATLASea, please ask Melanie van Weddingen at ambassadors@atlasea.fr. You will need one MNHN label per specimen (individual), one ATLASea label per batch of specimen(s) (one batch corresponds to a sampling event at the same time and the same place), and one ATLASea label per cryotube.
Please do not use any other tube than the recommended model (see Appendix).

A. Taxonomic identification and status verification

The Genome on a Tree (GoaT) database centralises information generated by various genome sequencing projects. It enables researchers to monitors the evolution of the status of the species that are being sequenced worldwide and deposited in the INSDC (International Nucleotide Sequence Database Collaboration) repositories (Genbank, ENA, DDBJ). All projects affiliated to the Earth Biogenome Project (EBP), including ATLASea, update GoaT regularly with the status of the species they are proceeding with. This ensures transparency, facilitates tracking of genomic progress, and helps prevent overlaps or duplication of effort. It is crucial to confirm the taxonomic identification of the species by a qualified taxonomist familiar with its distinctive morphological traits. The name of the taxonomist responsible for identifying the species will need to be recorded later. Once the specimen name is confirmed, please verify in the World Register of Marine Species (WoRMS) that you are using the accepted name, not a synonym. Following this, you may verify its status in GoaT (https://goat.genomehubs.org/):

1. Type the name of the species (*Binomial nomenclature*) in the “Type to search GoaT taxon index” box.

2. Select the option “results columns” and ensure that the “sequencing status” option is activated. Update your option



3. Click on “TAXON” on the pulldown menu to the right of the entry box.
4. If no results are returned (0 hits), you may have misspelled the species name. GoaT contains ~1.5 million species described to date and their synonyms, so you should find your species. However, some genus may be poorly represented, especially for overseas species.
5. Click on the name of your species of interest in the table containing the results. If the genome is already sequenced at “reference” of “chromosome” level, a yellow badge “EBP standard” will be shown above a “snail plot” summarising the assembly’s characteristics. Next to the plot, there should be at least “1 chromosomal” assembly shown.
6. If the genome is not sequenced at chromosome level, it may be sequenced at a lower level of contiguity. Look further down the table and check the “assembly_level” attribute, which may show “Scaffold” or “Contig”.
7. If the genome is not sequenced at all, it may however be underway in a sequencing centre. Look further down the table and check the “sequencing_status” attribute, which shows the status at the centre that is the furthest advanced in the sequencing process. Statuses include, from least to most advanced in the sequencing process, “sample collected”, “sample acquired”, “in progress”, “in assembly”, “insdc open”.

By default, ATLASea will only sequence the genome of species for which no high-quality genomic data has been produced, or those not already marked as "in progress" by other projects. Exception will be made for species who have been sequenced at low contiguity levels (Scaffold or Contig) but this will be decided by the ATLASea Species Selection Committee. The ATLASea SSC will systematically verify the status of a species before proceeding with DNA extraction.

B. Standard Operating Procedures for Specimen Photography

B.1 Introduction

Why take photos of specimen? Photos of specimen in ATLASea are extremely important for several reasons. First, for logistical reasons, they connect a recognisable physical specimen with its labels, which greatly facilitates traceability later on in the procedure. Second, when individuals are too small to be dissected and kept as voucher in MNHN collections, the photo is the only way to go back to the sample and perform detailed taxonomical identification. In effect, the photo becomes the Museum voucher. Finally, the genome that will be sequenced from the sample will probably become the reference genome for all genomic studies involving this species. Metadata accompanying the sample therefore become crucially important, including its photo. Taking photos of specimen is becoming standard practice in all projects that sample specimen from biodiversity for reference genome sequencing.

B.2 General Guidelines

- Specimens should be photographed while alive.

- Seawater must be changed between each specimen and must be clean.
- Avoid using transparent plastic containers; instead, use glass containers or solid white/black containers. If the animal is dark, use a light/white background.

- It is possible to place the specimen directly on a black/white background without a container, for example Asteroidea, Echinoidea, Ophiuroidea. Return the specimen to seawater from time to time (between adjustments, information collection, etc.) to avoid animal stress and deterioration (refer to their sections in relevant SOPs).

- The duration of the photography process may vary depending on the activity level of the specimen.
- Do not use anesthesia during photography, except for specific groups such as annelids, crustaceans, and fish (refer to their sections in relevant SOPs).
- Save all photos in JPEG format with the highest possible resolution (varies depending on the camera type, e.g., phone, professional camera).
- Name photos using the ATLASea identifier assigned to the specimen, with incremental numbering if there is more than one photo.
    - Example:
        - Reference photo: A-765YDNQJ7
        - Specimen photo: A-765YDNQJ7_01
        - Photo 02: A-765YDNQJ7_02
        - Photo 03: A-765YDNQJ7_03

Photography Planning:

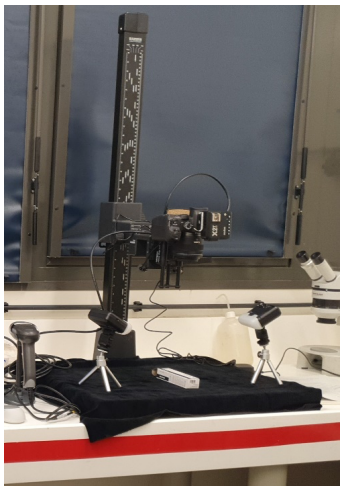
If you are processing a single specimen, you need to take one Reference Photo (see Section B.4), one Specimen photo (without labels) and possibly several others (without labels) to focus on anatomical details that are critical for taxonomic identification.

1. **Reference Photo**: Specimen with labels (ATLASea, MNHN).
2. **Specimen photo**: Entire specimen in greatest possible details / resolution.
3. **Anatomical Detail Photos**: Close-ups of specific anatomical features.

If you are processing several specimens from the same species, the Reference Photo (see Section B.4) should contain all specimen together, then designate one as the voucher for the Specimen Photo and other photos focusing on details.

Once the photos are taken, the specimen can proceed to cold-chain processing.

B.3 Photography Equipment



Ideally, please use high quality material used for scientific photography, with a flash, installed on a stand and with dedicated lightning (Left). If this equipment is not available where you are, you can also use a smartphone attached to a support for stability, and good *ad hoc* lightning.

Always use a scale, such as a ruler graduated in mm and cm, and ensure that it is visible on the photo (no reflection, blurry, etc.) across the length of the specimen.

If using professional cameras, here are some general guidelines:

- **Shutter Speed (1/100)**: Use fast speeds for live specimens to freeze motion, especially when using flashes.
- **Aperture (F14)**: Higher values decrease light entry but increase depth of field, resulting in sharper images.
- **ISO (125)**: Adjusts brightness. Higher values increase brightness but add noise, reducing image sharpness.
- Avoid lenses that cause distortion, such as wide-angle lenses.
- Use **50 mm or 100 mm macro lenses** for specimens larger than 1 cm.
- Use a **65 mm macro lens** for specimens smaller than 1 cm.

B.4 Reference Photos

Single Specimen

If you are processing a single specimen, you need to take one Reference Photo, which will be used to connect the specimen with its identification labels in case of doubt later in the procedure. The specimen need not be photographed in great details, but the photo MUST include a ruler, an ATLASea label and an MNHN label corresponding to the correct phylum (see taxon-specific SOP for more details).

Multiple Specimens

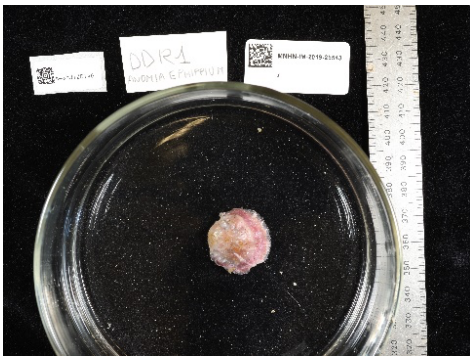
When multiple specimens represent the same species, designate one as the voucher for the Specimen Photography.

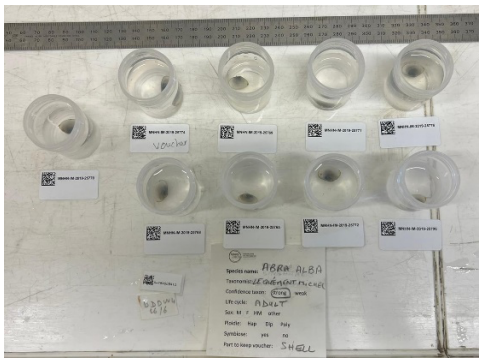
Procedure for the Reference Photo:

- Separate specimens into containers.

- Assign an MNHN label to each specimen.
- Assign a single ATLASea label to the entire collection.
- Mark the MNHN label of the voucher specimen with "Voucher" or a cross for identification.
- Include a ruler.

Proceed with further photos of the voucher specimen (Specimen Photo and additional close-ups).





Typical Reference Photos for single specimen (left) and multiple specimen (right). Additional labels (Station labels e.g. DDR1, species identification labels) are shown but are only relevant for ATLASea field missions.

B.5 Sending photos

All photos should be sent by email to ambassadors@atlasea.fr in one or several batches, or made available on a local server for download with the link sent to the above address. Please remember to use JPEG format, and to name the photos with the ATLASea ID of the Reference Photo with an incremented suffix for all ensuing photos.

C. Preparation of tissues

Prior to the preparation of tissues, the operations carried out on the Vertebrata and Cephalopoda (collection, anesthesia, euthanasia) must be carried out in accordance with European directive n°2010/63/UE, transposed in France under decree n°2013-118 of February 1, 2013 relating to the protection of animals used for scientific purposes.

1. For each taxon, provide enough aliquots:
   - one for taxonomic identification vouchering (in ethanol, formalin, etc., depending on the specimen)
   - at least 15 for High Molecular Weight (HMW) DNA extraction, RNA extraction and long-range data production (Flash-frozen or in ethanol depending on the organisms, see taxon-specific SOP)

2. Each vial should be handled with gloves and correctly labelled:


- Only use labels provided by ATLASea:
- Labels must be stuck along the tube (not around the tube).
- Be sure that tubes and gloves are dry before handling the labels (the labels can detach from the tubes during the flash freezing if wet).

3. Fresh material is critical for obtaining HMW-DNA. If possible, specimens should be sampled alive. However, specimens will deteriorate even in these conditions so sampling should occur as soon as possible once the specimen is out of its natural environment.
4. Anaesthetic methods can be applied to calm animals. . See specifics SOPs for more details.
5. Absolutely avoid freeze-thaw cycles to prevent unwanted cell disruption or degradation.
6. Samples should be flash-frozen in several aliquots as soon as they are prepared.
7. Always handle input material in a manner that minimizes nuclease exposure and activity, e.g. wear clean gloves (change regularly) when handling input material, storage tubes etc., use nuclease-free buffers and filtered and autoclaved seawater bottles and keep the input material as cold as possible during handling.
8. Before dissection and preservation remove all visible contaminants and epibionts as much as possible (washes, brushing… with stereomicroscope).
9. **Never pool different individuals** (even from the same species) in the same tube.
10. Dissection should be done as quickly as possible, maintaining the cold chain until flash-freezing (for example, by dissecting individuals on a glass board placed on an ice tray).
11. Decontaminate instruments (forceps, scalpels, etc.) between **EACH** organism by wiping with wet absorbent paper, then immersing them for 1 min in sterile water, transferred for 30 minutes in a bleach bath (2.6% active chlorine solution diluted ¼), then rinsing them for 5 minutes in a sterile water bath, finish with ethanol immersion to dry the instrument quickly.
    Wash surfaces (Aniospray + water…) and change gloves between each organism.
12. Collect "fleshy" parts when possible (avoiding shell, digestive contents, embryos/brooded progeny, ...).
13. Dissect **at least 15 pieces** (approx. **300 mg** each for animals, ideally **1 g** each for algae and plants). More details for each phylum in dedicated SOPs. Cut each piece into smaller fragments before putting them in separate tubes (with unique identification labels). For small individuals, put 1 specimen per conical-bottom tube.
14. Avoid compacting the sample in the bottom of the tube, and never fill the tube to the top (to facilitate sample recovery for subsequent handling and to prevent the cap from popping off during thawing).
15. In the case of very small specimens, make sure that it is placed in the bottom of the tube.
16. After conditioning the sample in the tubes, screw the cap of the tube properly (to prevent the cap from opening when immersed in liquid nitrogen), then weigh the tube with the cap. Use a tare with the same model of tube already labelled to deduce the weight of the sample inside the tube.
17. Scan the labels on the vials and fill in the log sheet dedicated to the ATLASea sampling. Ask ambassadors@atlasea.fr for the latest version. The barcode must be scanned

(before flash freezing) to avoid typos. Ensure that all tissues from the same individual are correctly identified on the log sheet.

18. Once scanned, the sample must be immediately preserved. Most biological samples should be flash-frozen (FF) in liquid nitrogen to minimize nucleic acid degradation by nucleases, then stored at -80°C and shipped on dry ice (see Section D). Depending on the sample, it can also be preserved in ethanol 80%, stored at -20°C and shipped in cold box.
19. Vials should be grouped (around 10 tubes) and placed in a nylon bag identified with a unique number. When possible, place tubes corresponding to the same taxon in the same bag. Bags should be properly sealed using twist-ties to avoid loss of tubes during the transportations. Sealed bags should be then immersed in liquid nitrogen (in Dewar).


(Left) typical nylon bag used to group samples from the same species, if several species will be provided. (Right) Dewar container used to hold liquid nitrogen and flash freeze samples.

20. If immediate storage of the samples in a -80°C freezer is not feasible due to field conditions, an alternative is to place them in a larger liquid nitrogen container specifically designed for storage. This container should have a large opening to facilitate sample retrieval. The samples can then be transported to a facility where they can be stored under ultra-freezer conditions.
21. Place the nylon bags into a Zip-Seal bag, label it with the campaign information, and prepare it for shipping.

D. Voucher

1. It is very important to have as many taxonomic vouchers as possible (correction/validation of identification post-mission, collection duty), minimally damaged or with interesting taxonomic parts.
2. Once the specimen dissection is finished, the rest of the animal must be fixed, for example in ethanol 80%, or 96% ethanol for molluscs, or formalin for ascidians. See taxon-specific SOP for more details for conservation.
3. Fish must be frozen at -20°C or -80°C and sent frozen at the same temperature when stored.
4. Vouchers must be labelled properly while fixed; labels must be ethanol resistant if put inside the container. The MNHN ones are ethanol resistant, put them with the corresponding specimen. A specimen must always have a label to be able to identify it.

5. If the specimen is too small and nothing is left after dissection and sample preservation, another specimen can be fixed as species representative. **WARNING**: ensure that they have the same morphological traits of the preserved specimen.
6. If nothing remains of the specimen after dissection and there is no other specimen to be called as voucher, you need to keep the labels (ATLASea, MNHN). These labels should be placed in a bag labelled “ATLASea specimen labels WITHOUT VOUCHER”. Never throw away a label. Send them to the ATLASea team with or without the others vouchers.
7. All the permits are mandatory; we cannot include a species in the MNHN collection without the permit.
8. **Contact ambassadors@atlasea.fr to arrange details for voucher shipment**:

E. Shipment of Genomic Samples

1. Always arrange shipping details with a staff member of Genoscope SeqLab and send the ATLASea log sheet via e-mail.
2. Please contact:

- **Janaina RIGONATO**: jrigonat genoscope.cns.fr
- Karine LABADIE: klabadie@genoscope.cns.fr
- Pedro OLIVEIRA: pcoutool@genoscope.cns.fr

3. Shipment of **frozen samples** should be performed either in dry-shippers or on dry ice via a suitable carrier (e.g.: Transportéo, Cryoexpress, etc).
4. The samples preserved in -80°C should be placed in the **middle of dry ice** and the box completely filled with dry ice, samples preserved in ethanol -20°C should be placed on top of dry ice.
5. Shipment should be favoured for a delivery on Mondays or Tuesdays between 8.30 a.m. and 5.00 p.m. Outside this time frame, please contact **Janaina RIGONATO** to arrange for the reception.

6. The package should be sent to the following address:

   SITE CEA EVRY
   Réception Marchandises
   To : **Janaina Rigonato**, Emmanuelle Petit or Pedro H Oliveira
   31, Boulevard des Coquibus
   91000 EVRY
   FRANCE

7. Each shipment should include a letter mentioning:
   - The name ATLASea
   - The name of the sender
   - A partial description of the samples
   - Permits for species requiring CITES permits
   - Printed copy of the email which was sent to ambassadors@atlasea.fr prior to the shipment of the samples, and that had the ATLASea log sheet as attached file (do not print the actual log sheet).

Appendix: material references

Tubes:

- ClearLine® 1.2 ml cryotube, skirted with external thread and natural screw cap [ref Dutscher # 390700]
- ClearLine® 2 ml Cryotube, skirted, external thread with natural screw cap [ref Dutscher # 390701]
- 5 ml screw tube sterile (Eppendorf) [ref Dutscher # 934683]
- 25 ml screw tube sterile (Eppendorf) [ref Dutscher #934685]
- 50 ml Conical Centrifuge Tubes (Greiner) [ref Dutscher #227261]

Bags:

- Nylon protection net, reusable with drawstring (15x10 cm) [Amazon #https://www.amazon.fr/Anti-Insectes-Protection-Plantes-R%C3%A9utilisables-Anti-Oiseaux/dp/B08XXJMY4C]
- Nylon protection net, reusable with drawstring (15x25 cm) [Amazon #https://www.amazon.fr/AMZMUKAUP-Protection-Raisins-R%C3%A9utilisables-Rangement/dp/B09N7PV2LD/ref=sr_1_1_sspa?crid=2VTI1EVYUW654&keywords=sachet+filet+nylon&qid=1690200116&sprefix=sachet+ny%2Caps%2C213&sr=8-1-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1]
- Twist attaches [Amazon #Twist Attaches Or, 800 Pcs Métallique Pince pour Fermeture Sachet Alimentaire, Attache Sac pour Paquet de Bonbon, Sachet Plastique, Transparent Cello]
- Zip-Seal Bags (grands sachets) 350x250 mm [ref VWR #129-0307]
- Zip-Seal Bags (petits sachets) 170x120 mm [ref VWR #129-0297]

Scan:

- Bluetooth QR & Barcode to PC application to scan labels with your phone and import the code to the Excel sheet via Bluetooth: https://play.google.com/store/apps/details?id=dev.fabik.bluetoothhid
- Honeywell Voyager Extreme Performance 1470g-2D [Ref. Fabricant #1470G2D-2USB-1-R]