**Extractions and Database *detailed*-Workflow**

Only the Albatross lots that will be used for the Philippines PIRE Project (PPP) will be reflected on the PPP-Database. However, all contemporary collections/lots should be included. As you work in a lot, please follow the following steps to record your progress:

**ODU**

1. **Select a Lot**
2. **check the “Species\_sheet”**

Is the species that you are working on in the Species\_sheet?

* + 1. YES – check that all the info is complete and correct.
    2. NO – add a new record and:
       1. check the current valid name in the Eschmeyer’s Catalog of Fishes
       2. create a new “Species\_code” after the valid name. This is a 3-letter code consisting of the first letter of the genus and the two first letters of the species name. If this code is already taken by another species, keep the genus letter but modify the two letters from the species to create a UNIQUE code.
       3. Fill as much as info as possible about this species.

1. **check the “Lot\_sheet”**

Is the Lot (i.e. this species/site collection) that you are working on in the Lot\_sheet?

* + 1. YES – check info and update the “Lot\_status” accordingly
    2. No – add a new record, populate all cells, and:
       1. Create a unique “Lot\_ID” following the same format as other Lot\_IDs, i.e. no parenthesis, etc. DO NOT confuse the Lot\_ID with the “ODU\_collection\_catalog\_number”
       2. Create a unique “Site\_ID”. Check if your site already exists for another species. If so, use the same “Site\_ID”. If not, create a new UNIQUE 3-letter Site\_ID. Check that your site is not in such geographic proximity to an already existing one that it would make sense to keep the same name. Collection sites can have different “Site\_IDs” even when they will have the same “Match\_ID”.
       3. Find the “Match\_ID”. If this is for the PPP, this lot should have an Albatross/Contemporary matching lot. Make sure this is in the list. If for connectivity study, enter “None” as the Match\_ID.

1. **update the “Individual\_sheet”**
   * 1. enter each individual in a new row and populate all columns. Enter all the individuals at once if you know how many will be done.
     2. create a UNIQUE “Individual ID” for each individual:

i.e. the ID for the 1st individual of a contemporary lot of *Taeniamia zosterophora* from Port Matalvi is:

* + - 1. Species\_ID: Tzo
      2. a dash “-“
      3. Collection era: A, for Albatross or C, for contemporary.
      4. Site\_ID: Mvi
      5. Underscore ”\_”
      6. a sequential number for each individual

Individual\_ID: Tzo-CMvi\_001

1. **update the “Extractions\_sheet”**
   * 1. enter each individual as one extraction in a new row and populate corresponding columns, i.e. if you are only subsampling, populate the first 4 columns. Enter all the individuals at once if you know how many will be done. The number of rows you have entered in the Individual\_sheet should equal the number of row you are entering here.
        1. Copy the Individual\_IDs from the Individual\_sheet and paste here.
     2. create a UNIQUE “Extraction\_ID” for each extraction with:
        1. Individual\_ID
        2. Underscore “\_”
        3. “Ex1” if this is the 1st extraction (this is the usual), “Ex2” if 2nd, etc.
           1. search if your lot has already been extracted and adjust
2. **Label spin columns with “Plate\_ID”, elution plates with “Elution ID”, and ADD the range of Individual\_IDs and the date to columns and all plates.**
   * 1. Plate\_ID consitst of:
        1. Species\_ID
        2. a dash “-“
        3. Collection era: A, for Albatross or C, for contemporary
        4. Underscore “\_”
        5. a sequential number for each extracted plate of this species.
           1. Note: there is not site ID or individual number.
           2. Check that we have not already sent previous plate of this species to TAMUCC. If not, then this plate will be “001”, otherwise add the next number if previous plates exist.

i.e. Plate ID for our example: Tzo-C\_001

i.e. Fifth Albatross Adu plate sent: Adu-A\_005

* + 1. Elution\_ID consist of:
       1. Plate\_ID,
       2. Underscore “\_”
       3. “E” and the number of corresponding elution (1, 2, 3, or 4) = i.e. “E1” (for elution 1, etc.)

i.e. Elution\_ID for our example: Tzo-C\_E1-4

* + 1. Add range of Individual\_IDs. If plate contains 96 individuals:

i.e. For our example: “Tzo-CMvi\_001-0096”

* + 1. Add the date to everything

1. **Assess extractions. Nanodrop and Gel DNA**
   * 1. Nanodrop the first 12 samples in each elution and update the Extractions\_sheet
     2. Gel the first 12 samples in each elution
        1. See the “Electrophoresis protocol”
        2. See “Gel Image Editing protocol”
           1. Label gel with:

Sample IDs

Elutions

Nanodrop values

Gel procedure

DNA/dye volume ratio and conc.

Amount of ladder\_voltage\_time\_gel\_type(1%)

Image\_edits, i.e. Fiji=green, Inv\_Enh0.6

1. **Update SLACK, and if needed, the Lot\_sheet**
   * 1. Update in the corresponding species slack channel after:
        1. Subsampling
        2. Extracting
        3. Gelling (post the labelled gel pic)
     2. May need to update the “Lot\_status” and “Frezeer\_location”
2. **Ship DNA to TAMUCC – Update “Shipping\_sheet” and SLACK**
   * 1. Create a new record in the Shipping\_sheet, populate all cells, and:
        1. Check that each row you enter represents one shipment/box
        2. Create a UNIQUE “Shipment\_ID” with:
           1. The date, separated with “\_”
           2. Sequential number for all shipments.

Try to ship the spin columns and 4 elutions of a species in the same box so they have the same “Shipment\_ID”

* + 1. Update SLACK in the corresponding species channel and post the Tracking\_number.

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1. **Update the date received in the “Shipping\_sheet”**
2. **Update “Lot\_status”**
   * 1. after starting the library preparation for a lot as “lib\_prep”
     2. after sending for sequencing as “sequencing”
     3. after receiving data as “with\_data”
3. **Populate “Library\_Prep\_sheet”**
   * 1. Populate the “Extraction\_ID”.
        1. If the unit of each row in the “Library\_Prep” is the individual/extraction, then you can copy the Extraction\_IDs from the “Extractions\_sheet”. If the unit is the plate, then you can copy the plate’s Extraction\_ID ( “Extraction\_ID” in the Shipment\_sheet)
     2. Create UNIQUE “Library\_IDs”

Note: we need to decide the unit for each row in this sheet. Either per plate or per well/individual. I could see how each could work but let me know what people think would be most convenient/informative for all.

In the “Extractions\_sheet”, each row represents one extraction of DNA from an individual. For instance, If we perform a second extraction from a lot bc the first one didn’t work, then there will be two rows for each of the individuals in this lot.

1. **Update the species SLACK channel with each step**

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1. **Populate “Sequence\_sheet”**
   * 1. Copy the Library\_IDs from the “Library\_sheet”
     2. Populate the UNIQUE “Sequence\_ID”

Note: here too, we need to decide the unit for each row. One row per sample? Two rows per sample, i.e forward and reverse IDs?

**ODU Extractions and Database *brief*-Workflow**

1. **Select a Lot**
2. **Check the “Species\_sheet”**

Is the species that you are working on in the Species\_sheet?

1. **Check the “Lot\_sheet”**

Is the Lot (i.e. this species/site collection) that you are working on in the Lot\_sheet?

* + 1. update the “Lot\_status” or
    2. add a new record

1. **Update the “Individual\_sheet”**
   * 1. enter each individual in a new row and populate all column.
     2. create a UNIQUE “Individual ID” for each individual
2. **Update the “Extractions\_sheet”**
   * 1. Copy the Individual\_IDs from the Individual\_sheet and paste here. Number of rows must match. Populate the first 4 columns only, if subsampling only.
     2. Create a UNIQUE “Extraction\_ID” for each extraction

1. **Label spin columns with “Plate\_ID”, elution plates with “Elution ID”, and ADD the range of Individual\_IDs and the date to columns and all plates.**

i.e. PlateID: Tzo-C\_001 or Tzo-C\_001\_E1, Tzo-CMiv\_001-096, 2019-12-15

1. **Assess extractions. Nanodrop and Gel DNA**
   * 1. Nanodrop and Gel the first 12 samples in each elution and update the Extractions\_sheet
2. **Update SLACK, and if needed, the Lot\_sheet**
   * 1. Update in the corresponding species slack channel after:
        1. Subsampling
        2. Extracting
        3. Gelling (post the labelled gel pic)
     2. May need to update the “Lot\_status” and “Frezeer\_location”
3. **Ship DNA to TAMUCC – Update “Shipping\_sheet” and SLACK**
   * 1. Create a new record per box in the Shipping\_sheet, populate all cells, and:
        1. Create a UNIQUE “Shipment\_ID” with:
     2. Update SLACK in the corresponding species channel (tracking\_number)