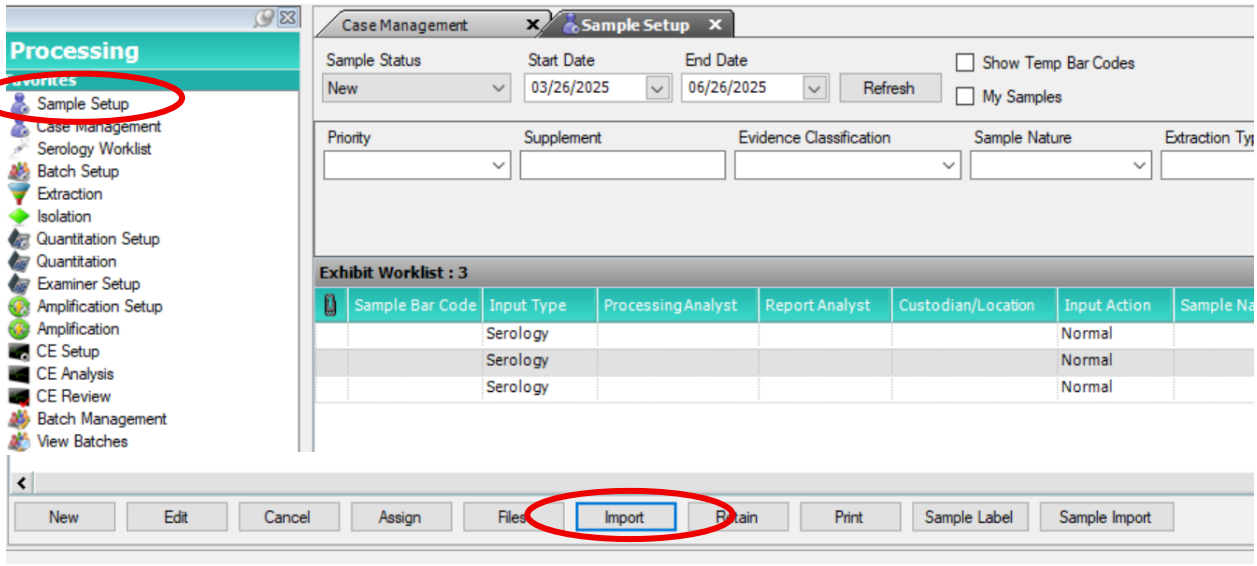
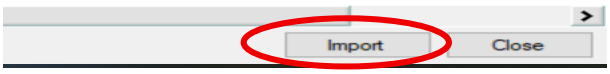
Knowns Examination

1. In JusticeTrax (i.e., LIMS): In lieu of the typical DNA knowns request, create a **DNA-STACS-Sero** request. The Sero request is for any item that must be examined like knowns. Associate the **parent** item **#X** to the request**.** See STACS SOP-2 for details involving Justice Trax (LIMS).
2. Transfer the **#X** parent item to yourself in LIMS.
3. Open the submission packaging.
4. Itemize the contents in **JusticeTrax**.
    a. **Note**: When processing in STACS, the (*) designations will no longer be used.
    b. **Itemize only the #X-1 subitem** in Justice Trax. Further cuttings will be completed in STACS.
5. Associate the newly created sub-item **#X-1** to the existing **DNA-STACS-Sero request.**
6. Import the request to STACS: both the parent **#X** (for examination documentation) and sub-item **#X-1** (sample from packaging) are imported.
    a. In the **Processing** module, open **Sample Setup** → select the **Import** button.



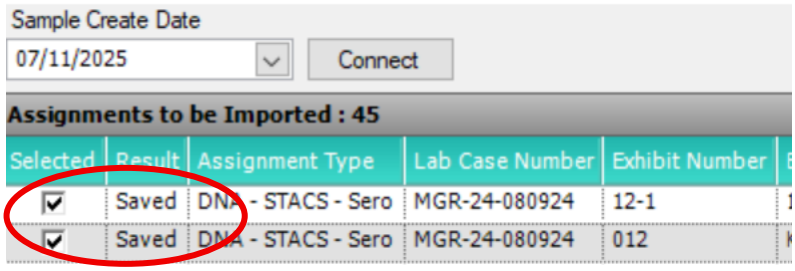
    b. In the popup window, select the appropriate date and click **Connect**. Note that the Sample Create Date, shown below, refers to the date the STACS request was created in JusticeTrax.



c. Select the evidence sub-item to import into STACS and click **Import**.

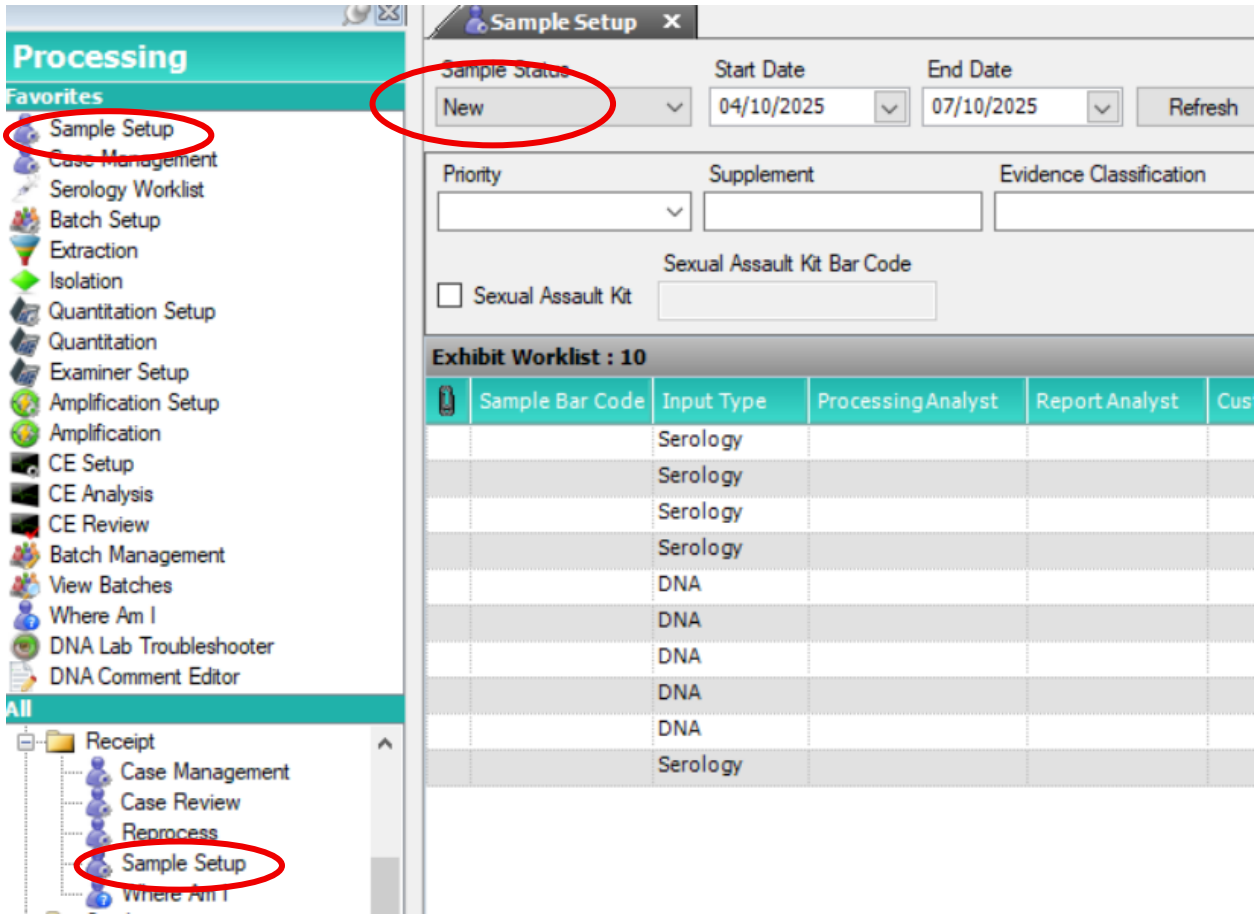
Note: If duplicates of the sample are listed, pick the sample with the primary agency's name and agency case number that the sample was submitted under. Ensure only 1 line for each sample or packaging is imported into STACS.

d. Once imported, the '**Results**' column will change to '**Saved**'. Already imported items will say **'LIMS Identifier Already Exists'**.



e. Select **Close.**

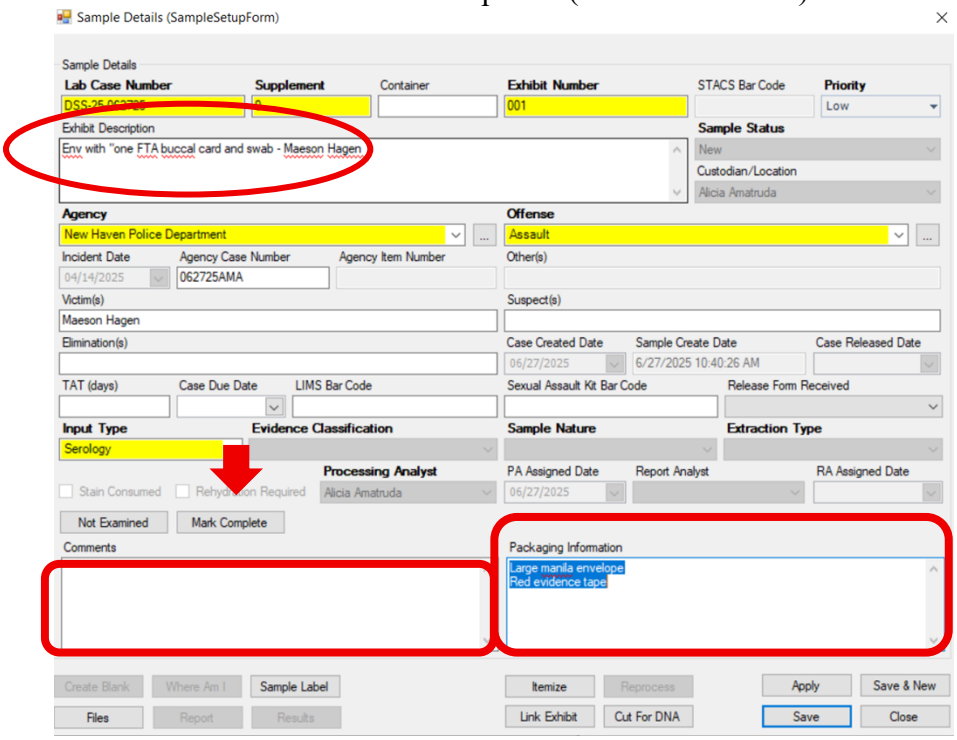
7. Open **Sample Setup (Processing → Receipt → Sample Setup).** Under the **Sample Status** dropdown, choose '**New'.**



8. The samples just imported will be here with **Serology Input Type**. If not, select refresh at the top and ensure the dates are correct.
9. Select the newly imported case exhibit(s) **(#X and #X-1)** and click **Assign** at the bottom of the screen. Select yourself as the Analyst. The Reporting Analyst does not need to be selected at this time.
10. In **Justice Trax**, ensure the parent item **#X** of the known is transferred into your custody for examination. The sub-item **#X-1** will also stay in your custody at this point.
11. To define what the item designations are in relation to STACS; see below:
    - **#X-1** for an FTA: is the FTA card in a foil envelope. Imported from LIMS
    - **#X-1-1** for an FTA: is the punch on the BSD. Made in STACS (made by the processor)
    - **#X-1** for a buccal swab is the entire swab. Imported from LIMS. COC in LIMS to Freezer Storage.

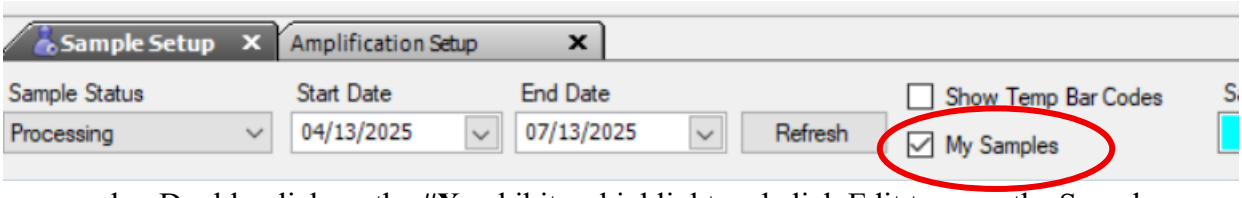
- **#X-1-1** for a buccal swab: is the ½ of cut swab to be tested. Made in STACS (made by the processor)

12. **Document the packaging examination of #X:**
    a. Double-click the parent item **#X** or highlight the exhibit and click **Edit** to open the **Sample Details** window. Ensure the yellow highlighted areas are filled out appropriately.
        - **Supplement** will typically be '0'. Subsequent numbering will be used for supplemental testing on the same exhibit number.
        - **Priority** should be 'Low', unless the case is an expedite
    b. Fill out all applicable examination notes in the **Packaging Information** section of the **Sample Details** window. This may be done using the **Autofill** options (Right click; Auto-fill; Knowns Packaging options). Free text is also an option. See image below.
    c. If the submission contains additional evidence that will not be examined and containerized into the packaging, document these contents in the **Comments** section to the left of the window.
    d. Additional auto-fill options (Knowns NEATT) are available for these statements.



    e. Once all notes are added, click **Save**. It will disappear and move to **Processing**.
    f. Now the **#X** can be closed out.

g. In the Sample Setup module, select **'Processing'.** It is easier to select 'My Samples' to select the new items.



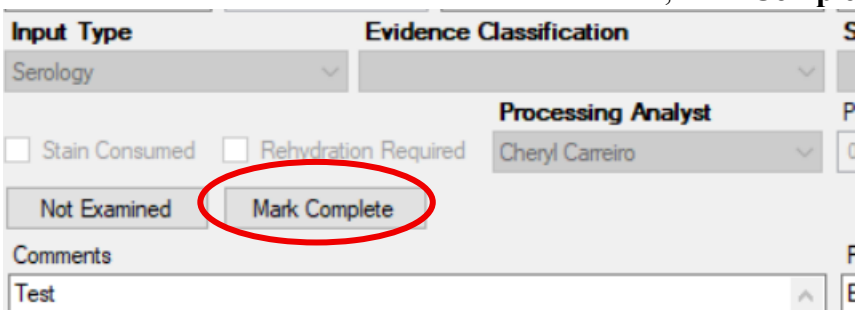
h. Double-click on the **#X** exhibit or highlight and click Edit to open the Sample Details window.

i. If all the information is complete, and no further action is needed for the **#X**, click **Mark Complete** (arrow above). The **#X** will be removed from the Processing list, however, there is one more step to finishing the completion of the packaging exam.

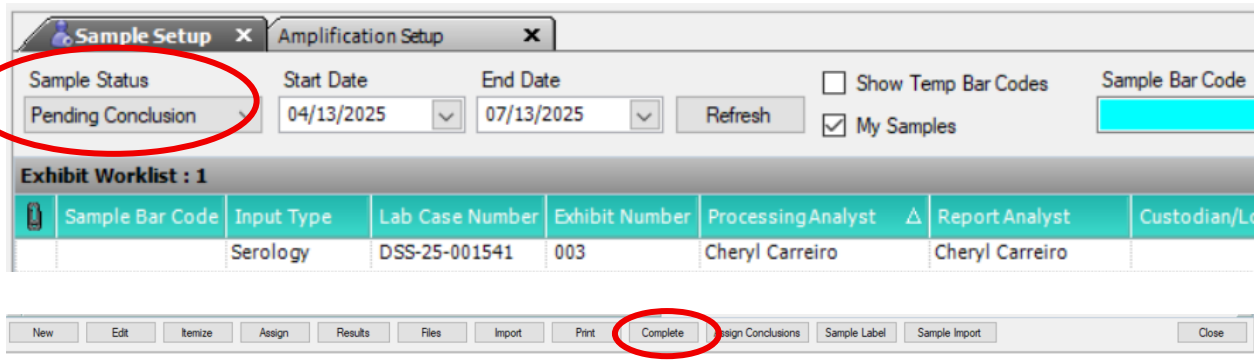
   a. Select **Close**.

      1. In **Sample Setup** select '**Pending Conclusion'** from drop down and select the **sample #X** (parent/packaging) that was just completed.

   b. At the bottom of the screen, click **Complete** for **#X**.

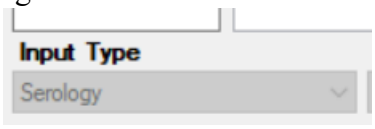


   c. The sample will stay in the **'Pending Conclusion'** status <u>if it is not also **'completed'** on this screen</u>. See below.



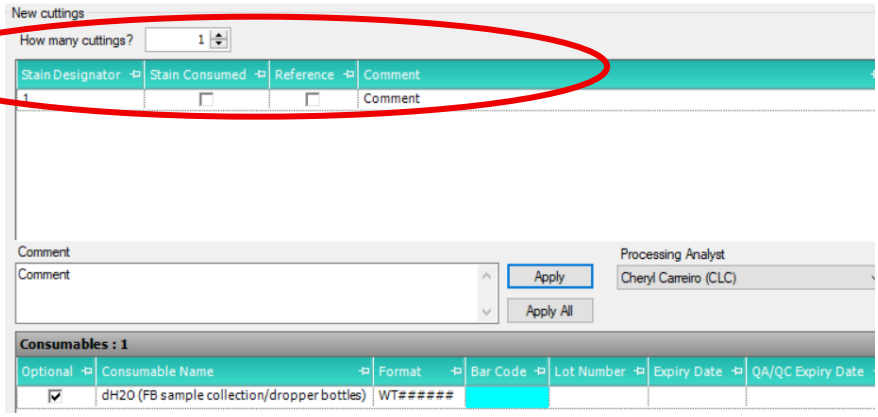
13. Document the evidence examination (Subitem **#X-1**):

a. Go to '**Sample Setup' → 'New';** the subitem (serology input) will be listed here after import.
b. Double click the exhibit **#X-1** or highlight the evidence item and click **Edit** to open the **Sample Details** window. Ensure the yellow highlighted areas are filled out appropriately.
   - Supplement will typically be '0'. Subsequent numbering will be used for supplemental testing on the same exhibit number.
   - Priority should be 'Low', unless the case is an expedite.
c. **Fill out all applicable examination notes** in the Sample Details window. This may be done using the autofill options (Right click; Auto-fill; Knowns/Staining options).
d. Click **Save**. The **#X-1** item will disappear from **'New'** and move to the **'Processing'** dropdown as a Sero input. A DNA input will be made at later steps.
e. After an FTA card is examined, the FTA card can be moved in **LIMS** to **'Knowns to Be Tested'.** *The FTA card in the envelope is a Sero request and cannot be transferred in STACS.*
f. Make sure **#X** is completed. FTA card is done until punching by the processor.
g. **For Swabs continue below.**



h. Examination of buccal known submisions containing **swabs or filter paper** that will **not** go through punching, will proceed to the next step below.

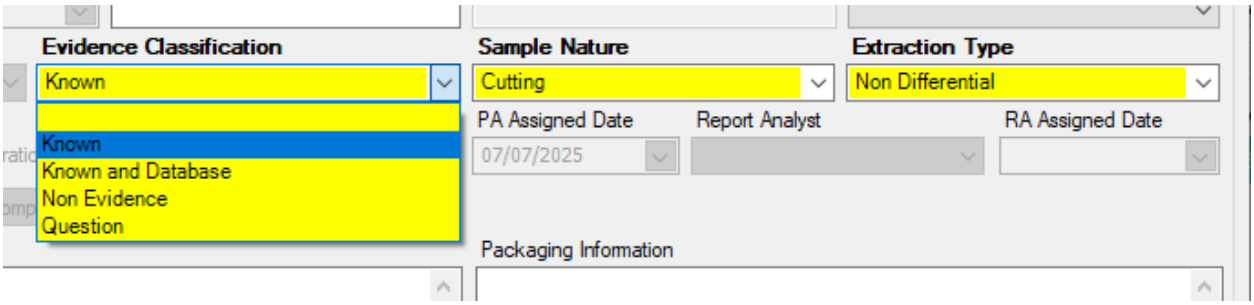
i. In the Sample Details window for the **#X-1**, click **'Cut for DNA'** to create the testable portion of the sample **(#X-1-1).** Fill out the **Stain Designator** (typically 1). **Do not** check off **Stain Consumed**.



j. A comment may be added to indicate the amount of sample cut (e.g., ½ cut; ½ retained). After making a comment click **Apply** and the comment will go to the top comment bar.

i. Click **Save**. A new **Sample Details** window will populate related to the Swab Cutting. It will be titled **#X-1-1**.

ii. In the new **Sample Details** window of **#X-1-1**, ensure the Supplement = 0 and select the **Known** option in the **Evidence Classification** in the drop-down.



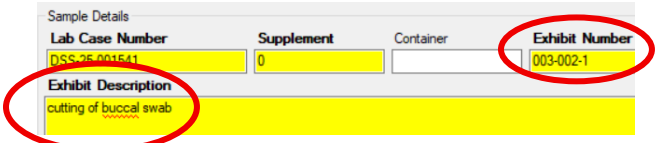
iii. Click **Save**. A unique STACS barcode will be generated for the cutting sample.

iv. Scan the barcode in the **'Barcode Verification'** window and affix to the sample. Scan the sample barcodes in the window that pops up.

v. See below that once '**Cut for DNA'** is selected the input field is now DNA. This signifies that this sample is the testing portion of the sample.

vi. The cutting **#X-1-1** is moved automatically to **Sample Setup → 'Received'.**

vii. Click **Save**.



k. Once **#X-1-1 (cutting of swab)** is created, the examination on the item **#X-1 (swab)** is complete.

l. In the **'Sample Details'** window for **#X-1** click **'Mark Complete'**. An additional **'Mark Complete'** is needed in **Sample Setup** dropdown **'Pending Conclusions'** similar to closing out **#X**.

14. Going back to Justice Trax (that will have the COC for **#X-1)** move **#X-1** to **freezer storage**. If the other ½ of the swab needs to be tested in the future, it can be imported into STACS and "cut for DNA" with the consumption button selected.

15. To summarize:

d. **Parent Item** = Transfer in LIMS to the **'Knowns Completed'** storage to be returned. COC is completely tracked in JusticeTrax.

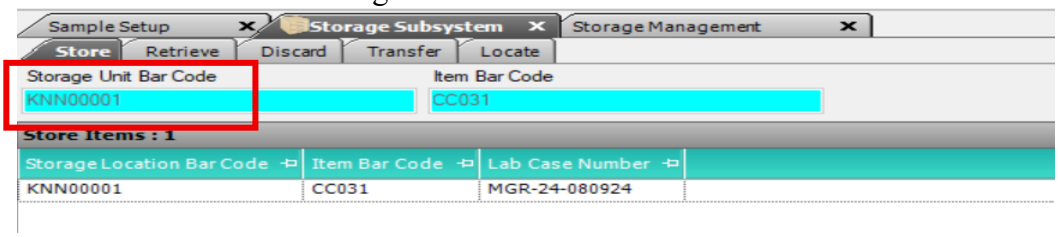
e. **Subitem (#X-1)** = After cutting made, transfer the rest in LIMS to **Freezer Storage;** Examination COC is <u>completely tracked in LIMS</u>.

1. If the other ½ of the swab needs to be tested, it can be imported into STACS and "cut for DNA" with the consumption button selected.

f. **Subitem Cutting (#X-1-1)** = **In STACS** transfer the cutting to '**Knowns To Be Tested**' **(KNN00001)**. The cutting does **not exist** in LIMS/JusticeTrax. All applicable COC for the cutting is <u>tracked in STACS</u>.

a. Open **Utilities → Storage → Storage Subsystem.**

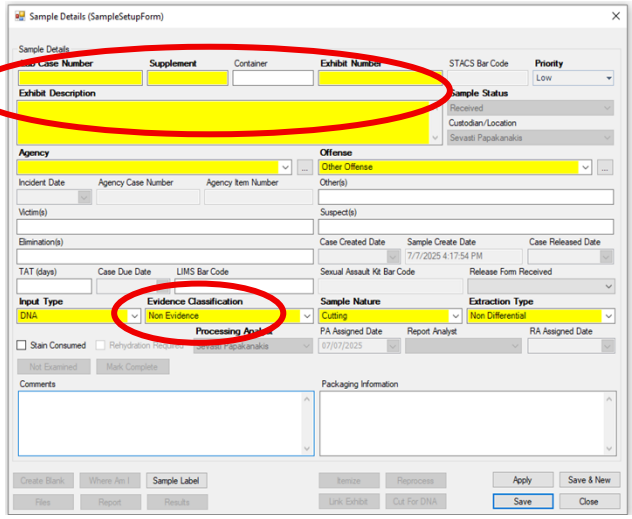
1. Under the **Store** tab, scan the storage location bar code and the item bar code to be put into '**Knowns to be Tested'**. Select **Save.** Storage Location below is "Knowns To Be Tested" in STACS.



14. If Database or Staff search samples need to be added to a knowns processing batch, see step below.

a. **Staff search/other samples:**

b. Samples are not being imported through JusticeTrax, so they must be manually entered into STACS.
c. In **Sample Setup**, click **New**. Enter the mandatory information into the yellow highlighted boxes.
d. It is critical to select **Non-Evidence** for the Evidence Classification. The Offense is put as **Other**. See below.
e. For the **Lab Case Number** field**:**
    i. **Database Cards:** DB cards will be the **DB-XXXXX** (DB identifier).
    ii. **Staff/Visitor:** For these samples the format is **Other-[Name of the Person].**
f. For the **Exhibit** and **Offense** Fields**:**
    i. For Exhibit: Enter number 1.
    ii. For Offense: Other
    iii. For Agency: z- My Test Agency



g. Transfer the non-evidence samples to **'Knowns to be Testied'** in STACS.

Known – Pre Processing

15. First, make sure the Processor has taken custody of the sample(s) to be processed.
    a. **FTA blood/buccal cards,** will be transferred into their custody in JusticeTrax by normal procedures (DNA SOP-12). FTA cards are in the LIMS storage location 'Knowns To Be Tested'. The LIMS barcode is keeping the COC for this sample.
        i. The **FTA Sero Input** will still be in **'Processing'** as seen below, but without a custody/location or barcode. See highlights below.

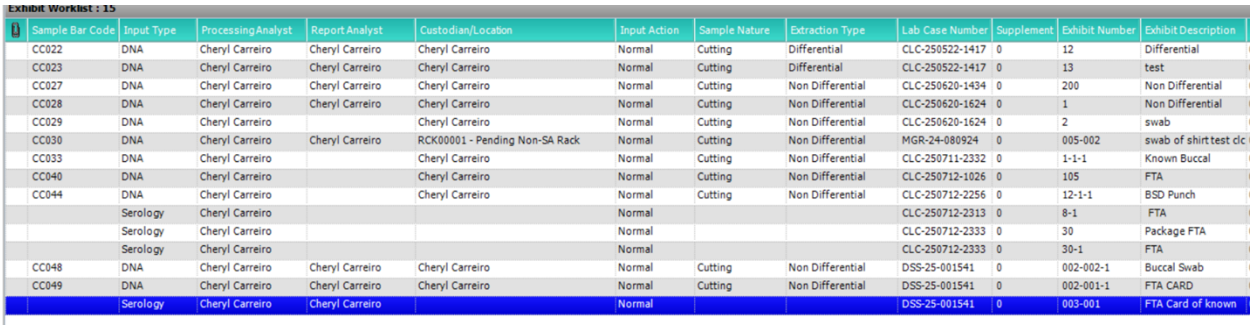
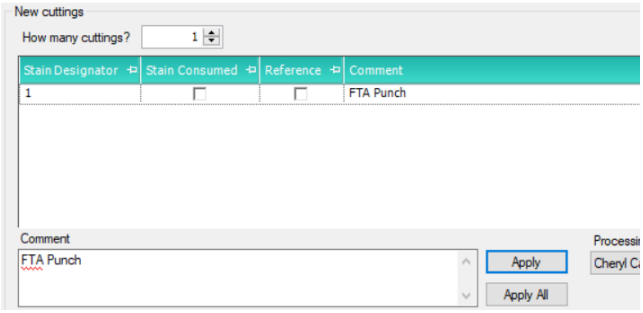


Exhibit Worklist : 15

Sample Bar Code	Input Type	Processing Analyst	Report Analyst	Custodian/Location	Input Action	Sample Nature	Extraction Type	Lab Case Number	Supplement	Exhibit Number	Exhibit Description
CC022	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Differential	CLC-250522-1417	0	12	Differential
CC023	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Differential	CLC-250522-1417	0	13	test
CC027	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250620-1434	0	200	Non Differential
CC028	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250620-1624	0	1	Non Differential
CC029	DNA	Cheryl Carreiro		Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250620-1624	0	2	swab
CC030	DNA	Cheryl Carreiro	Cheryl Carreiro	RCK00001 - Pending Non-SA Rack	Normal	Cutting	Non Differential	MGR-24-080924	0	005-002	swab of shirt test clc
CC033	DNA	Cheryl Carreiro		Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250711-2332	0	1-1-1	Known Buccal
CC040	DNA	Cheryl Carreiro		Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250712-1026	0	105	FTA
CC044	DNA	Cheryl Carreiro		Cheryl Carreiro	Normal	Cutting	Non Differential	CLC-250712-2256	0	12-1-1	BSD Punch
	Serology	Cheryl Carreiro			Normal			CLC-250712-2313	0	8-1	FTA
	Serology	Cheryl Carreiro			Normal			CLC-250712-2333	0	30	Package FTA
	Serology	Cheryl Carreiro			Normal			CLC-250712-2333	0	30-1	FTA
CC048	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Non Differential	DSS-25-001541	0	002-002-1	Buccal Swab
CC049	DNA	Cheryl Carreiro	Cheryl Carreiro	Cheryl Carreiro	Normal	Cutting	Non Differential	DSS-25-001541	0	002-001-1	FTA CARD
	Serology	Cheryl Carreiro	Cheryl Carreiro		Normal			DSS-25-001541	0	003-001	FTA Card of known

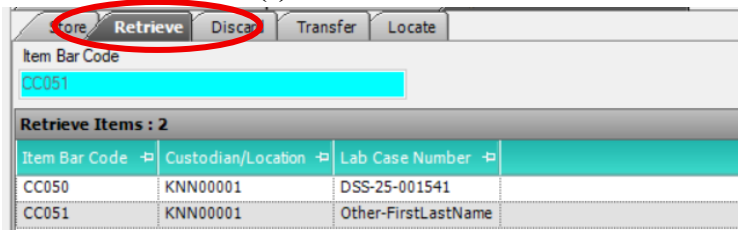
b. Double click the **#X-1 FTA** sample to open the **Sample Details**.
c. In the **Sample Details** window for item **#X-1 (FTA card)**, click **'Cut for DNA'**. This will generate the "punch" **#X-1-1** for further testing.



d. Click **Save**. A new **Sample Details** window will populate related to the cutting.
e. In the new **Sample Details** window, ensure the Supplement = 0 and select the **Known** option in the **Evidence Classification** in the drop-down.
f. Click **Save**. A unique STACS barcode will be generated for the cutting. Scan the barcode in the **'Barcode Verification'** window.
g. The barcode can be affixed to a sheet of paper since "the punch" is tracked with the LIMS BSD barcode.
h. At this point while having the **#X-1 Sample Details** open, mark the sample **'Complete'**. Also mark the sample **#X-1** complete in the **'Pending Conclusion'** dropdown.
i. The **#X-1-1 FTA Punch** will be in **Sample Setup → Received** as a DNA Input.
j. Click **Start Processing** at the bottom for this sample.

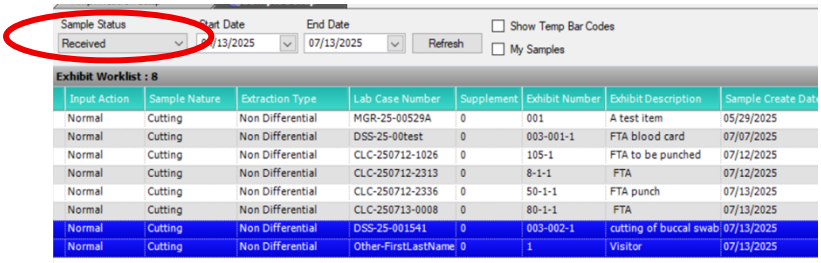
16. The **#X-1-1 Swabs** in **Storage → Subsystem →'Knowns to Be Tested'** in STACS have a barcode already affixed on the item from the examination step. The physical swab sample is in a rack **'Knowns to Be Tested'**.
    a. Open **Utilities → Storage → Storage Subsystem.**

b. For swabs or non-evidence samples, under the **Retrieve** tab, **scan the item bar code(s)** that are to be tested in 'Knowns to be Tested' to be put into your custody.



c. Select **Save.**

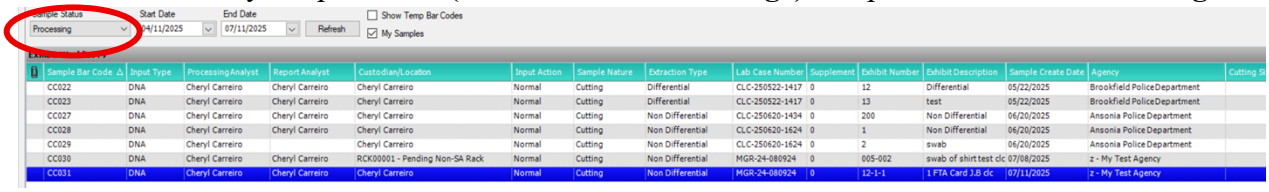
d. The samples will move to **'Received'.**



e. Swab cuttings will be found in the **'Received'** dropdown.

f. For swabs, select the sample and at the bottom click **'Start Processing'.**

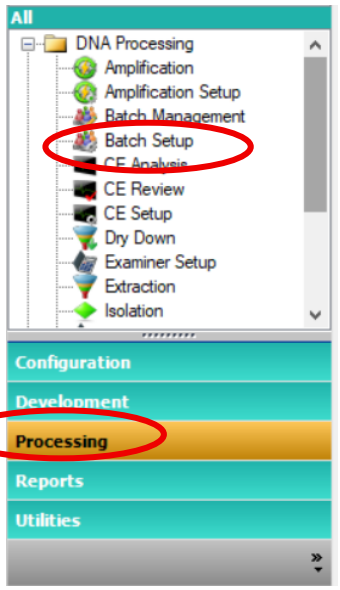
Note: A swab cut from FB will come in as a 'DNA input'. These will only need to be received by the processor **(STACS Knowns Storage)** and put into **'Start Processing'**.



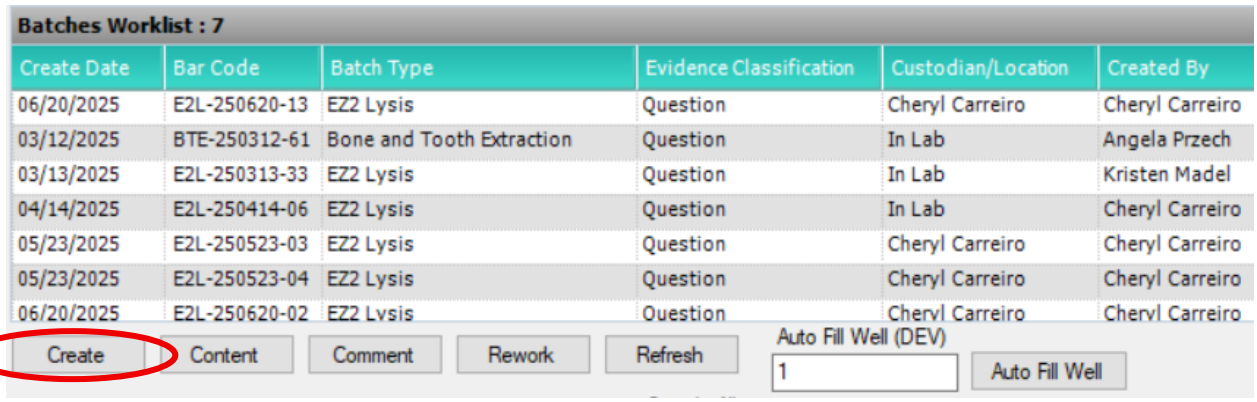
Knowns – Processing Continued

Direct Amplification – Batch Setup

1. Use the **Batch Setup** module to create an Extraction Batch and assign samples and controls to it. You select the Batch Type here as well.
2. Open **Processing → DNA Processing → Batch Setup.**



3. Click **Create**.

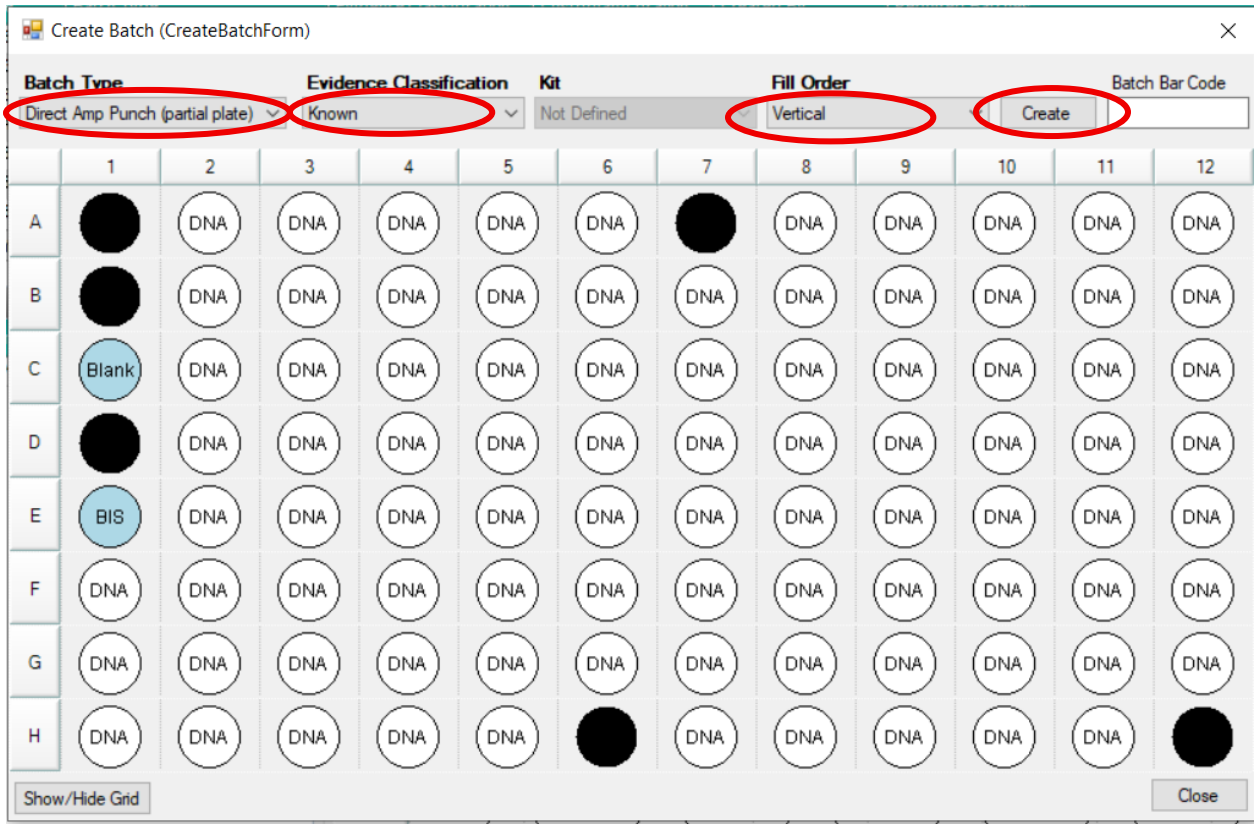


4. Select the appropriate extraction under Batch Type, the corresponding Evidence Classification, and the Fill Order.
    a) Select the Batch Type as appropriate:
    b) **Direct Amp Punch** (partial or full)
    c) **Direct Amplification Swabs (requires Prep and Go Buffer)**
    d) **Direct Amplification Swabs – No BIS;** Use when combining GFE Swabs with FTA Punch samples on a single amplification set.
    e) **It is important to note here that Punches and Swabs will be combined at the Amplification step.
        - For example, a batch of **Direct Amp Punch (partial or full)** will be made for the FTA cards and a **Direct Amplification Swabs-No BIS** for Swabs will be made.

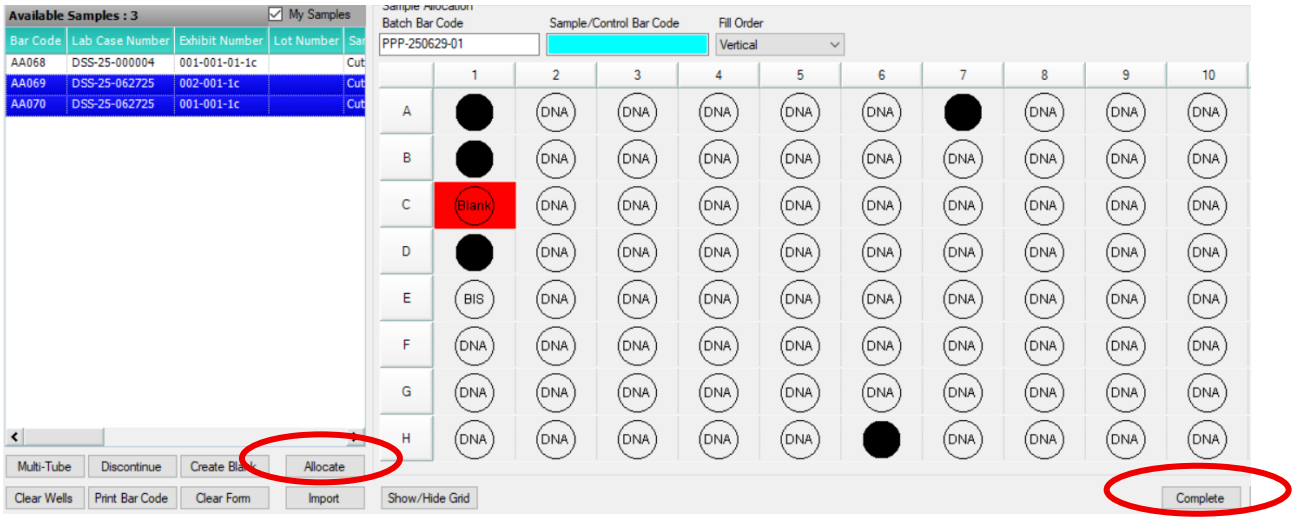
- After the batches are made, it is at the **Amplification** step the two batches will be merged. Details are below further down in this SOP.

f) Evidence Classification **Known,** unless non-evidence (i.e. staff) samples are to be tested.

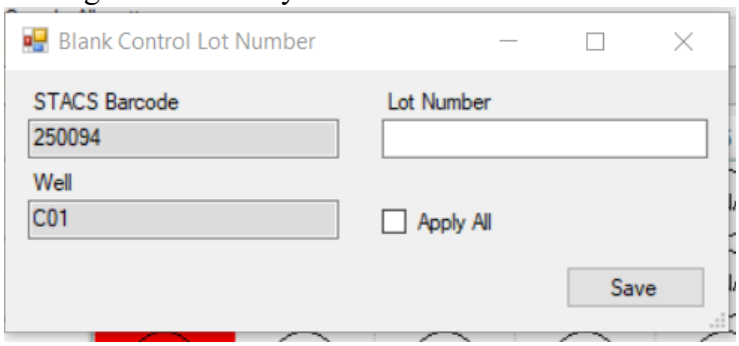
g) Fill Order = **Vertical. See Below.**



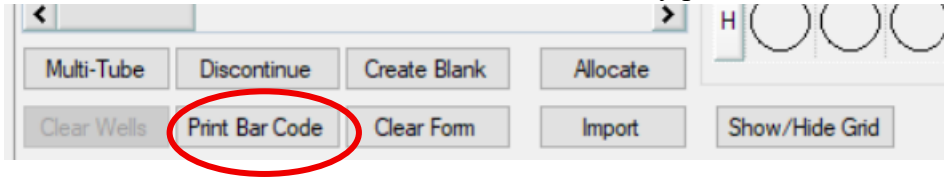
5. Click **Create**. A unique barcode associated with the extraction will be generated and printed.
6. Moving forward, this barcode may be used to transfer the samples as a whole set.
7. To begin sample allocation, scan the extraction barcode into the blue-highlight or double-click the batch in the upper Batches Worklist window.
8. Allocate the sample(s). Highlight the sample(s), select a destination well labeled “DNA” and click “**Allocate**”.



9. To add the BIS (positive controls) to the worksheet, select the designated BIS well and scan the barcode for the control.
10. When all samples and controls are added, click **Complete** in the **Batch Setup** window.
11. By completing the batch, the **Blank** sample(s) will automatically be generated. In the pop-up window, click **Save**.
    a) The barcode will automatically print. Initials/date may be entered as a lot number, although not necessary.



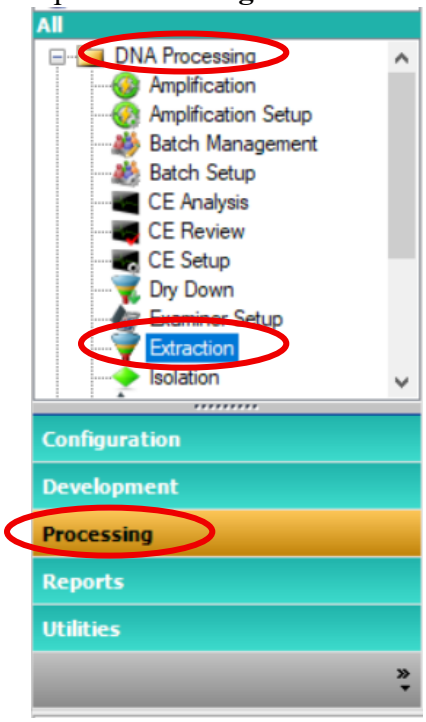
    b) If for some reason a bar code does not automatically print; see below:



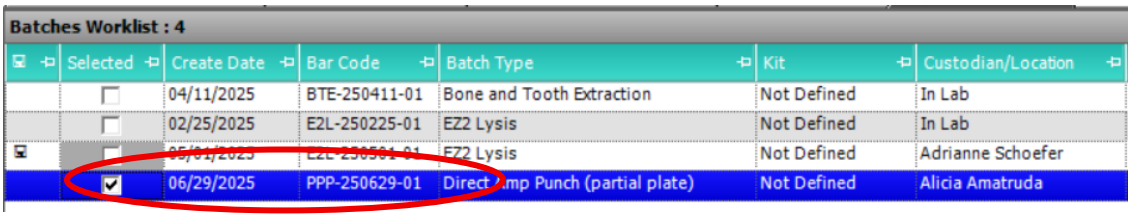
12. The BSD Punchbot file will open to be saved on the Udrive: U:\STACS share\BSD. This file can be uploaded to the instrument for punching of the cards.

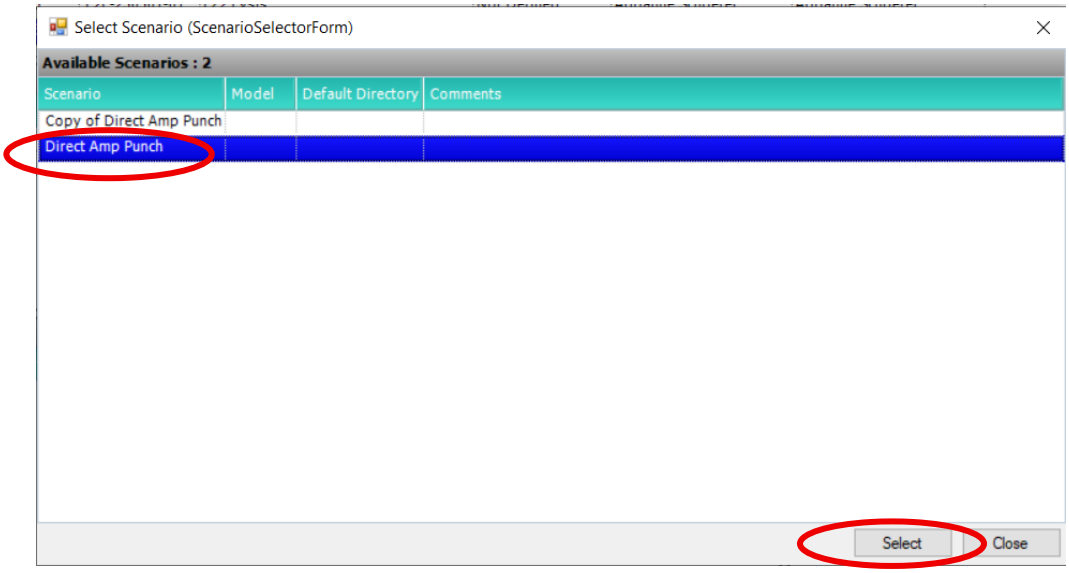
Direct Amplification – Extraction Step

1. Open **Processing → DNA Processing → Extraction.**

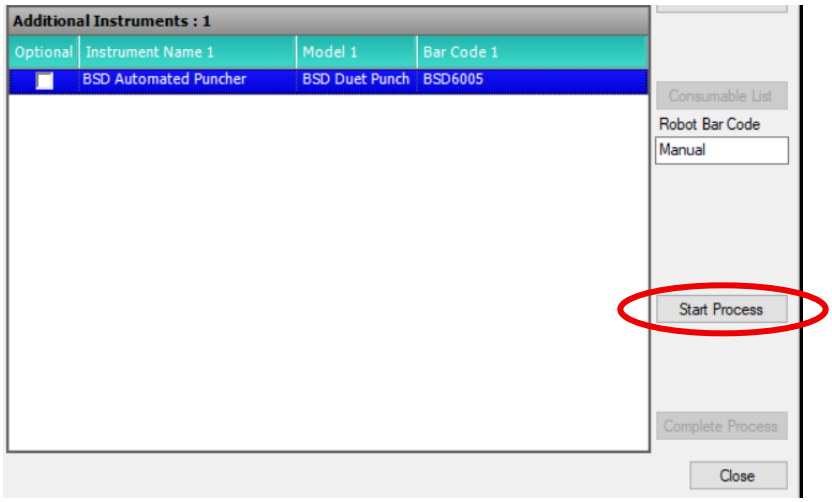


2. Select the batch to be processed from the worklist and click **Select Scenario**. Select the appropriate corresponding scenario:
    - **Direct Amp Punch**
    - Note: This scenario for **Direct Amp Punch** will skip the isolation step and lead straight into **Amplification Setup**.

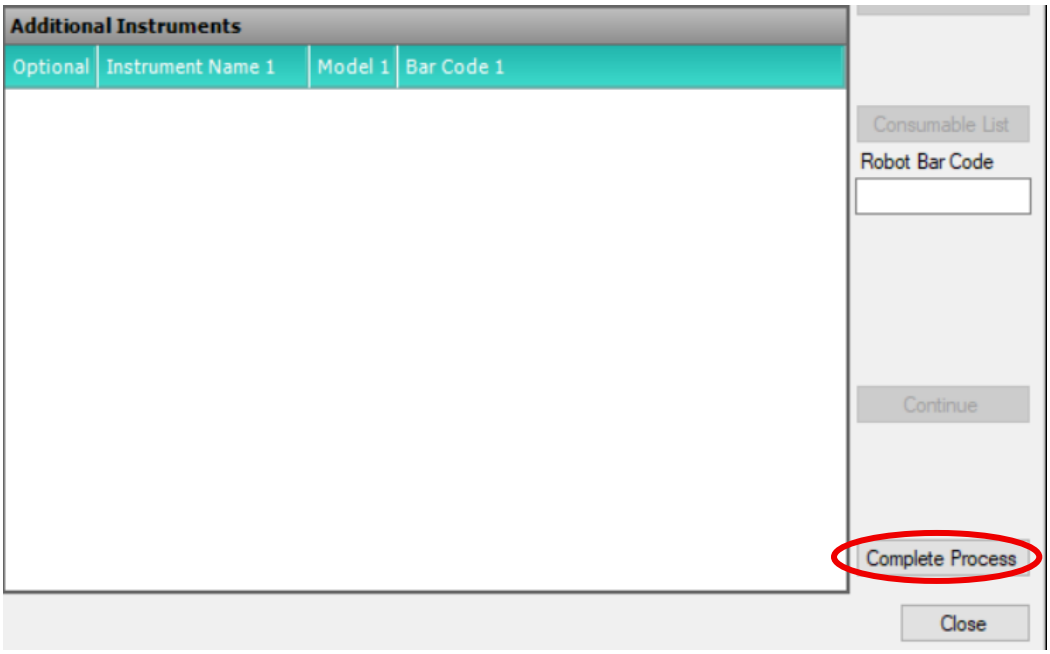


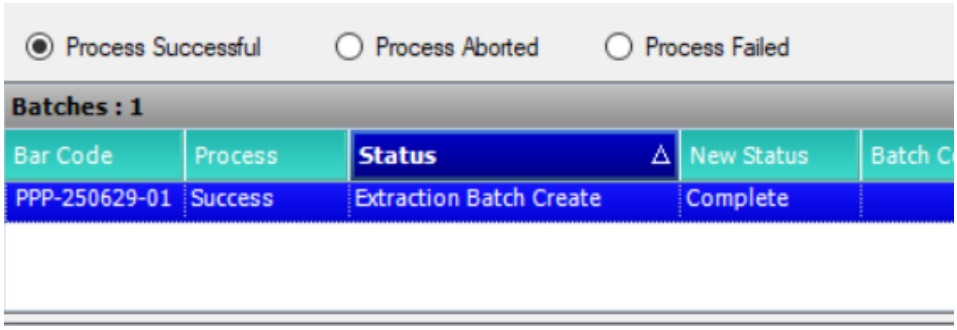


3. Scan the required barcodes highlighted blue. This will include the batch barcode, BSD barcode and/or Prep and Go Buffer (for swabs) barcode, as applicable.
4. Click **Start Process**.



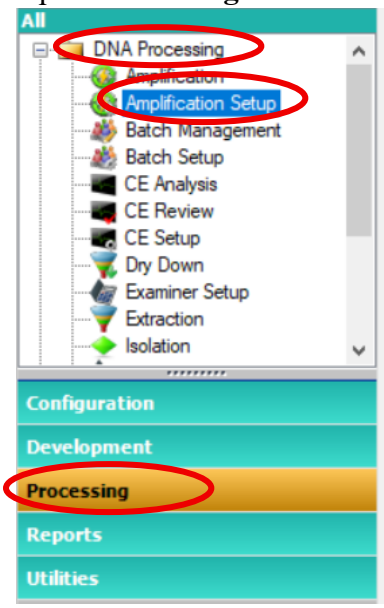
5. Complete the tube check by scanning the sample barcodes.
6. A window will pop up to save the BSD file. Save in the folder BSD in the STACS Share folder on the U:drive. It should automatically open up at that folder.
7. When done, click **Complete Process** and record the results using the Complete Batch Activity screen.
    - **Process Successful**: the batch advances to the next processing step.
    - **Process Aborted:** the batch remains on the Extraction Batches worklist. A **Batch Comment** is required with this option.
    - **Process Failed:** the batch is abandoned and all samples return to Batch Setup. A **Batch Comment** is required with this option.


Additional Instruments
Optional
Instrument Name 1
Model 1
Bar Code 1
Consumable List
Robot Bar Code
Continue
Complete Process
Close

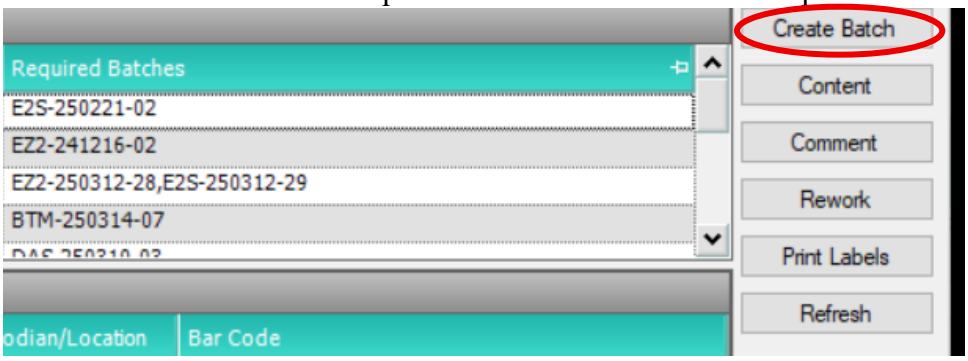

Process Successful
Process Aborted
Process Failed
Batches : 1
Bar Code
Process
Status
New Status
PPP-250629-01
Success
Extraction Batch Create
Complete

Direct Amplification – GFE

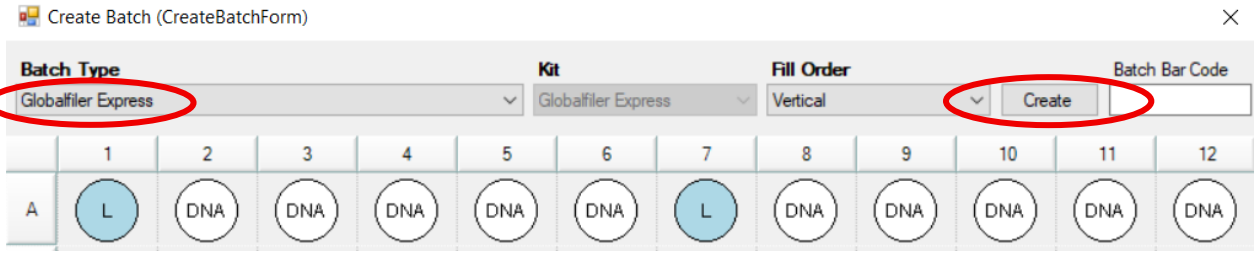
1. Open **Processing → DNA Processing → Amplification Setup**.



2. Click **Create Batch**. The Amplification Batch Create screen opens.



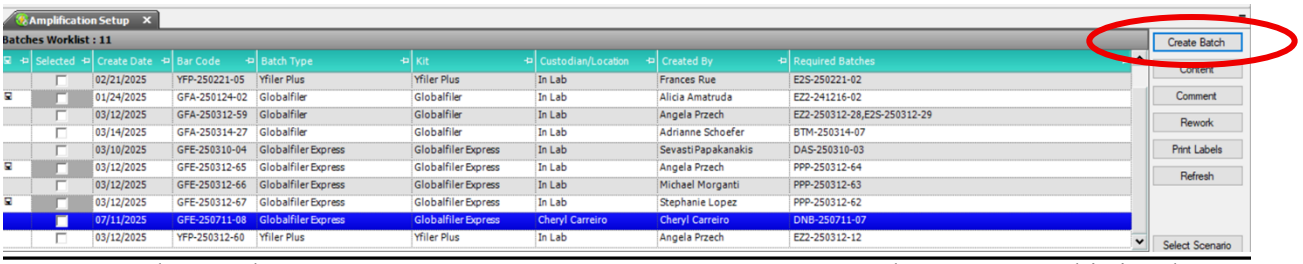
3. In the **New Batches** window, click **Create**.
4. Select a Batch Type (typically GFE) and click **Create**.
5. **Note: If no swabs are part of the set, only punches, select the 'no TE' which means no TE will be needed for the addition to the NEG.**



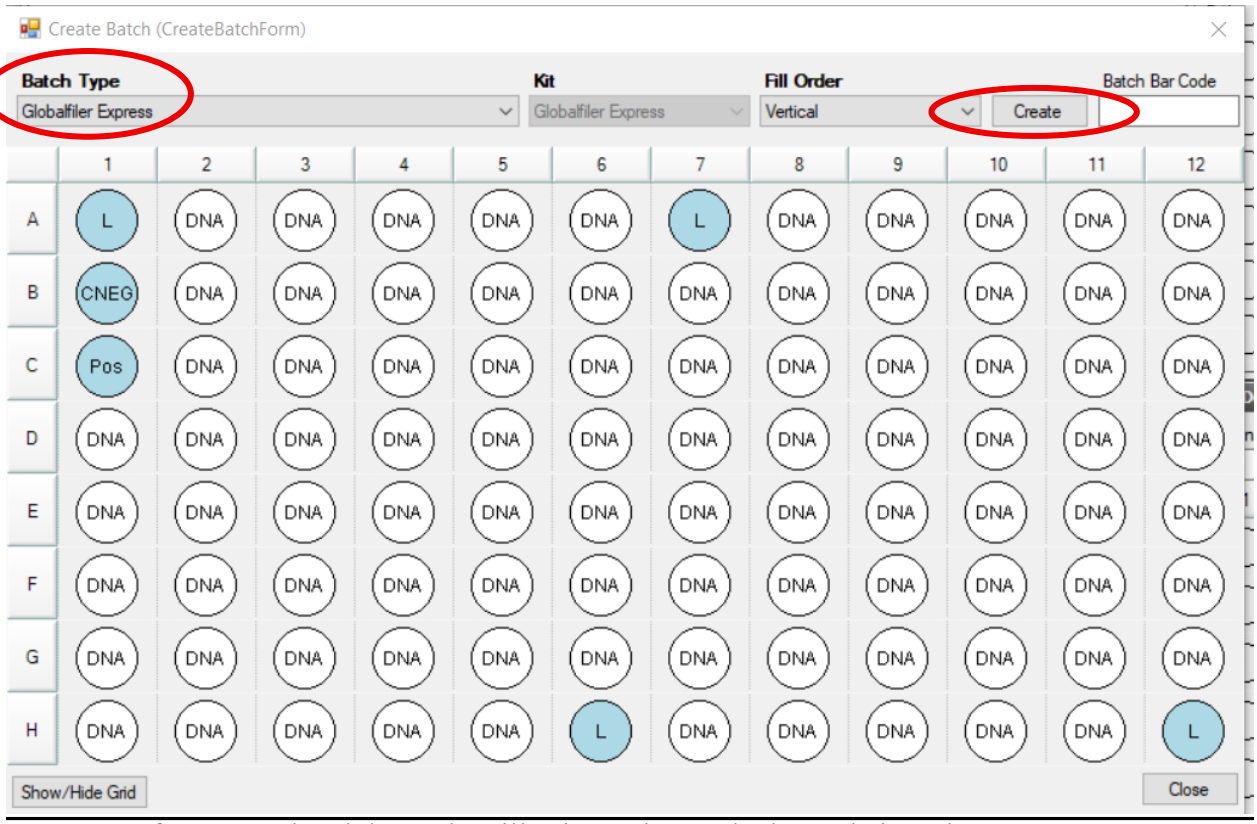
6. Scan the newly created batch barcode into the **Destination Batch** field.
7. Scan the **Extraction Batch** barcode(s) in the **Source Batch** field.
8. Select Samples and Allocate onto the **Destination Batch.**
9. Select **Close**. If completed, please see **Step 16**.

10. Below will be a section of directions for when **Multiple Extraction Batches** can be merged together at this step.

11. <u>Below is where Swabs can be merged with Punches.</u>
    a. Batch Type **'Direct Amplification Swabs-No BIS'** should be for the **Swab Batch Type** to merge with punches as described in the beginning of the SOP.
    b. The "**Direct Amplification-Swabs**" could also be merged if picked accidently, but an extra BIS will be included.
    c. Select **Create Batch**.



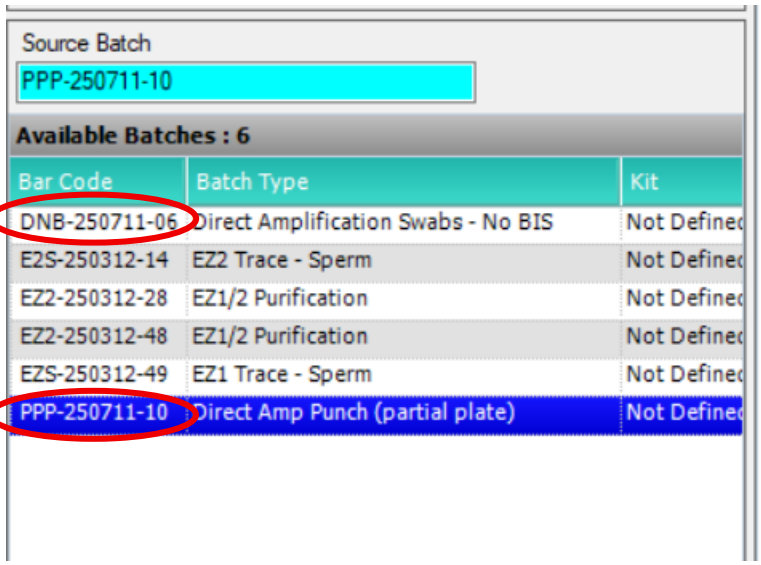
    d. On the next screen, **Amplification Batch Create** – Select **Create.** This is where you will select the batch type.
    e. If you are merging punches and swabs together; Select GlobalFiler Express. Click **Create** on the right.



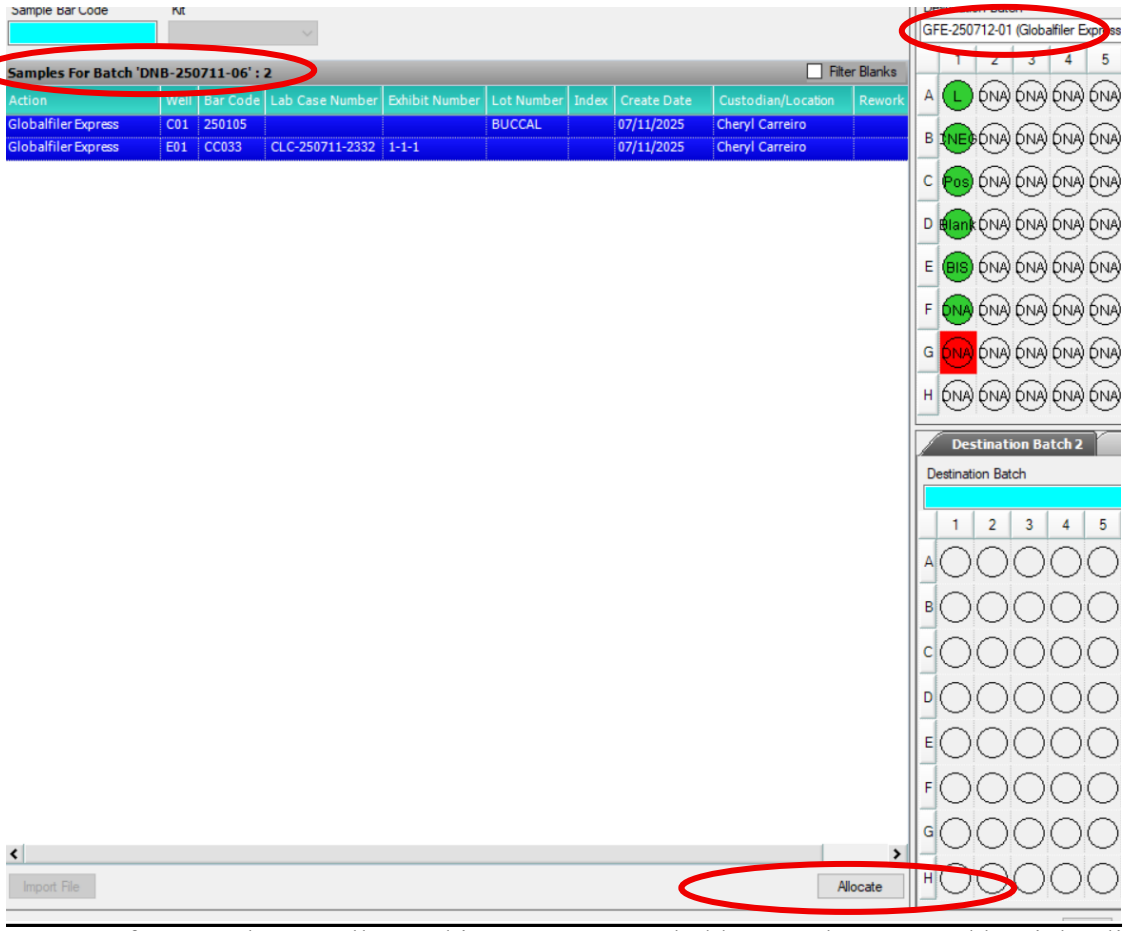
f. A new batch barcode will print and scan the barcode into the **Destination Batch** field on the top right.

g. Scan the Source Batch barcodes of the samples that are to be merged will be added one batch at a time.

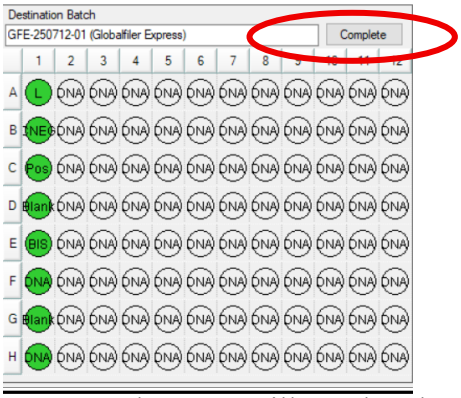
   i. **Scan 1st Source Batch.**
   ii. Select Samples and click **Allocate**.
   iii. Then **Scan 2nd Source Batch.**
   iv. Select Samples and click **Allocate**.
   v. Continue this process until all Batches are on the Amp Plate.



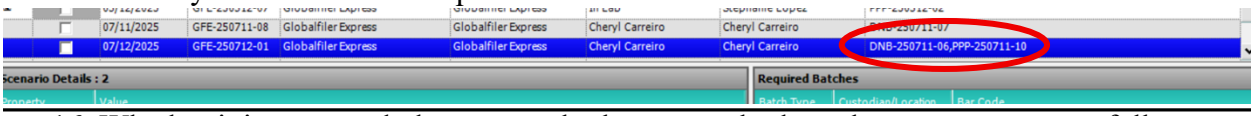
12. In the image below the first source batch (PPP-250711-10) above has been added and the second source batch (DNB-250711-06) is ready to be allocated to that same plate.



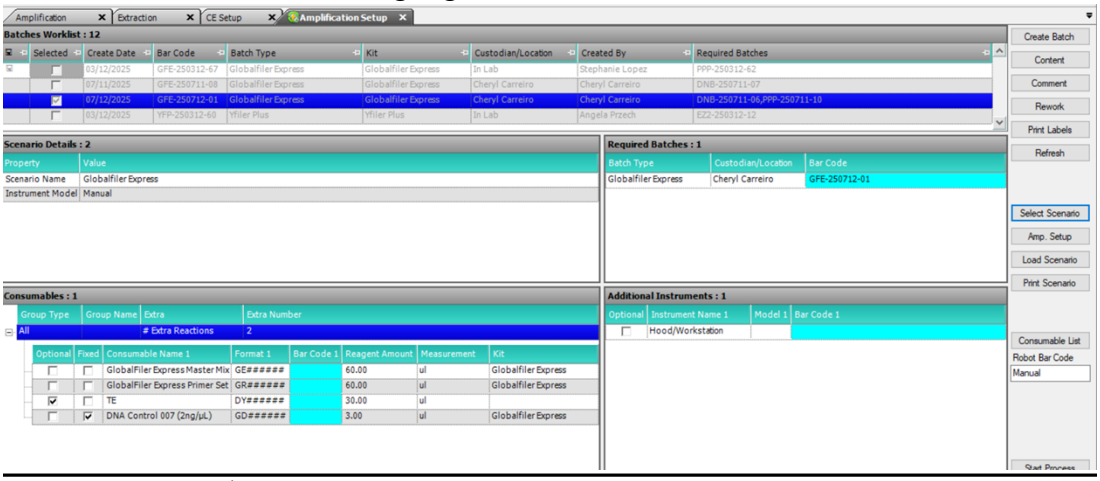
13. After samples are allocated in STACS extra ladders can be removed by right clicking the green **L** and select "**clear well'**. This also can be done with plates that are not merged together.
14. When finished; **Save** the batch and click **Complete** at the top. The below image is a plate with a punch sample and a swab sample. Remember to click **Complete.**



15. Below you will see that the plate above has **both Source Batches** listed.



16. Whether it is a merged plate, a punch plate or swab plate, the next steps are as follows:
    a. In **Amplification Setup;**
    b. Select the newly made batch.
    c. Select **Scenario**. It will pre-populate the scenario with the GFE process.
    d. Scan all blue highlighted fields as shown below:

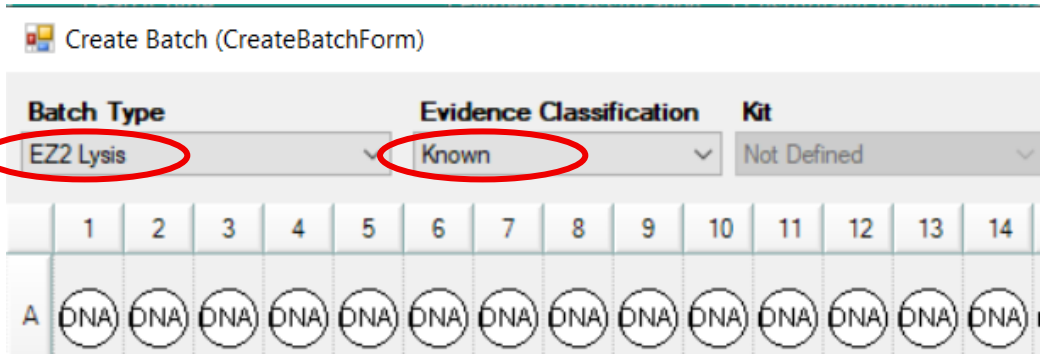


    e. Select **Start Process.**
    f. When finished with Amplification select **Complete Process**.

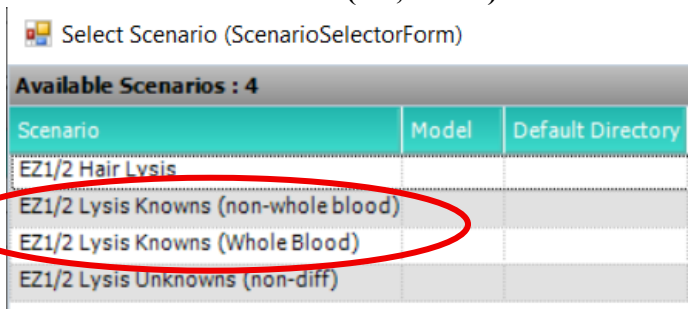
17. The remaining steps of Amplification and CE can proceed as described in the DNA Unknown Processing sections (e.g., STACS SOP-6 and SOP-7).

Knowns – EZ2

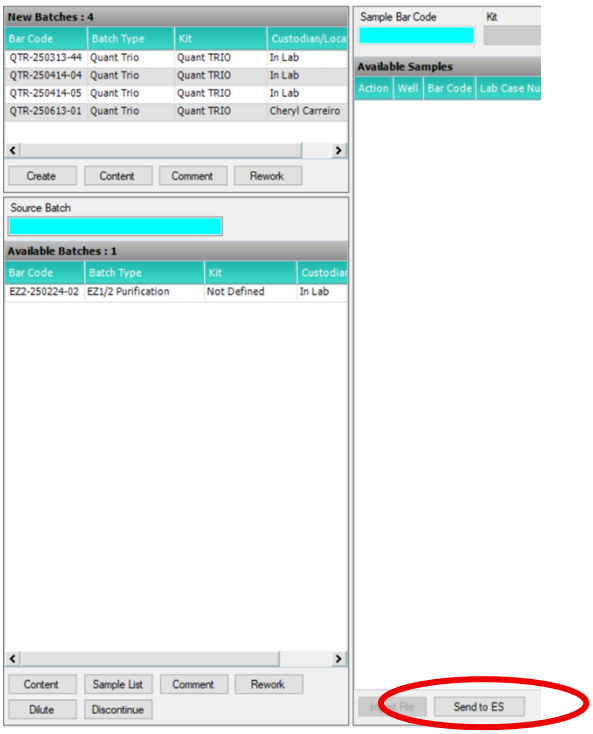
1. **Except as noted below**, Known EZ2 processing is identical to the DNA Unknown Processing sections (STACS SOP-3, -5).
2. **Batch Setup**: Select **EZ2 Lysis** as the Batch type. Select **Known** for Evidence Classification.



3. **Extraction**: When selecting a scenario, select **Knowns Non-whole Blood (i.e., buccals)** or **Knowns Whole Blood (i.e., blood).**



4. **Quantitation Setup**: In the 'create batch' window, select all the known samples and click **Send to ES (Examiner Setup)** to bypass quantitation**.**


New Batches : 4
Bar Code
Batch Type
Kit
Custodian/Loca
QTR-250313-44
Quant Trio
Quant TRIO
In Lab
QTR-250414-04
Quant Trio
Quant TRIO
In Lab
QTR-250414-05
Quant Trio
Quant TRIO
In Lab
QTR-250613-01
Quant Trio
Quant TRIO
Cheryl Carreiro
Create
Content
Comment
Rework
Source Batch
Available Batches : 1
Bar Code
Batch Type
Kit
Custodia
EZ2-250224-02
EZ1/2 Purification
Not Defined
In Lab
Content
Sample List
Comment
Rework
Dilute
Discontinue
Sample Bar Code
Kit
Available Samples
Action
Well
Bar Code
Lab Case Nu
Send to ES