12.1 PURPOSE

This SOP defines the procedures for performing rework in STACS, including sample and batch-level rework (re-quantitation, re-amplification, re-injection), routine batch management actions (dilutions, discontinuations, volume edits), handling of process outcomes (Successful, Aborted, Failed), and the controlled intake of pre-extracted DNA into the DNA workflow. It applies to all analysts performing bench work and data processing in STACS.

12.2 RESPONSIBILITIES

DNA Analysts**:** Follow steps as written; record reasons for any rework; ensure chain-of-custody and barcode scanning at each step.

Technical Reviewers: Verify that rework and outcomes are appropriate and documented.

Bench Work Only Personnel: Follow steps as written; record reasons for any rework; ensure chain-of-custody and barcode scanning at each step.

12.3 DEFINITIONS

Rework (batch): Remove an entire batch from its current stage and return all included samples to a prior entry point to rebuild the batch.

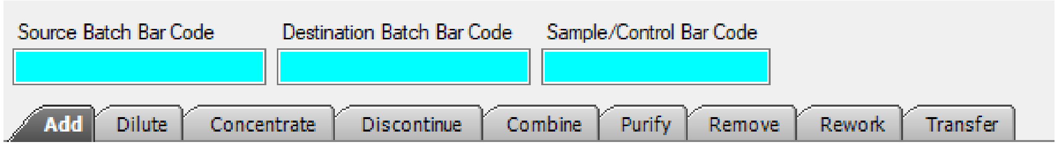
Rework (sample): Return one or more selected samples (not the whole batch) to an earlier entry point (e.g., Quantitation Batch Create, Examiners Setup, CE Analysis) for targeted reprocessing.

Discontinue: Stop further processing of a sample (e.g., when using a dilution instead of the neat extract).

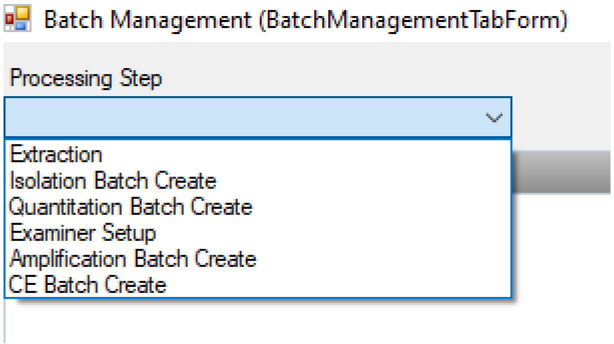
Outcomes for Completion: Process Successful (advances), Process Aborted (rescan/retry at the same step), Process Failed (returns batch to a previous step).

12.4 UTILIZING BATCH MANAGEMENT

The Batch Management Module drives sample decision making and can be found in DNA Processing (**Processing → DNA Processing → Batch Management**). Each action described below is a tab within the Batch Management module.



Source batches can be accessed by selecting the appropriate step in the 'Processing Step' dropdown. Once a batch is selected, the available samples will auto-populate.



<u>ADD TAB</u>

Allows for individual samples to be added to a batch. This will typically be used to add 're-work' or 'pre-extracted' samples/controls to existing progress batches.

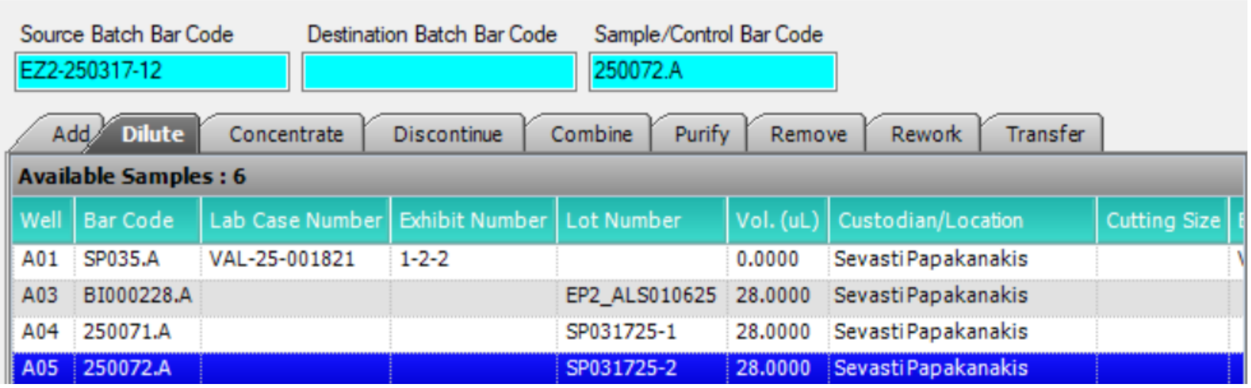
1. Scan the destination batch. Select a destination in the Wells worklist.
2. Add the sample(s) and associated controls from the Samples worklist to destination batch, by double-clicking or scanning the barcode.
3. Click Save.

<u>DILUTE TAB</u>

Will typically be used to add water to extracts after isolation if they elute at a volume slightly lower than the reagent blank(s):

1. Open Processing → DNA Processing → Batch Management; set Processing Step to where samples are waiting (e.g., Quantitation Batch Create).
2. Select the Dilute tab. Double-click the relevant isolation batch to display Available Samples.
3. Select the source batch.
4. Scan the Water to be used. Highlight a target well under Available Wells.

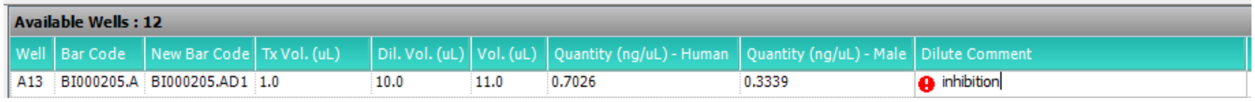
5. Double-click each sample or scan to create a dilution; STACS prints a new dilution label with an added suffix to the name that will replace the original barcode label.
6. Enter dilution parameters (Tx = current extract volume; Dil Vol = How much H2O to add; Vol. = final volume). Save.
7. There is no “destination” batch for this step, however an available well must be selected in the right-hand window.
8. Fill out the ‘Dilute Comment’, Tx. Vol and Dil. Vol. columns in the Available Wells window. The new human and male quant values are automatically calculated. Save.
    a. TX. Vol. = neat extract volume
    b. Dil. Vol. = added dH2O



Source Batch Bar Code: EZ2-250317-12
Destination Batch Bar Code:
Sample/Control Bar Code: 250072.A

Available Samples : 6

Well	Bar Code	Lab Case Number	Exhibit Number	Lot Number	Vol. (uL)	Custodian/Location	Cutting Size
A01	SP035.A	VAL-25-001821	1-2-2		0.0000	Sevasti Papakanakis	
A03	BI000228.A			EP2_ALS010625	28.0000	Sevasti Papakanakis	
A04	250071.A			SP031725-1	28.0000	Sevasti Papakanakis	
A05	250072.A			SP031725-2	28.0000	Sevasti Papakanakis	



Available Wells : 12

Well	Bar Code	New Bar Code	Tx Vol. (uL)	Dil. Vol. (uL)	Vol. (uL)	Quantity (ng/uL) - Human	Quantity (ng/uL) - Male	Dilute Comment
A13	BI000205.A	BI000205.AD1	1.0	10.0	11.0	0.7026	0.3339	inhibition

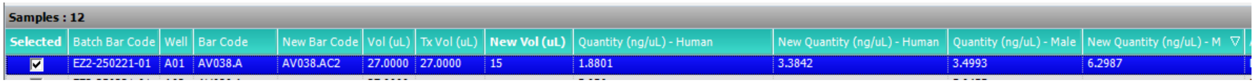
9. Discontinue the original extract by following the steps below under DISCONTINUE.

CONCENTRATE TAB

1. Open Processing → DNA Processing → Batch Management → Concentrate.
2. Select the source batch.
3. Double-click or scan sample(s) from the Samples worklist.

4. This will create and print a new barcode label, adding a ‘C1’ designation to the end of the existing barcode.

5. Adjust the New Volume column to equal the desired elution volume. The new quantitation values are automatically calculated.



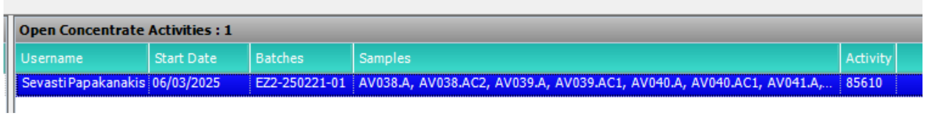
Samples : 12

Selected	Batch Bar Code	Well	Bar Code	New Bar Code	Vol (uL)	Tx Vol (uL)	New Vol (uL)	Quantity (ng/uL) - Human	New Quantity (ng/uL) - Human	Quantity (ng/uL) - Male	New Quantity (ng/uL) - M
☑	EZ2-250221-01	A01	AV038.A	AV038.AC2	27.0000	27.0000	15	1.8801	3.3842	3.4993	6.2987

6. Click Start Process.

7. Scan the consumables.

8. Processing Analyst:
   a. Select the Source Batch.
   b. Click the “worksheet: in the Open Concentrate Activities window.



Open Concentrate Activities : 1

Username	Start Date	Batches	Samples	Activity
Sevasti Papakanakis	06/03/2025	EZ2-250221-01	AV038.A, AV038.AC2, AV039.A, AV039.AC1, AV040.A, AV040.AC1, AV041.A,...	85610

   c. Conduct the concentration and click Complete Process to disposition samples according to entry point.
   d. Document in comments.

DISCONTINUE TAB

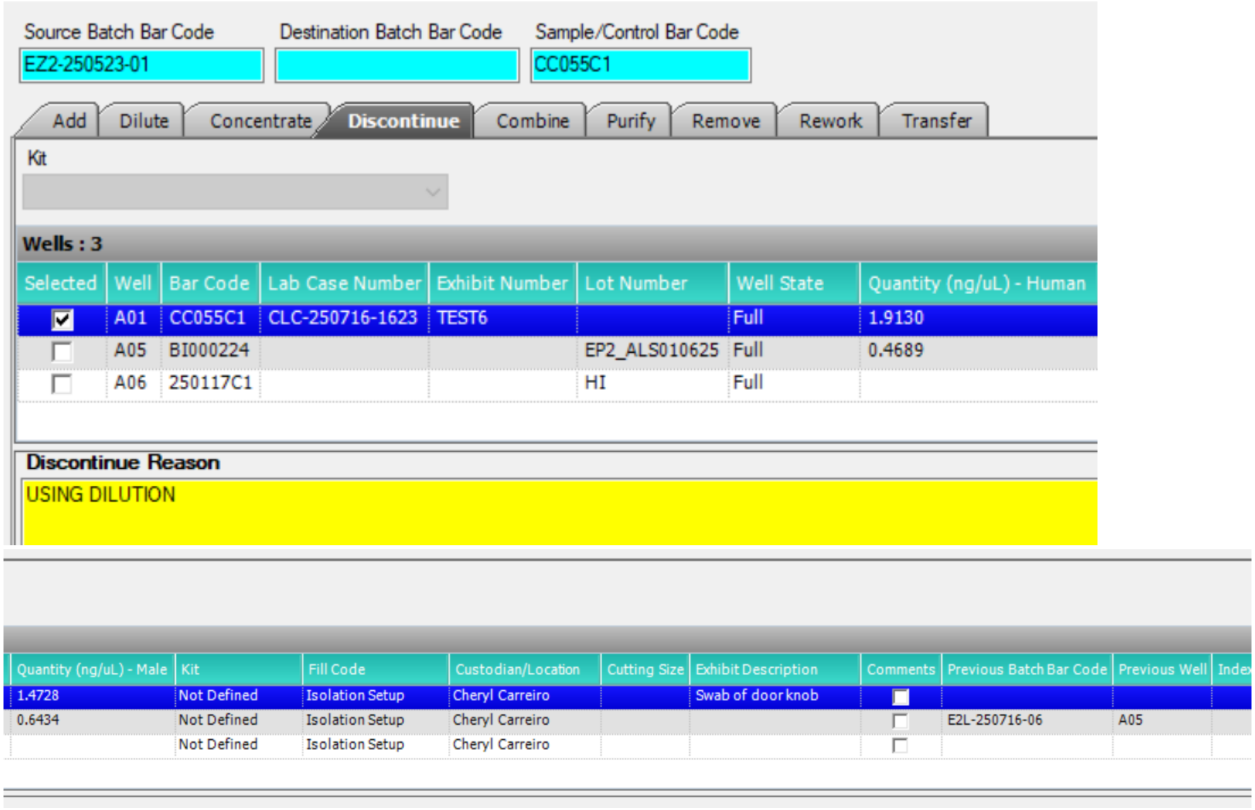
Assigns the discontinue sample (e.g., Stop processing ‘neat’ extracts when using dilutions):

1. Batch Management → Discontinue tab → Select Processing Step accordingly.

2. Scan the batch or select from the appropriate Processing Step dropdown.

3. Open the batch, check or scan the sample(s), enter reason (e.g., “using dilution”).

4. Enter the Discontinue Reason and click Save.



Source Batch Bar Code: EZ2-250523-01
Destination Batch Bar Code:
Sample/Control Bar Code: CC055C1

Wells : 3

Selected	Well	Bar Code	Lab Case Number	Exhibit Number	Lot Number	Well State	Quantity (ng/uL) - Human
☑	A01	CC055C1	CLC-250716-1623	TEST6		Full	1.9130
☐	A05	BI000224			EP2_ALS010625	Full	0.4689
☐	A06	250117C1			HI	Full	

Discontinue Reason

USING DILUTION

Quantity (ng/uL) - Male	Kit	Fill Code	Custodian/Location	Cutting Size	Exhibit Description	Comments	Previous Batch Bar Code	Previous Well	Inde
1.4728	Not Defined	Isolation Setup	Cheryl Carreiro		Swab of door knob	☐			
0.6434	Not Defined	Isolation Setup	Cheryl Carreiro			☐	E2L-250716-06	A05	
	Not Defined	Isolation Setup	Cheryl Carreiro			☐			

COMBINE TAB

Typically used to combine tubes after Isolation (e.g., Merge multiple extracts or controls into a single extract).

1. Open Processing → DNA Processing → Batch Management.
2. Scan the source batch or set processing step to the stage where the items reside (Isolation Batch Create, Quantitation Batch Create, Amplification Batch Create, or Examiner Setup).

3. Sample selection: Select only the samples that will combine into a single sample, not the comprehensive sample set. Double-click or scan each sample to be combined from the ‘Available Samples’ section.

4. Scan each sample to be merged; they move to 'Samples to Combine'.

5. In the ‘Samples to combine’ section check the first sample. This will replace the sample “position” with the new combined one.

6. Scan consumables and click Save. A new barcode will be generated and printed for the combined sample.

7. Note in comments.

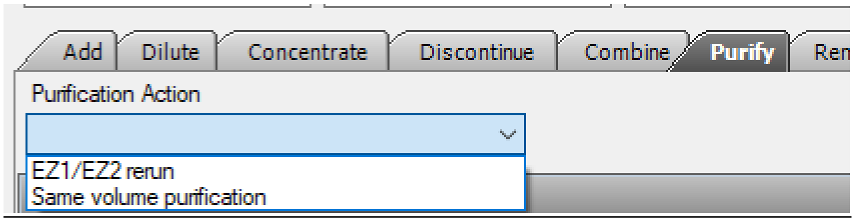
PURIFY TAB

1. Open Processing → DNA Processing → Batch Management → Purify.

2. Scan the source batch (left wells populate) or select from the appropriate Processing Step dropdown.

3. Scan the destination batch (right wells populate).

4. Select a Purification Action – EZ1/EZ2 rerun vs. Same volume purification (microcon).



5. Select a destination well; double-click or scan the sample(s) from Wells worklist to move into the Samples window.
   Note: the destination well must be a sample well and not a control or empty well.

6. Repeat as needed.

7. Scan consumables and click Save upon process completion.

RERUN ON EZ2:

If you have a sample that does not elute properly (below 20uL) or has an issue during isolation.

1. Processing→ DNA Processing → Batch Management.
2. In the Processing Step, choose Quantitation Batch Create.
3. Click on the Purify tab and then double click your batch on the left.
4. In the Purification Action dropdown, select EZ1/EZ2 rerun.
5. Double click on the sample(s) you want to reprocess and the reagent blank controls.
6. Rescan any the reagents you used in the process and the instrument you are using.
7. Save.

TO ADJUST VOLUME

View Batches→ find your isolation batch→ press content at bottom→ press "edit volume" button on bottom right and adjust volume in the "extract remaining" column and then press save volume on the bottom right.

REMOVE TAB

Remove incorrectly assigned or duplicate items from a batch prior to completion. The removed sample will repopulate into the Add tab.

1. Open Processing → DNA Processing → Batch Management → Remove.
2. Scan the batch; select the samples to remove.
3. Enter a Remove reason and click Save.

STACS will remove the item from the well list and place it onto the Add tab's sample list; the reason noted will appear on the sample history.

TRANSFER TAB

Use this to transfer samples from one or more batches to a destination batch. If the sample being transferred contains linked samples such as differential fractions or multi-tube extractions, STACS Casework will display a notification informing you that these samples should also be transferred to the destination batch.

1. Open Processing → DNA Processing → Batch Management → Transfer.
2. Scan the source batch (left wells populate) and the destination batch (right wells populate).
3. Select a destination well; scan the sample from the source wells to move it.

4, Repeat as needed; Save.

5. Destination position must be a DNA sample well (not a control/empty position).

PRE-EXTRACTED SAMPLES

Samples and corresponding controls extracted prior to STACS implementation can be brought into STACS for further processing using the 'pre-extracted' feature.

1. Import the sample and associated controls from LIMS to STACS.
2. In Samples Setup, identify the Sample Nature:
    a. Select 'Pre-extracted' for samples that need to be quanted.
    b. Select 'Pre-extracted Q' for samples that are using previous quantification values.
    c. Extraction Type = Non-Differential (or Differential for fractions as applicable).
    d. Fill case identifiers and any notes.
    e. Save.
3. Once a barcode is generated and scanned, a pop-up window will ask to create blanks now. As you already have a reagent blank select 'NO' unless a manipulation blank is needed.
4. Assign the sample(s) and mark as Start Processing.

5. In Batch Management, select either Quantitation Batch Create or Examiner Setup, as appropriate for the pre-extracted type.

6. In the Add tab, add the sample to a batch. If rework is needed prior to the quant/amp, (concentration, dilution, etc.) create a ‘dummy’ batch and add the sample there.

7. The samples can be found on the designated batches for further processing or re-work.

8. Place a Sample into the Workflow by option A or B:

OPTION A: Preferred (batch-based insertion):

1. Processing → DNA Processing → Batch Management; set Processing Step to Quantitation Batch Create for Previously Extracted samples needing Quant or Examiner Setup for Previously Quanted samples.

2. Click Create under Batches to create a placeholder Isolation batch and print its label.

3. Assign the pre-extracted sample (and control) to an available well and save.

4. Open Quantitation Batch Create or Examiner Setup; the placeholder Isolation batch will now be selectable to allocate the sample onto a quant plate.

OPTION B: Reprocess from Receipt (for discontinued items):

1. Processing → Receipt → Reprocess (or from Case Management/Sample Setup → Reprocess).

2. Enter lab case or item; choose Entry Point (e.g., Amplification Batch Create) → Check sample(s) → Save and comment.

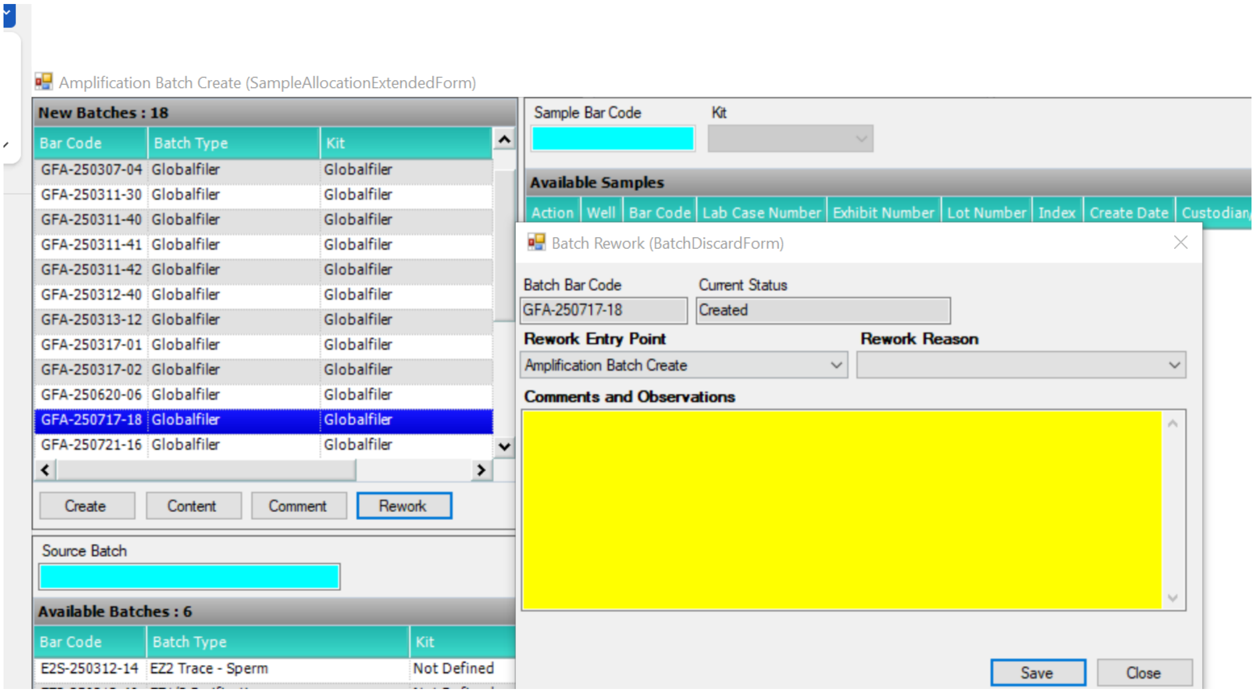
3. Proceed at the selected entry point.

12.5 GENERAL REWORK GUIDANCE

Use this to perform rework of previous steps. A Rework Entry Point and Rework Reason must be selected. Use Batch Rework when the entire batch layout or process needs to be reworked. Use Sample Rework for targeted re-quantitation, re-amplification, or re-analysis of specific samples.

Reworking an Entire Batch:

1. Open the step your samples are at. In all modules except Batch Setup and Extraction, highlight your batch in the upper worklist and click 'Rework' on the right-hand side.
2. Alternately, if your batch creation hasn't been completed yet, you can click Create Batch on the right and highlight the existing batch → select Rework.
3. Choose the Rework Entry Point (e.g., send back to previous create step) → Enter a Reason in the yellow box → Save.
4. Recreate the batch at the chosen entry point; samples return to Available Batches for allocation.



New Batches : 18

Bar Code	Batch Type	Kit
GFA-250307-04	Globalfiler	Globalfiler
GFA-250311-30	Globalfiler	Globalfiler
GFA-250311-40	Globalfiler	Globalfiler
GFA-250311-41	Globalfiler	Globalfiler
GFA-250311-42	Globalfiler	Globalfiler
GFA-250312-40	Globalfiler	Globalfiler
GFA-250313-12	Globalfiler	Globalfiler
GFA-250317-01	Globalfiler	Globalfiler
GFA-250317-02	Globalfiler	Globalfiler
GFA-250620-06	Globalfiler	Globalfiler
GFA-250717-18	Globalfiler	Globalfiler
GFA-250721-16	Globalfiler	Globalfiler

Available Batches : 6

Bar Code	Batch Type	Kit
E2S-250312-14	EZ2 Trace - Sperm	Not Defined

Batch Bar Code: GFA-250717-18
Current Status: Created
Rework Entry Point: Amplification Batch Create

Rework Tab from Isolation/Quant/Amp (before processing):

Processing→ DNA Processing → Batch Management → Rework

1. From Isolation Complete, Process Failed returns samples to Isolation Batch Create; otherwise use Rework prior to completion.
2. From Quantitation or Quantitation Setup, use Rework to abandon the batch and return samples to Quantitation Batch Create.
3. From Amplification, use Rework to abandon the batch and return samples to the selected entry point.

12.6 MISCELLANEOUS GUIDANCE

Quantitation

1, After reviewing results, saving a Failed quant sends samples back to Quantitation Batch Create for repeat setup.

2, Before saving a failed quant, ensure samples are not flagged 'No Amp' unless the intent is to stop them.

Examiner Setup:

1. Add only samples you intend to amplify; set the kit class (autosomal STR or Y-STR) and adjust reaction DNA volumes to hit the target input.
2. Avoid clicking Save on samples in the Amplification Setup Sheet unless ready; instead, mark Complete so samples appear at Amplification Batch Create.
3. If a sample must be allocated to multiple amplification batches (e.g., autosomal + Y-STR), ensure the action allows multi-assignment before allocation; otherwise, rework will be required.

Amplification Setup & Amplification:

1. Default outcome is Process Successful with comments as needed.
2. If setup errors occur after Save/Complete, use Rework to correct.
   Note: Once saved/completed, removing samples from a layout requires Rework.

CE Analysis

1. Process Aborted returns the batch to CE Analysis (e.g., to rescan instrument or address typo/illegal character in sample sheet).
2. Process Failed returns the batch to CE Batch Create to rebuild the plate.
3. Process Successful advances to next step.

Appendix 1:

Rework Entry Point Cheat-Sheet:

Goal	Rework Entry Point to Select
Repeat quantitation of sample(s)	Quantitation Batch Create
Change reaction DNA volumes/targets; re-add to amp	Examiners Setup (then rework in Amp Setup)
Rebuild amplification plate layout	Amplification Batch Create
Re-inject or rework CE plate	CE Analysis (re-injection) or CE Batch Create (rebuild)

Outcomes by Step Cheat-Sheet:

Step	Aborted	Failed
Quantitation	—	Returns to Quantitation Batch Create
Quant, Amplification, or CE Setup	Use Rework if setup was interrupted	Use Rework to re-do

Amplification (thermocycler)	Document and Rework as needed	Document and Rework as needed
CE Analysis	Returns to CE Analysis (rescan/retry)	Returns to CE Batch Create (rebuild)

Common Batch Management Tasks:

Task	Where	Notes
Make dilution(s)	Batch Management → Dilute	Scan diluent; set Tx and Dilution volume; Save
Discontinue a sample	Batch Management → Discontinue	Enter reason (e.g., using dilution)
Edit remaining volume	Utilities → View Batches → Content → Edit Volume	Save with justification
Allocate to multiple amp batches	Examiner Setup / Amp Setup	Enable multi-assignment before allocation; otherwise, Rework