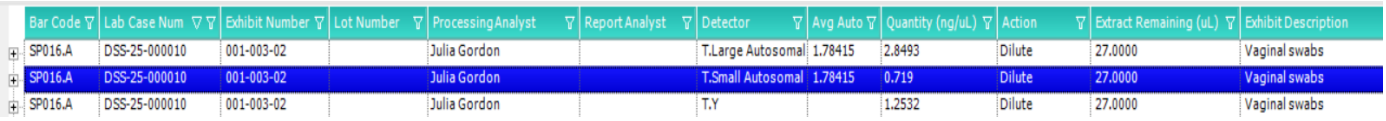
Amplification – Examiner Setup

The **'Examiner Setup'** module is used to make amplification decisions and will be completed by an authorized DNA analyst.

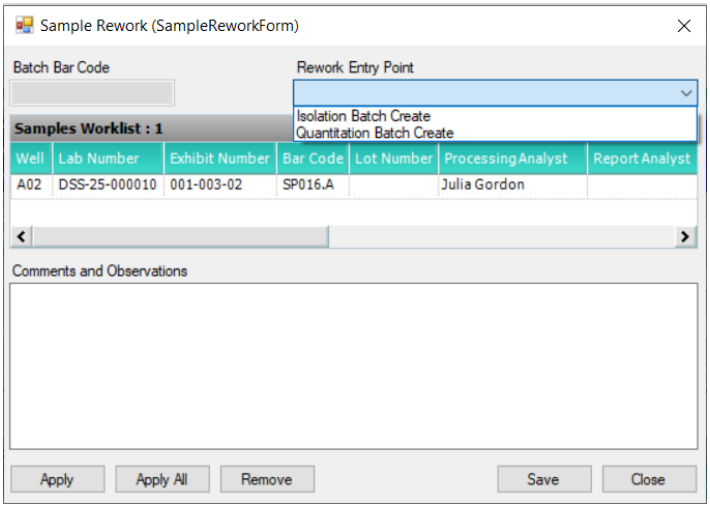
1. Open **Processing → DNA Processing → Examiner Setup**
2. The **Examiner Setup** module can be filtered for ease of view. Suggested viewing is as follows:
    a. Right click → Select Show Group By. Drag the Batch Barcode column to the top. This will separate samples by Extraction batch ID.
    b. Right click → Select Filter Type header. Use this feature to select one detector to view at a time (particularly step 4).
    c. Right click → Select Save Layout to maintain this view for all future viewings.
3. Isolation batches available for amplification will be displayed in the **Quantitation Results** worklist.
4. Each sample will be displayed 3 times – once for each detector value. However, only one detector value needs to be selected for subsequent testing. The detector selected will be the quantification value used for the amplification calculations. RBs that were not quanted will be listed only once with no quant value.

    The Action column recommends "use as is" vs. "dilution" based on target amplification values

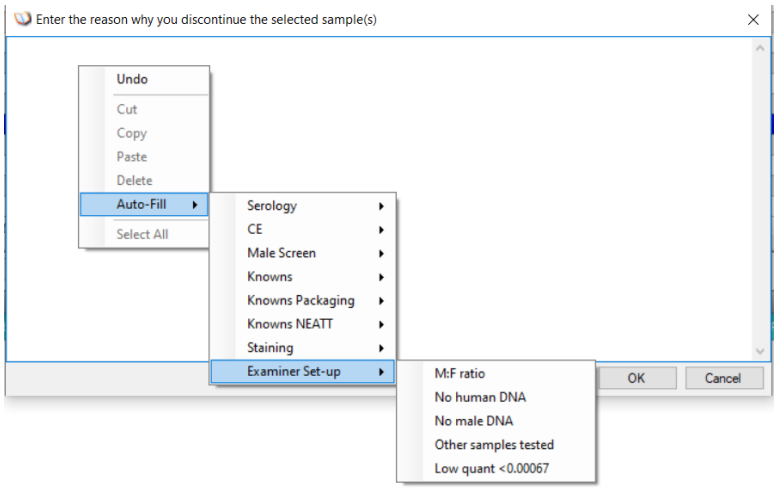


Bar Code	Lab Case Num	Exhibit Number	Lot Number	Processing Analyst	Report Analyst	Detector	Avg Auto	Quantity (ng/uL)	Action	Extract Remaining (uL)	Exhibit Description
SP016.A	DSS-25-000010	001-003-02		Julia Gordon		T.Large Autosomal	1.78415	2.8493	Dilute	27.0000	Vaginal swabs
SP016.A	DSS-25-000010	001-003-02		Julia Gordon		T.Small Autosomal	1.78415	0.719	Dilute	27.0000	Vaginal swabs
SP016.A	DSS-25-000010	001-003-02		Julia Gordon		T.Y		1.2532	Dilute	27.0000	Vaginal swabs

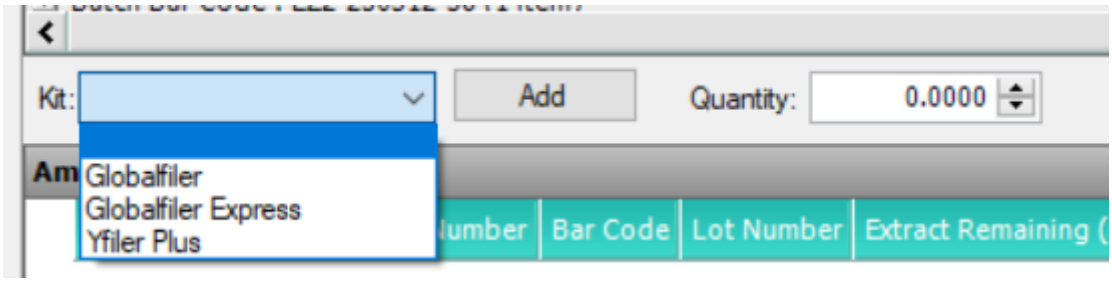
5. Samples highlighted in crimson (IPC=Yes) are an indication of a possible inhibited sample.
6. Samples highlighted brown are samples with a Degradation Index ≥3. Select the large autosomal quant values for these samples.
7. If necessary, any samples may be reworked by selecting the sample and clicking **Rework**. Select the appropriate entry point and add comments.



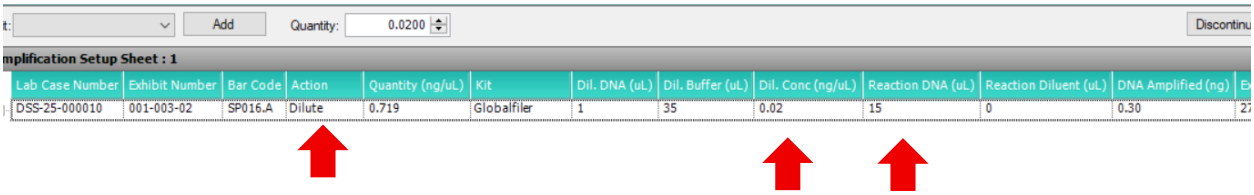
8. To halt a sample, select the sample (any detector line) and click the **Discontinue** button. A discontinue reason will be added using the autofill option (Right click → Autofill → Examiner Setup comments).



9. Adding a sample to an amplification kit: While selecting the appropriate detector for calculations, highlight the samples for amplification. (ex: highlight the large autosomal value for degraded samples, Y value for Y-STR testing, etc.)
10. Select the amplification kit from the kit dropdown and click **Add**. Do not forget to add the appropriate blanks and EP1 as well.



11. Adjust the **Action** column, **Dilution** and/or **Reaction DNA** columns as needed. Calculations will be set to target 15uL of input DNA volume. Leaving the target dilution concentration at 0.02 ng/uL. This can be changed to 0.3ng/ul to decrease the volume of total dilution if necessary.



12. Samples may be added to the Amplification Setup Sheet window multiple times (ex: amplifying a sample in both GF and YFP) by selecting different detector values.

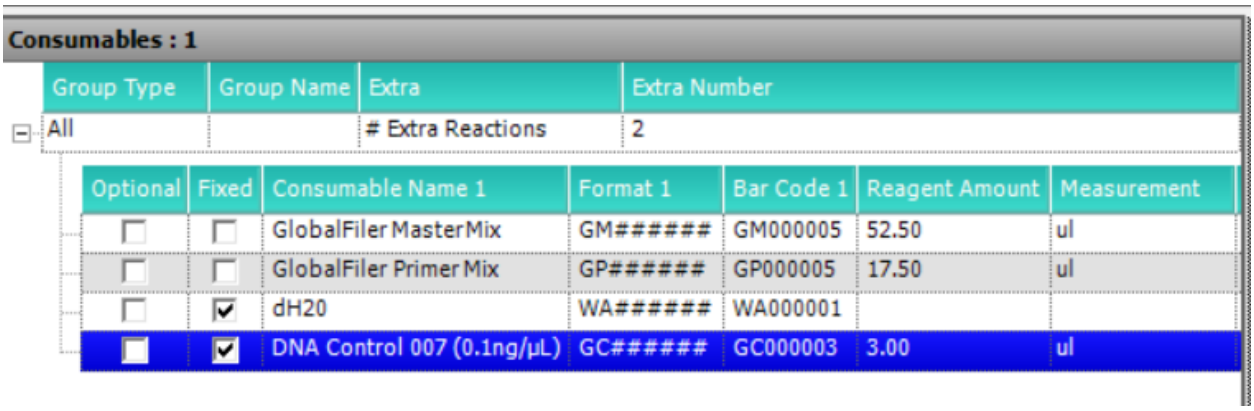
    Note: If a sample is to undergo multiple amplifications, this must be done prior to marking the sheet complete.
    Select a sample choose the other kit and click add.

13. Click **Mark Complete**.

Amplification – Setup & Processing

1. Open **Processing → DNA Processing → Amplification Setup**.
2. Click **Create Batch**. The **Amplification Batch Create** screen opens (another window).
3. Click **Create**. Select a **Batch Type**. The Fill order is not relevant to manual processing.
4. Click **Create** and scan the generated amplification batch bar code.
5. Select the **Source Batch** and **Allocate** to the **Destination Amplification Batch**. Samples can be moved around after hitting save. Be sure if moving the negative control, you choose “consumable negative control” and not “amplification blank”.
6. Click **Complete** when all samples are added.

7. In the **Amplification Setup** module which is already open, select/scan the batch to be processed and click **Select Scenario**.
8. Scan in all consumables, batch barcodes and hood/workstation. The consumables window will automatically calculate the reagent volumes needed to make the master mix.

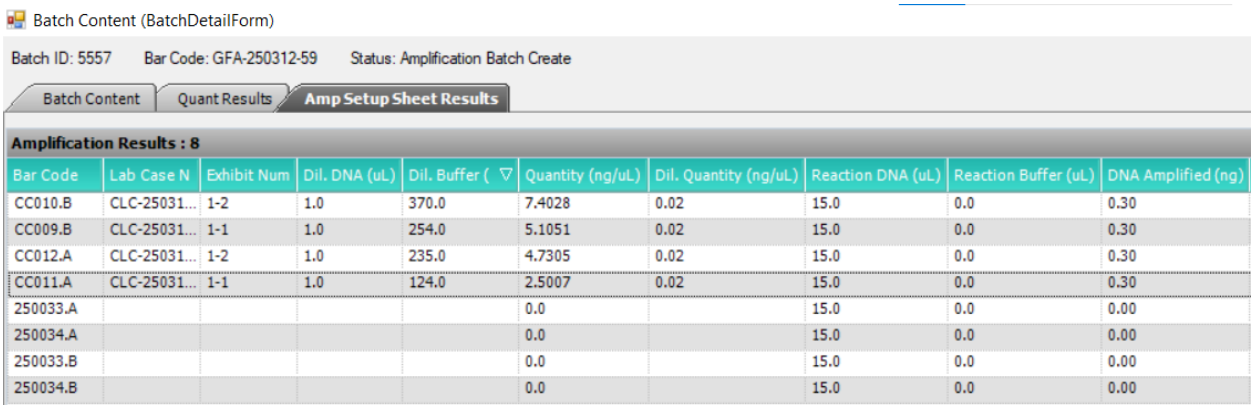


Consumables : 1

Group Type	Group Name	Extra	Extra Number
All		# Extra Reactions	2

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measurement
☐	☐	GlobalFiler MasterMix	GM######	GM000005	52.50	ul
☐	☐	GlobalFiler Primer Mix	GP######	GP000005	17.50	ul
☐	☑	dH20	WA######	WA000001		
☐	☑	DNA Control 007 (0.1ng/µL)	GC######	GC000003	3.00	ul

9. For ease of aliquoting, volumes and dilutions can be viewed by clicking on the Content button on the right-hand column. Select the **Amp Setup Sheet Results** tab and organize columns as needed. This window can remain open throughout the process, separate from the Amplification Setup window.



Batch Content (BatchDetailForm)

Batch ID: 5557 Bar Code: GFA-250312-59 Status: Amplification Batch Create

Batch Content | Quant Results | **Amp Setup Sheet Results**

Amplification Results : 8

Bar Code	Lab Case N	Exhibit Num	Dil. DNA (uL)	Dil. Buffer (▽	Quantity (ng/uL)	Dil. Quantity (ng/uL)	Reaction DNA (uL)	Reaction Buffer (uL)	DNA Amplified (ng)
CC010.B	CLC-25031...	1-2	1.0	370.0	7.4028	0.02	15.0	0.0	0.30
CC009.B	CLC-25031...	1-1	1.0	254.0	5.1051	0.02	15.0	0.0	0.30
CC012.A	CLC-25031...	1-2	1.0	235.0	4.7305	0.02	15.0	0.0	0.30
CC011.A	CLC-25031...	1-1	1.0	124.0	2.5007	0.02	15.0	0.0	0.30
250033.A					0.0		15.0	0.0	0.00
250034.A					0.0		15.0	0.0	0.00
250033.B					0.0		15.0	0.0	0.00
250034.B					0.0		15.0	0.0	0.00

10. When ready to aliquot, click **Start Process**.
11. Complete the tube check by scanning the tube labels.

    Note: Scan the DNA Control 007 and dH2O lot numbers as the positive and negative, respectively.

12. When the amplification setup has been completed, click **Complete Process** and record the results using the Complete Batch Activity screen. Click **Save**.
    - **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 Quant Setup. A batch comment is required with this option.
13. Document the thermalcycler used. Open **Processing → DNA Processing → Amplification.** This can be done in the post-amp lab.
14. Scan the batch from the Batches Worklist.
15. Scan the thermalcycler bar code.
    Note: if a C-1000 is used, indicate in the batch comments which block is used.
16. Click **Start Process.**
17. Fill out the **Complete Batch Activity** window by completing the process when done.
18. Next step is STACS SOP-7 CE.