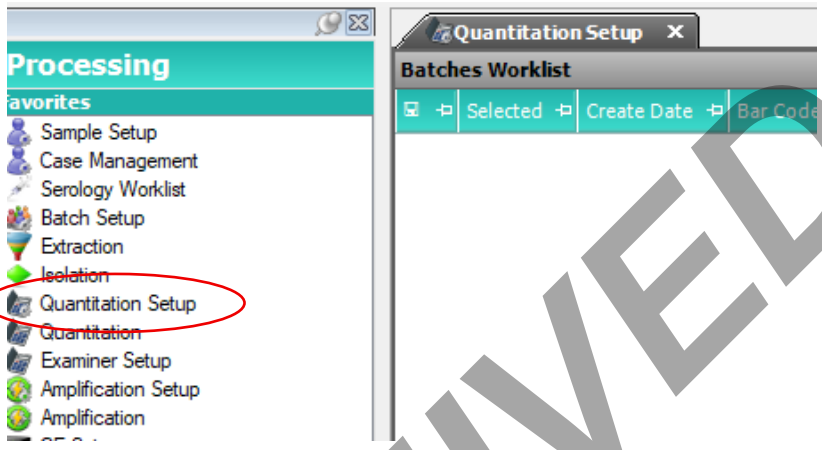
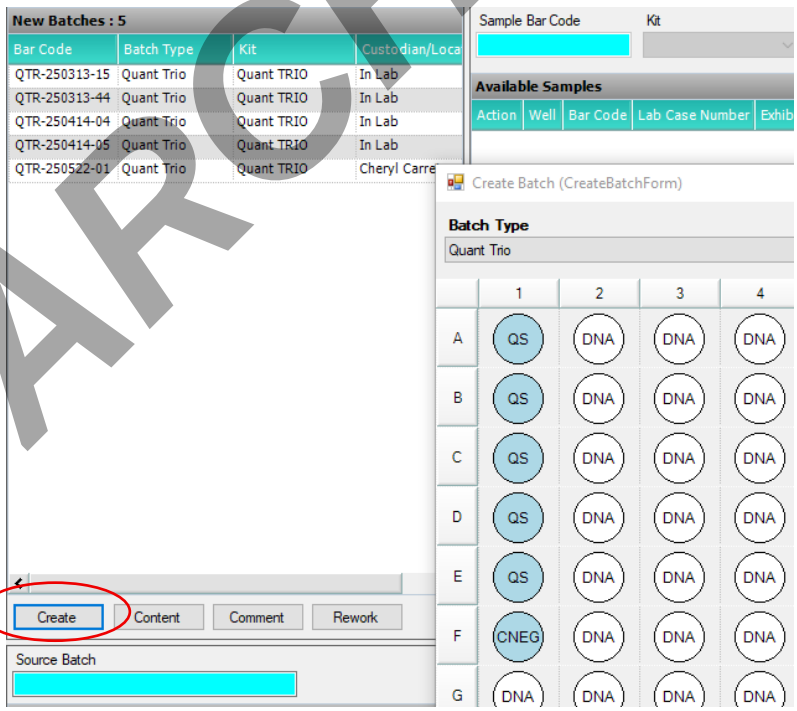
I. Quantitation Setup

1. Open **Processing → DNA Processing → Quantitation Setup**.
2. Click **Create Batch**.



3. To create a new batch, click the **Create** button located under the **New Batches** worklist(upper-left corner).



a) The batch type (Quant Trio) and Kit (Quant Trio) are the default option. Select 'Vertical' sample allocation if not already selected.

b) Click **Create**. A unique Batch Bar Code will be generated and printed. This barcode should be affixed to the quant plate.

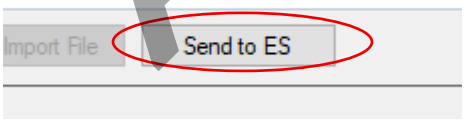
4. Allocate Samples to a quant batch:

a) Scan the extraction batch bar code into the **Source** box. Individual samples barcodes may be scanned into the sample bar code box as well. This will place the samples into the 'Samples for Batch' box.

b) Scan the quantification plate's Batch Bar Code into the **Destination Batch**.

c) Click **Allocate** to allocate all qualifying samples from Samples for Batch box.

Note: If the reagent blank will not be quanted, select the RB(s) and click **'Send to ES'**. This will send the RB to **Examiner Setup**. This must be done after all other samples have been allocated. If doing this, you will have to allocate each sample individually by double clicking on them instead of allocating the whole batch.

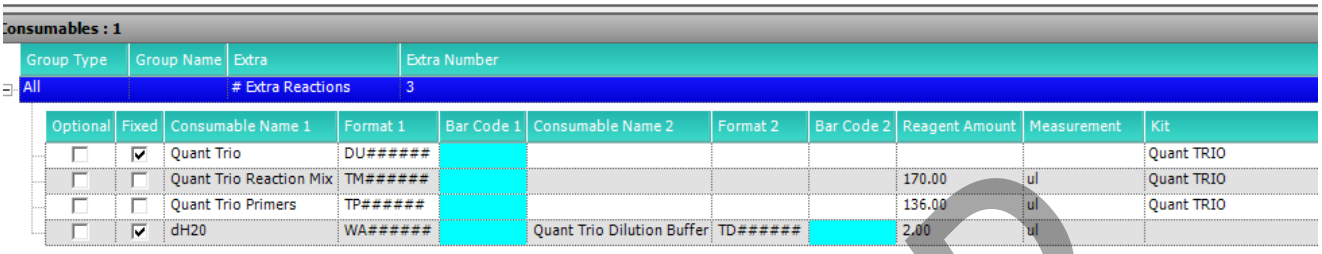


5. To move samples within the allocated plate, the allocation must be saved. Samples may then be dragged to different wells to balance the plate, if necessary. Standards can't be moved from the first column. Balance the rest of the plate accordingly.

6. Once all samples have been allocated and no further changes are required, click **Complete** (next to Destination Batch Barcode). Close the window.

7. On the Quantitation Setup tab select/scan the batch to be processed from the worklist and click **Select Scenario**.

8. Scan the hood/workstation barcode and consumable barcodes to enter the lot numbers. The volumes needed to make the mastermix are shown in the reagent amount column. Either water or Dilution Buffer can be scanned to be used for the Neg Control.



Consumables : 1

Group Type	Group Name	Extra	Extra Number
All		# Extra Reactions	3

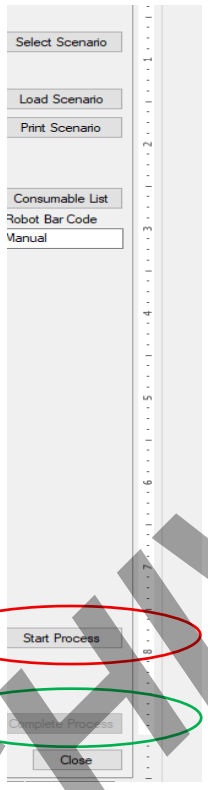
Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Consumable Name 2	Format 2	Bar Code 2	Reagent Amount	Measurement	Kit
☐	☑	Quant Trio	DU######							Quant TRIO
☐	☐	Quant Trio Reaction Mix	TM######					170.00	ul	Quant TRIO
☐	☐	Quant Trio Primers	TP######					136.00	ul	Quant TRIO
☐	☑	dH20	WA######		Quant Trio Dilution Buffer	TD######		2.00	ul	

9. Click **Start Process**.

10. Complete the tube check by scanning the sample tube labels.

11. For ease of processing in the hood, the quant worksheet may be printed or viewed from the **Content** button.

12. When the plate has been aliquoted, click **Complete Process.** Choosing **Process Successful** will generate the 7500-import file. Save the file to **U:\STACS share\Quantitation\7500 import files**. If not already generated click **Generate** to generate the 7500 Import File. You can then import this file into the instrument.



Complete Batch Activity (CompleteBatchActivityForm)

Process Successful Process Aborted Process Failed

Batches : 1

Bar Code	Process	Status	New Status	Batch Comment
QTR-250523-09	Success	Quantitation Batch Create	Quantitation Setup Complete	

Samples : 8

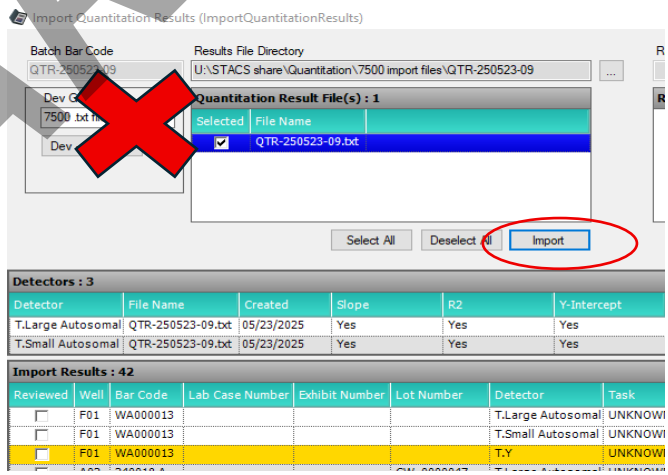
Batch Bar Code	Sample	Kit	Lab Case Number	Exhibit Number	Transferred (uL)	Source Sample F
QTR-250523-09	CC022.A	Quant TRIO	CLC-250522-1417	12	2.0	28.0
QTR-250523-09	BI000193.A	Quant TRIO			2.0	28.0
QTR-250523-09	240018.A	Quant TRIO			2.0	28.0
QTR-250523-09	240019.A	Quant TRIO			2.0	28.0
QTR-250523-09	CC023.A	Quant TRIO	CLC-250522-1417	13	2.0	28.0
QTR-250523-09	BI000193.A	Quant TRIO			2.0	28.0
QTR-250523-09	250086.A	Quant TRIO			2.0	28.0
QTR-250523-09	250087.A	Quant TRIO			2.0	28.0

II. **<u>Quantitation</u>** – This section pertains to the documentation related to the instrument run. STACS steps should be completed in the post-amp lab.

1. Open **Processing → DNA Processing → Quantitation**
2. Scan the Batch Bar Code from the Batches Worklist.
3. Scan the barcode for the 7500 instrument to be used.
4. Click **Start Process**.
5. Once the runs is complete, completing batch activity in STACS
    a) Scan the bath from the Batches Worklist.
    b) Click **Complete Process** and select the appropriate option, this step should be done by the person analyzing the quant and making the amp sheet.

III. **<u>Post-Quantitation</u>**

1. Export the quantitation results from instrument. Click **Import** and select the appropriate file.
    a) Run the 7500 per normal process.
    b) Export from the instrument and upload the file into the Trio Software.
    c) "Analyze" the data and save the .xls file in the STACS 7500 results folder **U:\STACS share\Quantitation\7500 Results**.
    d) In STACS import the .xls file



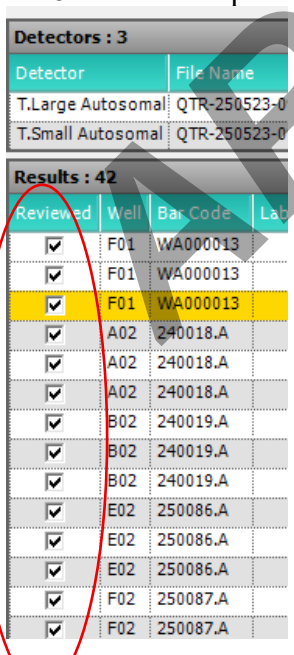
2. The **Action** column will auto-populate with suggested actions based on system configurations (highlighting the sample).

3. Adjust the actions for each detector for each sample. **No Amp** is for sample/detector that will not be used for amp. **Use As Is** is for sample/detector whose quant value will be used. **Dilute** is for sample/detector that will be diluted prior to amplification. **Concentrate** is for sample/detector that will require concentration prior to amplification (if choosing this option, be sure to do the same for the blank control associated with the sample). For samples going to YFP amplification, be sure to choose the Y detector to move forward. For samples with high degradation index, be sure to choose the Large Autosomal detector to move forward.

   Note: Samples with no detected values in any detector category will be flagged in red and 'No Amp' will automatically be assigned. These samples will not be available for amplification in the Examiner Setup module unless manually changed.

4. Review standards, curve thresholds and controls. If TL signoff is needed, add a batch comment and notify the TL with the batch name. Save the quant without reviewing. Once TL has reviewed, continue with steps below.

5. Mark samples as reviewed by selecting all samples and click **Save**.



6. Fill out the **Complete Batch Activity** window.

7. See SOP-6 for next step which is: **Examiner Setup**.