Quantifiler® Trio DNA Quantification Kit

1 LIMS Pre-Processing

1.1 In the ***Analytical Testing*** » ***Test Batches*** tram stop, select the appropriate quantitation assay and Click ***Edit.***

NOTE**:** If you are creating a new quantitation test batch, use the ***New Test Batch*** tram stop followed by the ***Create New Test Batch*** wizard. In that wizard, include the following information: description, functional group, analysis, batch configuration, and test batch type (case test batch).

1.2 If necessary, Click ***Add Unknowns*** and select any samples that need to be included on the test batch.

NOTE: Quanting exemplars and evidence samples may be done on the same plate; however, evidence extracts should be aliquoted into the plate before the exemplar samples. This precaution helps to prevent potential cross-contamination of exemplar extracts into evidence extracts.

1.3 Select All Input Samples » Click Add Output Sample » 🔍 » 1:1*» Click Select and Return » Click Ok » Click Create

1.3.1 * "1:1" signifies the dilution of the sample. Samples run at a 1:1 are being run neat. If a sample is scheduled for a dilution, assign the appropriate dilution (e.g., 1:10) when creating the output sample.

1.4 Select All Output Samples » Click Load Plate

1.5 In the ***Load Plate*** view, select all samples on the left side of the screen. Click on the next available well in the ***Plate Layout*** tab located on the right side of the screen.

1.6 Fill in the plate name » Click ***Save*** » Click ***Return to List***

NOTE**:** Do not use a period (.) in the plate name. Use an underscore for plate naming.

1.7 If you have created the output samples and loaded the plate, you must fill out the ***Performed By*** tab indicating you completed *Batch Setup Review.*

1.7.1 Select Batch Setup Review » Click Fill Perform By/Date

1.7.2 Assign the Run Name by choosing the plate from the dropdown. Do not assign Analysis Set.

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1.7.3 Click Save » Click Return to List

1.8 Select the test batch » Click Ready

1.9 When ready to perform a run, if not already in the test batch, go to the ***Analytical Testing*** » ***Test Batches*** tram stop, select the appropriate quantitation assay and click ***Edit***

1.10 In the Performed By tab, select Trio Run task » click Fill Perform By/Date » click Save

1.11 Using the data and time listed in the ***Performed By*** tab, update the Description in the ***main test batch*** tab (located at the top of the page) with the following format:

TU#Qdate_time (U# = instrument used) [e.g. TU4Q012115_0815]

1.12 Click *Save*

1.13 In the ***Plate/Analysis Set*** tab, Select the Pre-Loaded Plate » **Click *Load Plate***

1.14 Update the ***Plate Name*** to reflect the name listed in the ***Description*** field of the main ***Test Batch*** tab.

1.15 Click Save » click Download to Instrument.

1.16 In the ***Instrument*** tab, record the 7500 used for the quantitation assay.

1.17 If you are the analyst performing the quantitation assay, generate a ***Test Batch Pick List Report*** to help locate the samples needed in the laboratory.

1.17.1 Select the desired quantitation assay in the ***Analytical Testing*** » ***Test Batches*** tram stop.

1.17.2 On the side bar, click Choose Report » Test Batch Pick List Report

2 Assay Preparation

2.1 Retrieve the following reagents:

Quantifiler® THP PCR Reaction Mix
Quantifiler® HP Primer Mix
Quantifiler® DNA Dilution Buffer
Quantifiler® THP DNA Standard (100ng/µL)

2.2 Retrieve samples needed for quantitation from associated refrigerator and/or freezer.

2.3 Record lot numbers in LIMS » Click ***Save***

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2.4 Calculate the master mix need for the assay in the *Reagents* tab: Select *Quantifiler® THP PCR Reaction Mix* and *Quantifiler® HP Primer Mix* » Click *Calculate Amount* » Click *Save*

2.5 Create the Standard Curve:

2.5.1 **Briefly centrifuge** Quantifiler® THP DNA Standard (100ng/µL) for no more than 3 seconds at no greater than 3000rpm.

2.5.2 Label tubes for the standard curve as follows. If the standards will be used for an additional run, include the date that the standard was made:

100ng/uL [date], 50 ng/µL [date], 5 ng/µL [date], 0.5 ng/µL [date], 0.05 ng/µL [date], 0.005 ng/µL [date], and NTC [date]

2.5.3 Add 10µL of Quantifiler® DNA Dilution Buffer to tubes 50 and NTC.

2.5.4 Add **90µL** of Quantifiler® DNA Dilution Buffer to tubes **5**, **0.5**, **0.05**, and **0.005**.

2.5.5 Standards may be stored in a refrigerator and used for up to **two (2) weeks**. If you are making a standard curve for 2 assays, record the following information on the rack containing the standard curve tubes:

- Name
- Date
- Lot numbers of the Quantifiler Standard and Dilution Buffer (labels containing lot numbers may be printed from LIMS via the Reagent Tram stop)

NOTE**:** Each standard must be thoroughly mixed prior to the next step. Standards should be mixed by vortexing and briefly centrifuging for no more than 3 seconds at no greater than 3000rpm.

2.5.6 To make standards for one (1) assay:

- Aliquot **16µL** from the Quantifiler® THP DNA Standard (100ng/µL) into the **100ng/µL** tube.
- Add **10µL** from the **100ng/µL** tube to the **50ng/µL** tube, thoroughly mix contents.
- Add **10µL** from the **50ng/µL** tube to the **5ng/µL** tube, thoroughly mix contents.
- Add 10µL from the 5ng/µL tube to the 0.5ng/µL tube, thoroughly mix contents.
- Add 10µL from the 0.5ng/µL tube to the 0.05ng/µL tube, thoroughly mix contents.

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- Add 10µL from the 0.05ng/µL tube to the 0.005ng/µL tube, thoroughly mix contents.

2.5.7 To make standards for two (2) assays:

- Aliquot **20µL** from the Quantifiler® THP DNA Standard (100ng/µL) into the **100ng/µL** tube.
- Add **10µL** from the **100ng/µL** tube to the **50ng/µL** tube, thoroughly mix contents.
- Add **10µL** from the **50ng/µL** tube to the **5ng/µL** tube, thoroughly mix contents.
- Add **10µL** from the **5ng/µL** tube to the **0.5ng/µL** tube, thoroughly mix contents.
- Add **10µL** from the **0.5ng/µL** tube to the **0.05ng/µL** tube, thoroughly mix contents.
- Add **10µL** from the **0.05ng/µL** tube to the **0.005ng/µL** tube, thoroughly mix contents.

2.6 Prepare dilutions of extracted samples if necessary. The Quantifiler® DNA Dilution Buffer should be used to make the dilutions. Vortex and centrifuge samples prior to aliquoting for dilution.

2.7 **Vortex** all standards, extracted samples and NTC. **Briefly centrifuge** for no more than 3 seconds at no greater than 3000rpm.

2.8 Arrange samples in the order as they appear on the plate loading screen in LIMS in a vertical fashion starting at A1 down to H1 continuing at A2.

2.9 Witness step:

2.9.1 Confirm the input sample names and order by reading the complete sample ID.

2.9.2 The witness will confirm the sample order to be downloaded to the instrument by comparing the order of the output sample ID to the plate map on the Load Plate screen.

2.9.3 Fill out the Witness tab in LIMS.

2.10 **Gently vortex** Quantifiler® THP PCR Reaction Mix and Quantifiler® HP Primer Mix and **briefly centrifuge** for no more than 3 seconds at no greater than 3000rpm.

2.11 Prepare master mix as calculated by LIMS in a new tube.

NOTE**:** If the calculated master mix volume is ≥1400µL, use a 2.0mL dolphin tube for preparation.

2.12 **Gently vortex** and **briefly centrifuge** freshly made master mix for no more than 3 seconds at no greater than 3000rpm.

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2.13 Aliquot **18µL** of prepared master mix in each of the appropriate wells of a new Applied Biosystems® MicroAmp® Optical 96-Well Reaction Plate.

NOTE**:** For every 16 wells (i.e., 2 columns) **gently vortex** the master mix and **briefly centrifuge** for no more than 3 seconds at no greater than 3000rpm.

2.14 Aliquot **2µL** of each sample, including standards, NTC and extracted samples to the assigned well.

2.15 **Seal** the reaction plate using Optical Adhesive Film.

NOTE**:** When using the Optical Adhesive Film, use a straight edge or tube opener to eliminate bubbles which may otherwise interfere with detection.

2.16 **Centrifuge** sealed reaction plate for 1 minute at 3000rpm

NOTE**:** Check plate prior to loading on to instrument. If bubbles are still seen in the wells, repeat step 2.16 until they are no longer present.

3 Software Operations

3.1 Turn on the Applied BioSystems® 7500 Real-Time PCR System. Allow time for the instrument to warm up.

3.2 Press the tray door to open and load the plate on the instrument.

NOTE**:** Plate is correctly aligned when position A12 is in the top right corner of the tray.

3.3 Close the tray door by pushing the depressed imprint on the right side of the tray. Do not push from the center.

3.4 Double click icon HID Real-Time PCR Analysis Software

3.5 Click *Quantifiler® Trio icon* located in the upper left corner of the screen.

3.6 Inside the Experiment Menu on the left side of the screen, click ***Setup*** » ***Experiment Properties*****.**

3.7 Enter run name into the topmost field labeled Experiment Name.

3.8 Click Setup » Plate Setup » Assign Targets and Samples.

3.9 To import samples, click ***File*** **» *Import*.** Locate the file in the LIMS file share folder. Click ***Start Import***

NOTE**:** A warning will come up indicating your current plate set-up will be lost**.** Click ***Yes***

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3.10 Plate set-up imported successfully » click ***OK***

3.11 Check the top header and ensure the following:

3.11.1 **Experiment Name:** Current Run Name

3.11.2 **Type:** HID Standard Curve

3.11.3 **Kit Name:** Quantifiler® Trio

3.11.4 PCR Conditions for Quantifiler® Trio:

7500 Quantifiler Trio®	The Quantifiler file is as follows: Holding stage: 95°C for 2 minutes 40 cycles :Denature at 95°C for 9 seconds :Annealing at 60°C for 30 seconds

3.12 Click ***Start Run.*** Run time is ~1 hour.

3.13 Turn the instrument off when the run is complete.

4 Exporting Results

4.1 Open HID Real-Time PCR Analysis Software on the desktop, if needed.

4.2 If the assay that needs analysis is not currently open, click ***File » Open.*** Navigate to desired file, select the file, and click ***Open***.

4.3 In the ***Experiment Menu*** located on the left side of the screen, click ***Analysis.***

4.4 In the ***Analysis*** tab on the top right side of the screen, click ***Analysis Settings » C_T Settings***

4.5 Verify the settings below and click ***Cancel***

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Target	Threshold	Baseline Start	Baseline End
T. IPC	0.1	3	15
T. Large Autosomal	0.2	3	15
T. Small Autosomal	0.2	3	15
T. Y	0.2	3	15

4.6 Click Analyze

4.7 After analysis, results can be exported. Click View Plate Layout » Highlight All Wells.

4.8 Located on the top toolbar, click ***Export***

4.8.1 Select data to export » ***Results***

4.8.2 Select one file or separate files » ***One File***

4.8.3 Ensure the correct file name

4.8.4 In the Custom Export tab check the data is exporting columns (A1, B1, etc.)

4.8.5 Click Start Export

4.9 With all wells still highlighted, click ***Print Report*** located on the top toolbar. Select ***All Report Types.***

4.10 Click ***Print*** and choose to save as a ***.PDF***. Ensure the correct run name is listed. **Add reports** to the end of the file name.

4.11 Save file in appropriate LIMS folder and **Click *Save***.

4.12 Transfer the raw data .EDS files from the instrument PC to the Forensic Biology network drive. These files should be saved in the respective instrument folders that are in the "Quant Trio" folder.

NOTE**:** Open the PDF to ensure the file exported correctly.

5 LIMS Post Processing I

5.1 If not already in the test batch, go to the ***Analytical Testing » Test Batches*** tram stop, select the appropriate quantitation assay and click ***Edit***

5.2 In the ***Attachments*** tab located at the bottom of the page, attach***.PDF*** file for the associated test batch.

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5.3 In the Performed By tab, select Trio Run Review Task » click Fill Perform By/Date » click Save

5.4 Check the remaining tabs to ensure all have been filled out properly.

5.5 In the Plate/Analysis Set tab, select the Trio Run » click Data Entry

5.6 In the ***Data Entry*** screen, click ***Import Instrument Data*.*** Locate file in the LIMS file share folder by clicking ***Browse***; Once found click ***OK***

5.7 If a sample was quanted at a dilution, manually enter the dilution in the Data Entry screen.

5.8 Click Save

6 Interpretation

6.1 Use the reports generated and the data imported into LIMS to interpret the results for each assay.

6.1.1 Using the standard curve reports, ensure the following parameters are met for targets **T.Y.**, **T. Large Autosomal**, and **T. Small Autosomal** and record the **slope, Y-Intercept,** and **R^2** value. In LIMS, record the ***QCBatch Params*** located at the top of the screen. Make sure to *release* and save all data stored in the ***QCBatch Params*** tab:

- Standard Slope must be between **-3.0** to **-3.6**
- the Y-Intercept value (**T.Y and T. Small Autosomal only**) must be between ≥ 24.5 and ≤29.5
- the Y-intercept value (**T. Large Autosomal only**) must be between ≥ 24.3 and ≤29.5
- R^2 values must be **≥ 0.98**

6.2 All three targets must pass the above quality criteria in order for the quantitation to pass. Indicate "Pass" or "Fail" in the QC Batch Parameters.

6.3 All samples and controls should be reviewed to ensure that the data makes sense in the context of the samples being tested.

6.4 If the quantitation assay fails, the assay must be re-done. **Notify QA/QC if the repeating quantitation assays fails.**

6.5 To confirm that data was imported correctly, use the data entry screen in the LIMS test batch to ensure that all standards are listed in the correct order.

6.6 Check the "QC Summary" flagging guide at the end of the Quant Trio pdf. report. Follow the resolution column under Section 8 of this manual if a sample is flagged.

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6.7 For Y-screening negative controls, only the male target will be evaluated.

6.7.1 If the male target is 0.0 pg/µL, the assay passes.

6.7.2 If the male target is >0.0 pg/µL, the results of the associated samples may be used for triaging purposes (refer to section 7); however, any presumptively positive male screening results will be reported as inconclusive. Samples with a male target of 0.0 pg/µL will be reported as no male DNA indicated.

6.8 For all other negative controls, including extraction negatives, microcon negatives, and the NTC associated with the quantitation assay, a passing result is **≤ 0.2pg/µL**. Indicate "Pass" or "Fail" in the interpretation column for the NTC; do not use for extraction or microcon negatives.

6.8.1 The quantitation value is determined only by the small autosomal target under non-inhibitory conditions.

6.8.1.1 If inhibition is detected in an extraction negative, review the raw data and consider a microcon as described in the QC Summary Flagging Guide.

6.8.2 If there is a value shown only in the Y target and no value in the small autosomal under non-inhibitory conditions, the Y target value is not used as an indication of total DNA.

6.8.3 If there is a value shown only in the Y target and no value in the small autosomal under inhibitory conditions, the control should be re-quantified.

6.8.4 If the NTC associated with the quantitation assay fails, the entire assay must be re-done. **Notify QA/QC if the repeating quantitation assays fails.**

6.8.5 If an extraction or microcon negative control yields a value > 0.2 pg/µL, that negative control must be quantified a second time. If the control fails after two successive quantitation assays, then the associated extraction/microcon assay fails. Do not indicate "pass" or "fail" in the interpretation column for these samples.

6.8.5.1 Negative controls yielding high quantitation values (≥5pg/µL) must be evaluated to determine if there is a possible issue with the samples or assay (e.g. a sample switch). This evaluation must be documented in the batch or casefile.

6.9 IPC (internal positive control) is used to determine if inhibition is present within a non- standard. Use the following criteria to determine if inhibition is present.

No inhibition:	26 to 29
Low inhibition:	< 26 to 24 or > 29 to 31
High Inhibition:	< 24 or > 31 or blank

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6.9.1 If the non-standard is not being requanted for high quant value and inhibition is present, it **must** be noted in LIMS in the ***Interpretation*** column of the ***Data Entry*** tab for that associated non-standard.

NOTE**:** Inhibition is to be documented for all non-standards. As per the Quantifiler® HP and Trio DNA Quantification Kits User Guide, IPC flagging in the standards is not due to inhibition but is rather due to the competition between the human and/or male specific and IPC reactions.

6.10 The degradation index is used to determine if the non-standard exhibits signs of degradation. Use the following criteria to determine if degradation is present. If high degradation is present, and the non-standard is not being requanted for a high quant value, it must be noted in LIMS in the ***Interpretation*** column of the ***Data Entry*** tab for the associated non-standard.

No degradation:	< 1
Low Degradation:	1 to 10
High Degradation:	>10 or blank

NOTE: A "blank" value in the degradation column does not always indicate high degradation. If a non-standard contains a "blank" degradation value under non-inhibitory conditions, this typically indicates a very low or negative quantitation result (for example, extraction negatives often produce a "blank" value in the degradation column).

6.11 After the quality for each sample is assessed, determine if further testing is necessary.

6.11.1 If a sample or control is being sent for microcon or re-quantitation it must be noted in LIMS in the ***Interpretation*** column of the **Data Entry** tab for the associated sample or control.

6.11.2 If a sample exhibits high inhibition it should be submitted for microcon and sent for re-quantitation prior to amplification. It may also be useful to examine the Amplification Plot within the HID Real-Time PCR Analysis Software.

6.11.3 If bone or tissue samples exhibit high inhibition, consult the RA on the case or a Missing Persons team member to determine if microconning is necessary.

6.12 The Small Autosomal quantitation value must be used for samples sent for autosomal STR amplification.

6.12.1 The minimum sample concentration for PowerPlex Fusion® amplification is 5.00pg/uL (37.5pg total DNA).

6.12.2 When scheduling samples for autosomal STR amplification, samples may be tested using the following strategies:

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6.12.2.1 GS, S, PT, T, ST samples

- Samples with a concentration between 5 pg/µL to 70 pg/µL: auto-replicate after quant
- Samples with >70 pg/µL: amplify once after quant

6.12.2.2 BL, AM, CB, F1(EC), F2(SF), hair samples

- For F1(EC) and F2(SF) samples with M:F ratios between 1:50 and 1:100; auto-replicate after quant if male component is the target.
- All other samples labeled as BL, AM, CB, F1(EC) or F2(SF) should not be automatically amplified in replicate.

6.13 The Y quantitation value must be used for samples sent to Y-STR amplification.

6.14 Regarding samples to be amplified in PowerPlex Fusion®, if a male/female mixture is indicated and the ratio of M:F DNA is more extreme than 1:50 (i.e., 1:75) for samples to be run on the 3130xL or 1:100 (i.e., 1:150) for samples to be run on the 3500xL, that sample should not be amplified using Fusion initially if the male component is the target profile. Such samples that contain a sufficient amount of male DNA may be sent for Y-STR testing but must first be evaluated by the assigned reporting analysts (RA) for whether or not Y-STR testing is needed.

6.14.1 The minimum sample concentration for PPY23 amplification is 5.72pg/ µL (100pg total male DNA).

7 Interpretation of Y-screening Results

7.1 For SAKs, if no male DNA is indicated (0.00 pg/µL in the Y channel), small items/underwear are present that also tested negative or had no stains present, AND the case is not scheduled for Molecular Serology testing, send the case to QA for possible reanalysis.

7.2 If male DNA positive swabs or stains were found, be mindful that the primary goal in the majority of cases (especially stranger cases) is to develop a database eligible profile. This may be achieved by a single sample in a single assailant case. For single assailant cases, no more than two samples will routinely proceed to amplification.

7.3 **Cases with one male suspect and a female victim**: Following Y-screening, evaluate each case for male presumptively positive samples. The concentration of male DNA and male to female ratio can be used for triage as follows:

7.3.1 For a case with samples that have male quant values and/or male to female ratio as described below, referred to as high male criteria, schedule two samples from the case for recut to differential extraction from either of the below criteria.

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- Male quant value ≥ 11pg/µL that is majority male (50% or more male)
- Samples with Male DNA ≥ 50pg/µL.

When selecting samples that meet high male criteria for differential extraction:

- Orifice swabs and samples from underwear are preferred.
- Choose two samples from different areas of the body, if possible.
- If only one sample meets these criteria, send one sample.
- It is recommended to cut a larger piece of the swab or stain for differential extraction if the male quant is below 50pg/µL.

7.3.2 If a case does not have samples that meet high male criteria schedule all male presumptively positive samples from the case for recut for differential extraction.

- It is recommended to cut a larger piece of the swab or stain for differential extraction if the male quant is below 50pg/µL.

7.4 **Cases with multiple male suspects and/or consensual partner(s), and a female victim**: Following Y-screening, evaluate each case for male presumptively positive samples. The concentration of male DNA and male to female ratio can be used for triage as follows:

7.4.1 Schedule all male presumptively positive samples for recut for differential extraction.

- It is recommended to cut a larger piece of the swab or stain for differential extraction if the male quant is below 50pg/µL.

7.5 **For all sexual assault cases with male suspect(s) and a female victim:** Following differential extraction and/or EZ1 extraction, evaluate the concentration of male DNA and male to female ratio for each sample for each case.

7.5.1 Select the samples with the best (least extreme) male to female ratio to send to amplification, following the guidance in 6.14.

7.5.1.1 In some instances, concordance of the results may require additional amplification of samples:

7.5.1.1.1 If only F2 fraction(s) were chosen for amplification, choose a single F1 fraction for amplification.

7.5.1.1.1.1 Choose the F1 fraction associated with the same sample as an F2 fraction chosen for amplification.

7.5.1.1.1.2 If the F1 fraction associated with the same sample as the F2 fraction chosen for amplification is insufficient at quantification (< minimum total concentration needed for amplification), another F1 fraction can be chosen.

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7.5.1.1.1.3 If the M:F ratio of an F2 fraction that is being sent to amplification is 1:>1, it is not necessary to send an associated F1 fraction.

7.5.2 If possible, select one sample from an orifice swab or from underwear and select samples that are from a different area of the body.

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8 QC SUMMARY FLAGGING GUIDE

Flag	Possible Reason	Resolution
AMPNC	Not Used	-
BADROX	**No Master Mix Added**	**Requant as needed**
BLFAIL	Not Used	-
CTFAIL	Not Used	-
EXPFAIL	Not Used	-
HIGHQT	**Quant Value >99ng/µL**	**If SA value is >99ng/µL: for Y-screening samples that are 0.0 pg/ µL of Male DNA, a requant is not required; all other samples must be requanted at a dilution. If Male (Y) value is >99ng/ µL, a requant at a dilution is required if the sample is going to Y-STR amplification. If LA value is >99ng/ µL, a requant is not necessary.**
HIGHSD	Not Used	-
IPPCT	**IPC <26 or >29**	**Determine rate of inhibition**
	Reagent Inhibition	**Check raw data; consider microcon/sample recut***
LOWQT	Not Used	-
MTFR	**Extreme M:F where male DNA is the target of interest**	**Sample should not be amplified using Fusion; May send sample directly to YSTR amplification, if necessary**
NOAMP	Not Used	-
NOISE	**Sample Not Spun Down**	**Requant**
	Improper Seal	
	Condensation	
	Pipetting errors	
NOSIGNAL	Not Used	-
OFFSCALE	**Fluorescent Contaminant**	**Notify QA/QC**
OUTLIERRG	Not Used	-
R^2	**R^2 <0.98**	**Quant Assay Fails**
Slope	**Slope <-3.0 or >-3.6**	**Quant Assay Fails**
Spike	**Bubbles**	**Requant**
	Seal leak	
THOLDFAIL	**Not Used**	**-**
YINT	**Used**	**Check standard curve reports for targets T.Y, T.Large Autosomal and T.Small Autosomal. Contact QA if the quant passes. QA will need to check the programming of the flags.**

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*Check the raw data to determine if controls and/or samples appear to have amplification (other than the IPC (JUN)) in the MultiComponent Plot. Also check the Amplification Plot to see if any of the curves appear suppressed. If there is no amplification in the Multicomponent Plot and the curves suppressed in the Amplification Plot you may consider a microcon to clean or recutting of samples.

Notify QA/QC immediately if any of the flags that are not used give a value other "0".

9 LIMS Post Processing II

9.1 After all interpretations are made, in the ***Select Drop Down*** » ***Unreleased*** » Click the Release Icon » Click ***Save.***

9.2 In order to send the quantitation values for amplification, hold the ***Ctrl*** key and **Select the Quant Value** applied for each sample by **Clicking the Row**.

9.3 To push the Total Concentration (SA concentration), highlight all the applicable samples and click ***[Push Concentration]***. The screen will refresh and list a value in the ***Concentration*** Column.

9.4 To push the Total Male Concentration, (T.Y.) highlights all applicable samples and click ***[Push Male Concentration].***

9.5 In the Select Drop Down » Select *All* » Click *Test Approval*.

9.6 Click the Green Check Button in the ***Status*** column**.**

9.7 Assign the appropriate next process steps for each sample.

9.8 Click ***Save*** » Fill in E-Sig » Click ***OK*** » Click ***Close***

9.9 When adding samples to an amplification batch, refer to Abbreviations for appropriate suffixes.

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