

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
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MaxSuite Automated DNA IQ™ Extraction from Casework Samples

This extraction is applicable for exemplar samples and all casework evidence samples.

Follow all relevant processes in the [General Guidelines for Forensic Biology and DNA Casework procedure](#).

Follow all relevant processes in the [BEAST DNA Worksheet Setup Manual](#) for creating and adding to worksheets and [BEAST DNA Worksheet Processing Manual](#) for how to record all relevant information while processing the worksheets.

1 General Information

- 1.1 **WARNING:** THE LYSIS BUFFER IN THE DNA IQ KITS IS CORROSIVE AND TOXIC. IT CAUSES SEVERE SKIN BURNS AND EYE DAMAGE AND IS HARMFUL IF INHALED OR SWALLOWED. If on skin: take off all contaminated clothing and rinse with water and soap. If in eyes: rinse with copious amounts of water for several minutes. Keep lysis buffer bottle tightly closed.
 - 1.1.1 The waste tubes containing residual reagents can be discarded in the regular laboratory garbage.
 - 1.1.2 The remaining residual Elution buffer reagents in the reservoirs left over from the previous day can be disposed of in the laboratory sink flushed with copious amounts of water.
 - 1.1.3 However, the remaining residual Lysis Buffer must be collected in a properly labeled waste container and be properly discarded via a chemical waste vendor.
 - 1.1.4 Contact QA to collect waste containers and expired bottles for proper disposal.
- 1.2 **CAUTION:** DO NOT ADD BLEACH OR ACIDIC SOLUTIONS DIRECTLY TO ANYTHING CONTAINING LYSIS BUFFER INCLUDING SAMPLE WASTE. Exposure to strong acid or bleach will result in the generation of toxic gases.
 - 1.2.1 If liquid containing these buffers spills, clean with suitable laboratory detergent and water.
- 1.3 **CAUTION:** DO NOT ADD BLEACH TO ANY PART OF THE MAXPREP® ROBOT INCLUDING THE DECK. Bleach will cause the robot to rust. For any spills on the robot, use 70% ethanol and water for cleanup.
 - 1.3.1 Contact QA for any significant spills within the instrument.

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2 Daily Maintenance: Maxprep

- 2.1 Daily maintenance is to be performed prior to the first run of the day. If needed, refer to [QC191 - Maxprep Maintenance](#). If already completed, continue to Section 3.
- 2.2 Follow the prompts to complete the daily maintenance.
 - 2.2.1 Ensure the Tip Ejector Bar has been removed before maintenance is performed.
 - 2.2.2 Remove reagent reservoirs and discard reagents if present. Refer to step 1.1 for directions on residual reagent disposal. Rinse reagent reservoirs with deionized water and let them fully dry prior to reuse.
 - 2.2.3 If maintenance fails, contact the QA team.
- 2.3 Perform a UV sanitization run for ~20min
- 2.4 Record the daily maintenance in the instrument maintenance log.

3 Sample Incubation

- 3.1 Retrieve sample cuttings in 1.5 mL tubes. Compare the label on the tubes to the worksheet and confirm that you have the correct samples.
- 3.2 Take each sample into your custody.
- 3.3 Obtain one empty 1.5mL CW Microfuge Tube with CW Spin Basket for the extraction negative and label it with the associated extraction negative label. If both Maxwell deck sample trays are being used, also prepare Extraction Negative 2.
- 3.4 Retrieve reagents for the Digest Buffer Master Mix and record the lot numbers. Consult the Mixture Information table for the exact amount of Max CW Extraction Buffer, Max Proteinase K (18mg/ml), and Max 1-Thioglycerol needed to prepare the Master Mix.

NOTE: Vortex the Max Pro-K and Max 1-thioglycerol for 10-15 seconds before aliquoting.

Stock Solution	Per Sample
Max CW Extraction Buffer	286µL
Max Proteinase K (18mg/ml)	10µL
Max 1-Thioglycerol	4µL

Note: 1-Thioglycerol is viscous. Pipet slowly.

- 3.5 Vortex the master mix well.

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- 3.6 Add 300µL master mix to each sample tube and negative control. If necessary, take a clean pipette tip and push the substrate down into the digestion liquid.
- 3.7 Vortex all samples at high speed for five seconds.
- 3.8 Incubate all samples in a Thermomixer set to 56°C (+/-3°C) for 30 minutes without shaking. Record the thermomixer and the temperature reading on the temperature probe.
- 3.9 Record the 'Incubation Run By' review task.

4 Maxprep Pre-Processing Run Setup

- 4.1 Software screenshots are available in the [Manual Appendix for MaxSuite Software](#).
- 4.2 In the Maxprep software, press Start to access the 'Methods' screen. Select the '**Maxwell FSC DNAIQ – Tubes: 40µL Elution Volume v1.2.1**' method, and press Proceed.
- 4.3 Press the 'Run' button on the method run screen to start the run. After the instrument initializes, the door will unlock again and can be opened for loading.
- 4.4 Enter the number of samples using the slider on the software screen. Press 'Next'.

NOTE: If the gantry is blocking a carrier needed for instrument loading, use the 'Move Arm' function first before entering information on the software to prevent an error message. This will slowly move the gantry to the opposite end of the instrument.

- 4.5 If the MaxPrep will be used to place plungers, place the plunger rack onto the appropriate carrier on the instrument. At least 1 plunger per sample is needed. Skip to step 4.6 if plungers will be manually placed into the cartridges.
 - 4.5.1 Select the next available plunger when prompted to enter 'plunger count.' If a full plunger rack is being loaded, the plunger rack can just be checked off.
 - 4.5.2 The second plunger rack does not need to be loaded if the first rack has an adequate number of plungers. The rack will still need to be checked off in the software.
 - 4.5.3 Press 'Next' to proceed to the sample deck tray loading.
- 4.6 If the plungers will be manually placed in the cartridges, do not load plungers into the plunger rack, and load the empty plunger rack into the first deck position. When prompted to enter the 'plunger count', ensure that a full rack is checked on the software.
- 4.7 Set up the Maxwell deck sample tray(s) at your bench
 - 4.7.1 Wipe the sample tray(s) with 70% EtOH and a fresh lint-free wipe.

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- 4.7.2 Obtain one reagent cartridge for each sample and extraction negative and record the lot number.
- 4.7.3 Place the cartridges in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from where the elution tubes will be placed. Press down on the cartridge to snap it into position (the cartridge should audibly click into place). Carefully peel back the seal so that all foil comes off the top of the cartridge. Ensure that all sealing foil and any residual adhesive are removed before placing the sample trays in the instrument.

Caution: Buffers present in the cartridge wells may splash while removing the foil seals. Clean or change your gloves to remove any splashed buffer before continuing to the next step.

- 4.7.4 Place open, empty 0.5mL elution tubes into the elution tube position for each cartridge in the deck tray(s).
 - 4.7.5 Place all tubes loosely into position.
 - 4.7.6 Using a fresh, clean lint-free wipe, press down on the elution tubes to secure them in their position.
 - 4.7.7 Place the prepared deck tray(s) into the appropriate carrier(s) on the Maxprep. Scan the tray barcode(s) when prompted. Check off the tray as having been loaded. Press 'Next.' The scan box for the 'Maxwell RSC Kit Lot' will be left blank.
- 4.8 Retrieve the Lysis Buffer and Elution buffer and record the lot numbers.
- 4.8.1 Press 'Enter Reagent Details' for each buffer. The pop-up will provide the minimum required volume. Prepare the Lysis Buffer and Elution Buffer reservoirs following the required run-specific volumes provided in the Maxprep software. Place reservoirs in the appropriate carrier on the instrument deck.

NOTE: The reagent lots do not need to be recorded in the software, only on the worksheet. Reagent reservoirs should be labeled with the reagent name and reagent lot number. If reagents from a previous run from the same day are present in the reservoir, confirm the amount using a clean graduated cylinder and add the reagent of the same lot number to reach the required volume.

5 Sample Transfer and Maxprep Pre-Processing Run

- 5.1 Remove the tubes from the thermomixer.
- 5.2 **Extraction WITNESS:** Have another analyst verify the order of the CW Microfuge tubes by reading the label for each sample.
 - 5.2.1 Record the 'Extraction Witness'.
- 5.3 Centrifuge the substrates in spin baskets at 13,200 rpm to 15,000 rpm for 2 minutes.

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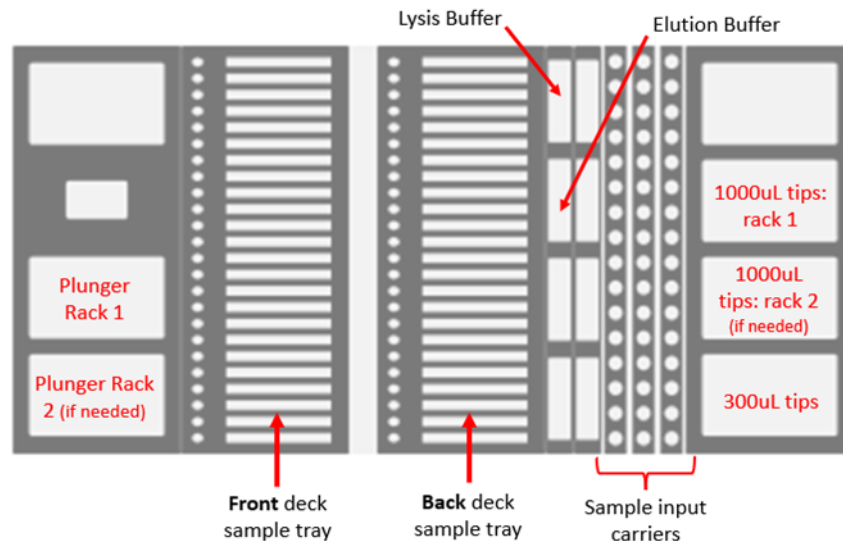
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- 5.3.1 If liquid is still present in the spin basket of any samples, centrifuge only those samples for another 2 minutes.
- 5.3.2 After the 2-minute spin, any liquid remaining in a spin basket can be manually pipetted from the basket to the sample tube.
- 5.4 Using a fresh lint-free wipe, remove and discard the spin baskets (including the swab remains), taking care to avoid bubbles at the rim of the open tube. Close the tube.
- 5.5 Proceed to the sample loading screen. Press ‘Scan,’ wait for the gantry to move into position and remove and replace the first sample carrier. Wait for the gantry to move and repeat removal/replacement for all carriers that will be used. Press ‘Stop’ or wait for the scanning function to time out.
 - 5.5.1 Select ‘**1.5mL Flip Cap Tube**’ tube type for all 3 sample carriers. The tube type must be selected for all sample carriers regardless of the number of samples being run.
 - 5.5.2 Remove the sample carrier to be loaded from the instrument deck. Loading back to front and starting in position S1, scan the label on each closed sample tube before placing it into the carrier. After scanning all samples for each sample carrier, quickly scroll through the carrier sample locations on the computer and verify that a Sample ID was scanned for all positions, and that no stray barcodes had been scanned by mistake.
 - 5.5.3 Once all sample tubes are in position and seated properly, fully open the caps with a tube opener. Replace the carrier with open samples onto the instrument. Repeat for all samples and carriers. Press ‘Next’.
- 5.6 Use the ‘Move Arm’ function to access the tip carrier if needed. Load tips according to software instructions, with the tip tray barcode facing to the left.
 - 5.6.1 At least one 1000uL tip is needed per sample. A total of four 300uL tips will be needed; this is independent of the number of samples in the specific run.
 - 5.6.2 The second rack of 1000uL tips does not need to be loaded if the first rack has an adequate number of tips (you will still need to check off the rack as if loaded in the software).
 - 5.6.3 **NOTE:** If the instrument runs out of tips mid-run, the run will pause, the instrument will unlock, and the software will prompt you to load a new, full tip rack.
- 5.7 **Pre-Processing Robot Setup WITNESS:** Have another analyst verify the instrument setup by confirming the following:
 - 5.7.1 The tip eject bar was properly replaced after Daily Maintenance.
 - 5.7.2 The deck sample trays are fully seated in the carriers.

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- 5.7.3 The sample tubes were opened after loading. Additionally, verify that the samples appear to have been loaded back to front in the sample carriers.
- 5.7.4 A sufficient number of the correct tip sizes were placed in the appropriate locations on the carrier (tip size will be printed on the tip tray barcode).
- 5.7.5 The plunger rack was placed in the appropriate location on the carrier.
- 5.7.6 The plunger rack contains at least one plunger for each sample being loaded on the instrument; or the second full rack is loaded, as necessary.
- 5.7.7 Record the 'Pre-Processing Robot Setup Witness'.

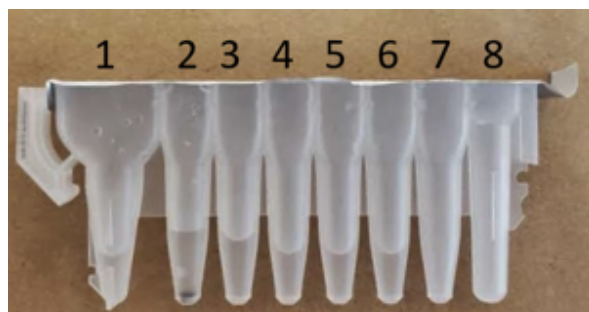


- 5.8 Close the instrument door and press the 'Next' button to start the automated preprocessing of samples.
- 5.9 After the run is finished, open the instrument door and remove the sample deck trays.
 - 5.9.1 Check that all cartridges have a plunger present in the last well (closest to the elution tube). If a plunger is missing, manually take a plunger from the plunger rack and place it in the appropriate cartridge well using clean gloves and a fresh lint-free wipe.
 - 5.9.2 Check that the 0.5ml elution tubes contain equal amounts of elution buffer. If less than ~40uL appears to be present in any of the tubes, manually add elution buffer to bring the volume up to 40uL. Notify the Lab Supervisor and QA if volumes appear inconsistent.

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- 5.10 If plungers were not loaded onto the MaxPrep for robotic placement, manually place a plunger into well 8 on every cartridge.



6 Maxwell RSC 48 Run

- 6.1 Start the Maxwell® software on the Tablet PC, if it is not already open. The instrument will proceed through a self-check and home all moving parts.
- 6.2 Press 'Start' to begin the process of running a method.
- 6.3 Scan the bar code(s) on the deck sample tray(s). Wait for the data to be returned from the Portal software, verify the correct information has populated (correct tray code and the date modified should match the date of extraction), and press 'Continue.'
- 6.4 Select the '**DNA IQ Casework**' method and press the 'Proceed' button.
- 6.5 On the 'Cartridge Setup' screen verify that both racks populated with all sample IDs. Press 'Proceed.'
 - 6.5.1 When using both deck trays, press the Front and Back buttons to switch between overviews of each deck tray.
- 6.6 The instrument door will open outwards and towards you. Ensure the benchtop in front of the instrument is clear.
- 6.7 After the door has been opened, confirm all points on the Extraction Checklist pop-up before loading the deck sample tray(s).
- 6.8 Insert the Maxwell deck sample tray(s) into the appropriate position.
 - 6.8.1 Hold the deck tray by the sides to avoid dislodging cartridges. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place it into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray

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to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

- 6.9 Make sure elution tubes are open and contain elution buffer before starting the run. Press the 'Start' button to begin the extraction run. The platform will retract, and the door will close.
- 6.10 Wait for the instrument Vision System check to complete. If an error occurs, the software will return to the Cartridge Setup screen.
 - 6.10.1 Press the cartridge position with a red error mark to view and correct the detected error.
 - 6.10.2 After correcting errors, press 'Start' again, and the instrument will repeat the Vision Check before beginning the run.

NOTE: During the run, proceed to section 7 and begin setting up for the Maxprep Post-Processing Run.

- 6.11 After the run, press 'Open Door', ensure no plungers remain on the bar in the Maxwell® Instrument, and remove sample deck tray(s) from instrument.
 - 6.11.1 If plungers are still present on the plunger bar, see Troubleshooting Section 8 'Clean Up' Procedure before removing the sample deck tray(s).
 - 6.11.2 Check that the 0.5ml elution tubes contain equal amounts of elution buffer. If volumes appear inconsistent, give the tray a gentle tap on the bench and proceed with the post-processing run.



- 6.12 Close the door and return to the home screen.
- 6.13 Select 'Sanitize' and tap 'Start'. This will run the UV lamp for 20 minutes.

7 Maxprep Post-Processing Run Setup

- 7.1 Remove all tip racks from the deck and store partial racks in their appropriate trays within the drawer.
- 7.2 Remove the plunger box, cover the top with parafilm, and store it in the drawer.
- 7.3 Ensure the reagent reservoirs and their caps are labeled with the buffer reagent name and lot number. Cap the reagent reservoirs and leave them on the instrument for future runs that day.
- 7.4 Discard flip cap sample tubes.

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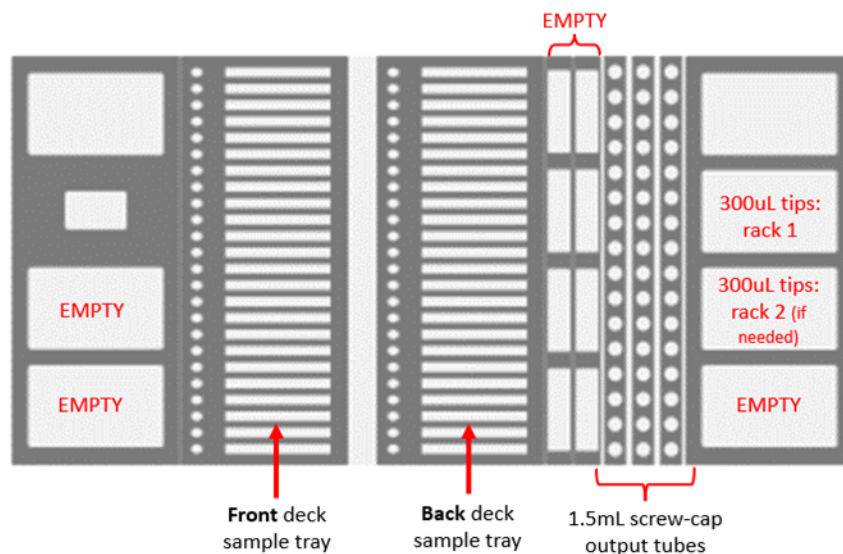
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- 7.5 Print a copy of the labels and prepare 1.5mL elution tubes with labels and no caps. Cover the open tubes with a fresh lint-free wipe, if needed.
- 7.6 Return to the home screen in the Maxprep software. Press 'Start'. Select the '**Sample Transfer: 40uL transfer**' v1.4.0 method. Press 'Proceed,' close the instrument door, and press 'Run.'
- 7.7 Enter the labware types:
- 7.7.1 Input Labware type: **Maxwell RSC 48 Trays**.
 - 7.7.2 Destination Labware type: **Samples in Tubes**.
 - 7.7.3 Press 'Next'.
- 7.8 Scan the barcode of the deck sample tray(s). Press 'Query Portal'.
- 7.8.1 Verify the number of samples imported at the top of the pop-up matches the number of samples being run.
 - 7.8.2 Press 'Exit'
- 7.9 Use the arrow keys on the keyboard to quickly scroll through the sample positions. Verify all positions imported a Sample ID and check that the Sample IDs are from the correct extraction. Press 'Next'.
- 7.10 Load the instrument:
- 7.10.1 The first carrier should be empty. Press 'Next'.
 - 7.10.2 Load the sample deck tray(s) into the appropriate Front/Back carrier position.
 - 7.10.2.1 Check off the tray on the software to verify it was loaded. Press 'Next'.
 - 7.10.3 Load the uncapped and labeled 1.5mL screw-top tubes onto the sample carriers:
 - 7.10.3.1 Press 'Scan', wait for gantry to move into position and remove and replace the first sample carrier, then wait for gantry to move back and repeat for all carriers that will be used. Press 'Stop' on the computer. Select '**Sarstedt 1.5mL tube**' type for all 3 sample carriers.
 - 7.10.3.2 Remove the first sample carrier. Loading back to front, manually scan each output tube and place it loosely into position. Once all output tubes for that carrier are scanned and placed, use a fresh lint-free wipe to press the tubes into the correct positioning in the carrier. Repeat for all samples and carriers. Press 'Next'.

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- 7.10.3.3 After scanning all samples for each sample carrier, quickly scroll through the carrier on the computer and verify that all sample IDs scanned, and no stray barcodes had been scanned by mistake.
- 7.10.4 Use 'Move Arm' if needed and then load tips.
- 7.10.4.1 Only one (1) 300uL tip is needed per sample. The second rack of 300uL tips does not need to be loaded if the first rack has an adequate number of tips (you will still need to check off the rack as loaded in the software).
- 7.10.4.2 The 50uL tips do not need to be loaded. Check off the tips in the software. Press 'Next'.
- 7.11 **Post-Processing Robot Setup WITNESS:** Have another analyst verify the instrument setup by confirming the following.
- 7.11.1 Front/Back sample deck trays are loaded in the correct carriers.
- 7.11.2 Screw-cap output tubes are loaded into the sample carriers. Additionally, verify that the samples appear to have been loaded back to front in the sample carriers.
- 7.11.3 A sufficient number of the correct tips were placed in their appropriate location on the tip carrier.
- 7.11.4 Record the 'Post-Processing Robot Setup Witness'.



- 7.12 Close the instrument door and press 'Next' to start the run.

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7.13 After the run is complete:

7.13.1 Remove one carrier at a time from the instrument deck. Leaving the samples in the carrier, place a clean cap onto each tube. Remove the tubes from the carrier and ensure the cap is fully closed before storing samples.

7.13.2 Create a reference tube with 30uL of 0.1X TE-4 and compare each extract to the reference tube.

7.13.2.1 If the volume appears lower than 30uL, check the corresponding 0.5ml elution tube to ensure the extract was properly transferred from the tube. If there is volume remaining in the 0.5ml tube, manually transfer the remaining volume to the storage tube.

7.13.2.2 If the 0.5ml elution tube is empty, measure the extract in the storage tube. Extraction volumes below 30ul should be documented in the Pre/Post Issue log and in the sample comments field on the worksheet . Samples with sufficient volume for quantification and amplification may be sent on as necessary. QA should be notified for volumes below 30uL.

7.13.3 Place the partial rack(s) of tips into the appropriate tray within the drawer.

7.13.4 Clear out the labware in the deck sample tray(s).

7.13.4.1 Discard the plungers into the sharps waste.

7.13.5 Carefully remove the cartridges and 0.5mL elution tubes. They can be discarded into regular waste.

NOTE: The cartridges may splash when being pulled from the tray. Place a lint-free wipe or paper towel over the open cartridges during removal if needed.

7.13.6 Wipe the deck sample tray(s) with 70% ethanol before storing them in the drawer.

7.14 Verify all labware and samples were removed from the instrument deck. Close the door and navigate to the home screen.

7.15 Record the 'Extraction Run By' review task and 'response' dropdown for the 'Pass or Fail?' step.

7.16 Transfer custody of all extracts to a cryobox and store in a refrigerator or freezer.

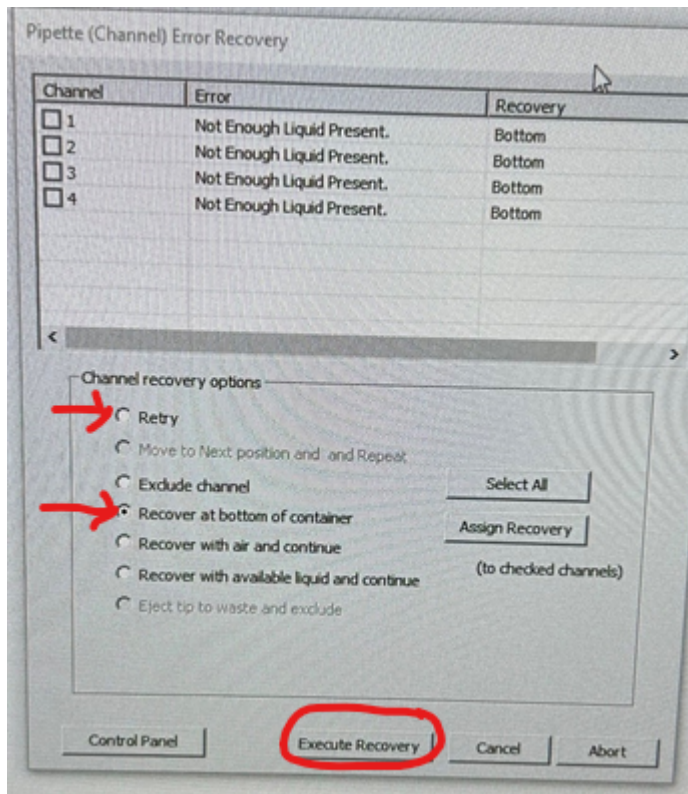
7.17 Assign samples to next process step.

8 Troubleshooting

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- 8.1 If the following error message occurs, ensure the proper amount of lysis buffer is present in the reservoir. Add more buffer, if necessary. Select 'Retry' and click 'Execute Recovery'. If the error continues to occur, select 'Recover at bottom of container' and 'Execute Recovery'. Alert the QA Team when these messages occur so their frequency may be monitored and recorded.



8.2 Pausing or Aborting a Maxprep Run

- 8.2.1 If for any reason you need to pause the instrument during a run, select the 'Pause' button. The instrument will finish the current step, and the door will unlock, allowing you access to the inside of the instrument. When you are finished with the pause, close the instrument door and select the 'Resume' button to resume the run.
- 8.2.2 If you need to abort the method for a run currently in progress, select the 'Abort' button. When aborting a run, it is recommended to first select the 'Pause' button to pause the instrument run, and then select the 'Abort' button.
- 8.2.2.1 The 'Abort' function used on its own will not immediately stop the run. The instrument will complete the step that is in progress before ending the run.

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- 8.2.2.2 If there is a need to perform an immediate stop of the Maxprep during a run, use the power button on the instrument to power it off. The method run will be aborted, and the doors will unlock.
- 8.2.3 If the 'Abort' function is used or the instrument is manually powered off during a run, QA should be contacted for troubleshooting.
- 8.3 Maxwell 'Clean Up' Procedure
- 8.3.1 If a method has been aborted, press the 'Open Door' button. The Vision system will determine whether plungers have been unloaded successfully, and if not, will attempt to unload them. Otherwise, the 'Clean Up' screen will be displayed.
- 8.3.2 The 'Clean Up' screen requests the user check if plungers are still engaged on either the front or back plunger bar. If the plungers are not engaged, remove the deck trays from the instrument and press the 'Skip Clean Up' button to continue. On pressing the 'Skip Clean Up' button, you will be presented with the extraction report.
- 8.3.3 If some or all the plungers are still engaged on the front or back plunger bar, press the 'Start Clean Up' button to eject the remaining plungers. Do not attempt to remove and reinsert the deck samples tray(s). Samples and cartridges have a risk of splashing during removal/insertion of the trays, so all unnecessary handling should be avoided.
- 8.3.4 After the 'Clean Up' is successful, you can press the 'Open Door' button and remove the deck tray. If the plunger 'Clean Up' fails, you should contact QA for further assistance.
- 8.4 If Maxwell tablet is not responsive, restart the tablet by holding down the power button. Once off, hold the power button until the tablet turns back on. Restart the Maxwell instrument by holding the power button for 3 seconds. Once off, push the power button to turn it back on.
- 8.5 Switching from MaxSuite to Maxwell Extraction Procedure
- 8.5.1 If the Maxprep instrument is offline for an extended period of time, and casework will be using the Maxwell Automated DNA IQ Extraction from Casework Sample protocol:
- 8.5.1.1 Turn off the Portal Access system on all Maxwells being used for the procedure.
- 8.5.1.2 Ensure the Maxwell Demonstration video is available in Qualtrax or on the network.
- 8.5.1.3 Have QA Make aliquots of the MaxElution Buffer reagent.