

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 1 OF 13

MaxSuite Automated DNA IQ™ Extraction from Casework Samples

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.
- 1.4 This extraction is applicable for exemplar samples and all casework evidence samples EXCEPT suspected semen samples. Refer to the Evidence Examination manual to determine the appropriate sample size to submit for extraction.
- 1.5 Do not extract evidence samples and exemplars together.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 2 OF 13

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](#).
- 2.2 If already completed, continue to Section 3.
- 2.3 Ensure the Tip Ejector Bar has been removed before maintenance is performed.
- 2.4 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.
- 2.5 Follow the prompts to complete the daily maintenance.
 - 2.5.1 If maintenance fails, contact the QA team.

2.6 Perform a UV sanitization run for ~20min

- 2.7 Fill in the maintenance log in LIMS for the instrument to reflect that the daily maintenance has been completed.

3 Sample Incubation

- 3.1 Obtain one empty 1.5mL CW Microfuge Tube with CW Spin Basket for the extraction negative and label as Extraction Negative 1. If both Maxwell deck sample trays are being used, also prepare Extraction Negative 2. Obtain labeled cuttings in 1.5mL CW Microfuge tubes with CW Spin Baskets.
- 3.2 Prepare digest buffer master mix as per the calculated amounts in the Reagents tab in LIMS. Vortex the Max Pro-K and Max 1-thioglycerol for 10-15 seconds before aliquoting. Record the lot# of each reagent in LIMS.

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.

- 3.3 Vortex the master mix well.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 3 OF 13

- 3.4 Add 300µL of digest buffer master mix to each of the sample tubes including the extraction negative tubes. If necessary, take a clean pipette tip and push the substrate down into the digestion liquid.
- 3.5 Vortex all samples at high speed for five seconds. Incubate at 56°C for 30 minutes without shaking in the thermomixer.
- 3.6 Record the temperature of the thermomixer in LIMS. The temperature should be within $\pm 3^{\circ}\text{C}$ of 56°C.

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. This will slowly move the gantry to the opposite end of the instrument.

- 4.5 Place plungers onto the appropriate carrier on the instrument. At least 1 plunger per sample is needed.
 - 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 Set-up the Maxwell deck sample tray(s) at your bench
 - 4.6.1 Wipe the sample tray(s) with 70% EtOH and a fresh lint-free wipe.
 - 4.6.2 Record the cartridge lot number in LIMS. 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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 4 OF 13

cartridge. Ensure that all sealing foil and any residual adhesive are removed before placing the deck 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.

- 4.6.3 Place open, empty 0.5mL elution tubes into the elution tube position for each cartridge in the deck tray(s).
- 4.6.4 Place all tubes loosely into position.
- 4.6.5 Using a fresh, clean lint-free wipe, press down on the elution tubes to secure them in their position.
- 4.7 Place the prepared deck tray(s) into their 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 Prepare the Lysis Buffer and Elution Buffer reservoirs, following the required run-specific volumes provided in the Maxprep software. 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. Place reservoirs in the appropriate carrier on the instrument deck.
 - 4.8.1 Press ‘Enter Reagent Details’ for each buffer. The pop-up will provide the minimum required volume. The reagent lots do not need to be recorded in the software – all reagents should be logged in LIMS.

5 Sample Transfer and Maxprep Pre-Processing Run

- 5.1 Remove the tubes from the thermomixer. Print one copy of the LIMS output labels. Save the label strip for the extraction witness step.
- 5.2 Have a witness verify the order of the CW Microfuge tubes by reading the entire input sample ID number for each sample.
 - 5.2.1 The witness also must verify the order of the LIMS output sample labels by reading the entire output sample ID number on each printed label, beginning at the bottom of the label strip.
 - 5.2.2 This is recorded as your “*extraction*” witness.
- 5.3 Cover the input sample labels with their corresponding output labels.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

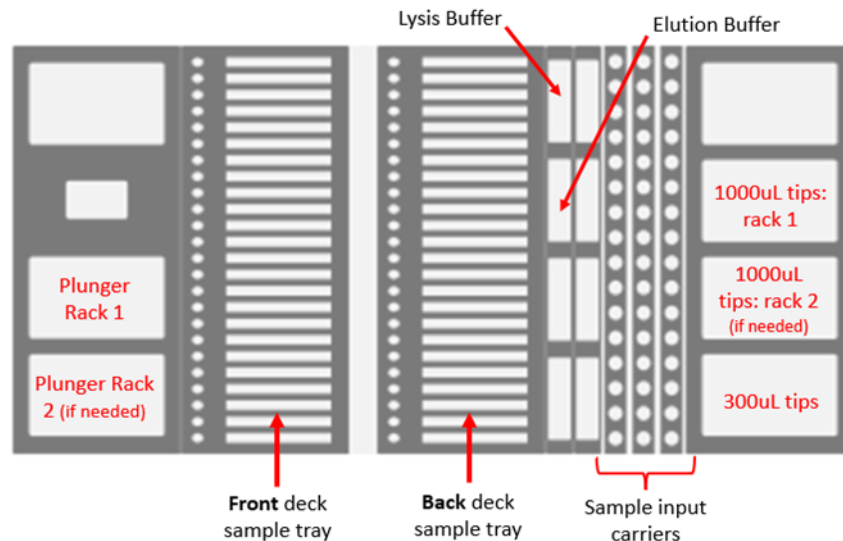
MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 5 OF 13

- 5.4 Centrifuge the substrates in spin baskets at 13,200 rpm to 15,000 rpm for 2 minutes.
- 5.4.1 If liquid is still present in the spin basket of any samples, centrifuge only those samples for another 2 minutes.
- 5.4.2 After the 2 minute spin, any liquid remaining in a spin basket can be manually pipetted from the basket to the sample tube.
- 5.5 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.
- 5.6 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.
- 5.6.1 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.
- 5.6.2 Remove the sample carrier to be loaded from the instrument deck. Loading back to front and starting in position S1, scan the LIMS output 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 an S-ID scanned for all positions, and that no stray barcodes had been scanned by mistake.
- 5.6.3 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’.
- 5.7 Load tips according to software instructions, with the tip tray barcode facing to the left. 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.
- 5.7.1 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).
- 5.7.2 Use the ‘Move Arm’ function to access the tip carrier if needed.
- 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.
- 5.8 Have a witness verify the “robot setup” by confirming the following:
- 5.8.1 The tip eject bar was properly replaced after Daily Maintenance.
- 5.8.2 The deck sample trays are fully seated in the carriers.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 6 OF 13

- 5.8.3 The sample tubes were opened after loading. Additionally, verify that the samples appear to have been loaded back to front in the sample carriers.
- 5.8.4 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).
- 5.8.5 The plunger rack was placed in the appropriate location on the carrier.
- 5.8.6 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.
- 5.8.7 This is recorded as your “*pre-processing robot setup*” witness.



- 5.9 Close the instrument door and press the ‘Next’ button to start the automated preprocessing of samples.
- 5.10 After the run is finished, open the instrument door and remove the sample deck trays.
 - 5.10.1 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.
 - 5.10.2 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.

6 Maxwell RSC 48 Run

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 7 OF 13

- 6.1 Start the Maxwell® software on the Tablet PC, if 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 S-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 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 setup for the Maxprep Post-Processing Run.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 8 OF 13

- 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 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 sample input flip cap tubes.
- 7.5 Print a copy of the LIMS output labels. Prepare 1.5mL elution tubes with labels and no caps. Cover 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’.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

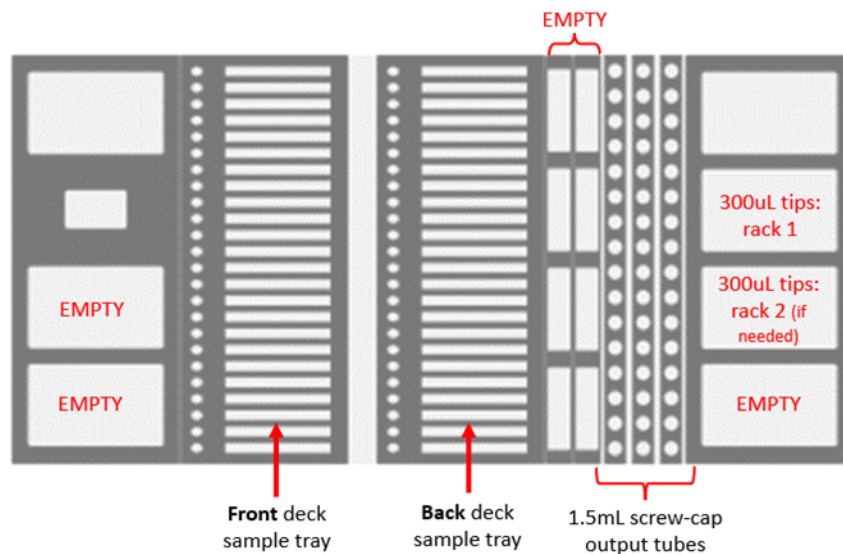
MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 9 OF 13

- 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 an S-ID and check that the S-IDs are from the correct date. 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 destination output tubes (uncapped and labeled 1.5mL screw-top tubes).
 - 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.'
 - 7.10.3.3 After scanning all samples for each sample carrier, quickly scroll through the carrier on the computer and verify that all S#s scanned, and no stray barcodes had been scanned by mistake.
 - 7.10.4 Load tips, pressing 'Move Arm' if needed.
 - 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 Have a witness verify the "robot setup" by confirming the following. This is recorded as your "**post-processing robot setup**" witness.
 - 7.11.1 Front/Back sample deck trays are loaded in the correct carriers.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 10 OF 13

- 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.



- 7.12 Close the instrument door and press 'Next' to start the run.
- 7.13 After the run is complete:
- 7.13.1 Remove one carrier at a time from the instrument deck. Leaving the output 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 PrePost Issue log and the batch comments. 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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 11 OF 13

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.

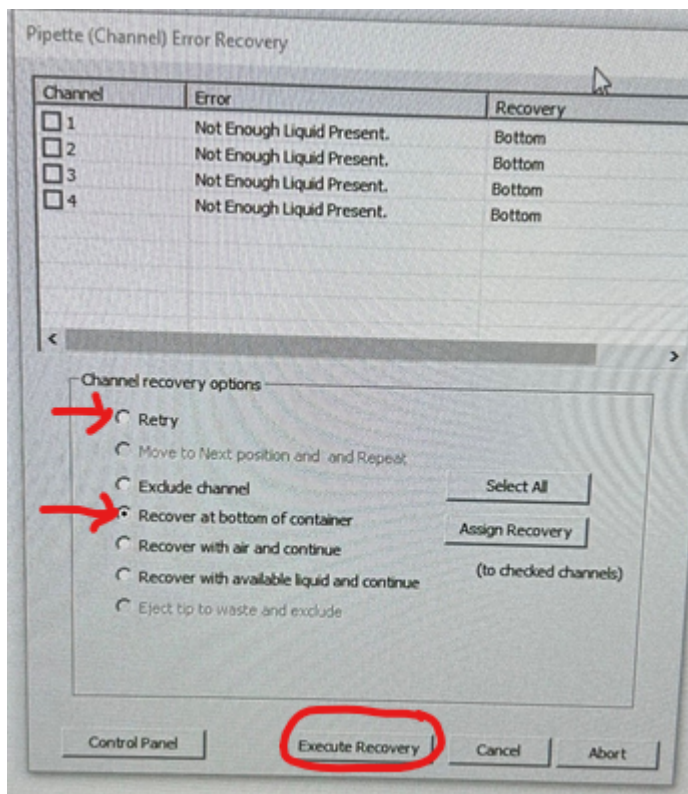
NOTE: The cartridges may splash when being pulled from the tray. Place a lint-free wipe or paper towel over the open cartridges during removal if needed.

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.

8 Troubleshooting

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.



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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 12 OF 13

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.
 - 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 of 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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

MaxSuite Automated DNA IQ Extraction from Casework Samples		
Status: Published		Document ID: 88338
DATE EFFECTIVE 11/12/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 13 OF 13

- 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.