Deviation Request Form (DRF)

Directions: The Initiator will complete Sections A through C. Additional continuation pages can be included if necessary.

Initiator	Timothy Baize	**Date**	2-7-2019

A. Requested deviation applies to (Technical Procedure – include specific section):

Procedure for DNA Extraction using the EZ1 Advanced XL Sections 5.4, 5.4.1, 5.7.1.2.6

B. Requested deviation:

5.4 Change title to "**Preprocessing of Samples with Identified/Possible Sperm"**
Adjust wording of **5.4.1 "**Aseptically place the sample into a labeled microcentrifuge tube. "
Adjust wording **5.7.1.2.6** Open the EZ1 Advanced XL door and wipe the piercing units and tray using isopropanol or other approved decontamination solution.

C. Necessity for the deviation:

The section title change is due to processing changes with direct to DNA cases. The wording in 5.4.1 allows for tubes other than 1.5 ml tubes to be used based on section needs and ordering. The wording change corresponds with the current cleaning solution and allows for a future change of decontamination solutions.

D. Technical review and Authorization (to be completed by the Quality Manager and/or Technical Leader)
Comments(to include merits and impacts):

No inherent negative impact and allows for multiple tube types to be utilized. It also allows for a change in decontamination solutions without further changes to the procedure.

Approved	☑	Yes	☐	No	Duration	1 year or revision
Signature	Mackenzie G DeHaan	Digitally signed by Mackenzie DeHaan DN: cn=Mackenzie DeHaan, o=NC SCL, ou=FB, email=mdehaan@ncdoj.gov, c=US Date: 2019.02.07 15:54:21 -05'00'			Date	2/7/19

E. Quality Assurance Authorization (to be completed by the Quality Manager, Forensic Scientist Manager or designee)

Acceptable within general QA guidelines and good laboratory practice?	☑	Yes	☐	No
Significant negative impact to Crime Laboratory Quality System?	☐	Yes	☑	No

Restrictions/limitations:

☑	Authorized	☐	Rejected	Signature	Jody H. West	Digitally signed by Jody H. West DN: cn=Jody H. West, o=DOJ, ou=State Crime Laboratory, email=jwest@ncdoj.gov, c=US Date: 2019.02.08 12:33:49 -05'00'	Date	2/8/19

Form approved for use by: Timothy G. Suggs — Digitally signed by Timothy G. Suggs DN: cn=Timothy G. Suggs, o=North Carolina State Crime Laboratory, ou=Quality Manager, email=tsuggs@ncdoj.gov, c=US Date: 2014.12.19 09:01:52 -05'00'

Procedure for DNA Extraction using the EZ1 Advanced XL

1.0 Purpose – This procedure specifies the steps for performing DNA extractions using the EZ1 Advanced XL BioRobot.

2.0 Scope – This procedure applies to Forensic Scientists in the Forensic Biology Section who perform DNA extractions for forensic casework.

3.0 Definitions – See Section Definition List

4.0 Equipment, Materials, and Reagents

- Stain Extraction Buffer (SEB)
- Tris/EDTA Solution (TE)
- Tris/EDTA/NaCl Solution (TEN)
- Dithiothreitol (DTT)
- 3M NaOAC, pH 5.0
- 0.5M EDTA
- Calibrated pipets (various sizes)
- ART Pipet Tips (or equivalent, various sizes)
- Qiagen EZ1 Advanced XL BioRobot
- Qiagen EZ1 DNA Investigator Kit (reagent cartridge, ProK, carrier RNA)
- Thermomixer
- Autoclaved microcentrifuge tubes (various sizes)
- Autoclaved Spin Ease Baskets (or equivalent)
- 2 mL tubes with a lyse & spin basket
- Sterile tubes (various sizes)
- Microcon 100 Filters and corresponding centrifuge tubes (or equivalent)
- Vortex mixer
- Various lab equipment (various disposable conical tubes, lab tape, lab coat, lab gloves, microcentrifuge tubes and racks, wipes, etc)
- 10 % Bleach solution

5.0 Procedure

5.1 Overview

5.1.1 All known samples shall be extracted separately from unknown samples. Thermomixers shall be designated exclusively for either “knowns” or “unknowns.”

5.1.2 For casework unknowns, a portion of each swab present shall be cut for analysis. The amount of sample taken shall be determined based upon the scientist's evaluation of the sample (e.g. amount of staining, sperm quantity recorded).

5.1.3 Make a master mix containing the pre-processing reagents using the volumes list for the sample type (allowing for 1-2 extra samples for pipetting variation). Reagents will be added to the sample tubes from this master mix and not from the stock or aliquot bottles.

5.1.4 No more than 30 total samples (knowns and unknowns) shall be extracted as a batch. Exemptions to this item limit shall be approved only with written documentation from the Technical leader.

5.1.5 Negative extraction control

5.1.5.1 For each case, a reagent blank shall be prepared each time an extraction set is begun (i.e., knowns and unknowns.) This blank will consist of the reagents used in the extraction process and shall be treated the same as other samples throughout the entire process. Also, the final volume of this control shall be the same as the forensic sample(s) brought up in the most minimal volume and amplified using the maximum volume.
Note: If hair roots are processed within an extraction set, a separate reagent blank shall be prepared.

5.1.5.2 Due to the amount of sample needed for an extraction (i.e. number of swabs, size of the cutting) it may be necessary to split the extraction of a sample across multiple extraction tubes. In such instances, multiple reagent blanks will need to be created. At the point when the separate extractions of the sample are recombined, prior to amplification, the same number of reagent blanks must also be combined. This ensures that the same quantity of reagents have been processed in the reagent blank as the given sample.

5.1.5.3 It is acceptable to run more than one reagent blank in anticipation of having to re-run or dilute samples for amplification.

5.1.5.4 If additional extractions are performed, the associated negative extraction controls shall have a unique identifier (different date will suffice as identifier).

5.1.6 All tubes shall be labeled with a unique identifier.

5.2 Preprocessing of Known Samples

5.2.1 Aseptically place the sample into a labeled 1.5 mL microcentrifuge tube or a lyse & spin basket with a 2 mL tube.

5.2.2 To the sample, add 480 µL of SEB, 20 µL of Proteinase K solution, and 1 µL of carrier RNA. Vortex briefly on low speed.

5.2.3 Incubate the samples for approximately 1 to 2 hours (based upon the type of sample and not to extend overnight) at 56 °C in a thermomixer set to approximately 700 rpm. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.2.4 If using a 2 mL tube with a lyse & spin basket, spin in a microcentrifuge at high speed for at least 30 seconds to activate the basket and force the extraction fluid into the tube. If any liquid remains in the basket, repeat spin. Remove the basket and discard it into a biohazard waste container.

5.2.5 If using a 1.5 mL tube, spin briefly in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the sample(s) into a basket insert. Place the basket back in the tube containing the stain extract and cap the tube. Spin in a microcentrifuge at high speed for at least 30 seconds, repeating if liquid remains in the basket. Remove the basket and discard it into a biohazard waste container.

5.2.6 Proceed to **5.7** to continue with Protocol: DNA Purification (Large Volume).

5.3 Preprocessing of Hair Roots

5.3.1 Hair roots are cut and placed in either a 1.5 mL tube or a 2 mL screw cap tube by the Trace Evidence Section and transferred to the Forensic Biology Section.

5.3.2 To the sample, add 160 µL of SEB, 20 µL of Proteinase K solution, 20 µL of DTT and 1 µL of carrier RNA. Vortex briefly on low speed and spin briefly in a microcentrifuge to force the cutting into the extraction fluid.

5.3.3 Incubate the samples for approximately 1-2 hours, not to extend to overnight (based upon the type of sample) at 56 °C in a thermomixer. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.3.4 Spin briefly in a microcentrifuge to force condensation into the bottom of the tube.

5.3.5 Proceed to **5.7** to continue with Protocol: DNA Purification (Trace).

5.4 Preprocessing of Samples with Identified Sperm

5.4.1 Aseptically place the sample into a labeled 1.5 mL microcentrifuge tube.

5.4.2 To the sample, add 480 µL of SEB, 20 µL of Proteinase K solution and 1 µL of carrier RNA. Vortex briefly on low speed.

5.4.3 Incubate the samples for approximately 2 hours, not to extend overnight at 56 °C in a thermomixer set to approximately 700 rpm. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.4.4 Spin briefly in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the material to a basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at high speed for 30 seconds and make sure all the liquid has transferred out of the catch basket. Remove the basket and discard it into a biohazard waste container.

5.4.5 If the Forensic scientist is authorized to use the QIACube instrument, they shall utilize the instrument for the separation and should continue with Step 5.4.14 . If the Forensic scientist is not authorized to use the QIACube instrument, they shall continue processing of the sample as below.

5.4.6 While being careful not to disturb the pelleted material, remove the supernatant fluid from the original tube and place it into a new labeled tube. This supernatant is the non-sperm fraction. Analysis of the non-sperm fraction shall then be continued in **5.7** following Protocol: DNA Purification (Large Volume).

5.4.7 Wash the sperm pellet by adding 1000 µL of TEN, vortex the suspension briefly, and spin the tube in a microcentrifuge at maximum speed for 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the sperm pellet.

5.4.8 Repeat the step above two to three times for a maximum of four washes to the sperm pellet. The number of washes depends on the quantity of sperm identified by the body fluid analysis.

5.4.9 To the tube containing the washed pellet, add:

160 µL SEB
10 µL Proteinase K
40 µL DTT
1 µL carrier RNA

5.4.10 Close the tube caps, mix thoroughly by vortexing for 10 seconds, and spin in a microcentrifuge to force all the fluid and material to the bottom of the tubes.

5.4.11 Incubate the samples for 10 minutes at 70 °C in a thermomixer set to approximately 700 rpm.

5.4.12 Spin the sample tube in a microcentrifuge to force all the fluid to the bottom of the tube. DNA from the sperm fraction can now be purified from this tube.

5.4.13 Proceed to **5.7** to continue with Protocol: DNA Purification (Trace).

5.4.14 QIACube Instrument

5.4.14.1 Label the adaptor, 1.5 mL microcentrifuge tube, and 2mL flat bottom tube with at least the QIACube centrifuge bucket number, which corresponds with the DNA workbook. Secure the 1.5 mL microcentrifuge tube and lid into the adaptor and make sure the tube and lid are flush.

5.4.14.2 While pipetting up and down to dislodge any pellet which may have formed, transfer the liquid to its clean corresponding 1.5 mL microcentrifuge tube within the adaptor.

5.4.14.3 Turn on the QIACube, ensure the current lot of STR-SEB is filled, lid is off and placed in well 1, DiH2O is filled, lid is off, and placed in well 2 in the reagent bottle rack, and the proper tips are filled.

5.4.14.4 Place the 2mL flat bottom tubes, without lids into the shaker which corresponds to the DNA workbook set up. Place the rubber stopper (or tape can be used) to cover the shaker slots. The 2mL flat bottom tubes will be reported as Fraction 1 (formerly the non-sperm fraction).

5.4.14.5 Add the adaptors to the corresponding buckets in QIACube centrifuge. Double check to make sure all tubes correspond with the workbook and bucket positions, all lids are off, adaptors are flush in the buckets, and tips are full. Close the QIACube lid.

Note: Once the lid is closed and a program has started DO NOT Open the lid. This will cause the program to stop and will have to be re-started back to the beginning. If the program is still running, note the message code and report it to the DNA technical leader for further direction.

5.4.14.6 Select either the 12A or 6 sample program. Follow touch screen prompts until the selected program starts. If the 12A program is selected, when the program stops, remove the 2mL flat bottom tubes and place in a rack. Refill the tips and select the 12B program.

5.4.14.7 Once Fraction 1 (formerly non-sperm fraction) has been removed, proceed to **5.7** to continue with Protocol: DNA Purification (Large Volume).

5.4.14.8 The samples located within the 1.5 mL microcentrifuge tube, within the adaptors, are considered Fraction 2 (formerly the sperm fraction). Aseptically remove the adaptors one at a time. Aseptically remove the 1.5 mL microcentrifuge tube from each adaptor, place in a tube rack, and discard the adaptor into a biohazard waste container.

5.4.14.9 Prepare a slide for microscopic examination. Slides will be made only for items that did not have an existing smear/slide.

5.4.14.9.1 Label slides and slide mailer with the case number and item number. Mark a circle on the slide with a wax pencil where the sample will be added.

5.4.14.9.2 Pipette up and down to disturb the pellet, and add 5µL of the liquid to the corresponding slide.

5.4.14.9.3 Place on a hot plate until dry and place it in the labeled slide mailer. Do not package slides from multiple cases in the slide mailer.

5.4.14.10 Make a master mix for the number of Fraction 2 tube samples using the volumes list (allowing for 1-2 extra samples for pipetting), vortex for approximately 10 seconds, and spin briefly in a microcentrifuge.

160 µL SEB
10 µL Proteinase K
40 µL DTT
1 µL carrier RNA

5.4.14.11 Aseptically pipette 211µL into each Fraction 2 tube, secure the lids, and vortex.

5.4.14.12 Incubate the samples for 10 minutes at 70 °C in a thermomixer set to approximately 700 rpm.

5.4.14.13 Spin the sample tube in a microcentrifuge to force all the fluid to the bottom of the tube. DNA from fraction 2 can now be purified from this tube.

5.4.14.14 Proceed to **5.7** to continue with Protocol: DNA Purification (Trace).

5.5 Preprocessing of Unknown Samples

5.5.1 Aseptically place the sample into a labeled 1.5 mL microcentrifuge tube or a lyse & spin basket with a 2 mL tube.

5.5.2 To the sample, add 480 µL of SEB, 20 µL of Proteinase K solution, and 1 µL of carrier RNA. Vortex briefly on low speed.

5.5.3 Incubate the samples for approximately 2 hours, not to extend overnight at 56 °C in a thermomixer set to approximately 700 rpm. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.5.4 If using a 2 mL tube with a lyse & spin basket, spin in a microcentrifuge at high speed for at least 30 seconds to activate the basket and force the extraction fluid into the tube. If any liquid remains in the basket, repeat spin. Remove the basket and discard it into a biohazard waste container.

5.5.5 If using a 1.5 mL tube, spin in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the cutting into a basket insert. Place the basket in the tube containing the stain extract and cap the tube. Spin in a microcentrifuge at high speed for at least 30 seconds, repeating if liquid remains in the basket. Remove the basket and discard it into a biohazard waste container.

5.5.6 Proceed to **5.7** to continue with Protocol: DNA Purification (Large Volume).

5.6 Preprocessing of Bone and Teeth

5.6.1 Sample Preparation – shall be performed in a Biological Safety Hood

5.6.1.1 Dried Bone (no marrow or associated tissue)

5.6.1.1.1 Cleaning the bone.

5.6.1.1.1.1 Small bones: If the bone is heavily soiled, it may be necessary to remove any debris or associated dirt from the bone or part of the bone prior to cutting/grinding. The bone shall be placed in a weigh boat and a new toothbrush and sterile dH2O or SEB used to physically remove any excess dirt.

5.6.1.1.1.2 Large bones: The area of the bone to be cut and used in analysis shall be ground off mechanically using a Dremel tool (cleaned with 10 % bleach) and a new grinding bit to remove soil and dirt.

5.6.1.1.2 Remove a cross-sectional wedge or rectangle of bone using a cutting tool (e.g., Dremel tool with a cut-off wheel). Do not cut the bone in half unless necessary due to size; this preserves the bone for further anthropological study.

5.6.1.2 Bone with Associated Tissue and Marrow

5.6.1.2.1 If the tissue and marrow are not too degraded, take a sample of tissue and marrow and place in separate labeled microcentrifuge tubes for DNA extraction.

5.6.1.2.2 Cleaning the bone

5.6.1.2.2.1 Using a cutting tool (e.g., sterile scalpel blade or Dremel tool with a cut-off wheel) remove any associated tissue on the bone to be processed. Note: It is helpful to remove the tough fibrous membrane, the periosteum, prior to processing because the removal aids in the extraction process.

5.6.1.2.3 Removal of bone section for DNA extraction using one of the following methods.

5.6.1.2.3.1 Remove a cross-sectional wedge or rectangle of bone using a cutting tool (e.g., Dremel tool with a cut-off wheel).

5.6.1.2.3.2 Using a decontaminated drill bit, drill 4 to 5 holes through the bone.

5.6.1.2.3.3 Collect the powder produced from the drilling for analysis.

5.6.1.3 Tooth

Wash the tooth. It may be necessary to remove debris and/or associated dirt from the tooth. The tooth should be placed in a weigh boat and thoroughly washed with dH2O or SEB. A new toothbrush shall be used to remove any debris or dirt.

5.6.2 Produce a Fine Powder from the Bone or Tooth

5.6.2.1 Obtain a new coffee grinder.

5.6.2.2 Wipe down inside and outside with a Kim-wipe wetted with fresh 10 % bleach. Wipe down a second time with 100 % alcohol. Allow to dry.

5.6.2.3 Place the sample into the cleaned grinder and grind the sample to a fine powder. Note: for small bones that are too small for the coffee grinder, a mortar and pestle may be used to grind the bone to a powder for DNA extraction.

5.6.3 DNA Extraction

5.6.3.1 Aseptically transfer 0.5 g to 3 g of powder to a 2 mL, 5 mL or 15 mL conical tube. Place any excess bone or tooth powder into a separately labeled tube and save.

5.6.3.2 To the sample add the following:

675 µL SEB
75 µL Proteinase K
750 µL 0.5M EDTA

5.6.3.3 Mix by inverting the 2 mL, 5 mL or 15 mL tube several times.

5.6.3.4 Incubate the tube containing the sample at 56 °C for 24 to 48 hours (based upon sample type), inverting the tube several times during incubation to mix the sample.

5.6.3.5 Divide the liquid from each digested sample into up to three (3) 2 mL screw cap tubes. Each tube should contain approximately 500 µL.

5.6.3.6 While the sample is still warm, add the following:

30 µL 3M NaOAC, pH 5.0
1 µL carrier RNA

5.6.3.7 Vortex\ and spin in a microcentrifuge to force the condensate to the bottom of the tubes.

5.6.3.8 Proceed to **5.7** to continue with Protocol: DNA Purification (Large Volume).

5.6.3.9 Once the DNA purification protocol has been completed on the EZ1 Instrument and the sample tubes have been removed, prepare a Microcon 100 concentrator (or equivalent) by labeling the concentrator and centrifuge tube and wet the membrane with 20 µL TE buffer.

5.6.3.10 Transfer the eluted sample from the sample tubes containing the sample to the concentrator.

5.6.3.11 Cap the concentrator and spin in a microcentrifuge at low speed (i.e., no greater than 4000 rpm) for 10 minutes.

5.6.3.12 Remove the spin cap and add 50 µL TE to the concentrator. Remove the concentrator from the filter cup and carefully invert onto a new labeled 1.5 mL flip-top

microcentrifuge tube. Discard the corresponding centrifuge tube containing the filtrate into a biohazard waste container.

5.6.3.13 Centrifuge the assembly at 4000 rpm for 5 minutes.

5.6.3.14 Remove and discard the concentrator.

5.7 Operation of the EZ1 Advanced XL BioRobot

5.7.1 Cleaning/Maintenance – All cleaning/maintenance tasks shall be documented on the Forensic Biology Section EZ1 Cleaning/Maintenance Form (located with the instrument). The QCO shall retain such information in the QC files with the specific instrument cleaning/maintenance records. The forensic scientist shall ensure that the Cleaning/Maintenance form is updated prior to use. If the instrument is not used for a week (e.g. due to being out of service) the Forensic Scientist assigned as instrument operator shall ensure that the reason is documented in the comments block on the Cleaning/Maintenance form.

5.7.1.1 UV Decontamination

5.7.1.1.1 Prior to use of the EZ1 Advanced XL for a batch, each Forensic Scientist shall perform a UV decontamination run as follows:

5.7.1.1.2 Switch on the EZ1 Advanced XL at the rear power switch.

5.7.1.1.3 Ensure the EZ1 Advanced XL door is closed. In the main menu, press "1" to select the UV light function.

5.7.1.1.4 Use the keys "0" through "9" to set the duration of the decontamination time to 30 minutes. Note: The default is 30 minutes. Pressing "ESC" will abort the procedure and return you to the main menu. After setting a valid time, press the "ENT" key.

5.7.1.1.5 Press "START" to switch on the UV lamp. The worktable will move slowly back and forth under the UV light. For user safety, the UV lamp cools for approximately 3 minutes. The EZ1 Advanced XL cannot be opened until after the cooling time has elapsed. After the cooling period, press the "ESC" key to return to the main menu.

5.7.1.2 Daily Cleaning (Post-Use) - After each protocol run, the piercing unit of the pipettor head and the worktable shall be cleaned by the Forensic Scientist as follows:

5.7.1.2.1 After removing the sample elution tubes, remove sample preparation waste (sample tubes, reagent cartridges, and filter tips), and discard in a biohazard waste container.

5.7.1.2.2 Close the EZ1 Advanced XL door.

5.7.1.2.3 Press "2" in the main menu to select the manual function.

5.7.1.2.4 Press "3" to choose the "Clean" operation.

5.7.1.2.5 Press "START". The EZ1 Advanced XL will lower the piercing unit.

5.7.1.2.6 Open the EZ1 Advanced XL door and wipe the piercing units and tray using ethanol.

5.7.1.2.7 Close the EZ1 Advanced XL door and press "ENT". The piercing unit returns to its original position.

5.7.1.2.8 Press "ESC" to return to the main menu.

5.7.1.2.9 Open the EZ1 Advanced XL door. Clean the racks with ethanol.

5.7.1.2.10 A new protocol can now be performed or the instrument can be switched off.

5.7.1.3 Weekly Maintenance

5.7.1.3.1 During weeks where the instruments are in use, , the Forensic Scientist shall apply silicon grease to the end of a filter-tip after ensuring the completion of the daily cleaning.

5.7.1.3.2 Apply silicon grease to the surface of the O-rings.

5.7.1.3.3 Place the tip onto the pipettor head, and rotate the tip on the pipettor head to distribute the silicon grease evenly. Note: The filter-tip should sit flush against the upper white plastic bar if the O-rings are properly greased. There should not be a gap.

5.7.2 Operation of the EZ1 Advanced XL for DNA Purification

5.7.2.1 If fewer than 14 samples are being purified, the reagent cartridges, sample tubes, elution tubes, and filter-tips may be loaded in any order on the rack. However, tips and tubes must be in line with the reagent cartridges.

5.7.2.2 Switch on the EZ1 Advanced XL at the rear power switch. After initialization, the main menu appears.

5.7.2.3 Setup the worktable to perform the appropriate DNA Purification Protocol.

5.7.2.3.1 Remove the tip rack and cartridge rack from the worktable.

5.7.2.3.2 Load the reagent cartridges.

5.7.2.3.2.1 Invert the reagent cartridges several times to mix the magnetic particles. Use the same number of reagent cartridges as the number of samples to be processed.

5.7.2.3.2.2 Tap the reagent cartridges until the reagents are deposited at the bottom of the wells.

5.7.2.3.2.3 Slide the reagent cartridges into the cartridge rack in the direction of the arrow until you feel resistance. Press down the cartridges until they click into place.

5.7.2.3.2.4 Return the cartridge rack to the worktable.

5.7.2.3.3 Load the elution tubes, filter-tips, and sample tubes.

5.7.2.3.3.1 Place the appropriate number of filter-tips into the tip holders.

5.7.2.3.3.2 Load the elution tubes, tip holders containing filter-tips and sample tubes into the sample rack in the following order:

Row 4: Sample Tube (containing digested sample)
Row 3: Empty
Row 2: Tip holder containing filter-tip
Row 1: Elution tube

Ensure that the elution tubes, tip holders containing filter-tips and sample tubes are loaded in the same order as the reagent cartridges.

Note: If the sample tube is a flip-top tube, the top shall be cut off prior to placing it onto the instrument.

5.7.2.3.3.3 Return the tip rack to the worktable.

5.7.2.3.3.4 Close the instrument door.

5.7.2.4 Starting and Finishing a Protocol Run.

5.7.2.4.1 Press "START" on the control panel. (If asked to "create a report file" choose "ESC".)

5.7.2.4.2 Press "1" (Trace Protocol) or "3" (Large Volume).

5.7.2.4.3 Press "2" to elute the samples in TE buffer. Then select the appropriate elution volume for the set of samples being run by pressing "2" (50), "3" (100) or "4" (200).

5.7.2.4.4 Press "ENT" to proceed through the text shown on the display, ensuring the worktable is setup as described.

5.7.2.4.5 Press "START" to begin the purification procedure.

5.7.2.4.6 When the protocol ends, the display shows "Protocol finished."

5.7.2.4.7 Press "ENT" to continue. Open the instrument door.

5.7.2.4.8 Retrieve the elution tubes containing the purified DNA and cap with screw caps. The DNA is ready to use, or can be stored refrigerated at 2-8 °C until quantitation.

5.7.2.4.9 Press "ESC" twice to return to the main menu. Clean the instrument following steps in section **5.7.1.2**.

5.8 Concentration of Extracted DNA

NOTE: This procedure may be used if (1) the original final volume of the DNA extract, based on the training and experience of the Forensic Scientist, leaves the extract too diluted to obtain a DNA profile or (2) if the sample amount for an item required the use of multiple tubes and the final product shall be concentrated into one final tube. If the final volume is less than the final volume of the associated negative extraction control, the control shall also be concentrated using the steps below.

5.8.1 Vortex the extracted DNA and centrifuge briefly at maximum speed (5 seconds).

5.8.2 Wet the membrane of a new, labeled Microcon 100 concentrator (or equivalent) with TE. Transfer the extracted DNA to the concentrator.

5.8.3 Cap the concentrator and spin in a microcentrifuge at 4000 rpm for 10 minutes.

5.8.4 Remove the spin cap and add a measured volume of TE. The TE cannot be less than 20 µL and is dependent on the results of the previous quantitation. If the sample could be processed for Y-STR analysis, bring it up in no less than 50 µL of TE unless there is original evidence remaining. Remove the concentrator from the corresponding microcentrifuge tube and invert the concentrator onto a labeled microcentrifuge tube. Discard the corresponding centrifuge tube.

5.8.5 Spin the assembly in a mirocentrifuge at 4000 rpm for 5 minutes.

5.8.6 Discard the concentrator. Cap the microcentrifuge tube.

5.9 Storage of DNA Extracts – Store the samples at 4 °C (short term) or frozen (long term.) Prior to use of samples after storage, they shall be vortexed and then centrifuged for 5 seconds.

6.0 Limitations – N/A

7.0 Safety

7.1 Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers may produce toxic fumes.

7.2 Use caution when performing the daily cleaning. Piercing units are sharp.

8.0 References

Forensic Biology Section Procedure for DNA Casework Training

9.0 Records

Forensic Biology Section Extraction worksheet (to be used in QC)

Forensic Biology Section EZ1 Cleaning/Maintenance Form

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/25/2013	1	Original Document
03/07/2014	2	5.7 – reorganized section to group cleaning/maintenance tasks; 5.7.1 – added documentation requirement; 5.7.1.3 - changed wording to maintenance; 9.0 – added EZ1 cleaning form as record
04/18/2014	3	3.0 – updated unknown definition; 5.1 – added wording to clarify setup of extractions; 5.7.1.1.1 – clarified when UV would be performed; 5.7.2.4.1 – added wording to match computer prompts; 5.8 – added wording to allow combining of sample tubes
08/29/2014	4	5.1.4.3 – added requirement to uniquely identify additional controls; 5.2.2, 5.4.2, 5.5.2 – clarified wording; 5.4.6 – removed specific tube type; 5.7.1.2.6, 5.7.1.2.9 – removed water wipe; 5.7.1.2.10 – removed wiping o- rings
02/27/2015	5	5.1.4.3 – clarified unique identifier; 5.2.2 – adjusted volumes for consistency; 5.2.6 – adjusted procedure protocol used; 5.4.2, 5.5.2- removed centrifugation step; 5.4.11 – removed minimum time; 5.7.1.3 – clarified weekly maintenance requirement
06/22/2017	6	3.0 – moved definitions to section list; 5.1.4.2 – added wording for combining of samples and reagent blanks; 5.4 – changed wording for baskets in use.

03/23/2018	7	5.1.2 – clarified amount of sample to consume; 5.1.3 – replace items with samples; 5.2.3, 5.2.4, 5.2.5, 5.3.3, 5.4.3, 5.4.4, 5.5.3, 5.5.4, 5.5.5 – update wording to make sample types consistent; 5.4.5, 5.4.14 – added wording to use Qiacube instrumentation for sperm sample processing; 5.6.3.12, 5.7.2.4.3, 5.8.4 – update extraction volume; 5.7.1 – added requirement for scientist to verify form has been completed
01/25/2019	8	5.1.3 – add master mix preparation for pre-processing reagents; 5.1.4 added written approval for sample limits; 5.4.14.9 – added wording for when slides are made;