20.1 PURPOSE

To purify genomic DNA from unknown biological samples using the EZ1 DNA Investigator Kit and the EZ1 DNA Tissue Kit on the EZ1 Advanced XL.

20.2 RESPONSIBILITY

DNA Section personnel.

20.2.1 Document the Extraction on **DNA QR-26 (non-differential & hair root) or DNA QR-27 (differential samples)**

20.3 CLEANING EZ1 ADVANCED XL

Note: The reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not use bleach on the EZ1 Advanced XL or to clean up EZ1 reagent spills. If reagents spill, completely soak up liquid with paper towel, then clean area with water, followed by bleach.

Note: When using the EZ1 Advanced XL DNA Investigator Flip Cap Card and Flip cap rack:
Elution tubes can either be 1.5ml flip cap tubes or the EZ1 Elution Tubes.
Sample tubes (used in the EZ1) can either be 2.0ml SPIN tubes or the EZ1 Sample Tubes.

20.3.1 Insert the EZ1 Advanced XL DNA Investigator Flip Cap Card (if using the flip cap racks completely into the EZ1 Advanced Card slot of the EZ1 Advanced XL. Switch on the EZ1 instrument. Prior to and after each extraction run, Press "1" on the main menu to select the UV function. Enter "20" for 20 minute duration. Press "ENT" and then press "START" to turn the UV lamp on.

20.3.2 After each extraction run, discard waste in proper receptacle. Close the instrument door. Press "2" in the main menu to select the manual function. Press "3" to choose the "clean" operation. Press "START". Open the instrument door and carefully wipe the piercing unit using a soft tissue moistened with ethanol or Proprietary Solvent (caution - piercing unit is sharp). Close the instrument door and press "ENT". Clean the cartridge rack and tip rack with a soft tissue moistened with ethanol or Proprietary Solvent. Document on **DNA QR-**

281 EZ1 Maintenance Log. Weekly (+/- 3 days) O-ring wiping/greasing will be documented on **DNA QR-223**.

20.4 SAMPLE PRETREATMENT

20.4.1 Pretreatment of Most Non-Semen Containing Body Fluid and Touch Samples on Solid Substrates

20.4.1.1 Cut out a sample of the stain. The size of the cutting depends upon the quantity and quality of the sample. As needed, cut the stain into smaller pieces and place these in a labeled SPIN tube. Samples are not limited to stains. Samples could be swabs, cuttings, scraping, etc.

20.4.1.2 Make a master mix of n+1 (n = number of samples and corresponding controls):

480µl	Buffer G2 or Extraction Buffer
20µl	Proteinase K (20mg/ml) (Qiagen or in-house)
1µl	cRNA (1µg/µl)

20.4.1.2.1 To each tube add 500µl of master mix.

20.4.1.3 Mix and centrifuge tubes as needed to force samples into extraction buffer.

20.4.1.4 Incubate sample tubes for 15 minutes to 18 hours on thermal shaker set to 56°C and 850rpm.

20.4.1.5 Pulse spin tubes as needed to collect sample condensation to bottom of tube after incubation.

20.4.1.6 After incubation, the liquid is separated from the sample substrate by transferring the solid substrate to a spin basket and centrifuging for 2 minutes at 15,000rpm in a microfuge. Discard spin basket (containing solid substrate). If debris is present in the tube, the liquid may need to be transferred to another tube (minimizing the amount of debris transferred). This will prevent the debris from potentially clogging tips during the extraction process.

20.4.1.7 Add 400µl of Buffer MTL to lysate in the SPIN tube. If using an EZ1 sample tube, immediately transfer lysate/Buffer MTL mixture into a labeled EZ1 Sample Tube. Alternatively, Buffer MTL can be added to the labeled EZ1 Sample Tubes shortly prior to the addition of the lysate. In order to help prevent a precipitant from forming, the Sample Tubes (containing the MTL buffer) may be temporarily stored in 56°C oven until use or Buffer MTL can be pre-heated to 56°C.

20.4.1.8 Go to step 20.5.

20.4.2 Pretreatment of Semen Containing Body Fluid Samples on Solid Substrates[1]

Note: DNA Tissue Reagent Cartridges are to be used for processing A fraction (Epithelial-rich) samples and DNA Investigator Reagent Cartridges are to be used for processing B fraction (Sperm-rich) samples on the EZ1 Advanced XL. A reagent blank will undergo differential separation as all evidentiary samples. You will need an extraction positive and reagent blank-A for the A fraction samples and an extraction positive and reagent blank-B for the B fraction samples. The extraction positive for the B fraction samples should be introduced at step 20.4.2.12.

Note: In the scenario of extracting non-differential samples (using the Investigator Kit) with differential samples, a separate extraction positive and reagent blank shall be used for the non-differential samples.

Note: 1M DTT may be added to a sample (and RB) at the analyst's discretion (e.g., samples that have a p30 positive result and are not undergoing a differential extraction). Depending on the sample type[2], follow protocol for A fraction samples (using the Tissue Kit) or B fraction samples (using the Investigator Kit).

Note: The Tissue Kit can be used at the analyst's discretion (e.g., vaginal or anal swab potentially containing a small quantity of DNA from a male contributor).

Note: For semen stains thought to be single source in origin (e.g., non-intimate samples), minute semen samples, or semen samples from putative aspermic males may be extracted without a differential where appropriate but with the addition of DTT (1M). These samples will follow protocol for B fraction samples using the Investigator Kit (go to step 20.4.2.12).

20.4.2.1 Follow step 20.3: CLEANING EZ1 ADVANCED XL for proper decontamination procedures of the EZ1 Advanced XL.

20.4.2.2 Cut out a sample of the stain. The size of the cutting depends upon the quantity and quality of the sample. As needed, cut the stain into smaller pieces and place these in a labeled SPIN tube. Samples are not limited to stains. Samples could be swabs, cuttings, scraping, etc.

20.4.2.3 Make a master mix of (n+1) for samples and corresponding controls:

[1] including some other sample types (see note 5)

[2] May use Tissue Kit for samples potentially containing a large quantity of female DNA and a small quantity of male DNA (e.g., vaginal or anal swab).

480µl Extraction Buffer
20µl Proteinase K (20mg/ml) (in-house)
1µl cRNA (1µg/µl)

20.4.2.3.1 To each tube add 500µl of master mix.

20.4.2.4 Mix and centrifuge tubes as needed to force samples into extraction buffer.

20.4.2.5 Incubate sample tubes for 15 minutes to 1 hour on thermal shaker set to 56°C and 850rpm.

20.4.2.6 Pulse spin tubes as needed to collect sample condensation to bottom of tube after incubation.

20.4.2.7 After incubation, the liquid is separated from the sample substrate by transferring the solid substrate to a spin basket and centrifuging for 2 minutes at 15,000rpm in a microfuge.

20.4.2.8 Discard spin basket (containing solid substrate). Carefully, transfer the supernatant to an EZ1 Sample Tube or SPIN tube (labeled A fraction), without disturbing the sperm cell pellet. A fraction samples can either be processed immediately (step 20.4.2.14), incubated in the oven or incubated on the thermal shaker with the B fraction.

20.4.2.9 Wash the sperm cell pellet by re-suspending the pellet in 500µl Extraction Buffer.

20.4.2.10 Centrifuge the tube for 2 minutes at 15,000rpm and carefully remove and discard the supernatant, without disturbing the sperm cell pellet.

20.4.2.11 Repeat steps 20.4.2.9 and 20.4.2.10 2-3 times.

20.4.2.12 Make a master mix of (n+1) for B faction samples and corresponding controls:

460µl Extraction Buffer
20µl Proteinase K (20mg/ml) (in-house)
20µl 1M DTT
1µl cRNA (1µg/µl)

20.4.2.12.1 To each tube add 500µl of master mix.

20.4.2.13 Re-suspend sperm pellet and incubate each tube at 56°C for 2 to 18 hours on a thermal shaker set to 56°C and at 850rpm.

20.4.2.14 Add 400µl of Buffer MTL to **ALL** sample tubes. If using EZ1 sample tubes, immediately, transfer each lysate/Buffer MTL mixture into labeled EZ1 Sample Tubes. Alternatively, Buffer MTL can be added to the labeled EZ1 Sample Tubes shortly prior to the addition of the lysate. In order to help prevent a precipitant from forming, the Sample Tubes (containing Buffer MTL) may be temporarily stored in 56°C oven until use or Buffer MTL can be pre-heated to 56°C.

20.4.2.15 To process the **B fraction samples and corresponding controls**, go to step 20.5.

20.4.2.16 To process the **A fraction samples and corresponding controls**, obtain **DNA Tissue Reagent Cartridge**.

20.4.2.17 Label side of EZ1 Elution Tube. Alternatively, if a 1.5ml flip cap tube is used for elution, the tube may be labeled on the side or cap.

20.4.2.18 After the UV process is complete, press "ESC" to return to the main menu. Press "START" to start the protocol setup. Press ESC when asked about data tracking.

20.4.2.19 Press "3" for Large Volume protocol.

20.4.2.20 Press "1" to elute into water and then press "2" for the 50µl elution volume.

20.4.2.21 Press any key to proceed through the text shown on the display and start worktable setup.

20.4.2.22 Open the instrument door.

20.4.2.23 Invert the reagent cartridges twice to mix the magnetic particles. Load the reagent cartridges into the cartridge rack. Ensure that you press down on the cartridge until it clicks into place after you slide it into the rack. Prior to extraction, if cartridges are left in rack for an extended period of time and resin pellets, remove cartridges and repeat this step.

20.4.2.24 Load opened elution tubes into the first row of the tip rack. Load tip holders containing filter-tips into the second row of the tip rack. Load opened sample tubes containing digested sample into the back row of the tip rack.

Note: **MAKE SURE CAPS ARE REMOVED (CUT OFF OF SPIN TUBES) FROM SAMPLE TUBES AND CAPS ARE REMOVED OR OPENED FROM ELUTION**

TUBES PRIOR TO CLOSING INSTRUMENT DOOR AND STARTING PROTOCOL OR INSTRUMENT WILL MALFUNCTION.

20.4.2.25 Close the instrument door.

20.4.2.26 Press "START" to start the purification procedure.

20.4.2.27 When the protocol ends, the display shows "Protocol finished", press "ENTER".

20.4.2.28 Open the instrument door.

20.4.2.29 Check the pipette tips. Make sure the filters are not wet, salty or discolored.

20.4.2.30 Replace elution tube caps (if using the EZ1 elution tubes) or close the caps (if using 1.5ml flip cap tubes) and remove samples from instrument.

20.4.2.31 Check the elution tubes. They should have approximately the same volumes. If no liquid is present in the tube, note on worksheet, and troubleshoot the situation. If necessary, consult a Lead or the Technical Leader.

20.4.2.32 Clean instrument according to 20.3 CLEANING EZ1 ADVANCED XL.

20.4.2.33 To the Microcon unit, add the eluted DNA sample and 400µl of dH_2O.

20.4.2.34 Centrifuge for 10 minutes, or longer as required, in a microfuge at 2300 – 4000rpm (500g-1500g).

20.4.2.35 Add approximately 50µl dH_2O to the sample. Remove the upper reservoir, invert, and place in a labeled tube. Centrifuge for 2 minutes at 2300rpm.

20.4.2.36 Using a sterile pipette tip, determine the volume of the DNA solution. The elution volumes shall be documented manually on DNA QR-26 or QR-27. The volume of the RB must not exceed the volume of any sample. If necessary, add dH_2O to bring samples up to volume of RB.

20.4.2.37 Store DNA at +4°C or -20°C for short-term storage.
Store DNA at -70°C for long-term storage.

20.4.3 Pretreatment of Hair Root Samples

Note: Hair can be cleaned if necessary. See DNA SOP-2 (2.6.1.1) and/or mtDNA SOP-2 (2.6.3).

20.4.3.1 Follow step 20.3: CLEANING EZ1 ADVANCED XL for proper decontamination procedures of the EZ1 Advanced XL.

20.4.3.2 Using a sterile scalpel blade, cut the root and some of the hair shaft for a total length of approximately ½ cm. Place the root portion in a labeled EZ1 Sample Tube or SPIN Tube.

20.4.3.3 Make a master mix of (n+1) for samples and corresponding controls:

180 µl	Buffer G2 or Extraction Buffer
10 µl	Proteinase K (20mg/ml) (Qiagen or in-house)
20 µl	1M DTT
1 µl	cRNA (1µg/µl)

20.4.3.3.1 To each tube add 210µl of master mix.

20.4.3.4 Mix and centrifuge tubes as needed to force samples into extraction buffer.

20.4.3.5 Incubate sample tubes for 1-18 hour on thermal shaker set to 56°C and 850rpm.

20.4.3.6 Pulse spin tubes as needed to collect sample condensation to bottom of tube after incubation.

20.4.3.7 Remove any remaining hair sample.

20.4.3.8 Obtain DNA Investigator Reagent Cartridge.

20.4.3.9 Label the side of the EZ1 Elution Tube. Alternatively, if a 1.5ml tube is used for elution, the tube may be labeled on the side or the cap.

20.4.3.10 After the UV process is complete, press "ESC" to return to the main menu. Press "START" to start the protocol setup. Press ESC when asked about data tracking.

20.4.3.11 Press "1" for Trace protocol.

20.4.3.12 Go to step 20.5.5

20.4.4 <u>Extraction Controls</u>

20.4.4.1 For each extraction set a reagent blank and extraction positive shall be used. If extracting a variety of substrates within a set, the reagent blank shall contain the largest volume of pretreatment buffer in comparison to the sample tubes. **The reagent blank shall be extracted in parallel and concurrently with its respective samples.**

20.5 DNA EXTRACTION ON THE EZ1 ADVANCED XL

20.5.1 Obtain **DNA Investigator Reagent Cartridge**.

20.5.2 Label side of EZ1 Elution Tube. Alternatively, if a 1.5ml tube is used for elution, the tube may be labeled on the side or the cap.

20.5.3 After the UV process is complete, press "ESC" to return to the main menu. Press "START" to start the protocol setup. Press ESC when asked about data tracking.

20.5.4 Press "3" for Large Volume protocol.

20.5.5 Press "1" to elute into water and then press "2" for the 50µl elution volume.

20.5.6 Press any key to proceed through the text shown on the display and start worktable setup.

20.5.7 Open the instrument door.

20.5.8 Invert the reagent cartridges twice to mix the magnetic particles. Load the reagent cartridges into the cartridge rack. Ensure that you press down on the cartridge until it clicks into place after you slide it into the rack. Prior to extraction, if cartridges are left in rack for an extended period of time and resin pellets, remove cartridges and repeat this step.

20.5.9 Load opened elution tubes into the first row of the tip rack. Load tip holders containing filter-tips into the second row of the tip rack. Load opened sample tubes containing digested sample into the back row of the tip rack.

Note: MAKE SURE CAPS ARE REMOVED (CUT OFF OF SPIN TUBES) FROM SAMPLE TUBES AND CAPS ARE REMOVED OR OPENED FROM ELUTION TUBES PRIOR TO CLOSING INSTRUMENT DOOR AND STARTING PROTOCOL OR INSTRUMENT WILL MALFUNCTION.

20.5.10 Close the instrument door.

20.5.11 Press "START" to start the purification procedure.

20.5.12 When the protocol ends, the display shows "Protocol finished", press "ENTER".

20.5.13 Open the instrument door.

20.5.14 Check the pipette tips. Make sure the filters are not wet, salty or discolored.

20.5.15 Replace/close elution tube caps and remove samples from instrument.

20.5.16 Check the elution tubes. They should have approximately the same volumes. If no liquid is present in a tube, note on worksheet, and troubleshoot the situation. If necessary, consult a Lead or the Technical Leader.

20.5.17 Using a sterile pipette tip, determine the volume of the DNA solution. The elution volumes shall be documented manually on DNA QR-26 or QR-27. The volume of the RB must not exceed the volume of any sample. If necessary, add dH_2O to bring samples up to volume of RB. Use the same dH_2O for amplification. Store DNA at +4°C or -20°C for short-term storage. Store DNA at -70°C for long-term storage.

20.5.18 Clean instrument according to 20.3 CLEANING EZ1 ADVANCED XL.

Note: Sample types not listed in protocol can be extracted with approval from TL.

20.6 CONCENTRATION OF DNA SAMPLES

20.6.1 Please refer to DNA WI-32 "DNA Sample Concentration" for detailed guidance.

20.6.2 The purified DNA extract may be concentrated for STR amplification using a Microcon-100 in instances where the limited quantity of DNA recovered from the evidence indicates that it would be appropriate to amplify > than the maximum amplification volume (e.g., 10 µl for IDP) of the purified DNA sample with Lead or TL approval. Document the sample concentration on QR-28 "Concentration Worksheet".

20.6.3 A reagent blank must be processed in the same fashion/at the same time as the evidentiary sample(s) to be concentrated, i.e., concurrent and parallel--the RB must have the same or greater stringency compared to the evidentiary sample.

20.6.4 When multiple RBs are used within an extraction set, each RB must be quantified and at least one must be amplified if any of the samples associated with the extraction set are amplified. If multiple RBs are used and quantified within an extraction set, at a minimum, the RB that demonstrates the greatest signal (if any) must be amplified and characterized.

20.6.5 In extraction sets where it is anticipated that samples could be concentrated, 2 RBs will be processed with the set, given that concentration may consume an entire RB.

20.6.6 Add the appropriate volume of sample/RB to the Microcon unit and ~ 400 µl of dH_2O to each Microcon.

20.6.7 Centrifuge for ~ 10 minutes, or longer as required for sample concentration at 2300-4000 rpm (500g-1500g). After sample concentration, elute in approximately 10-20 µl of dH_2O as appropriate for the amplification(s).

Note: Additional dH_20 wash(es) through the Microcon may be performed if PCR inhibitors are a concern.