20.1 PURPOSE

To purify genomic DNA from unknown biological samples using the EZ1 DNA Investigator 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. It is recommended that the piercing unit be clean prior to extraction as well. Weekly (+/- 3 days) O-ring wiping/greasing will be documented on DNA QR-223.

20.4 SAMPLE PRETREATMENT

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

Note: 20 µl 1M DTT will be added to any sample containing human tissue, being sure to also add to that samples corresponding reagent blank.

20.4.1.1 If not previously prepared, 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 labelled EZ1 Sample Tube. Alternatively, Buffer MTL can be added to the labelled 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 at 56°C until use or Buffer MTL can be pre-heated to 56°C.

20.4.1.8 To process the samples and corresponding controls, go to step 20.5.

20.4.2 Pre-treatment of Semen Containing Body Fluid Samples on Solid Substrates

Note: 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 with differential samples in the same set, the RB-A and EP1A will also serve as the reagent blank and extraction positive control for non-differential samples as well as for the epithelial-rich samples.

Note: 1M DTT may be added to a sample (and RB) at the analyst's discretion (e.g., samples that have a p30 or male screen positive result and are not undergoing a differential extraction).

Note: 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).

20.4.2.1 If not previously prepared, 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.

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

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

20.4.2.2.1 To each tube add 500µl of master mix.

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

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

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

20.4.2.6 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.

20.4.2.7 Discard spin basket (containing solid substrate). Carefully, transfer the supernatant to an EZ1 Sample Tube or SPIN tube (labelled 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.

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

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

20.4.2.10 Repeat steps 20.4.2.8 and 20.4.2.9 two to three times.

20.4.2.11 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)

20.4.2.11.1 To each tube add 500µl of master mix.

20.4.2.12 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.

20.4.2.13 Add 400µl of Buffer MTL to all sample tubes. If using EZ1 sample tubes, immediately, transfer each lysate/Buffer MTL mixture into labelled EZ1 sample tubes. Alternatively, Buffer MTL can be added to the labelled 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.

20.4.2.14 To process the samples and corresponding controls, go to step 20.5.

20.4.3 Pre-treatment of Hair Root Samples

Note: **Hair can be cleaned if necessary:** For hair samples which have been mounted or may indicate surface contamination other than semen, the hair should be cleaned as follows: Transfer the hair into a 1.5 mL plastic tube filled with approximately 1 mL of Histoclear and sonicate for 20 minutes. Briefly rinse the hair with approximately 1 mL 100% ethanol, followed by 1 mL dH_2O. Transfer the hair into a 1.5 mL plastic tube filled with approximately 1 mL of 5% Terg-a-zyme™ at room temperature and sonicate for 20 minutes. Briefly rinse the hair with approximately 1 mL dH_2O and repeat the previous sonication step in fresh 5% Terg-a-zyme™. Additional sonicated washes using fresh aliquots of Terg-a-zyme™ solution or Histoclear can be performed at the analyst's discretion. Briefly rinse the

hair with approximately 1 mL 100% ethanol, followed by 1 mL dH_2O. Unmounted hair samples which show no signs of surface contamination shall be cleaned by transferring the hair into a 1.5 mL plastic tube filled with approximately 1 mL of 5% Terg-a-zyme™ at room temperature and sonicated for 20 minutes then briefly rinsed with approximately 1 mL 100% ethanol and then by 1 mL dH_2O. Additional sonicated washes using fresh aliquots of Terg-a-zyme™ solution can be performed at the analyst's discretion.

For hairs submitted in cases where there is any indication of the presence of semen (i.e. sexual assault cases), the hair will be consumed, or the analyst believes it to be necessary the following cleaning procedure shall be used: place a portion of the hair (root end if available) in a 1.5 mL plastic tube filled with approximately 1 mL of 5% Terg-a-zyme™ pre-heated to 56°C and incubate at 56°C for 30 minutes. Briefly rinse the hair with approximately 1 mL 100% ethanol, followed by 1 mL dH_2O. Transfer the rinsed hair into a 1.5 mL plastic tube filled with approximately 1 mL of Histoclear and sonicate for 20 minutes. Briefly rinse the hair again with approximately 1 mL 100% ethanol, followed by 1 mL dH_2O. Transfer the hair into a 1.5 mL plastic tube filled with approximately 1 mL of 5% Terg-a-zyme™ preheated to 56°C and sonicate for 20 minutes. Briefly rinse the hair with approximately 1 mL dH_2O and repeat the previous sonication step in fresh warmed 5% Terg-a-zyme™. Additional sonicated washes using fresh aliquots of Terg-a-zyme™ solution at 56°C or Histoclear can be performed at the analyst's discretion. Briefly rinse the hair with approximately 1 mL 100% ethanol, followed by 1 mL dH_2O.

20.4.3.1 If not done previously, 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.

20.4.3.2 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)

20.4.3.2.1 To each tube add 210µl of master mix.

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

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

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

20.4.3.6 Remove any remaining hair sample.

20.4.3.7 Obtain DNA Investigator Reagent Cartridge.

20.4.3.8 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.

20.4.3.9 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.

20.4.3.10 Press "1" for Trace protocol.

20.4.3.11 Go to step 20.5.5

20.5 DNA EXTRACTION ON THE EZ1 ADVANCED XL

20.5.1 Obtain **DNA Investigator Reagent Cartridge**. Note: where appropriate, the cartridges have been pre-heated to minimize the formation of precipitates during the extraction process. Visually inspect each cartridge before use. Do not use the cartridge if you see a precipitate in any well and inform the Equipment/QC Coordinators.

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 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 rack. Load tip holders containing filter-tips into the second row of the rack. Load opened sample tubes containing digested sample into the back row of the 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 Visually check the volumes in the elution tubes. They should have the same volume (50µL) with the RB being at the most stringent volume. If a discrepancy is seen, manually check the volumes of all tubes with a pipette to ensure that the RB is at the most stringent volume and record each volume on the QR worksheet. If adjustments need to be made with dH_2O, note this on the QR worksheet. If no liquid is present in a tube, note on worksheet, and troubleshoot the situation (see below). Notify the Equipment Coordinator regarding any EZ1 issues. If necessary, consult a lead, the DNA Technical Leader, or Assistant Director/Deputy Director.

20.5.16.1 EZ1 troubleshooting. When the EZ1 elution tube volume is significantly reduced (e.g., 0-20 µl), and the DNA resides either somewhere in the cartridge or in the sample tube, the following approach may be employed to recover additional DNA:

A. Re-run the impacted sample in the EZ1 using the same cartridge and sample tube.

B. Make a master mix of extraction buffer, ProK, and cRNA as described above for an additional RB and EP1. Add master mix to the RB and EP1. MTL buffer is added to the controls, but not to the sample tube.

C. Run EZ1 using the standard protocol discussed above.

D. If troubleshooting steps are required, it will be documented on DNA QR-26 or QR-27.

Consult a lead, the DNA Technical Leader, or Assistant Director/Deputy Director should other EZ1 issues arise.

20.5.17 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.

20.6 CONCENTRATION AND PURIFICATION OF DNA SAMPLES

20.6.1 The following is general guidance for sample concentration. Other approaches may be employed as appropriate with TL approval. All approvals regarding sample concentration for STR amplification will go through the DNA TL, Assistant Director and Deputy Director. No approval is necessary for Y-STR concentration, since workflow in SOP-34 dictates when Y-STR testing is appropriate.

20.6.2 The analyst must evaluate the potential impact of sample concentration on the complete set of samples in the extraction set. Consider the number/volume of RBs, the number of different amplifications anticipated for the set and which amplification systems would be more critical/appropriate for the cases. Consult with your lead and/or TL as warranted and obtain approval as stated above. In general, no-suspect case samples should not be concentrated unless sample concentration is expected to lead to sufficient profile results for CODIS entry (SDIS or NDIS). Samples may be concentrated in suspect cases with approval from all parties as stated above.

20.6.3 To minimize the number of RBs, case management/the DNA analyst should batch samples and types of samples to be concentrated as much as possible.

20.6.4 To conserve as much DNA as possible, the samples to be concentrated will be quanted after extraction (before sample concentration). The amount of sample to be concentrated and the eluate volume after concentration is based on the quant results and the amplification(s) required.

20.6.5 The EZ1 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 of the purified DNA sample, with TL and or AD/DD approval. Document the sample concentration on

DNA-QR-28 Concentration Worksheet. Note; if potential PCR inhibitors are a concern and concentration is not indicated, the extract (known or question) may be purified using the Microcon-100 protocol listed below without sample concentration, i.e., elute in 50 µl of dH_2O.

20.6.6 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.

20.6.6.1 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.

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

20.6.6.3 In the event that another sample in an extraction set has previously been concentrated (e.g., to maximum stringency such that the RB has been consumed and amplified using the same STR system as the current concentration/amplification event), a manipulation blank must be created to account for the processing steps associated with the current concentration.

20.6.6.4 The sample shall not be concentrated if the RB is consumed (except as stated above) or missing. If the target volume of sample to concentrate is greater than the amount of RB remaining, the sample may be concentrated using up to the same volume as the RB. If it can be determined that evaporation/sublimation caused the volume of the RB to be less than the sample volume, the sample may still be concentrated. For this determination, the analyst must review the other cases in the extraction set to determine how much RB was consumed.

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

20.6.8 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.

20.7 SWAB MATERIALS FROM ONE SAMPLE SPLIT INTO TWO TUBES

20.7.1 In order for swab materials to be properly incubated in extraction buffer and to fit into a spin basket, evidentiary swab materials are processed in two separate tubes.

20.7.2 The sample tubes are extracted separately as described in 20.4 - 20.5.

20.7.3 To bring the final volume back to 50 µl for each sample, the pair of elutes is combined and re-eluted in 50 µl of dH_20 using a Microcon as described in 20.6 (final volume to ~50 µl).

20.7.4 The reagent blank within the batch is processed with the exact same additional manipulation steps, parallel and concurrent with the sample—2 reagent blanks are processed separately as described in 20.4 – 20.5 and the elutes are combined and the final elution volume is adjusted to 50 µl using a Microcon as described in 20.6 (final volume ~50 µl).

20.7.5 The reagent blank which will go through the Microcon step also serves as the reagent blank for the other samples in the same set/batch which will not go through the Microcon step, since it is more stringent than the other samples.