19.1 PURPOSE

To purify and amplify genomic DNA from known and single source biological samples using the EZ1/EZ2 DNA Investigator Kit on the EZ1 or EZ2 Advanced XL and appropriate amplification system. Quantification of known samples is not necessary using this protocol due to the validated normalization procedure.

Note: Single Source Samples can also be processed using the EZ1/EZ2 DNA Investigator Kit on the EZ1/EZ2 Advanced XL without the normalization procedure. Quantification of the DNA is necessary if not following the normalization protocol.

Note: When using the EZ1/EZ2 **Advanced XL DNA Investigator Flip Cap Card and Flip cap rack: Elution tubes can either be 1.5ml flip cap tubes or the** EZ1/EZ2 **Elution Tubes.**
Sample tubes (used in the EZ1 or EZ2) can either be 2.0ml SPIN tubes or the EZ1/EZ2 **Sample Tubes.**

Note: The Qiagen EZ1 and EZ2 instruments utilize the same reagents and tubes. Any reference to the EZ1 reagents and supplies may also be used on the EZ2.

19.2 RESPONSIBILITY

DNA Section personnel.

19.2.1 Document the Extraction on DNA QR-25. Refer to DNA WI-05 for control details.

19.3 CLEANING EZ1/EZ2 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 or EZ2 ADVANCED XL OR TO CLEAN UP EZ1 REAGENT SPILLS. If reagents spill, completely soak up liquid with paper towel, and then clean area with water, followed by bleach.

19.3.1 EZ 1 Pre-Run Setup

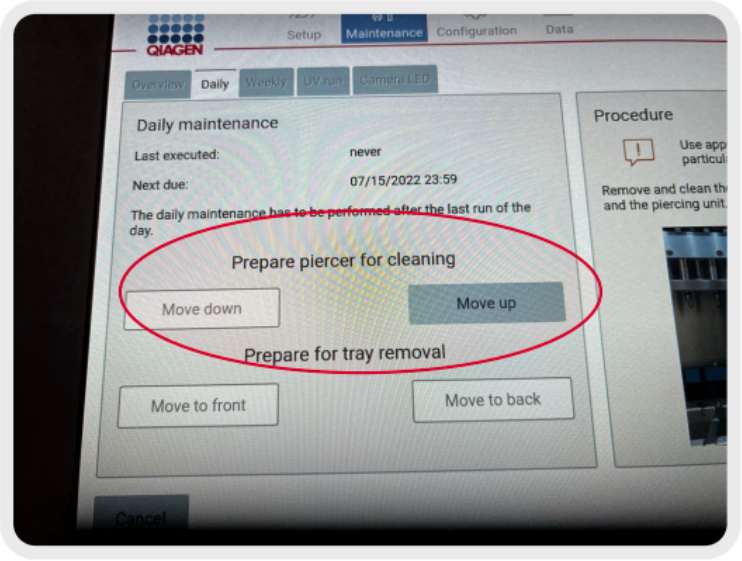
19.3.2 Prior to and after each extraction run on the EZ1, Press “1” on the main menu to select the

UV function. Select a 20-minute duration. Press “ENT” and then press “START” to turn the UV lamp on.

19.3.3 After each extraction run, discard waste in the 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 (the 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.

19.3.4 EZ2 Pre-Run Setup

19.3.5 Similar requirements in EZ1 maintenance, but different user pathway (see below). Select Maintenance to run the UV run. UV run is done before and after runs. Document cleaning on DNA QR-281 EZ1/EZ2 Maintenance Log. (May also refer to DNA SOP-20 for more on the EZ2)



19.4 SAMPLE PREPARATION

19.4.1 Buccal/Blood Swabs

19.4.1.1 Remove swab from applicator shaft.

19.4.1.2 Transfer swab to a labeled 2ml EZ1 Sample Tube or 2ml SPIN tube. Label the side of the tube.

19.4.2 Body Fluid Stains on Fabric, FTA Card or Filter Paper

19.4.2.1 Punch out four 3mm diameter discs or cut out ~1cm x 1cm area from the fabric, FTA card or filter paper. If needed, a larger area can be used with proper documentation.

19.4.2.2 Transfer discs or cutting to a labeled 2ml EZ1 Sample Tube or a SPIN Tube. Label the side of the tube.

19.5 SAMPLE PRETREATMENT

19.5.1 Pretreatment for up to 200µl of Whole Blood

19.5.1.1 Transfer up to 200µl of each blood sample into an EZ1 Sample Tube. Bring the volume up to 300µl with Buffer G2.

19.5.1.2 Go to step 19.6.

19.5.2 Pretreatment for Buccal/Blood Swabs and Body Fluid Stains on Fabric, FTA Card or Filter Paper (all samples besides whole blood)

19.5.2.1 Make 300µl master mix of Buffer G2, dH_2O and Proteinase K for N = n+ 1 samples (where n is the number of samples to be digested including RB and extraction positive).

Note: **For each extraction set a reagent blank and extraction positive shall be used. The reagent blank and extraction positive shall contain the same master mix and incubated in the same equipment as the samples.**

19.5.2.1.1 300µl master mix: Combine the following reagents in a tube for n + 1 samples:

145µl Buffer G2

145µl	dH_2O	Per sample
10µl	Proteinase K (found in kit)	

Mix well.

Add **300µl** of above master mix to each EZ1 Sample Tube or SPIN tube. Please note that although the extraction positive may be a whole blood aliquot, internal validation has shown that using the above master mix with whole blood extraction positive controls yields successful results.

19.5.2.2 Mix tubes gently and spin tubes briefly as needed to force substrate into buffer.

19.5.2.3 Incubate each tube for 60 minutes to 18 hours at 56°C or for 15 to 60 minutes on a thermal shaker at 56°C and at 850 rpm.

19.5.2.4 Press solid material against the inside of the tube to obtain maximum lysate volume and discard, or, if solid material is in a 2ml SPIN tube, transfer solid material to spin basket and place spin basket in tube. Centrifuge tube for 2 minutes at 15,000 rpm. Remove and discard spin basket containing the solid material and cut the cap off the tube (if using SPIN tube in the EZ1). Alternatively, transfer lysate to a labeled EZ1 Sample Tube.

19.6 DNA EXTRACTION ON THE EZ1/EZ2 ADVANCED XL

19.6.1 Obtain **DNA Investigator Reagent Cartridge**. Note: where appropriate, the cartridges may 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.

19.6.2 Label the side of the EZ1/EZ2 Elution Tube. If a 1.5ml tube is used for elution, the tube may be labeled on the side or the cap.

19.6.3 If not present already, insert the EZ1/EZ2 Advanced XL DNA Investigator Flip Cap Card completely into the EZ1/EZ2 Advanced Card slot of the EZ1 Advanced XL. Switch on the EZ1 instrument.

19.6.4 Refer to 19.3.1 for EZ1 UV process. For EZ2 see 19.3.3, or DNA SOP-20.

19.6.5 After the UV process is complete, press “ESC” to return to the main menu. Press “START”

on the main menu to start the protocol setup. Press ESC when asked about data tracking.

19.6.6 Press "4" for Norm protocol. Press "1" to elute into water and then press "2" for the 50µl elution volume. NOTE: The normalization protocol may be run on an EZ2 instrument for known samples, if ever necessary.

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

19.6.8 Open the instrument door.

19.6.9 Invert the reagent cartridges at least 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.

19.6.10 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 samples into the back row of the tip rack.

Note: **IF USING EZ1/EZ2 ELUTION TUBES, MAKE SURE CAPS ARE REMOVED FROM EZ1 SAMPLE TUBES AND ELUTION TUBES PRIOR TO CLOSING INSTRUMENT DOOR AND STARTING PROTOCOL OR INSTRUMENT WILL MALFUNCTION.**

19.6.11 Close the instrument door.

19.6.12 Press "START" to start the purification procedure.

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

19.6.14 Open the instrument door.

19.6.15 Check the pipette tips. Make sure the filters are not wet, salty or discolored, as this may indicate a run issue.

19.6.16 Visually check the volumes in the elution tubes. They should have approximately the same volume (50µL) with the RB being at the most stringent volume. If a discrepancy is seen,

manually check the volumes of the tubes with a pipette to ensure that the RB is at the most stringent volume. If adjustments need to be made with dH_2O, note this on the QR worksheet. If no liquid is present in a tube, note it on the worksheet, and troubleshoot the situation (see below). Notify the Equipment Coordinator regarding any EZ1/EZ2 issues. If necessary, consult a lead, DNA Technical Leader, Assistant TL or Deputy Director.

19.6.16.1 EZ1 troubleshooting. When the EZ1/EZ2 elution tube volume is significantly reduced (e.g., 0-20µl) 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/EZ2 using the same cartridge and sample tube.

B. Make a master mix of extraction buffer and ProK as described above for an additional RB and EP1. Add master mix to the RB and EP1.

C. Run EZ1/EZ2 using the standard protocol discussed above.

D. If the troubleshooting steps are required, it will be documented on the DNA-QR-25.

Consult a lead, the DNA Technical Leader, Assistant TL, or Deputy Director should other EZ1/EZ2 issues arise.

19.6.17 Replace/close elution tube caps and remove samples from instrument. Store DNA at +4°C or -20°C for short-term storage.

19.6.18 Clean the instrument according to **19.3 CLEANING EZ1/EZ2 ADVANCED XL.**

19.7 AMPLIFICATION OF SAMPLES

19.7.1 In the amplification hood, prepare reaction mix and pipet into plate wells or tubes as appropriate, using volumes listed on worksheet. Be sure to incorporate sufficient amplification POS and NEG controls. Please refer to DNA SOP-35 GlobalFiler Amplification and SOP-37 Y-STR Amplification for further details. For amplification of positive control samples, target ~0.3 ng total amount. For example, add 3 µL of the 0.1 ng/µL Positive Control found in the GlobalFiler kit. However, other target amounts are permitted for known processing due to the range of injection times allowed.

19.7.2 For GlobalFiler and Y-STR amplifications, suggested volumes of input DNA, based on the

sample type are as follows:

0.8µl	input DNA for FTA buccal samples
0.3-0.5µl	input DNA for blood samples on all substrates
0.3-0.5µl	input DNA for buccal/oral swab samples

Note: **Quality and quantity of sample may be taken into consideration when determining the volume of input DNA used.** For example, older bloodstains, older offender samples on FTA paper and samples having failed previously may require a larger input volume than those described.

19.7.3 Run amplification program (as appropriate) on thermal cycler.