19.1 PURPOSE

To purify and amplify genomic DNA from known and single source biological samples using the EZ1 DNA Investigator Kit or Tissue Kit on the EZ1 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 DNA Investigator Kit or Tissue Kit on the EZ1 Advanced XL following DNA SOP-20. Quantification of the DNA is necessary if processing samples following DNA SOP-20.

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.

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 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, and then clean area with water, followed by bleach.

19.3.1 Prior to and after each extraction run, 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.

19.3.2 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 issue moistened with ethanol or Proprietary Solvent (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**.

19.4 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.

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: In-house extraction buffer together with in-house Prot K may be used in place of Buffer G2 and Qiagen Prot K.

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	Per sample
145µl	dH_2O	
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 swab to spin basket and place spin basket in tube. Centrifuge tube for 2 minutes at 15,000 rpm. Remove and discard spin basket containing swab and cut cap off tube (if using SPIN tube in the EZ1). Alternatively, transfer lysate to a labeled EZ1 Sample Tube.

19.6 DNA EXTRACTION ON THE EZ1 ADVANCED XL

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

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

19.6.3 Refer to 19.3.1 for UV process.

19.6.4 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.

19.6.5 Press "4" for Norm protocol. Press "1" to elute into water and then press "2" for the 50µl elution volume.

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

19.6.7 Open the instrument door.

19.6.8 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.

19.6.9 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: IF USING EZ1 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.

19.6.10 Close the instrument door.

19.6.11 Press "START" to start the purification procedure.

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

19.6.13 Open the instrument door.

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

19.6.15 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 supervisor or the Technical Leader.

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

19.6.17 Clean the instrument according to **19.3 CLEANING EZ1 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 4 (Identifiler Plus and Yfiler) and DNA SOP 30 (Fusion 6C) for further details.

19.7.2

For Identifiler and Yfiler amplifications: suggested volumes of input DNA, based on the sample type:

1.5µl	input DNA for FTA buccal samples
1µl	input DNA for FTA blood samples
1µl	input DNA for buccal swab samples
0.5µl	input DNA for EP1 liquid blood samples

For Fusion 6C amplifications: suggested volumes of input DNA, based on the sample type:

0.8µl	input DNA for FTA buccal samples
0.5µl	input DNA for blood samples on all substrates
0.5µl	input DNA for buccal swab samples

Note: Quality and quantity of sample may be taken into consideration when determining the volume of input DNA used.

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