mtDNA SOP-15 Processing of Single Source Samples on EZ1 Advanced XL

15.1 PURPOSE

15.1.1 To purify and amplify genomic DNA from known and single source biological samples using the EZ1 DNA Investigator 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: 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.

15.2 RESPONSIBILITY

15.2.1 Mitochondrial DNA (mtDNA) Unit personnel.

15.2.2 Document the Extraction on **DNA QR-25**.

15.3 Controls

15.3.1 An extraction positive control, EP1 will be extracted for each run (batch). It will be taken forward if the samples are tested for nuclear DNA (nDNA). The EP1 will not be tested for mtDNA.

15.3.2 At least, one extraction negative control, RB (Reagent Blank) will be extracted for each Run (batch).

15.4 Quality Control

15.4.1 Quality Control of the EZ1 Investigator Kit for mtDNA known extractions can be performed one of two ways:

1. The extract that is generated from the nuclear DNA QC process will be utilized to demonstrate that the correct/expected mtDNA sequence/results is obtained for the EP1 and Reagent Blank (RB).

2. A new EZ1 extraction of an EP1 and RB will be performed specifically for the mtDNA Quality Control test.

Please refer to mtDNA WI-16 “QC of Critical Reagents” and

mtDNA SOP-01 "General Procedures" for further guidance.

15.5 Prior to beginning extraction

15.5.1 Stratalink EZ1 elution tubes, EZ1 sample tubes (or 2.0ml SPIN tubes), dH_2O, and 15ml Falcon tube for a minimum of 15 minutes.

15.5.2 Clean extraction hood and any necessary utensils (i.e. scissors, tweezers) with an appropriate bleach solution and ethanol.

15.5.3 UV hood and utensils for a minimum of 15 minutes.

15.6 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.

15.6.1 Prior to and after each extraction run, Press "1" on the main menu to select the UV function. Select 20 minute duration. Press "ENT" and then press "START" to turn the UV lamp on.

15.6.2 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**.

15.7 SAMPLE PREPARATION

Note: Amount of sample used is a recommendation. The type and quality of the sample maybe taken into consideration when determining the amount of sample used.

15.7.1 Buccal/Blood Swabs:

15.7.1.1 Remove approximately 1/4 to 1/2 of the swab from applicator shaft.

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

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

15.7.2.1 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.

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

Note: Other items deemed to be a known sample, may be processed in this manner.

15.8 SAMPLE PRETREATMENT

15.8.1 Pretreatment for up to 200µl of Whole Blood

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

15.8.1.2 Go to step 15.9.

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

15.8.2.1 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 Proteinase K may be used in place of Buffer G2 and Qiagen Proteinase K.

Note: For each extraction run (batch) 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.

15.8.2.1.1 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.

15.8.2.1.2 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 yield successful results.

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

15.8.2.3 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.

15.8.2.4 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.

15.9 DNA EXTRACTION ON THE EZ1 ADVANCED XL

15.9.1 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.

15.9.2 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 EZ1instrument.

15.9.3 Refer to 15.6.1 for UV process.

15.9.4 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.

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

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

15.9.7 Open the instrument door.

15.9.8 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.

15.9.9 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.

15.9.10 Close the instrument door.

15.9.11 Press "START" to start the purification procedure.

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

15.9.13 Open the instrument door.

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

15.9.15 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 on worksheet, and troubleshoot the situation. If necessary, consult a Lead, DNA Technical Leader, or Assistant Director.

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

15.9.17 Clean the instrument according to 15.6 CLEANING EZ1 ADVANCED XL.

15.10 AMPLIFICATION OF SAMPLES

Note: Extraction batches may be broken down into sets for ease of amplification.

15.10.1 For Mitochondrial DNA processing: suggested volumes of input DNA, based on the sample type:

1µl input DNA for samples and controls
1µl input DNA for RB samples
Note: The volume used for the RB should always reflect the highest volume used by the samples and/or controls in the set.

15.10.2 One HL60 will be amplified with each set. Each sample and the HL60 will have its own sequence range. The sequence range reported out will be the smaller of the two ranges between the sample and the HL60.

15.10.3 One NC (Negative control) will be amplified with each set.

15.10.4 Standard mtDNA protocols are followed for amplification through injection.

15.10.5 Please refer to DNA SOP 4 (Identifiler Plus and Yfiler) and DNA SOP 30 (Fusion 6C), for further details pertaining to nDNA amplification.

15.10.6 Suggested volumes of input DNA for STR testing, 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.