Procedure for Casework Direct System

1.0 Purpose – This procedure specifies the steps for rapid processing of casework samples using the Promega Casework Direct System

2.0 Scope – This procedure applies to Forensic Scientists in the Forensic Biology Section who perform DNA extractions for forensic casework.

3.0 Definitions – See Section Definition List

4.0 Equipment, Materials, and Reagents

- Calibrated Pipets (various sizes)
- ART Pipet Tips (or equivalent, various sizes)
- Promega Casework Direct System (Casework Direct Reagent, 1-Thioglycerol, 5X AmpSolution Reagent, Water, Amplification Grade)
- Thermomixer
- 2 mL tubes with a lyse & spin basket (or equivalent)
- Sterile tubes (various sizes)
- Vortex Mixer
- Various lab equipment (various disposable conical tubes, lab tape, lab coat, lab gloves, microcentrifuge tubes and rack, wipes, etc)
- 10% Bleach solution

5.0 Procedure

5.1 Overview

5.1.1 All known samples shall be processed separately from unknown samples. Thermomixers shall be designated exclusively for either “knowns” or “unknowns”.

5.1.2 For casework unknowns, a portion of each swab present shall be cut for analysis. The amount of sample taken shall be determined based upon the scientist’s evaluation of the sample (e.g., amount of staining, sperm quantity recorded) and the number of swabs present.

5.1.3 Make a master mix containing the reagents using the volumes listed for the sample processing (allowing for 1-2 extra samples for pipetting variation). Reagent will be added to the sample tubes from this master mix and not from the stock or aliquot bottles.

5.1.4 If a batch of samples being extracted is in excess of 30 total samples (knowns and unknowns) then witnessing steps shall be added when multiple tubes are open simultaneously during the transfer of tubes (e.g., Qiagility setup). This witnessing will be documented in the case notes. Additional exceptions shall be approved only with written documentation from the Technical leader.

5.1.5 Negative extraction control

5.1.5.1 For each batch of samples, at least two reagent blanks for each set of questioned samples and at least one reagent blank for each set of known samples shall be prepared each time an extraction set is begun. This blank will consist of the reagents used in the extraction process and shall be treated the same as other samples throughout the entire process. Also, the final volume of this control shall be the same as the forensic sample(s) brought up in the most minimal volume and amplified using the maximum volume.

5.1.5.2 Due to the amount of sample needed for an extraction (i.e., number of swabs, size of cutting) it may be necessary to split the extraction sample across multiple extraction tubes. In such instances, multiple reagent blanks will need to be created. At the point when the separate extractions of the sample are recombined, prior to amplification, the same number of reagent blanks must also be combined. This ensures that the same quantity of reagents have been processed in the reagent blank as the given sample.

5.1.5.3 It is acceptable to run more than two reagent blanks in anticipation of having to re-run or dilute samples for amplification.

5.1.5.4 If additional extractions are performed, the associated negative extraction controls shall have a unique identifier (different date will suffice as identifier).

5.1.5.5 Reagent blanks must be processed concurrently with the associated samples, not consecutively. Sample incubation must be occurring at the same time to be considered concurrent. For example, incubations occurring at the same time on multiple thermomixers would be considered concurrent while consecutive incubations on the same thermomixer would not be concurrent.

5.1.6 All tubes shall be labeled with a unique identifier.

5.2 Processing of Samples

5.2.1 Aseptically place the sample into a labeled lyse & spin basket with a 2 mL tube or a 1.5 mL flip cap tube.

5.2.1.1 Swab(s) should be sampled such that no more than the equivalent of approximately one quarter of a swab is present in the microcentrifuge tube.

5.2.1.2 If an item is to be swabbed for DNA analysis (e.g., underwear, condoms, tampons, pads, or similar items), approximately one quarter of the representative swabbing should be cutw for analysis. The remainder of the swab shall be placed back into the container with the original item.

5.2.1.3 If an item is to be cut for analysis, the cutting must be made such that the entirety of the cutting is submerged in the reagent volume present.

5.2.2 To the sample, add 200 µl of Casework Direct Reagent and 1 µl of diluted 1-Thioglycerol. Vortex for 5-10 seconds.

Note: 1-Thioglycerol is viscous. To facilitate accurate pipetting, warm the diluted 1-Thioglycerol to room temperature, pipette slowly, and avoid pipetting small volumes.

5.2.3 Incubate the samples for approximately 30 minutes at 70 °C in a thermomixer set to approximately 700 rpm. Vortex for 5-10 seconds after removing sample(s) from thermomixer.

Note: The thermomixer must reach 70 °C prior to the incubation start time.

5.2.4 If using a 2.0 mL tube with a lyse & spin basket, spin in a microcentrifuge at high speed for 5 minutes to activate the basket and force the extraction fluid into the tube. If any liquid remains in the basket, repeat spin. Remove the basket and discard into a biohazard waste container. Close the tube.

5.2.5 If using a 1.5 mL tube, spin briefly in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the sample(s) into a basket insert. Place the basket back in the tube containing the stain extract and cap the tube. Spin in a microcentrifuge at high speed for 5 minutes, repeating if liquid remains in the basket. Remove the basket and discard it into a biohazard waste container. Close the tube.

5.2.6 Vortex the samples to ensure that a homogeneous sample is ready for quantitation setup.

5.2.7 The DNA is ready to use or can be stored refrigerated at 2-8 °C until quantitation. If storing prior to quantitation, cap the elution tubes/for storage.

5.3 Sample Purification of Casework Direct Lysates

5.3.1 Casework Direct Lysates may be cleaned up using the DNA Investigator Kit in some situations where a full DNA extraction may not be needed (e.g., based on quantitation results).

5.3.2 Prior to sample purification, Casework Direct Lysates shall be allowed to come to room temperature, vortexed, and then centrifuged for 5 seconds.

5.3.3 If using the EZ1 Advanced XL, see Procedure for DNA Extraction, follow the protocol DNA Purification: (Trace Protocol).

5.3.4 If using the QIAsymphony, see Procedure for DNA Extraction, follow the protocol using Assay Control Sets: CW200ADVHE_CR24547_ID5606 or CWD200_ADV_HE_V10".

5.4 Storage of Casework Direct Lysates – Store the samples at 2-8 °C. Prior to use of samples after storage, they shall be vortexed and then centrifuged for 5 seconds.

6.0 Limitations – Casework Direct Lysates can be used up to twelve (12) months from the date of generation. Use beyond this time period requires the written approval of the DNA Technical Leader.

7.0 Safety -N/A

8.0 References

Forensic Biology Section Procedure for DNA Casework Training

Forensic Biology Section Procedure for DNA Extraction using the EZ1 Advanced XL

Forensic Biology Section Procedure for DNA Extraction using the QIAsymphony.

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control

9.0 Records

DNA workbook or equivalent (to be used for QC and training).

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
05/24/2024	2	5.1.4 – added witnessing steps for sample handling processing; 5.2.6 – add vortex step after spin; 5.3.3., 5.3.4 – updated procedure name; 6.0 extended lysate expiration