Procedure for PCR Amplification for Casework

1.0 Purpose - This procedure specifies the steps for performing PCR amplification with AmpFℓSTR® Identifiler® Plus.

2.0 Scope - This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform amplification with AmpFℓSTR® Identifiler® Plus.

3.0 Definitions - N/A

4.0 Equipment, Materials and Reagents

- Centrifuge
- GeneAmp® PCR System 9700 Thermal Cycler
- Calibrated Pipets
- ART Pipet Tips (or equivalent, various sizes)
- Sterile microcentrifuge tubes (various sizes)
- PCR reaction tubes (including individual tubes or strip tubes with caps)
- Optical plates and strip caps
- QIAgility liquid handler
- AmpFℓSTR® Identifiler® Plus Reagents
- Extracted DNA
- STR TE
- Biosafety amplification cabinet
- Bleach
- Bleached and clean amp trays
- Vortex

5.0 Procedure

5.1 No more than 30 total items (including controls) per analyst may be processed simultaneously.

5.2 Thaw the AmpFℓSTR® Identifiler® Plus Master Mix, Primer Set and Control DNA 9947A. Vortex each tube thoroughly before using and keep cold. New kits shall be kept in the freezer; once a kit has been thawed, it may be stored at 4 °C.

5.3 Preparation of the Reaction (Manual Setup)

5.3.1 Prepare the Amplification Worksheet. Determine the number of samples to be amplified. Include 9947A and the negative amplification control. The Forensic Scientist may amplify a lower or higher amount based on training and experience if inhibition is noted.

5.3.2 Calculate the required amount of each component for the PCR Master Mix. Multiply the volume (µL) per sample by the total number of reactions to obtain the final volume (µL). To account for pipetting variation, add 2 µL for every set of 16 samples. The volumes for the PCR Master Mix

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are 10 µL per sample for the Identifiler® Plus Master Mix and 5 µL per sample for the Primer Set.

5.3.3 Label the appropriate number of PCR reaction tubes (individual or strip) and place them in an amplification tray for the 9700 thermal cycler. If strip tubes are used, the first and last tube in each strip that contain sample shall be labeled using a sticker-type label. The label shall contain the distinguishable portion of the case number (e.g., R11-12345) and the item number or sample contained within (e.g., 9947A, Ladder). The intermediate tubes shall be labeled to permit the Forensic Scientist to determine the sample contained in each tube. This determination can be made in conjunction with the plate map (3130XL set-up sheet). If individual tubes are used, each tube shall be labeled using a sticker-type label with the distinguishable portion of the case number (e.g., R11-12345) and the item number or sample contained within (e.g., 9947A, Ladder).

5.3.4 Add the final volume of each reagent to make a PCR Master Mix in a sterile 1.5 mL microcentrifuge tube and mix. Spin down the tube to remove any liquid from the lid.

5.3.5 Add 15 µL of the PCR Master Mix to each sample tube.

5.3.6 Add the amount of sterile TE to each tube as calculated per the Amplification Worksheet.

5.3.7 Pipette DNA samples and controls into each tube as calculated per the Amplification Worksheet.

5.3.7.1 Make dilutions for DNA samples as required by the DUO Dilution Calculation Worksheet. If dilutions are made, use the same TE for the negative amplification control as used for the dilutions.

5.3.8 Cap tubes and spin amplification tray containing tubes to ensure all sample is seated at the bottom of the tubes and no bubbles are present.

5.3.9 Place amplification tray containing tubes onto the thermal cycler.

5.4 Preparation of the Plate Document

5.4.1 Forensic Scientists shall use the Microsoft Excel “Amp Setup Worksheet” (Setup Worksheet) to calculate the amount of reagents necessary for the number of samples to be amplified per plate and to enter the concentration of each sample. Note: For any sample which had a quant value of greater than 25 ng/µl, that sample shall be diluted to 1 ng/µl prior to setup on the QIAgility. The QIAgility will include one 9947A and one Negative Amp for the entire plate.

5.4.1.1 The Amp Export info tab of the Setup Worksheet shall be saved as a .CSV file.

5.4.1.2 The CE bank numbers tab of the Setup Worksheet shall be saved as a separate .CSV file.

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5.5 Preparation of the Reaction (QIAgility Setup)

5.5.1 The sides of the 96 well trays used for amplification shall be labeled with the Forensic Scientist's initials and the date. The columns used for a particular case shall be labeled by writing the distinguishable portion of the case number on the side of the tray and aligned with the specific columns.

5.5.2 Vortex the Identifiler Plus Primer Set and Reaction Mix and then centrifuge before opening each tube. Add the volumes as provided in the Setup Worksheet to a 2 mL tube. This combination of Primer and Reaction Mix is now the Master Mix.

5.6 Operation of the QIAgility

5.6.1 Turn on the QIAgility instrument. The switch is located on the lower back left corner of the instrument. Forensic Scientists shall open the QIAgility software by double clicking the "Shortcut to IDF+ norm&amp.QAS" Protocol located on the computer desktop.

5.6.2 The worktable for Amplification setup shall be set up as is appears on the screen. Holding the cursor over any colored well will give information about the contents of that well.

5.6.3 Within the IDF+ norm&amp Protocol, left-click on "Sample block B2." The right hand side of the screen will populate with information for that section. Click the "Import" button. The "Import Well Data" dialog box will appear.

5.6.4 Under Import File in the top left hand corner, click the browse button. Locate the .CSV file and click open. Note: The Forensic Scientist will have to change the file type to CSV files.

5.6.5 On the left hand side under Import Options, change the Start importing FROM box to 2. Click the "Import" button at the bottom of the screen to import the samples and then click "Finish."

5.6.6 Left-click on "Reaction block C1." In the upper right hand side of the screen, highlight the first line. If the line is grayed out (therefore containing no data), click "Delete." If it is not grayed out, then right-click and choose "Create Sample bank from Target wells." Click the button next to "Existing Bank," highlight "Diluted to 1.0ng," click "Add Selection" and then click "Close."

5.6.7 Repeat step **5.6.6** for the next line in the list.

5.6.8 Highlight any additional grayed out line and click "Delete."

5.6.9 Left-click on well B (9947A) in the Reagent block R1. On the right hand side, the concentration shall be adjusted if instructed to do so by the Section QC officer.

5.6.10 Grasp the handle and pull up to open the Instrument lid. Inspect the worktable to ensure that the tip holders and plate holder are in the position which appears on the screen.

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5.6.11 Place the following materials on the worktable:

Position	Reagent/Item
E of Reagent block R1	2 mL tube containing Master Mix (green well in software)
I of Reagent block R1	1.5 mL tube containing TE (orange well in software)
A of Mix Plate block M1	5 mL tube containing TE (blue well in software)
B of Reagent block R1	2 mL tube containing 9947A (red well in software)
Reaction block C1	Empty 96 well optical plate
Reaction block B1	Strip tubes into any columns with colored wells

5.6.12 Place the samples into the sample holder(s) according to the Setup sheet and deck layout. Place the sample holder(s) onto the Instrument deck into Sample blocks B2.

5.6.13 Check to ensure that there are enough tips to process the run. To do this, left-click on either Tip Holder A1 or A2. On the right hand side of the screen it will indicate if there are enough tips. If there are not enough tips, insert a new set of tips into Tip Holder A1 or A2 (whichever is empty). Right click on the Tip holder that was just filled and choose "Set all tips on current plate to Available." Close the instrument lid.

5.6.14 Click the green arrow to start the run. The "Save as" dialog box will appear. Save the run file to the Forensic Scientist's folder under C:\Program Files\QIAgility\Data. The file shall be named in the following format: IDF+ norm&amp_MMDDYYY_ABC (IDF+ norm&amp_full date_Forensic Scientist initials.) This file shall also be imported into the Forensic Scientist's case record repository.

5.6.15 After the Forensic Scientist clicks "Save," the pre-run Checklist screen will appear. If the run has been set up correctly, the checklist will not list any warnings or errors other than the default messages listed below. If other messages are listed, user intervention is required before the run can be started. After completing the listed tasks, select the boxes next to the warnings to continue. Click "OK."

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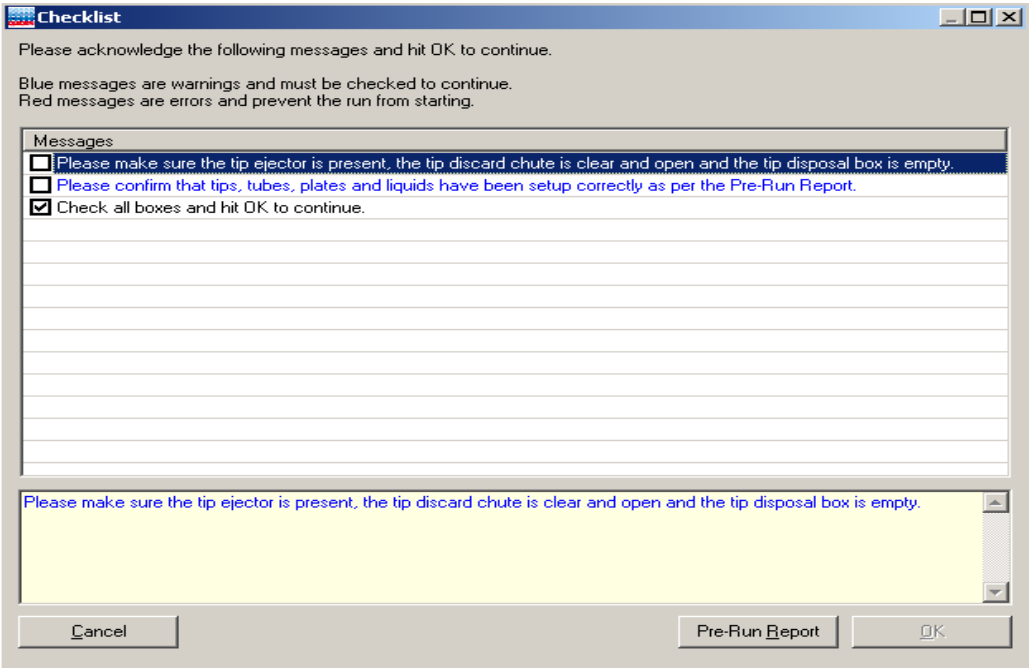

5.6.16 A run can be paused and aborted at any time by clicking the red X . Pauses will be logged in the post-run report. If the run is aborted, the instrument will discard the tip and return to its resting position. **Note:** A run will also be paused when the instrument lid is opened.

5.6.17 The instrument will complete the current operation and will then pause. A dialog box will appear indicating that the run was paused and whether the run will be continued or aborted. **Note:** The instrument does not stop immediately when the lid is opened. Therefore, wait until the Y-arm has stopped moving and the warning screen is displayed before continuing.

5.6.18 The instrument lid must be closed to abort the run. **Note:** An aborted run cannot be restarted from the point at which it was aborted.

5.6.19 If an error occurs or a warning is detected during a run, a dialog box which contains information about the error will appear. The Forensic Scientist will have 4 options: “Abort run,” “Retry operation,” “Ignore error,” and “Skip step.” Determine the error and the appropriate option.

5.6.20 Upon completion of each run, a support package is constructed and saved automatically to the program. This package contains the log file for the run and is used when reporting a problem to QIAGEN Technical Services. Click OK. **Note**: The Forensic Scientist will see a message concerning the support package only if an exception occurred during the run.

5.6.21 Upon completion of each run a Post-Run Report will also be displayed. This report shall be saved within the Forensic Scientist’s folder on the C drive (C:\ Program Files\QIAgility\Data\) on the computer associated with the QIAgility. Forensic Scientists shall also print a copy of this report using the print to pdf function to add to the case record repository.

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5.6.22 Open the instrument lid and remove the optical plate. Seal the optical plate with strip caps. Press down firmly using the Cap Installation tool. Centrifuge the plate to ensure that there are no bubbles at the bottom of the wells.

5.6.23 Remove the sample holder(s) and samples, TE, Master Mix and 9947A tubes from the deck. Empty the biohazard box.

5.6.24 Close the software by clicking the X in the top right corner. Follow the prompts to shutdown the instrument. Click "Yes" to quit QIAgility. Click "Move to Safety Position (recommended)." Click "OK."

5.6.25 Turn off the instrument.

5.6.26 Place the amplification plate onto the thermal cycler.

5.7 Turn on the thermal cycler. To select the appropriate cycle on the 9700 thermal cycler, press F1, use the arrows on the thermal cycler and move them until "if plus 28cyc" is highlighted. All amplifications shall have a 25 µL volume set on the thermal cycler. Start the thermal cycler run. The program is pre-recorded as follows:

95 °C for 11 minutes, then:

94 °C for 20 seconds
59 °C for 3 minutes

For 28 cycles, then:

60 °C for 10 minutes, then;

4 °C for infinite hold (to refrigerate until the Forensic Scientist takes the samples out of the thermal cycler).

5.8 Maintenance of the QIAgility

5.8.1 Refer to the Forensic Biology Section Procedure for Calibration and Equipment Maintenance

6.0 Limitations - Amplified products shall not be used after one month of being generated without the written approval of the DNA Technical Leader. Additionally, if AmpFlSTR® Identifiler® Plus kit lot numbers change during the one month period (due to expiration or supply exhaustion), the Forensic Scientist shall re-amplify the DNA extracts. If there is not enough DNA extract to re-amplify, the Forensic Scientist shall discuss with the DNA Technical Leader and proceed as instructed.

7.0 Safety - N/A

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8.0 References

Forensic Biology Section Procedure for DNA Casework Training

Forensic Biology Section Procedure for Calibration and Equipment Maintenance

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control

9.0 Records

- Amplification set-up worksheet (to be used for QC and training)

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/31/2012	1	Original Document
05/30/2013	2	5.5 – clarified labeling requirement
07/31/2013	3	5.1 – changed 4 case batch limit to sample limit of 30 samples at a time
9/25/2013	4	Header – added issuing authority titles; 4.0 – added various size tubes, optical plates, liquid handler to equipment list; 5.3 – changed to manual set-up – steps renumbered; 5.4 – added preparation of plate document (QIAgility setup); 5.5 – added preparation of reaction (QIAgility setup); 5.6 – added operation of QIAgility; 5.8 – added QIAgility maintenance reference

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