Procedure for PCR Amplification using PowerPlex® Y23

1.0 Purpose - This procedure specifies the steps for performing PCR amplification with PowerPlex® Y23.

2.0 Scope - This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform amplification with PowerPlex® Y23.

3.0 Definitions - N/A

4.0 Equipment, Materials and Reagents

- Centrifuge
- AB ProFlex Thermal Cycler (or equivalent)
- Calibrated Pipets
- ART Pipet Tips (or equivalent, various sizes)
- Sterile microcentrifuge tubes (various sizes)
- 96 well plates and strip caps (or equivalent)
- QIAgility liquid handler
- PowerPlex® Y23 Reagents
- Extracted DNA
- STR TE
- Biosafety amplification cabinet
- Bleach
- Vortex
- Forensic Biology workbook or equivalent

5.0 Procedure

5.1 No more than 30 total items (question and known samples) per analyst may be processed simultaneously. Exemptions to this item limit shall be approved only with written documentation from the Technical leader. Positive and negative amplification controls shall be run with each batch of samples. These controls must be set up and amplified simultaneously (i.e., same thermal cycler) with the items.

5.2 Amplification plates shall be set up utilizing the QIAgility instrument, unless instrumentation is unavailable (e.g. due to maintenance). If an individual sample needs to be added to an amplification plate (e.g. dilution sample) this sample may be added manually. If manual setup is required, it may be done with written supervisory approval.

5.3 The amount of positive control added shall be determined based upon the QC results of each kit and will be provided by the Section Quality Control Officer.

5.4 Thaw the PowerPlex® Y23 5x Master Mix, PowerPlex® Y23 10x Primer Pair Mix and Amplification Grade Water. Vortex each tube for 5-10 seconds before each use and keep cold. Vortex the final master mix for 5-10 seconds. **Do not centrifuge the 5X Primer Pair Mix or 10X Master Mix after vortexing**, as this may cause the reagents to be concentrated at the bottom of the tube. New kits shall be kept in the freezer; once a kit has been thawed, it may be stored at 2-10 °C for up to 6 months.

Note: The Positive Control DNA must be stored at 2-10 °C, for at least 24 hours before use.

5.5 Preparation of the Plate Document

5.5.1 Forensic Scientists shall use the Amp setup (qiagility) tab of Biology workbook 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. The worksheet shall be saved and printed to .pdf.

5.5.2 For manual setup, any sample which had a quant value greater than 1ng/µL shall be diluted per Dilution Calculation worksheet prior to setup.

5.5.3 For liquid handler setup, any sample which had a quant value greater than 25 ng/µl shall be diluted per Dilution Calculation worksheet prior to setup.

5.5.3.1 The Amp Export info tab of the workbook shall be saved as a CSV file (when setting up using the QIAgility robot) and also printed as a pdf file.

5.5.3.2 The CE bank numbers tab of the workbook shall be saved as a separate CSV file (when setting up using the QIAgility robot). Note: For both 5.5.3.1 and 5.5.3.2 when saving the .csv files, select “ok”, then “no”, then “cancel” in Excel.

5.6 Preparation of the Reaction (Manual Setup)

5.6.1 Prepare the Amplification Worksheet using values from the quantitation dilution worksheet and add the positive and the negative amplification controls. The Forensic Scientist may enter an additional value, based on training and experience, for amplification.

5.6.2 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 samples for every set of 24 samples. The volumes for the PCR Master Mix are 5 µL per sample and 2.5 µL per sample for the Primer Set.

5.6.3 The sides of the 96 well trays used for amplification shall be labeled with the initials of one of the Forensic Scientists with samples in the batch, the date and the distinguishable portion of the case number(s) on the side of the tray.

5.6.4 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 briefly to remove any liquid from the lid.

5.6.5 Add 7.5 µL of the PCR Master Mix to each sample tube.

5.6.6 Add the amount of sterile amplification grade water to each tube as calculated per the Amplification Worksheet.

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

5.6.7.1 Make dilutions for DNA samples as required by the Dilution Worksheet. If dilutions are made, use the same amplification grade water for the negative amplification control as used for the dilutions.

5.6.7.2 The positive amplification control volume shall be set as designated by the QCO.

5.6.8 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.

5.6.9 Place amplification tray containing tubes onto the thermal cycler.

5.7 Preparation of the Reaction (QIAgility Setup)

5.7.1 The sides of the 96 well trays used for amplification shall be labeled with the initials of one of the batching Forensic Scientists, the date and the distinguishable portion of the case number(s) on the side of the tray.

5.7.2 Vortex the Primer Set and Reaction Mix. Briefly spin down the 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.

5.8 Operation of the QIAgility

5.8.1 Ensure the QIAgility instrument is on. The switch is located on the lower back left corner of the instrument. Ensure that the deck is properly set up for amplification. Open the QIAgility software by double clicking the "Shortcut toY23.QAS" protocol located on the computer desktop.

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

5.8.3 Within the Y23 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.

5.8.4 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.

5.8.5 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."

5.8.6 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," click "Add Selection" and then click "Close."

5.8.7 Repeat step **5.8.6** for the next 3 lines in the list.

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

5.8.9 Left-click on well I (2800M) in the Reagent block R1. The concentration shall be adjusted based upon the results of QC Testing by the QCO.

5.8.10 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.

5.8.11 Place the following materials on the worktable:

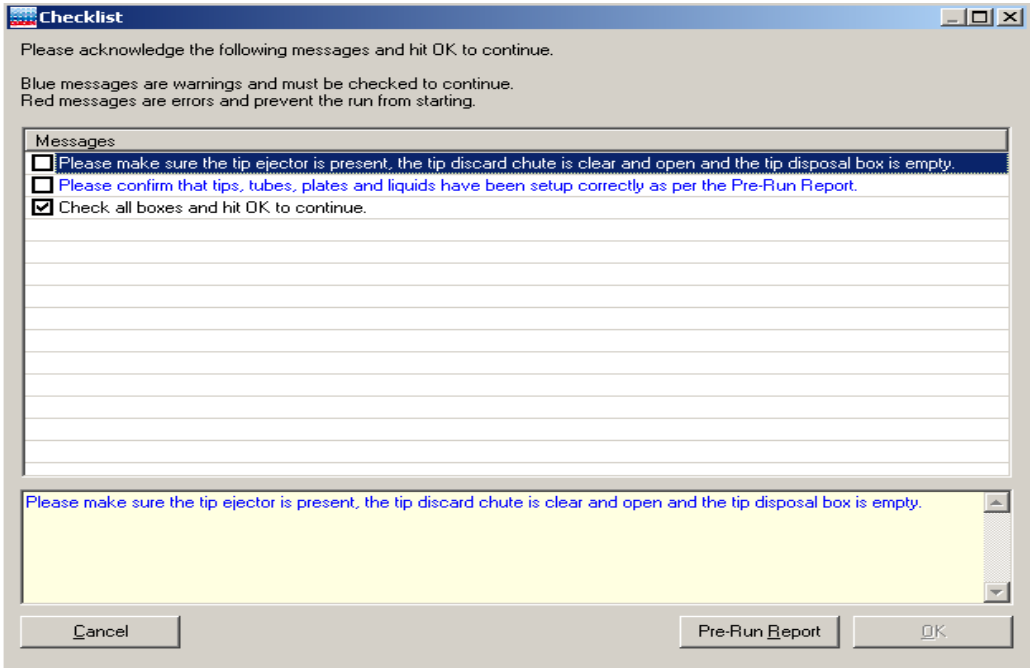
Position	Reagent/Item
A of Mix Plate block M1	5 mL tube containing amplification grade water (blue well in software)
H of Reagent block R1	2 mL tube containing Master Mix (green well in software)
I of Reagent block R1	1.5 mL tube containing 2800M (red well in software)
Reaction block C1	Empty 96 well optical plate
Reaction block B1	Strip tubes into any columns with colored wells

5.8.12 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.

5.8.13 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.

5.8.14 Click  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. This file shall also be imported into the Forensic Scientist's case record repository. The file name shall contain the initials of the operator for the run.

5.8.15 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."



5.8.16 A run can be paused and aborted at any time by clicking  . 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.

5.8.16.1 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.

5.8.16.2 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.

5.8.17 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.

5.8.18 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.

5.8.19 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.

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

5.8.21 Remove the sample holder(s) and samples, amplification grade water, Master Mix and positive amplification control tubes from the deck. Empty the biohazard box.

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

5.8.23 Place the amplification plate onto the thermal cycler.

5.9 Operation of the ProFlex Thermal Cycler

5.9.1 Turn on the thermal cycler.

5.9.2 To select the appropriate cycle on the thermal cycler, press "Open Method" from the Home Screen. Press "Y23 30 cycles."

5.9.2.1 All amplifications shall have a 25 µL volume set on the thermal cycler.

5.9.2.2 The program is pre-recorded as follows:

96°C for 2 minute, then:

94 °C for 10 seconds
61°C for 1 minute
72°C for 30 seconds

For **30 cycles**, then:

60 °C for 20 minutes

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

5.9.3 To start the run, press "Verify Block", then "Start Run."

5.9.4 When the run is completed, press "Stop Run."

5.9.5 When the display on the home screen reads "Done Remove Samples," the thermal cycler can be turned off.

Note: If turned off prior to this dispay reading, an error will appear the next time the thermal cycler is used. The error screen will state that there was a power failure during the previous run.

5.10 Maintenance of the QIAgility - 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 PowerPlex® Y23 kit lot numbers change during the

one month period (due to expiration or supply exhaustion), the Forensic Scientist shall re-amplify the extracted DNA. If there is not enough extracted DNA to re-amplify, the Forensic Scientist shall discuss with the DNA Technical Leader and proceed as instructed.

7.0 Safety - N/A

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

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

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/20/2016	1	Original Document
03/23/2018	2	5.5.1, 5.6.3, 5.6.7.1, 5.8.14 – update due to batching and standardization; 5.8.7 – update numbering
01/25/2019	3	4.0 – updated equipment; 5.1 – added written approval for sample limit; 5.6.3, 5.7.1 – update labeling due to batch processing; 5.8.14 – add initials to file name; 5.9 – Update steps for ProFlex thermal cycler use