Procedure for PCR Amplification with PowerPlex® Fusion

1.0 Purpose – To specify the steps for performing PCR amplification using PowerPlex® Fusion and manual set up.

2.0 Scope – This procedure applies to analysts in the DNA Database Section who perform PCR amplification using Promega PowerPlex® Fusion.

3.0 Definitions – See Section Definitions List

4.0 Equipment, Materials, and Reagents

- Centrifuge
- ProFlex PCR System Thermal cycler
- Calibrated Pipettes
- ART Pipette Tips (or equivalent, various sizes)
- Sterile microcentrifuge tubes
- 96-well reaction plates with strip caps
- Promega PowerPlex® PunchSolution Kit Reagents
- Promega PowerPlex® Fusion Amplification Kit Reagents
- Punch Tool with 1.2 mm Punching Head
- Heat block
- Biosafety amplification cabinet
- 10 % Bleach
- 70 % Isopropyl Alcohol
- Vortex

5.0 Procedure

5.1 PowerPlex® PunchSolution Kit Storage – PowerPlex® PunchSolution Kit reagents shall be stored and used as follows:

5.1.1 For long-term storage, store at -30°C to -10°C in a manual defrost freezer. For daily use, components can be stored at 2°C - 10°C.

5.2 PowerPlex® Fusion Amplification Kit Storage – PowerPlex® Fusion reagents shall be stored and used as follows:

5.2.1 Pre-Amplification Components Storage – Pre-amplification components include the 5X Master Mix, 5X Primer Pair Mix, Amplification Grade Water, and 2800M Control DNA.

5.2.1.1 For long-term storage, store 5X Master Mix, 5X Primer Pair Mix, and Amplification Grade Water at -30°C to -10°C in a manual defrost freezer. For daily use, these components can be stored for up to one month at 2°C - 10°C.

5.2.1.2 Store 2800M Control DNA at 2°C -10°C.

5.2.1.3 Primer Pair Mix is light-sensitive and shall be stored in the dark.

5.2.2 Post-Amplification Components Storage – Post-amplification components include the Allelic Ladder Mix and WEN Internal Lane Standard 500.

5.2.2.1 For long-term storage, store at -30°C to -10°C in a manual defrost freezer. For daily use, components can be stored for up to one month at 2°C - 10°C.

5.2.2.2 These reagents are light-sensitive and shall be stored in the dark.

5.3 Labeling

5.3.1 The side of a 96 well plate used for amplification setup shall be labeled with the DNA Database Forensic Scientist's initials. The columns used for a particular amplification shall be labeled by writing the project name on the front of the plate (e.g., analyst initials_CH_date of punching, COS-2013-00004 (COS-2013-4)). If subsequent amplifications are performed, the project name may be extended to designate the additional amplification (e.g., COS-2013-1_reamp).

5.4 PowerPlex® PunchSolution Kit and PowerPlex® Fusion Amplification Kit Usage

5.4.1 Prepare a punch/amplification setup worksheet. Determine the number of samples to be amplified. Include a reagent blank, a negative amplification control, and a positive amplification control. A NIST-TS may also be added.

5.4.2 Each amplification reaction shall contain 2.5 µL of 5X Master Mix, 2.5 µL of 5X Primer Pair Mix, 5 µL of Amplification Grade Water, and 2.5 µL of 5X AmpSolution. The PowerPlex Fusion Punch/Amplification Worksheet will use the number of samples in the amplification to calculate the required amount of each component for a PCR amplification mix as well as incorporate an additional two reactions.

5.4.3 Label a 96-well reaction plate as per the Labeling section of this procedure.

5.4.4 Obtain the PunchSolution from the refrigerator. Mix by gentle inversion. Pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Do not centrifuge longer than 1-2 seconds after mixing as this may cause the reagent to form a gradient.

5.4.5 In each well of a 96-well reaction plate where a Reagent Blank will be located or a punch will be placed, add 10 µL of PunchSolution.

5.4.6 Following the plate layout of the punch/amplification setup worksheet, place one 1.2 mm punch per sample into the corresponding well of the 96-well reaction plate. When punching, use a manual punch tool with a 1.2 mm tip to manually create sample disks from a blood card or buccal collector. Punchers are designated to be used with a specific sample type (blood or buccal); use the puncher that correctly corresponds with the sample that is to be punched. Before and after use of a 1.2 mm puncher for punching a plate, clean the puncher with a 10 % bleach solution followed by alcohol. Make sure to depress the plunger and clean the punch head. Two cleaning strikes shall be punched and discarded before and between each sample. Place the puncher tip on the desired sampling area, and with a twisting or pressing action, cut a 1.2 mm sample disk. Use the plunger to eject the disk into the appropriate well of the 96-well reaction plate. No more than one punch per well is permitted. Ensure that each punch is submerged in the PunchSolution.

5.4.7 Using a heat block, incubate the plate at 70 °C for 30 minutes or until dry.

5.4.8 Obtain the 5X Master Mix, 5X Primer Pair Mix, Amplification Grade Water, and 5X AmpSolution Each of these reagents shall be centrifuged briefly to bring contents to the bottom, then vortexed for 15 seconds before each use. Pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Do not centrifuge each tube longer than 1-2 seconds after vortexing as this may cause reagents to form a gradient.

5.4.9 Add the final volume of each reagent to make a PCR amplification mix in a sterile microcentrifuge tube. Vortex the PCR amplification mix 5-10 seconds. Pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Do not centrifuge the tube longer than 1-2 seconds after vortexing as this may cause some reagents to be concentrated at the bottom of the tube.

5.4.10 Add 12.5 µL of the PCR amplification mix to each reaction well that corresponds with a sample, reagent blank, negative control, or positive control.

5.4.11 Prior to the initial use of a tube of 2800M positive amplification control, dilute the 2800M Control DNA to 5ng/µL. Vortex for 5-10 seconds and pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. For a 25µL tube of 10ng/µL 2800M, add 25µL of Amplification Grade Water. Note the dilution on the tube.

5.4.12 Obtain a 5ng/µL tube of 2800M. Vortex for 5-10 seconds and pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Pipette 1.0 µL of 2800M positive control into the specified well(s) as indicated by the punch/amplification setup worksheet.

5.4.13 Cap used wells in the 96-well reaction plate, ensuring caps are properly seated.

5.4.14 Spin the plate in a centrifuge for one minute at 2000 rpm to ensure all punches and reagents are at the bottom of the wells and no bubbles are present.

5.4.15 Place the 96-well reaction plate onto a thermal cycler.

5.4.16 Turn on the thermal cycler. To select the appropriate cycle on the ProFlex PCR System thermal cycler, select “Open Method” and choose the “PPF24c” program. All database amplifications shall have a thermal cycler reaction volume set for a 13 µL reaction. Verify the block and start the thermal cycler run. The program is pre-recorded as follows:

96°C for 1 minute, then:

94°C for 10 seconds
59°C for 1 minute
72°C for 30 seconds
For 24 cycles, then:

60°C for 20 minutes, then:

4°C for infinite hold (until DNA Database Forensic Scientist takes samples out of thermal cycler)

5.4.17 After completion of the thermal cycling protocol, store amplified samples at -20 °C and away from light.

6.0 Limitations – Amplified products have an expiration date of one month after they are generated; however, if it is necessary for the DNA Database Forensic Scientist to use amplified product longer than one month, the approval of the Technical Leader shall be obtained for both the use of the amplified product and the resulting data. Additionally, if PowerPlex® Fusion Amplification Kit lot numbers change during the one month period (due to expiration or supply exhaustion), the DNA Database Forensic Scientist shall reamplify the DNA samples. If there is not enough DNA sample to reamplify, the DNA Database Forensic Scientist shall consult with the Technical Leader and thereafter proceed as directed.

7.0 Safety - N/A

8.0 References

DNA Database Administrative Policy and Procedure

DNA Database Administrative Policy and Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for DNA Reagent Quality Control

DNA Database Section Procedure for Instrument and Equipment Quality Control

DNA Database Section Procedure for Sample Accessioning and Processing

DNA Database Section Procedure for Sample Processing Quality Control

PowerPlex Fusion System: Instructions for Use of Product DC2402 and DC2408. 2012 Promega Corporation. Part Number TMD039 Rev.10/12. (or most recent revision)

Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program

9.0 Records

- Punch Amp & 3500xL PPF Plate Map and Import Sheet

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
07/01/2020	6	Definitions-Added reference to section definitions list; 5.4.8-Removed 2800M from list; Added 5.4.11; 5.4.12-Added instructions to obtain diluted tube, vortex, and spin down