Forensic Biology Section

Fusion Direct-Amp and Detect on 3500

1. **Scope**

1.1. The Promega PowerPlex Fusion System is a Short Tandem Repeat (STR) DNA typing technology that produces a DNA profile from biological material without extracting, purifying, or quantifying the DNA. This procedure uses half-volume reactions (12.5 □L) to obtain DNA profiles from known reference material, such as saliva on FTA cards. Small cuttings/punches of a sample are added directly to the amplification master mix, eliminating the extraction and quantification steps.

1.2. PowerPlex Fusion amplifies 24 locations (loci) throughout the human genome: the gender marker **Amelogenin** and 23 STR loci (**D3s1358, D1s11656, D2s441, D10s1248, D13s317, Penta E, D16s539, D18s51, D2s1338, CSF1PO, Penta D, TH01, vWA, D21s11, D7s820, D5s818, TPOX, DYS391, D8s1179, D12s391, D19s433, FGA,** and **D22s1045**).

1.3. Using the Polymerase Chain Reaction (PCR), all 24 loci are amplified in a single reaction using fluorescent tags (blue, green, yellow, and red) on the PCR primers to label the amplified DNA.

1.4. A portion of each amplified sample is combined with an Internal Lane Standard (ILS) labeled with orange and run on a capillary electrophoresis 3500 Genetic Analyzer (3500), injecting samples at 1.2 kV for 10 seconds. The peaks are detected based on the color of their fluorescent tags and the time it takes for the peak to reach the detector. Allelic ladders are run at the same time to define bins for the alleles.

2. **Safety**

2.1. Chemical Hazard: Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use chemical-resistant gloves and work in a well-ventilated area when handling.

3. **Specimen**

3.1. The recommended range of input of saliva on FTA is **one to two 1.2 mm Harris Micro-Punches** (or equivalent). The amount of 'input DNA' can be changed by adjusting the number or size of the punches (a single 1.2 mm punch has 1.13 mm^2 of surface area, two 1.2 mm punches have twice as much area, and a single 2.0 mm punch has almost three times as much area).

3.2. For saliva on oral swabs or blood on any substrate, the smallest size of cutting is often enough biological material for a successful DNA profiling reaction. Typically, blood has an abundance of DNA, but oral swabs vary in how much material is on any given part of a swab.

3.3. For Positive Controls or other extracted and quantified DNA extracts, approximately **4-5 ng of DNA** (or more) can be profiled with the Direct-Amp protocol.

3.4. More substrate/DNA can be added if a sample is degraded (to increase peak heights of larger loci), and less template can be added if PCR inhibitors are thought to be present (lowering the amount of inhibitor).

4. **Reagents**

- PowerPlex Fusion System (Promega)
- WEN Internal Lane Standard (ILS) 500 (Promega)
- 2800M Control DNA (0.25 ng/□L) (Promega)
- Hi-Di Formamide (Life Technologies)
- Anode and Cathode Buffer Containers (Life Technologies)
- Performance Optimized Polymer 3500 Series (POP-4, 384) (Life Technologies)
- 3500 Capillary Array, 36-cm (Life Technologies)

5. **Instrumentation**

- ProFlex Thermal Cyclers (Life Technologies)
- 3500 Genetic Analyzer (Life Technologies)

6. **Procedural Note**

6.1. This procedure uses half-volume reactions (12.5 ul total reaction volume instead of 25 ml).

6.2. When amplifying FTA punches with Direct-Amp, there is no need for a Reagent Blank. The Negative Control is the only non-template control required.

6.3. Master mix should be prepared in a laminar flow hood to protect the stock reagents. PCR setup and run setup may be performed in a laminar flow hood or on the bench top. When working outside of the hood, extra care should be taken around open tubes/plates.

6.4. Monitor and replace the 3500 Genetic Analyzer instrument consumables when needed. See "Operation of the 3500 Genetic Analyzer" protocol for specific time frames for each consumable.

6.5. Hi-Di reagent does not expire. Ladder and Pos Control are indicators of effective denaturation.

6.6. Applied Biosystems recommends that the oven be pre-heated for approximately **30 minutes** before starting a run. Pre-heating helps mitigate subtle first-injection migration rate differences.

6.7. It is recommended that the computer on the 3500 be restarted **once a week**.

7. **Programming the Thermal Cycler**

- 96 °C for 1 minute.
- **25 cycles** of: 94 °C for 10 seconds, 59 °C for 1 minute, 72 °C for 30 seconds.
- 60 °C for 20 minutes.
- 4 °C soak.
- Ramp rate = "Max".

8. **PCR Setup for CO Samples**

8.1. Complete a "Direct-Amplification Setup: PowerPlex Fusion" worksheet.

8.2. Label each microamp tube or a 96-well plate in a manner that corresponds with the Sample Number(s) or Batch ID on the amplification worksheet.

8.3. Thaw and vortex the amplification reagents (5X Master Mix, 5X Primer Pair Mix, Amplification Grade Water, 2800M Control DNA). Before use, gently tap to remove any liquid from the tube lids. Do not spin reagent tubes after vortexing.

8.4. Prepare the master mix (+1 for every 10 samples), then vortex for at least 10 seconds:

8.4.1.(# of reactions) x (**2.5 ul** of **5X Master Mix**)

8.4.2.(# of reactions) x (**2.5 ul** of **5x Primer Pair Mix**)

8.4.3.(# of reactions) x (**7.5 ul** of **Amplification Grade Water**)

8.5. Add **12.5 ul** of prepared **master mix** to each microamp tube or well in the 96-well plate. *Pipette tips do not need to be changed between additions.*

8.6. Take **one 1.2 mm punch** from a CO sample. Add this punch to a corresponding microamp tube or well. Sample placement should correspond to what is on the amplification worksheet. Repeat for each CO sample in the batch.

8.7. For the **positive control**, add **0.4 ul of the 2800M Control DNA** (10 ng/ul stock tube). This equates to 4.0 ng of Positive Control DNA.

8.8. The **negative control** should only contain **12.5 ul of the master mix**.

8.9. When all samples have been prepared, make sure all caps on the microamp tubes are closed, or if using a 96-well plate, seal the plate with a MicroAmp Clear Adhesive Film.

9. **PCR Amplification**

9.1. Place the rack of microamp tubes, or the 96-well plate, in the thermal cycler.

9.2. Open the direct-amp amplification method, "**Fusion Direct-Amp 25 Cycles**" (or similarly named). The total cycling time is approximately 1 hour and 15 minutes.

9.3. If a thermal cycler is not available, the samples can be stored at room temperature in a dark place (e.g. in a drawer) for several hours.

9.4. After amplification, samples should be run on the 3500 or stored at -20 °C. NOTE: Long term storage of amplified samples at 4 °C or higher may produce artifacts.

9.5. PCR product (amplicons) should be retained until the data has been technically and administratively reviewed.

10. **Run Plate Setup**

10.1. Complete a "3500 CE Plate Setup: PowerPlex Fusion (Direct-Amp)" worksheet.

10.2. Thaw (if necessary) and vortex the reagents (Hi-Di Formamide, WEN Internal Lane Standard, Fusion Allelic Ladder). Before use, gently tap to remove any liquid from the tube lids. Do not spin reagent tubes after vortexing.

10.3. Prepare the master mix (+1 for every 8 samples, be sure to include allelic ladder(s) and the remaining wells in a partially filled column in the total sample count), then vortex for at least 10 seconds:

10.3.1. (# of reactions) x (**10.0 ul** of **Hi-Di Formamide**)

10.3.2. (# of reactions) x (**0.5 ul** of **Internal Lane Standard**)

10.4. Add **10.0 ul** of prepared **master mix** to each well in the 96-well plate. *Pipette tips do not need to be changed between additions*.

10.5. Transfer **1.0 ul** of **allelic ladder** or **amplicon** to the corresponding well. Sample placement should correspond to what is on the CE Plate Setup worksheet.

10.6. Seal the 96-well plate with a plate septum, then briefly centrifuge the plate to remove any bubbles present in the sample wells.

10.7. Denature and snap cool the samples:

10.7.1. Place the 96-well plate in the thermal cycler, open the "**Denature**" (or similarly named) method, and denature (at 95°C) the samples **for 3 minutes**.

10.7.2. Plate the 96-well plate in a frozen metal plate holder, and **snap cool** for **at least 3 minutes**.

11. **Loading Samples onto the 3500 Instrument**

11.1. Place the 96-well plate into the blue plastic base of the 3500 plate tray, snap a white plate retainer over the plate and base, and verify the holes in the plate retainer and septum are lined up.

11.2. Press the "tray" button on the front of the instrument so the autosampler moves to the front, open the instrument door, place the assembled plate tray on the autosampler into position "A". Make sure the plate tray is secured to the autosampler with the side clips and close the instrument door.

12. **3500 Instrument Setup**

12.1. On the dashboard, click "Refresh" and check the status of the consumables. If necessary, replace any expired or near empty consumables. Refer to the "Operation of the 3500 Genetic Analyzer" for more information.

12.2. Pre-heat the oven, set the oven temperature to 60.0˚C and click "Start Pre-heat". The instrument takes ~30 minutes to pre-heat. When the temperature is displayed in green, the oven is pre-heated.

12.3. Fill out the "3500 Use and Maintenance Log".

13. **3500 Plate Record Setup**

13.1. If a 3500 Import file is used: On the "Dashboard" or "Workflow" screen, create a new plate record using the "**Create Plate from Standard File Format**" option, choose the .txt import file associated with your run. Proceed to step 12.8.

13.2. If a 3500 Import file is not used: On the "Dashboard" or "Workflow" screen, create a new plate record using the "**Create Plate from Template**" option, choose the plate template "**HID_36_POP4_Maine**".

13.3. On the "Define Plate Properties" screen, enter your "Plate Name" to match your "Batch ID" from your CE Plate Setup worksheet. The remaining plate properties are auto-populated based on the template used.

13.4. Click the "Assign Plate Contents" button at the bottom of the screen.

13.5. Enter the names of your samples, controls, and ladder. Sample, control, and ladder placement should correspond to what is on the injection list worksheet.

13.6. Assign the "Sample Type" to the controls and ladder(s) on your plate:

13.6.1. In "Plate View" expand the "Customize Sample Info" pane at the bottom-right corner of the screen, click on any/all relevant wells, and then assign "Allelic Ladder", "Positive Control", or "Negative Control".

13.6.2. In "Table View" choose the corresponding "Sample Type" from the drop-down list next to each allelic ladder, positive control, and negative control.

13.7. Assign the "Assay", "File Name Convention", and "Results Group" by selecting/highlighting all samples on the plate and then clicking the checkbox next to the assay, naming convention, and results group desired.

13.7.1. "**Assay"** = "**FUSION_HID_POP4_10sec**"

13.7.2. "**File Name Convention**" = "**Casework**"

13.7.3. "**Results Group**" = ***your initials***

13.7.4. If no selections are listed at the bottom of the "Assign Plate Contents" screen, you may need to add them to the plate from the library. Select "Add from Library" and search for what you need.

13.8. Save the plate by clicking "Link Plate for Run". If not previously loaded, the instrument will prompt you to load your plate onto the autosampler.

13.9. Verify the run information and click on "Start Run". The "Monitor Run" screen will then be displayed.

13.10. When the run has completed, use a USB drive to copy the results to the relevant user's data folder on "H:\Crimelab\DNA\3500 runs\" for analysis and archiving.