

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

PowerPlex® Fusion Direct Amplification of DNA from Bloodstain Card Punches		
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PowerPlex® Fusion Direct Amplification of DNA From Bloodstain Card Punches

1 Purpose

1.1 The PunchSolution™ Kit is designed for processing punches from exemplar bloodstain cards prior to direct amplification using PowerPlex® Fusion. (5X AmpSolution™ Reagent must be added to amplification reactions when performing direct amplification from blood punches using half volume amplification reactions (12.5µl)).

1.2 The sample size for this extraction is one 1.2mm card punch.

Note: Samples extracted via this method can only be amplified in PowerPlex Fusion 5C.

2 Extraction Procedure

2.1 Retrieve the following reagent from the associated refrigerator:

PunchSolution™ Reagent

2.2 Retrieve sample tubes with bloodstain punches. Label an empty 0.2ml tube with the associated tube top label for your Extraction Negative.

2.3 Have a **Witness** verify the labels of the tubes.

2.4 Add **10 µL** of the Promega PunchSolution™ Reagent to each 0.2 ml tube containing a punch in order to lyse the samples before adding PCR master mix. Make sure to add this reagent to the extraction negative control tube as well. (You do not need to vortex; if liquid remains on the tube wall or if the punches are not submerged, however, briefly centrifuge.)

2.5 Open each tube and place in a thermal cycler in the pre-amp laboratory. **Do not close the thermal cycler cover.**

2.6 Run the program “Fusion Direct Ext” on the thermal cycler in order to incubate the tubes at 30 minutes at 70°C. The tubes must be kept open in order to allow for complete evaporation. Tubes should be evaluated at the end of the 30 minute incubation to ensure that they are dry. If not, samples can be incubated for an additional 5 minutes and a note made in the batch comments. This process can be repeated until complete evaporation has occurred.

2.6.1 Record the thermal cycler in the LIMS usage log.

2.7 Complete the LIMS test batch.

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- 2.7.1 Perform data entry for all samples indicating if they were completed (Yes/No.)
 - 2.7.2 Fill out the performed by tab for the Extraction Run Review.
 - 2.7.3 Select all output samples and click Review to perform the test batch approval.
 - 2.7.4 Schedule the samples for Fusion Direct amplification and create the test batch.
- 2.8 Proceed with the amplification of the samples (see Section 3). Samples must proceed to amplification on the same day they are extracted.

3 Amplification Procedure

- 3.1 Retrieve the following reagents:

PowerPlex® Fusion 5X Primer Pair Mix
PowerPlex® Fusion 5X Master Mix
5X AmpSolution™ Reagent
Water, Amplification Grade
2800M Control DNA, 0.25ng/μl

- 3.2 After incubation in the thermal cycler is complete, add **8μL of Promega® Amplification Grade water** to each sample tube and the negative control tube. Re-suspend each sample by pipetting up and down with a new tip for each sample.
- 3.3 Prepare Master Mix
- 3.3.1 Centrifuge reagent tubes briefly to bring contents to the bottom and then vortex for 15 sec before use.

Note: After vortexing, do not re-centrifuge the Master Mix or Primer Pair Mix as this may cause the reagents to be concentrated at the bottom of the tube.

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- 3.3.2 In LIMS select the following reagents in the Reagent section of the test batch
- 3.3.3 Enter the volume of Amplification Grade Water required per the table below.
- 3.3.4 Click “Calculate” to determine the volumes to add to the master mix.

Amounts per reaction is as follows:

Reagent	Per reaction
Water, Amplification Grade	3.0 µL**
5X Master Mix	2.5 µL
5X Primer Mix	2.5 µL
5X AmpSolution Reagent	2.5 µL
Mastermix total:	10.5 µL

****CAUTION: Please make sure to enter the correct volume of water per reaction (3.0 µL) in LIMS prior to clicking “Calculate” when calculating the master mix amounts.**

- 3.3.5 When making the master mix, add the Amplification Grade water to the tube first. Next, add the PowerPlex® Fusion 5X Master mix, PowerPlex® Fusion 5X Primer Mix and 5X AmpSolution™ Reagent. Vortex prepared Master Mix for 5-10 sec.
 - 3.4 Label the 96 well plate with LIMS test batch ID and the time of the amplification in parentheses on the plate [i.e. DUSMMDDYY-XXX (HHMM)].
 - 3.5 Add 10.5 µL of the prepared master mix to each well in the 96-well plate. Skip the odd A wells (A1, A3, A5, etc.). These wells are reserved as place holders for allelic ladders downstream.
 - 3.6 Select the output samples and click “load plate” in LIMS. Click on well position B1 to “load” samples. Load samples into the LIMS screen only 15 at a time so that wells A1, A3, A5, etc can remain empty.
- Note: The samples may re-order themselves in the list on the left side of the load plate screen. Ensure that the samples are loaded into the LIMS plate in the order that you plan to physically add samples into the 96-well plate.
- 3.7 **Witness Step.** Have another analyst witness to verify that the samples are in the correct order by hovering the cursor over each well in the Load Plate screen and verifying that the tube labels are correct. The witness must also verify that the odd A wells are empty.
 - 3.8 Positive Control (**Well B1**)

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- 3.8.1 Vortex the **2800M Control DNA (0.25ng/ul)** and **add 2µL** of the positive control into **well B1**.
- 3.9 Amplification Negative (*Well C1*)
- 3.9.1 Add **2µl** Promega Amplification Grade Water to **well C1**.
- 3.10 Extraction Negative
- 3.10.1 Add **2µl** of the re-suspended extraction negative control into the appropriate well.
- 3.11 Add 2µl of re-suspended blood card extracts to their corresponding wells for amplification.
- 3.12 Seal the 96-well plate with strip caps using the capping tool prior to sending the samples to the post-amplification laboratory. Do not crush the caps.
- 3.13 The tubes with the blood punch re-suspensions should be discarded.

4 PowerPlex® Fusion Direct PCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

- 4.1 Briefly centrifuge the plate to remove air bubbles at 1000 rpm for 1 min.
- 4.2 Turn on the ABI 9700 Thermal Cycler in post-amp.
- 4.3 Place plate onto the Thermal Cycler and close the lid.
- 4.4 Choose the following program to amplify these samples.

PowerPlex® Fusion

user: casework

file: PPFusion-29

- 4.4.1 PowerPlex® Fusion PCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

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<p>9700</p> <p>PowerPlex® Fusion</p> <p>user: casework file: ppfusion-29</p>	<p>The PowerPlex® Fusion file is as follows:</p> <p>Soak at 96°C for 1 minutes</p> <p>29 Cycles : Denature at 94°C for 10 seconds : Anneal at 59°C for 60 seconds : Extend at 72°C for 30 seconds</p> <p>10 minute incubation at 60°C.</p> <p>Storage soak indefinitely at 4°C</p>
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- 4.5 Press Start and ensure the run begins.
 - 4.5.1 The run will start when the heated cover reaches temperature. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.
- 4.6 Record instrument in LIMS.
- 4.7 Upon completion of the amplification:
 - 4.7.1 Remove samples and press the STOP button repeatedly until the “End of Run” screen is displayed.
 - 4.7.2 Select the EXIT option (F5).
 - 4.7.3 Wipe any condensation from the heat block with a lint free wipe and pull the lid closed to prevent dust from collecting on the heat block.
 - 4.7.4 Turn the instrument off.
- 4.8 Place any racks used to set-up the plate for PCR in the container of 10% bleach container in the Post-Amp area.
- 4.9 After completion of the thermal cycling protocol, store amplified product at 4°C and proceed with fragment analysis for exemplars.
 - 4.9.1 Complete the LIMS test batch

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- 4.9.1.3 Select all output samples and click Review to perform the test batch approval.
- 4.9.2 Schedule the samples to the appropriate STR test batch and create the test batch.

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