

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

PowerPlex Fusion Direct Amplification of DNA from Buccal Swabs		
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PowerPlex® Fusion Direct Amplification of DNA From Buccal Swabs

1 Purpose

- 1.1 The SwabSolution™ Kit is designed for processing swabs prior to direct amplification using PowerPlex® Fusion. The SwabSolution™ Reagent is used to generate a buccal swab extract that is added directly to the PowerPlex® Fusion amplification reaction.
- 1.2 Sample size for this extraction should be approximately ½ of a cotton oral swab or one “triangle” of an OmniSwab™.
- 1.3 Note: Samples extracted via this method can only be amplified in PowerPlex Fusion 5C.

2 Extraction Procedure

- 2.1 Make sure a thermomixer is set to 70°C.
- 2.2 Retrieve the following reagent:

SwabSolution™ Reagent
- 2.3 Retrieve labeled 1.5mL microcentrifuge sample tubes. Label an empty 1.5mL microcentrifuge tube with the associated input label for your Extraction Negative.
- 2.4 Have a **Witness** verify the input labels of the tubes.
- 2.5 Add **500 µL** of the SwabSolution™ Reagent to each buccal swab cutting and negative control. (Ensure the cuttings are submerged in the liquid by briefly centrifuging if necessary.)
- 2.6 Place tubes in thermomixer and incubate samples at 70°C for 30 minutes in the pre-amp laboratory.
 - 2.6.1 Record the temperature and usage log for the instrument in LIMS.
- 2.7 Print the output sample labels and replace the existing input labels on the sample tubes after the incubation is completed.
- 2.8 Complete the LIMS test batch
 - 2.8.1 Perform data entry for all samples indicating if they were completed (Yes/No.)
 - 2.8.2 Assign a storage location for the extracts.

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- 2.8.3 Fill out the performed by tab for the Extraction Run Review.
 - 2.8.4 Select all output samples and click Review to perform the test batch approval.
 - 2.8.5 Schedule the samples to the Fusion Direct amplification test batch and create the test batch.
- 2.9 Store buccal swab extracts at 4°C, or proceed with the amplification of the samples (see Section 3)

3 Amplification Procedure

- 3.1 Retrieve the following reagents:

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

- 3.2 Make a 0.1 dilution of each sample and extraction negative by following the steps below:
- 3.2.1 Briefly vortex and centrifuge all swab extracts.
 - 3.2.2 Label empty 1.5mL tubes to be used in making dilutions of samples.
 - 3.2.3 Add 18ul of Promega amplification grade water and 2ul of sample extract into each output tube. Vortex and briefly centrifuge the diluted samples.
- 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 are as follows:

Reagent	Per reaction
Water, Amplification Grade	5.5 µL**
5X Master Mix	2.5 µL
5X Primer Mix	2.5 µL
Mastermix total:	10.5 µL

****CAUTION: Please make sure to enter the correct volume of water per reaction (5.5 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 and PowerPlex Fusion 5X Primer Mix. 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 The LIMS batch and Plate Name description should be written in the format of DFUSMMDDYY_HHMM.

3.5 Add **10.5 µL** of the prepared master mix to the 96-well plate, where needed. **Skip the first well of each injection (A1, A4, A7, A10).** These 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 **23** at a time so that wells **A1, A4, A7, and A10** 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 verify the tube set-up by reading the tube-top labels and complete INPUT sample IDs and having witness check the samples and order on the batch screen. Tube top labels should also be verified against the load plate screen by having the witness hover the cursor over each well in the Load Plate screen as the analyst re-reads each tube top label. The witness must then verify that **the appropriate Allelic Ladder wells** are empty on the plate.

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3.8 Positive Control (*Well B1*) – total input amount of 375pg.

3.8.1 Vortex the 2800M Control DNA (0.25ng/ul) and add 1.5µL of the positive control into well B1.

3.9 Amplification Negative (*Well C1*)

3.9.1 Add 2µL of Promega Amplification Grade Water to well C1.

3.10 Extraction Negative

3.10.1 Add 2µL of the diluted extraction negative control to the appropriate well.

3.11 Add 2µL of the 1/10 diluted sample extract to the corresponding wells in the 96-well plate.

3.12 Seal the 96-well plate with strip caps prior to sending the samples to the post-amplification laboratory with the cap tool. Do not crush the caps.

3.13 The 1/10 dilution tubes should be discarded.

3.14 Extraction tubes should be placed in an assigned cryobox.

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 1000rpm 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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9700 PowerPlex® Fusion	The PowerPlex® Fusion file is as follows: Soak at 96°C for 1 minutes 29 Cycles : Denature at 94°C for 10 seconds : Anneal at 59°C for 60 seconds : Extend at 72°C for 30 seconds 10 minute incubation at 60°C. Storage soak indefinitely at 4°C
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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.
- 4.7.5 NOTE: The 4°C storage soak step is not meant to store samples for an extended period. Samples should be removed from the instrument and placed in the 4°C refrigerator at the earliest convenience.
- 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.
- 4.10 Complete the LIMS test batch:

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- 4.10.1 Perform data entry for all samples indicating if they were completed (Yes/No.)
- 4.10.2 Fill out the Performed By tab for the Test Batch Review.
- 4.10.3 Select all output samples and click Review to perform the test batch approval.
- 4.10.4 Schedule the samples to the appropriate STR test batch and create the test batch.
- 4.10.5 A batch reviewer will then complete the Test Batch Tech Review.