

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Amplification using the Power Plex Fusion System		
Status:Published		Document ID: 5980
DATE EFFECTIVE 08/27/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 1 OF 4

PowerPlex® Fusion Sample Preparation for Amplification

1 Procedure

Fusion Sample Input Amount
Optimal – 525pg*
Minimum – 37.5pg

*The option for amplification with a greater input amount is available if determined appropriate for the sample by the analyst.

- 1.1 Retrieve the following reagents from the associated refrigerator and/or freezer.

PowerPlex Fusion® 5X Primer Pair Mix
PowerPlex Fusion® 5X Master Mix
Water, Amplification Grade
2800M Control DNA, .250ng/μl

- 1.2 Retrieve sample(s) needed for amplification from associated refrigerator and/or freezer.
- 1.3 Prepare dilutions **using Promega Amplification Grade Water**, for each sample, if necessary, according to Table 1. Vortex and centrifuge samples prior to aliquoting for dilution.

TABLE 1: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of Promega® Water (uL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2	38
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

- 1.4 Centrifuge reagent tubes briefly to bring contents to the bottom and then vortex for 15 seconds before use. 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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Amplification using the Power Plex Fusion System		
Status:Published		Document ID: 5980
DATE EFFECTIVE 08/27/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 2 OF 4

- 1.5 Consult the Reagents tab in LIMS for the exact amount of PowerPlex Fusion® 5X Primer Pair Mix and PowerPlex Fusion® 5X Master Mix to add.

Reagent	Per reaction
5X Primer Pair Mix	2.5 µL
5X Master Mix	2.5 µL
Mastermix total:	5 µL
DNA	7.5 µL

- 1.6 Vortex prepared Master Mix and samples created in Step 3. After vortexing, **briefly centrifuge** master mix and samples.
- 1.7 Add **5 µL** of the prepared master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.
- 1.8 **Witness Step.** Have another analyst witness the sample set-up.
- 1.8.1** For the input samples, confirm the tube label and sample ID for each sample. For the output samples, **the entire amp tube label must be read for each sample.**
- 1.9 Positive Control – total input amount of 500pg.
- 1.9.1 Aliquot positive control according to amplification sheet
- 1.10 Amplification Negative
- 1.10.1 7.5 uL of Water, Amplification Grade
- 1.11 Samples
- 1.11.1 Aliquot samples according to amplification sheet
- 1.12 Ensure that all caps are properly closed prior to sending the samples to the post-amplification laboratory.
- 1.13 Spin down samples at 1000 RPM for one minute.

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Amplification using the Power Plex Fusion System		
Status:Published		Document ID: 5980
DATE EFFECTIVE 08/27/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 3 OF 4

2 PowerPlex® FusionPCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

- 2.1 Turn on the ABI 9700 Thermal Cycler.
- 2.2 Choose the following program in order to amplify these samples:

PowerPlex® Fusion
user: casework
file: PPFusion-29

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

9700	The PowerPlex® Fusion file is as follows:
PowerPlex® Fusion	Soak at 96°C for 1 minutes
user: casework	29 Cycles : Denature at 94°C for 10 seconds
file: ppfusion-29	: 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

- 2.4 Record instrument in LIMS
- 2.5 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.
- 2.6 Upon completion of the amplification:
 - 2.6.1 Remove samples and press the STOP button repeatedly until the “End of Run” screen is displayed.
 - 2.6.2 Select the EXIT option (F5).
 - 2.6.3 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.

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Status:Published		Document ID: 5980
DATE EFFECTIVE 08/27/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 4 OF 4

- 2.6.4 Turn the instrument off.
- 2.7 Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the Post-Amp area.
- 2.8 After completion of the thermal cycling protocol, store amplified product at 4°C and proceed with fragment analysis for exemplars.
- 2.9 Complete the LIMS test batch
 - 2.9.1 Fill out the performed by tab for the Amplification Run Review.
 - 2.9.2 Select all output samples and click Review to perform the test batch approval.
- 2.10 Schedule the samples to the appropriate STR test batch and create the test batch.