

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Amplification using the Power Plex Fusion System		
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PowerPlex® Fusion Sample Preparation for Amplification

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.

- 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/ul

- Retrieve sample(s) needed for amplification from associated refrigerator and/or freezer.
- 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)

- Vortex **PowerPlex Fusion® 5X Primer Pair Mix and PowerPlex Fusion® 5X Master Mix**. Briefly centrifuge, for no more than 4 seconds, or tap down mastermix and primers.

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- 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

- Vortex prepared Master Mix and samples created in Step 3. After vortexing, **briefly centrifuge** master mix and samples.
- Add **5 µL** of the prepared master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.
- Witness Step.** Have another analyst witness the sample set-up.
- Positive Control – total input amount of 500pg.
 - Aliquot positive control according to amplification sheet**
- Amplification Negative
 - 7.5 uL of Water, Amplification Grade**
- Samples
 - Aliquot samples according to amplification sheet**
- Ensure that all caps are properly closed prior to sending the samples to the post-amplification laboratory.
- Spin down samples at 1000 RPM for one minute.
- Turn on the ABI 9700 Thermal Cycler.

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15. Choose the following program in order to amplify these samples:

a.

PowerPlex® Fusion
user: casework
file: PPFusion-29

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	: Denature at 94°C for 10 seconds
file: ppfusion-29	29 Cycles : 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