

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Amplification using the Power Plex Fusion System		
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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 and record the lot numbers.

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 in 1.5 mL tubes according to the values listed on the test batch data entry screen or the “FBAmplificationSheet”, **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 Label amp tubes using the values generated by LIMS. These values can be found in the test batch output samples or on the “FBAmplificationSheet”.
- 1.5 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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- 1.6 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

- 1.7 Vortex prepared Master Mix and all samples to be aliquoted. After vortexing, **briefly centrifuge** master mix and samples.
- 1.8 Add **5 µL** of the prepared master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.
- 1.9 **Witness Step.** Have another analyst witness the sample set-up.
- 1.9.1 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.**
- 1.10 Positive Control – total input amount of 375pg.
- 1.10.1 Aliquot positive control according to amplification sheet
- 1.11 Amplification Negative
- 1.11.1 7.5 uL of Water, Amplification Grade
- 1.12 Samples
- 1.12.1 Aliquot samples according to amplification sheet
- 1.13 Ensure that all caps are properly closed prior to sending the samples to the post-amplification laboratory.
- 1.14 Spin down samples at 1000 RPM for one minute.
- 1.15 For thermal cycler usage see [the Using the Mastercycler X50s manual.](#)

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1.16 The Fusion PCR program is as follows:

Soak at 96°C for 1 minute	
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	

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.

1.17 Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the Post-Amp area.