

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

PowerPlex® Fusion SAMPLE PREPARATION FOR AMPLIFICATION

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- 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

- 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.**
- Consult the Reagents tab in LIMS for the exact amount 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.

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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.
8. **Witness Step.** Have another analyst witness the sample set-up.
9. Positive Control – total input amount of 500pg.
 - a. **Aliquot positive control according to amplification sheet**
10. Amplification Negative
 - a. **7.5 uL of Water, Amplification Grade**
11. Samples
 - a. **Aliquot samples according to amplification sheet**
12. Ensure that all caps are properly closed prior to sending the samples to the post-amplification laboratory.
13. Spin down samples at 1000 RPM for one minute.
14. Turn on the ABI 9700 Thermal Cycler.
15. Choose the following program in order to amplify these samples:

a.

PowerPlex® Fusion

user: **casework**

file: **PPFusion-29**

