

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

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

Follow all relevant processes in the [General Guidelines for Forensic Biology and DNA Casework procedure](#).

Follow all relevant processes in the [BEAST DNA Worksheet Setup Manual](#) for creating and adding to worksheets and [BEAST DNA Worksheet Processing Manual](#) for how to record all relevant information while processing the worksheets.

1 Procedure

Fusion Sample Input Amount
Optimal – 375pg*
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 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 and take each sample into your custody

1.3 Vortex and centrifuge samples prior to aliquoting for dilution. Prepare dilutions in 1.5 mL tubes according to the values listed on the Amp Calc view, **using Promega Amplification Grade Water**, for each sample, if necessary, according to Table 1.

TABLE 1: Dilutions

Dilution	Amount of DNA Template (μL)	Amount of Promega® Water (μL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2	38
0.04	2	48
0.02	2	98
0.01	2	198
0.002	2	998
0.005	2	398
0.008	2	248

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- 1.4 Label amp tubes. These identifiers can be found on the PowerPlex Fusion Amplification Worksheet, listed after the item number in each sample well (i.e. F24-00010_4.8.2_2114).
- 1.5 Ensure reagents are completely thawed; 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.
- 1.6 Consult the Mixture Information table for the exact amount of PowerPlex Fusion® 5X Primer Pair Mix and PowerPlex Fusion® 5X Master Mix needed to prepare the Master Mix.

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

- 1.7 Vortex prepared Master Mix and all samples and controls to be aliquoted. After vortexing, briefly centrifuge master mix and samples.
- 1.8 Add 5 µL of the prepared master mix to each amp tube that will be utilized, changing pipette tips and remixing master mix as needed.
- 1.9 **Tube Setup WITNESS:** Have another analyst verify the tube setup.
 - 1.9.1 Confirm the sample label and amp tube label for each extract.
 - 1.9.2 Record the 'Tube Setup Witness'.
- 1.10 Aliquot Controls and Samples
 - 1.10.1 Aliquot Positive Control (375pg), Amplification Negative, and samples according to amplification sheet.
- 1.11 Ensure that all caps on the amp tubes are securely closed prior to sending the samples to the post-amplification laboratory.
- 1.12 Centrifuge samples at 1000 RPM for one minute.
- 1.13 Place the samples on the MasterCycler and start the Fusion PCR program. For MasterCycler usage, see [the Using the Mastercycler X50s manual](#).

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1.14 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.

- 1.15 Record the MasterCycler instrument.
- 1.16 Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the post-amp area.
- 1.17 Record the 'Run By' review task and 'Response' dropdown for the 'Pass or Fail?' step.
- 1.18 Transfer custody of each extract back to the original storage container.
- 1.19 Assign samples to next process step.