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.

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 500pg.

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.

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 **PowerPlex® Fusion** user: **casework** file: **ppfusion-29**	The PowerPlex® Fusion file is as follows: Soak at 96°C for 1 minutes 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

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.

2.6.4 Turn the instrument off.

2.6.5 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.

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.

2.9 Complete the LIMS test batch:

2.9.1 Fill out the Performed By tab for the Test Batch Review.

2.9.2 Select all output samples and click Review to perform the test batch approval.

2.9.3 A batch reviewer will then complete the Test Batch Tech Review.

2.10 Schedule the samples to the appropriate STR test batch and create the test batch.