PowerPlex® Y23 Sample Preparation for Amplification

1 Procedure

PPY23 Sample Input Amount
Optimal - 500pg of male DNA*
Minimum – 100pg of male DNA

*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® Y23 10X Primer Pair Mix
PowerPlex® Y23 5X Master Mix
Water, Amplification Grade for PPY23
2800M Control DNA for PPY23, 10ng/µl

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

1.3 Prepare dilutions (if necessary) 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 PPY23**, for each sample, according to Table 1. Vortex and centrifuge samples prior to aliquoting for dilution.

TABLE 1: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of 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	1 or (2)	99 or (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® Y23 10X Primer Pair Mix and PowerPlex® Y23 5X Master Mix to add.

Reagent	Per reaction
10X Primer Pair Mix	2.5 µL
5X Master Mix	5.0 µL
Mastermix total:	**7.5 µL**
DNA	17.5 µL

1.7 Vortex prepared Master Mix and all samples to be aliquoted for 5-10 seconds. After vortexing, **briefly centrifuge** master mix and samples.

1.8 Add **7.5 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 for PPY23 (PPY23 2800M Control in LIMS) – total input amount of **250pg**.

1.10.1 Perform dilution and aliquot positive control according to amplification sheet

1.11 Amplification Negative

1.11.1 17.5 uL of Water, Amplification Grade for PPY23 (PPY23 H2O in LIMS)

1.12 Samples

1.12.1 Aliquot samples according to amplification sheet

1.13 All amplification tubes should have a total final volume of 25uL.

1.14 Ensure that all caps are properly closed prior to sending the samples to the post-amplification laboratory.

1.15 Spin down samples at 1000 RPM for one minute.

2 PowerPlex® Y23 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® Y23
user: **casework** file: **ppy23**

2.3 PowerPlex® Y23 PCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

	The PowerPlex® Y23 file is as follows:
PowerPlex® Y23 user: **casework** file: **ppy23**	Soak at 96°C for 2 minutes 30 Cycles : Denature at 94°C for 10 seconds : Anneal at 61°C for 60 seconds : Extend at 72°C for 30 seconds 20-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.