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. Scan each sample into your custody.

1.3 Prepare dilutions (if necessary) in 1.5 mL tubes according to the values listed on Amp Calc view, **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 on the PowerPlex Fusion Amplification Worksheet, listed after the item number in the sample well (ie. F24-00010_4.8.2_**2114**)

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 Mixture Information table for the exact amount of PowerPlex® Y2310X 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 tube set-up.

1.9.1 Confirm the tube LIMS label and sample ID (Case # and Item #) for each sample extract and confirm the entire amp tube label for each sample..

1.10 Positive Control for PPY23 – 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

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.

1.16 For thermal cycler usage see the Using the Mastercycler X50s manual.

1.17 Add the thermal cycler instrument to the 'Instrument' box at the top of the worksheet

1.18 Fill out the 'Run By' review task and 'Response' dropdown.

1.19 Scan each extract back into the cryobox storage container.

1.20 The PPY23 PCR program is as follows:

Soak at 96°C for 2 minutes	
	: Denature at 94°C for 10 seconds
30 Cycles	: Anneal at 61°C for 60 second
	: Extend at 72°C for 30 seconds
20-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.

1.21 Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the post-amp area.