

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>Amplification using the PowerPlex Y23 System</b>		
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## PowerPlex® Y23 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

<b>PPY23 Sample Input Amount</b>
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 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 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 Amp Calc view, using Promega Amplification Grade Water for PPY23, for each sample, according to Table 1.

**TABLE 1: Dilutions**

Dilution	Amount of DNA Template (μL)	Amount of Promega® Water for PPY23 (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 Prepare the dilution for the Positive Control according to amplification sheet
- 1.5 Label amp tubes. These identifiers can be found on the PowerPlex Y23 Amplification Worksheet, listed after the item number in the sample well (i.e. F24-00010\_4.8.2\_2114).
- 1.6 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.
- 1.7 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	2.5µL
5X Master Mix	5.0µL
<b>Master mix total:</b>	<b>7.5µL</b>
DNA	17.5µL

- 1.8 Vortex the Master Mix and all samples and controls to be aliquoted. After vortexing, briefly centrifuge master mix and samples.
- 1.9 Add 7.5 mL of the prepared master mix to each amp tube that will be utilized, changing pipette tips and remixing master mix as needed.
- 1.10 Tube Setup **WITNESS**: Have another analyst witness the tube set-up.
  - 1.10.1 Confirm the sample label and amp tube label for each extract .
  - 1.10.2 Record the 'Tube Setup Witness'.
- 1.11 Aliquot Controls and samples
  - 1.11.1 Aliquot Positive Control for PPY23(250pg), amplification negative, and samples according to the amplification sheet.
- 1.12 Ensure that all caps on the amp tubes are securely closed prior to sending the samples to the post-amplification laboratory.
- 1.13 Centrifuge samples at 1000 RPM for one minute.
- 1.14 Place the samples on the MasterCycler and start the PPY23 program. For thermal cycler usage see the Using the Mastercycler X50s manual.

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1.15 The PPY23 PCR program is as follows:

Soak at 96°C for 2 minutes
30 Cycles : Denature at 94°C for 10 seconds : 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.

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