



Forensic Biology Section

Y23 Amp and Detect on 3500

1. Scope

- 1.1. The Promega PowerPlex Y23 System is a Y-Chromosome Short Tandem Repeat (Y-STR) DNA typing technology that produces a male DNA profile from the non-recombining region of the Y-chromosome. Non-recombining means a Y-STR profile is unchanged as it passes from father to son unless there is a random mutation. All the male descendants from a common ancestor have identical Y-STR profiles until there is a mutation.
- 1.2. PowerPlex Y23 amplifies 22 locations (loci) on the Y-chromosome (**DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456, YGATAH4**) producing a DNA profile with up to 23 alleles.
- 1.3. Using the Polymerase Chain Reaction (PCR), all 22 loci (with up to 23 alleles because DYS385a and DYS385b are amplified together as “DYS385”) are amplified in a single reaction using fluorescent tags (blue, green, yellow and red) on the PCR primers to label the amplified DNA.
- 1.4. A portion of each amplified sample is combined with an Internal Lane Standard (ILS) labeled with orange and run on a capillary electrophoresis 3500 Genetic Analyzer (3500), injecting samples at 1.2 kV for **15 seconds**. The peaks are detected based on the color of their fluorescent tags and the time it takes for the peak to reach the detector. Allelic ladders are run at the same time to define bins for the alleles at every locus.
- 1.5. Y-STR profiles can detect male DNA in overwhelming amounts of female DNA, may provide additional data from samples with very low levels of male DNA, can show relatedness between paternal relatives, and may indicate the number of males in a convoluted mixture.

2. Safety

- 2.1. Chemical Hazard: Formamide is a teratogen and is harmful by inhalation, skin contact and ingestion. Use chemical resistant gloves and in well-ventilated area when handling.

3. Specimen

- 3.1. The recommended amount of input DNA is **approximately 0.4 ng** for a single-source, high quality, **male** DNA extract.
- 3.2. With Rotor Gene qPCR, samples that are a mixture of male and female DNA, the male-specific quantitation can be estimated by using the X and Y peak height ratio to calculate the percentages of male and female DNA in the extract. Otherwise, the maximum volume of template can be amplified, but this could result in too much male DNA being added (any female DNA present will not amplify).
- 3.3. More DNA can be used if a sample is degraded (to increase peak heights of larger loci), or to increase the peak heights of a minor contributor in a mixture (even if it overloads the major contributors).
- 3.4. Less DNA can be used if PCR inhibitors are thought to be present (to lower the amount of inhibitor being added to the amplification reaction), or if a sample’s quantity is limited (to conserve DNA extract for additional amplifications).



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4. Reagents

- PowerPlex Y23 System (PROMEGA)
- Y23 WEN Internal Lane Standard 500 (PROMEGA)
- Hi-Di Formamide (LIFE TECHNOLOGIES)
- 2800M Control DNA (0.25 ng/μl) (PROMEGA)
- Hi-Di Formamide (LIFE TECHNOLOGIES)
- Stabilizer Reagent (PROMEGA)
- Cathode Buffer Container (LIFE TECHNOLOGIES)
- Anode Buffer Container (LIFE TECHNOLOGIES)
- Performance Optimized Polymer 3500 Series (POP-4, 384) (LIFE TECHNOLOGIES)
- 3500 Capillary Array, 36-cm (LIFE TECHNOLOGIES)

5. Instrumentation

- 9700 or ProFlex Thermal Cycler (LIFE TECHNOLOGIES)
- 3500 Genetic Analyzer (LIFE TECHNOLOGIES)

6. Procedural Note

- 6.1. If PCR polymerase inhibitors are thought to be present and significantly impacting the quality of data, a smaller volume of extract may be amplified, or the extract can be run through the “Cleanup of DNA Extracts” procedure in the ‘Extraction of DNA with QIAamp’ protocol.
- 6.2. Replace POP-4 if it has been in the instrument more than **14 days**. Replace the Anode and Cathode Buffer containers every **14 days** or as needed. The DataCollection Dashboard tracks the number of days.
- 6.3. The capillary arrays have a manufacturer’s expiration date, but this date can be exceeded if the allelic peaks are not broadening, and if the peak heights of the ILS are not decreasing. The capillary array should be replaced when the quality of these peaks begins to degrade.
- 6.4. Hi-Di reagent does not expire. Ladder and Pos Control are indicators of effective denaturation.
- 6.5. Applied Biosystems recommends that the oven be pre-heated for approximately **30 minutes** before starting a run. Pre-heating helps mitigate subtle first-run migration rate effects.
- 6.6. Perform a spatial calibration (alignment of capillaries) if the array or detection block have been moved.
- 6.7. Perform a spectral calibration (“matrix”) each time the capillary array is replaced or if pull-up or unexplained artifacts appears in numerous runs.



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7. Programming the Thermal Cycler:
 - 96 °C for 2 minutes.
 - **30 cycles** of: 94 °C for 10 seconds, 61 °C for 1 minute, 72 °C for 30 seconds.
 - 60 °C for 20 minutes.
 - 4 °C soak.
8. **PCR Setup**
 - 8.1. Complete a “PowerPlex Y23 Amplification” worksheet. Label each 0.2 ml microamp tube with its case number and specimen number, or derivation thereof.
 - 8.2. Thaw the 5X Master Mix, 10X Primer Pair Mix, and Amplification Grade Water **completely**. The tubes may be centrifuged briefly to bring the contents to the bottom. Vortex tubes for 15 seconds before each use and tap gently to remove any liquid from the lid (do NOT spin reagent tubes after vortexing).
 - 8.3. Prepare a final Master Mix (adding approximately 1 for every 10 samples to be amplified):
 - (# of reactions) x (**5.0 µl** of Y23 **5x Master Mix**).
 - (# of reactions) x (**2.5 µl** of Y23 **10x Primer Pair Mix**).
 - 8.4. Vortex the final Master Mix for at least 10 seconds, and then gently tap the tube on the bench top to remove liquid from inside the lid (do NOT spin after vortexing).
 - 8.5. Add **amplification grade water** to each tube so the **total volume of water + DNA will equal 17.5 µl**, and 17.5 µl of amp-grade water to the negative control. *Pipette tip does not have to be changed between additions.*
 - 8.6. Add **7.5 µl of final Master Mix** to each tube. *Pipette tip does not have to be changed between additions.*
 - 8.7. Add the **appropriate volume of extract or control DNA** to the reagent blank, specimen, and PCR positive control tubes. *The Positive Control DNA 2800M (0.25 ng/µl) is in the PCR Setup refrigerator.*
9. **PCR Amplification**
 - 9.1. Place the amp tubes in the thermal cycler (in the PCR Lab). Select the thermal cycler program for PowerPlex-Y23 (e.g. “**ppy23**”) and start the program. The total cycling time is approximately 1 hour.
 - 9.2. If a thermal cycler is not available, the reactions can be stored at room temperature in a dark place (such as a drawer) for several hours.
 - 9.3. Tubes may be removed when the program is done cycling and is going to, or has reached, the 4 °C hold. Do not leave samples in thermal cycler longer than 24 hours.
 - 9.4. After amplification, samples should be run on the 3500 or store the amplified samples at -20 °C. NOTE: Long term storage of amplified samples at 4 °C or higher may produce artifacts.
 - 9.5. PCR product should be retained until the data has been technically and administratively reviewed.



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10. Setting Up the 3500

- 10.1. In the dashboard, click “Refresh” and check consumables status to ensure the consumables are not expired and that buffer levels are adequate (i.e. at or near the fill lines).
- 10.2. Set the oven temperature to 60.0 °C, click “Start Pre-heat”, and wait **approximately 30 minutes** before starting the run to help mitigate subtle first-injection migration rate variations.
- 10.3. Check the instrument status in the Dashboard. The temperature is displayed in **red** as the instrument warms to the set-point. The temperature is displayed in **green** when it reaches the set point.
- 10.4. See “Operation of the 3500 Genetic Analyzer” method for detailed instructions.



11. Filling out Plate Record (before or after Adding Samples to Plate)

- 11.1. In the ‘Dashboard’ screen, create a new plate record by clicking on the “Create Plate from Template” button at the top of the screen, choose the plate template “**HID_36_POP4**”, and click “Open”.
- 11.2. In the Define Plate Details screen, type the Plate Name to include the run date, the kit name, and your initials (e.g. 030220_Y23_tc). The next 4 plate details should self-populate as follows:
 - Number of wells: **96**
 - Plate Type: **HID**
 - Capillary Length: **36**
 - Polymer: **POP4**
- 11.3. Click “Assign Plate Contents”.
- 11.4. Note: “Plate View” is automatically displayed, but “Table View” is also an option. Plate View shows the layout of samples as they appear on the plate, and multiple wells can be chosen by clicking-and-dragging or control-clicking to select and assign sample types, assays, or results groups to several samples simultaneously. Table View shows samples in column format with pulldown option lists.
- 11.5. Type the unique identifier for each sample, ladder, positive control, and negative control in their corresponding well location on the microamp plate (A1 through H12).



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- 11.6. Assign the Sample Type to the “Allelic Ladder”, “Positive Control”, or “Negative Control” to each sample in its corresponding well:
 - In “Plate View”: expand the ‘Customize Sample Info’ pane at the bottom-right corner of the screen, click on any/all relevant wells, and then assign “Allelic Ladder”, “Positive Control”, or “Negative Control” by choosing the corresponding Sample Type in the Customize Sample Info pane.
 - In “Table View”: choose the corresponding Sample Type from the drop-down list next to each “Allelic Ladder”, “Positive Control”, or “Negative Control”.
- 11.7. Right click in the “Assays” box, choose the “**Y23_HID_POP4**” assay from the library, click “Add to Plate”, and then click “close”.
- 11.8. Right click in “File Name Conventions” box, choose the “**Casework**” from the library (well position-sample name-capillary #), click “Add to Plate”, and then click “close”.
- 11.9. Right click in “Results Group” box, choose the various folders (Analyst’s initials) that the samples will be saved into from the library, click “Add to Plate”, and then click “close”.
- 11.10. Lastly, highlight the associated sample well positions and assign the appropriate Assay, File Name Convention and Results Group for the highlighted samples. Do this for all of the wells on the plate.
- 11.11. Save the plate.

12. Preparing Samples and Allelic Ladders

- 12.1. Prepare Hi-Di formamide, Y23 Internal Lane Size Standard (ILS), and Stabilizing Reagent:
 - Aliquot (**10.0 µl**) x (# injections, plus extra) of **Hi-Di** into a tube.
 - Add (**0.5 µl**) x (# injections, plus extra) of **Y23 WEN ILS** to the Hi-Di.
 - Add (**0.5 µl**) x (# injections, plus extra) of **Stabilizer Reagent** (*FOR Y23 SET-UP ONLY*).
 - Vortex the Hi-Di/WEN-ILS/Stabilizer Reagent mixture, then gently tap tube on bench top.
 - Record lot numbers on the 3500 Injection worksheet.
- 12.2. Aliquot **10 µl of Hi-Di/ILS/Stabilizer Reagent mix** into a 96-well plate, filling one well for each sample and ladder, including any blank wells within the sets of injections.
- 12.3. Transfer approximately **1.0 µl of each sample or allelic ladder** into corresponding wells in the plate (can be mixed by pipetting up and down).
- 12.4. NOTE: if pull-up or spectral overlap is seen in the ladders, using less volume of ladder (such as 0.5 µl) may alleviate the problem. Likewise, if the ILS is too low, a larger volume of ILS (such as 1.0 µl per sample) can be added to the Hi-Di/ILS/Stabilizing Reagent mix.
- 12.5. Seal the plate with a 96-well plate septum (may be cut to size for small runs), then **briefly centrifuge** the plate and verify that no bubbles are present in the sample wells.
- 12.6. Heat the plate in a thermal cycler or heat block at 95 °C for **3 minutes**. Do NOT close the thermal cycler lid or the septum may melt and/or adhere to the heated lid.
- 12.7. Transfer the plate to a frozen metal plate holder and chill for at least 3 minutes.




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12. Loading Samples into the Instrument

- 12.1. Place the 96-well plate into the plastic base for the 3500, snap a plate retainer over the plate and base (should be an audible “click”), and verify the holes in the plate retainer and septum are lined up.
- 12.2. Press the tray button so the autosampler moves to the front, open the instrument door, place the assembled plate on the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler, and close the instrument door.
- 12.3. Link the plate by clicking on “Link Plate for Run”. Verify the Run Information.
- 12.4. Check the instrument status in the Dashboard. The temperature is displayed in **red** as the instrument warms to the set-point. The temperature is displayed in **green** when it reaches the set point.
- 12.5. Click on “Start Run” after the **instrument status light turns green**. The Monitor Run Screen will then be displayed.
- 12.6. Check that the dates and lot numbers are entered on the 3500 Use and Maintenance Log.
- 12.7. When the run has completed, use a USB drive to copy the run to the relevant user’s data folder on H:\Crimelab\DNA\3500 runs\ for analysis and archiving.

13. Instrument Communication Reset (restarting computer)

- 13.1. Power OFF the computer and then power ON the computer.
- 13.2. Log into the computer as INSTR-ADMIN.
- 13.3. Right click on the ‘Server Monitor’ taskbar icon (at bottom of the monitor) and wait until all **four** services have launched (confirmed with “Y”). In approximately 1 minute, there should be green check mark icons  next to each service.
- 13.4. Lastly, launch the 3500 Software using the 3500 icon on the desktop or taskbar.

14. Instrument Communication Reset (restarting instrument and computer)

- 14.1. Perform these steps in this exact order!
- 14.2. Power OFF the computer.
- 14.3. Make sure the instrument door is closed, then power OFF the instrument.
- 14.4. Wait 1 minute, and then power ON the computer.
- 14.5. Wait until the Windows login screen is displayed, but do not login to Windows.
- 14.6. Power ON the instrument, wait for the front panel light status to turn **green**.
- 14.7. Log into the computer as INSTR-ADMIN.
- 14.8. Hover the cursor over the ‘3500 Server Monitor’ taskbar icon at bottom of the monitor. Wait until all of the services have a “Y” next to their names, then launch the 3500 Software using the icon on the desktop.

13. Analysis and Interpretation

- 13.1. Follow the “Y23 Analysis and Interpretation with GeneMapper ID-X” method.