TECHNICAL MANUAL

VersaPlex™ 27PY System for Use on the Applied Biosystems® Genetic Analyzers

Instructions for Use of Product **DC7020**

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VersaPlex™ 27PY System for Use on the Applied Biosystems® Genetic Analyzers

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

1. Description

Short tandem repeat (STR) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–9). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using fluorescence detection following electrophoretic separation.

The VersaPlex™ 27PY System[a–f] is a 27-locus multiplex for human identification applications including forensic analysis, relationship testing and research use. This six-color system allows co-amplification and fluorescent detection of the 20 autosomal loci in the expanded CODIS core loci (CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11 and D22S1045) as well as Amelogenin and DYS391 for gender determination. The Penta D, Penta E and D6S1043 loci are also included to increase discrimination and allow searching of databases that include profiles with these loci. Finally, two rapidly mutating Y-STR loci, DYS570 and DYS576, are included in the multiplex. This extended panel of STR markers is intended to satisfy both CODIS and ESS recommendations.

The VersaPlex™ 27PY System and all system components are manufactured in accordance with ISO 18385:2016. All necessary materials are provided to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the VersaPlex™ 27PY 5X Master Mix. This manual contains a protocol for use of the VersaPlex™ 27PY System with the ProFlex® PCR System in addition to protocols to separate amplified products and detect separated material on the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. Protocols to operate the fluorescence-detection instruments should be obtained from the instrument manufacturer. Amplification and detection instrumentation may vary. You may need to optimize protocols including amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

Information about other Promega fluorescent STR systems is available upon request from Promega or online at: **www.promega.com**

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
VersaPlex™ 27PY System	**200 reactions**	**DC7020**

Not For Medical Diagnostic Use. This system contains sufficient reagents for 200 reactions of 25µl each. Includes:

Pre-amplification Components Box

- 1ml VersaPlex™ 27PY 5X Master Mix
- 1ml VersaPlex™ 27PY 5X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 5 × 1,250µl Water, Amplification Grade

Post-amplification Components Box

- 100µl VersaPlex™ 27PY Allelic Ladder Mix
- 2 × 200µl WEN Internal Lane Standard 500

The VersaPlex™ 27PY Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening. The Water, Amplification Grade, is provided in a separate, sealed bag for shipping. Store this component with the pre-amplification components after opening.

Storage Conditions: Upon receipt, store all components at –30°C to –10°C in a nonfrost-free freezer. Make sure that the 2800M Control DNA is stored at 2–10°C for at least 24 hours before use. After the first use, store the VersaPlex™ 27PY System components at 2–10°C, where they are stable for 6 months. Do not refreeze. The VersaPlex™ 27PY 5X Primer Pair Mix, VersaPlex™ 27PY Allelic Ladder Mix and WEN Internal Lane Standard 500 (WEN ILS 500) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

The proper panels, bins and stutter text files for use with GeneMapper® *ID*-X software are available for download at: **https://www.promega.com/resources/software-firmware/versaplex-27py-genemapper-id-x-software-panels-and-bin-sets/**

Matrix standards are required for initial setup of the color separation matrix. The VersaPlex™ 6C Matrix Standard is provided separately and is compatible with the Applied Biosystems® 3500 and 3500xL Genetic Analyzers (VersaPlex™ 6C Matrix Standard, Cat.# DG4960).

3. Before You Begin

3.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (10,11). Guidelines for the validation process are published in the *Internal Validation Guide of Autosomal STR Systems for Forensic Laboratories* (12).

The quality of purified DNA, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and validation are required if any modifications to the recommended protocols are made.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (VersaPlex™ 27PY 5X Master Mix, VersaPlex™ 27PY 5X Primer Pair Mix, 2800M Control DNA and Water, Amplification Grade) are provided in a separate box and should be stored separately from those used following amplification (VersaPlex™ 27PY Allelic Ladder Mix and WEN Internal Lane Standard 500). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

Some reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

3.B. Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument.

For protocols and additional information on spectral calibration on these instruments, see the *VersaPlex™ 6C Matrix Standard Technical Manual* #TMD056. This manual is available at: **www.promega.com/protocols/**

4. Protocol for DNA Amplification Using the VersaPlex™ 27PY System

The VersaPlex™ 27PY System is designed for amplification of extracted DNA samples in a 25µl reaction volume.

The VersaPlex™ 27PY System is compatible with the ProFlex® PCR System, the GeneAmp® PCR System 9700 thermal cycler with a silver- or gold-plated silver sample block and the Veriti® 96-Well Thermal Cycler. This system has not been tested with the Veriti® 96-Well Fast Thermal Cycler or GeneAmp® PCR System 9700 with an aluminum block.

Note: It may be possible to use thermal cyclers other than those listed in this technical manual. The use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. The use of thermal cyclers with an aluminum block is **not** recommended with the VersaPlex™ 27PY System.

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 8.

4.A. Amplification of Extracted DNA in a 25µl Reaction Volume

Materials to Be Supplied by the User

- ProFlex® PCR System, GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block or Veriti® 96-Well Thermal Cycler (Applied Biosystems®)
- centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems®)
- aerosol-resistant pipette tips

We routinely amplify 1.0ng of template DNA in a 25µl reaction volume using the protocol detailed below.

Amplification Setup

1. At the first use, thaw the VersaPlex™ 27PY 5X Master Mix, VersaPlex™ 27PY 5X Primer Pair Mix and Water, Amplification Grade, completely. After the first use, store the reagents at 2–10°C.

   Note: Centrifuge tubes briefly to bring contents to the bottom, and then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after vortexing, because this may cause the reagents to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

4. Add the final volume of each reagent listed in Table 1 to a clean tube.

4.A. Amplification of Extracted DNA in a 25µl Reaction Volume (continued)

Table 1. PCR Amplification Mix for Amplification of Extracted DNA.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
VersaPlex™ 27PY 5X Master Mix	5.0µl	×		=	
VersaPlex™ 27PY 5X Primer Pair Mix	5.0µl	×		=	
template DNA (1.0ng)[2,3]	up to 15µl				
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, and then add VersaPlex™ 27PY 5X Master Mix and VersaPlex™ 27PY 5X Primer Pair Mix. The template DNA will be added at Step 6.

[2]Store DNA templates in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of DNA added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

[3]Apparent DNA concentrations can differ depending on the DNA quantification method used (13). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

5. Vortex the PCR amplification mix for 5–10 seconds, and then pipet PCR amplification mix into each reaction well.

! Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

Note: Do not store the PCR amplification mix for a prolonged period. Add the mix to the wells of the reaction plate as soon as the mix is prepared. Add DNA as soon as possible to each well and follow immediately by thermal cycling.

6. Add the template DNA (1.0ng) for each sample to the respective well containing PCR amplification mix.

   Note: The VersaPlex™ 27PY System was optimized and balanced using 1.0ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 1.0ng in the desired template DNA volume. Add 1.0ng of diluted DNA to a reaction well containing PCR amplification mix.

8. For the negative amplification control, pipet Amplification-Grade Water or TE^{-4} buffer instead of template DNA into a reaction well containing PCR amplification mix.

9. Seal or cap the plate, or close the tubes. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 29 cycles works well for 1.0ng of purified DNA templates.

1. Place the reaction plate or tubes in the thermal cycler.
2. Select and run the recommended protocol, which is provided below and in Figure 1. The total cycling time is approximately 1 hour.

   Notes:

   1. When using the ProFlex® PCR System use the default ramping mode (no emulation).
   2. When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%.
   3. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a silver or gold-plated silver sample block. The ramp speed is set after the thermal cycling run is started. When the Select Method Options screen appears, select "Max" for the ramp speed and enter the reaction volume.

Thermal Cycling Protocol
96°C for 5 minutes, then:
96°C for 5 seconds 60°C for 1 minute for 29 cycles, then:
60°C for 10 minutes
4°C soak

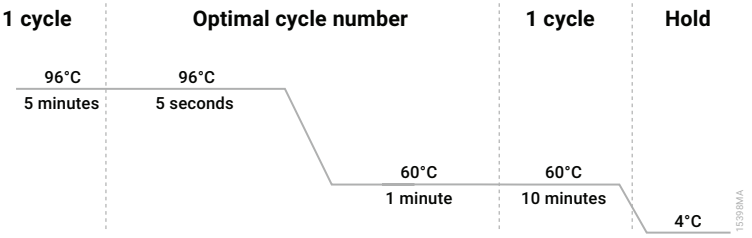


Figure 1. The thermal cycling protocol for the ProFlex® PCR System, GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler.

3. After completion of the thermal cycling protocol, proceed with fragment analysis or store amplified samples at –20°C protected from light.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

4.A. Amplification of Extracted DNA in a 25µl Reaction Volume (continued)

PCR Optimization (optional)

Cycle number should be optimized based on the results of an initial experiment to determine the sensitivity with your collection method, sample types and instrumentation.

1. Choose several samples that represent typical samples you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Prepare four identical reaction plates with these samples.
3. Amplify the samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (27–30 cycles).
4. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number from these samples.

5. Instrument Setup and Sample Preparation

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or a freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- plate retainer and base set (standard)
- POP-4® polymer for the 3500 or 3500xL
- anode buffer container
- cathode buffer container
- MicroAmp® optical 96-well plate and septa, or equivalent
- Hi-Di™ formamide (Applied Biosystems® Cat.# 4311320)

The quality of formamide is critical. Use only the recommended formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read warning label; take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Prepare a loading cocktail by combining and mixing WEN ILS 500 and formamide as follows:

   [(0.5µl WEN ILS 500) × (# samples)] + [(9.5µl formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be adjusted to change the intensity of the size standard peaks based on laboratory preferences.

2. Vortex for 10–15 seconds to mix.

3. Pipet 10µl of formamide/internal lane standard mix into each well.

4. Add 1µl of amplified sample (or 1µl of VersaPlex™ 27PY Allelic Ladder Mix) to each well. Cover the wells with appropriate septa.

   Notes:

   1. Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time in the run module, select "Instrument Protocol" from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.
   2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

5. Centrifuge the plate briefly to remove air bubbles from the wells.

6. Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

7. Place the plate in the 3500 series 96-well standard plate base and cover with the plate retainer.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

Instrument Preparation

Refer to the Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide for the instrument maintenance schedule and instructions to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide*.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 2). To ensure that you are viewing the most up-to-date information, press the Refresh button. Ensure that the Consumables Information and Maintenance Notifications are acceptable.

   Set the oven temperature to 60°C, and then select "Start Pre-Heat". When the Oven Temperature and Detection Cell Temperature turn green, you may proceed with the first injection.

Figure 2. The Dashboard.

2. Prior to the first analysis using the VersaPlex™ 27PY System, an Instrument Protocol, Size Standard, QC Protocol, Assay, File Name Convention and Results Group must be created.

2.a. To create a new Instrument Protocol, navigate to the Library, select "Instrument Protocols", and then select "Create". Alternatively, a previously created Instrument Protocol may be used.

Figure 3 shows the settings used at Promega for the Applied Biosystems® 3500xL Genetic Analyzer for the application type, dye set, capillary length, polymer, run module and appropriate protocol information.

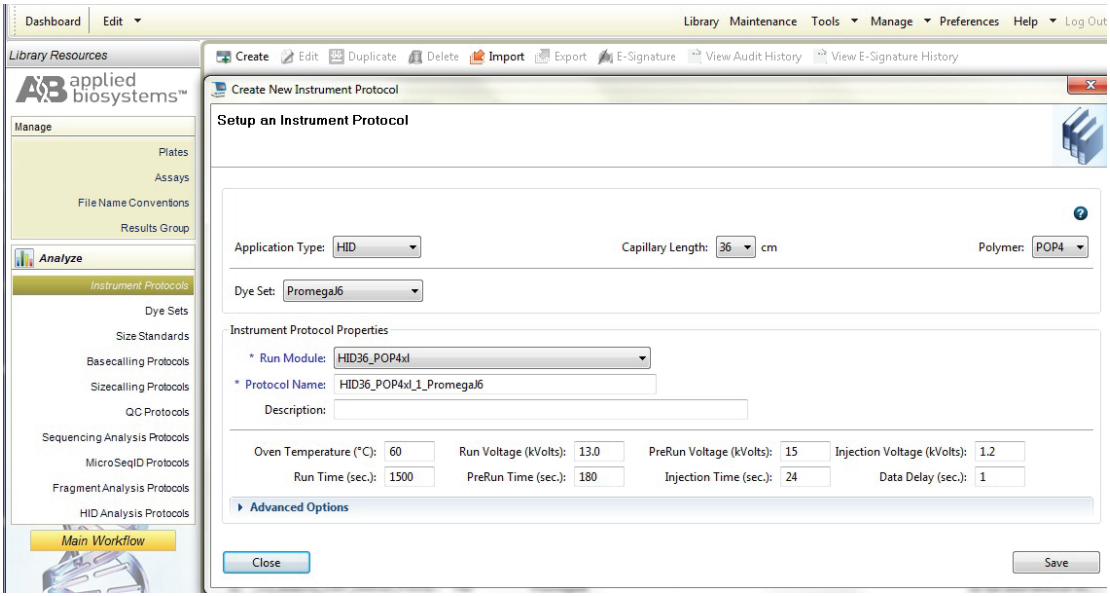


Figure 3. The Create New Instrument Protocol window.

The recommended settings are:

Application Type	HID
Capillary Length	36cm
Polymer	POP-4®
Dye Set	Promega J6
Run Module	HID36_POP4(xl)
Injection Time[1]	15 seconds for the Applied Biosystems® 3500 Genetic Analyzer 24 seconds for the Applied Biosystems® 3500xL Genetic Analyzer
Injection Voltage	1.2kV
Run Voltage	13kV
Run Time	1,500 seconds

[1]Injection time may be modified to increase or decrease peak heights.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

When creating an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega J6 spectral calibration.

Run time and other instrument settings should be optimized and validated in your laboratory.

When optimizing injection conditions in your laboratory, you may choose to create specific Instrument Protocols for each condition tested. If a single Instrument Protocol is used, follow the instructions in the *Applied Biosystems® 3500/3500xL Genetic Analyzers User Guide* to edit a library entry.

Assign a descriptive protocol name.

Note: For more detailed information refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzers User Guide*.

2.b. To create a new Size Standard for the QC protocol, navigate to the Library. Select "Size Standards", and then select "Create". Alternatively, a previously created Size Standard may be used.

Assign the Size Standard a descriptive name such as "WEN ILS 500". Choose "Orange" as the Dye Color. The fragments in the size standard are 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 4.

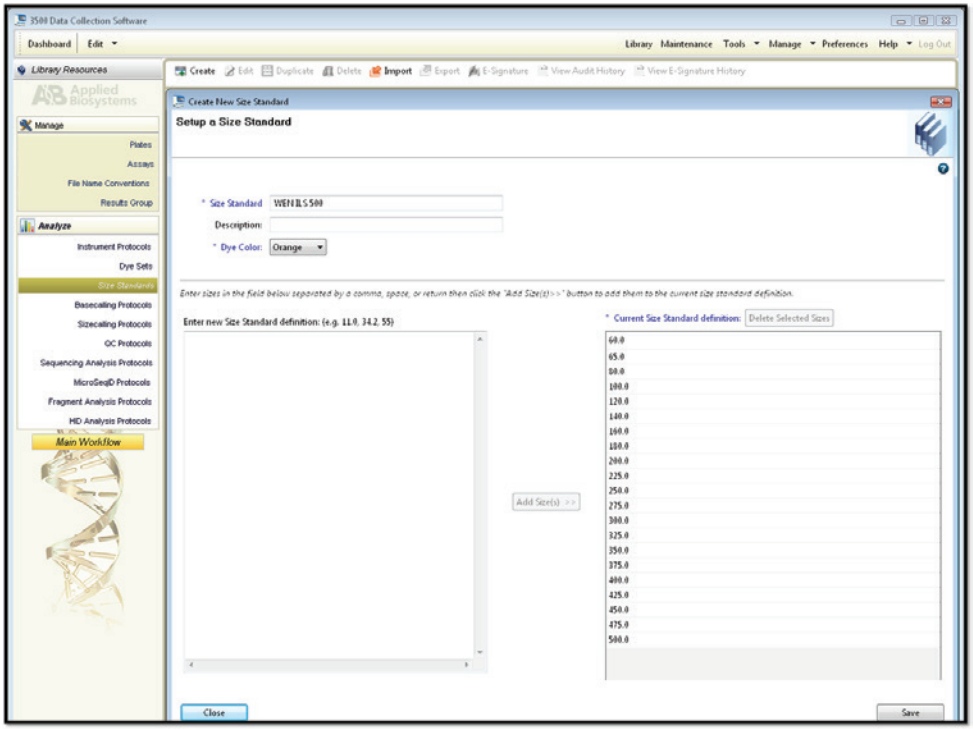


Figure 4. The Create New Size Standard window.

2.c. To create a new QC Protocol, navigate to the Library. Select "QC Protocols", and then select "Create". Alternatively, a previously created QC Protocol may be used.

Assign a descriptive protocol name such as WEN ILS 500. Select the size standard created in Step 2.b. The settings for the QC protocol should be based on the internally validated conditions for the VersaPlex™ 27PY System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 5 shows one option for these settings.

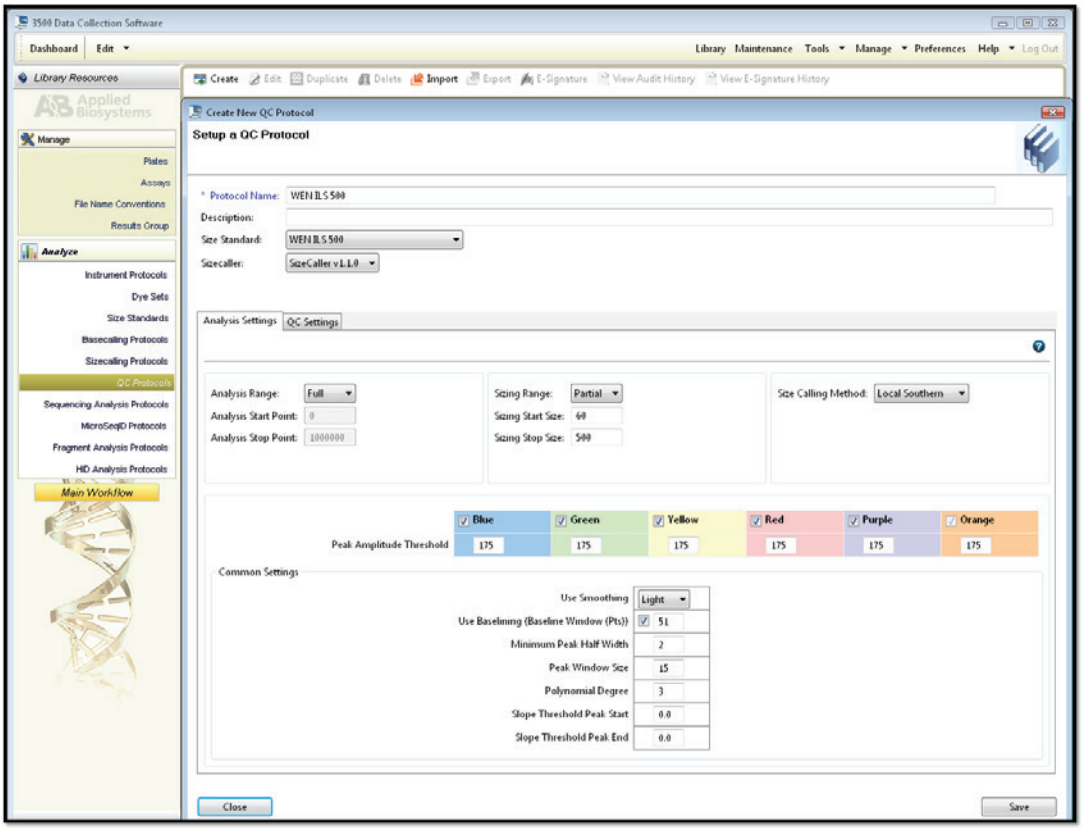


Figure 5. The Create New QC Protocol window.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

2.d. To create a new Assay, navigate to the Library. Select "Assays", and then select "Create". Alternatively, a previously created Assay may be used.

In the Create New Assay window (Figure 6), select the Instrument Protocol created in Step 2.a and the QC Protocol created in Step 2.c. Assign a descriptive assay name. Select the application type "HID". An Assay is required for all named samples on a plate.

Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.

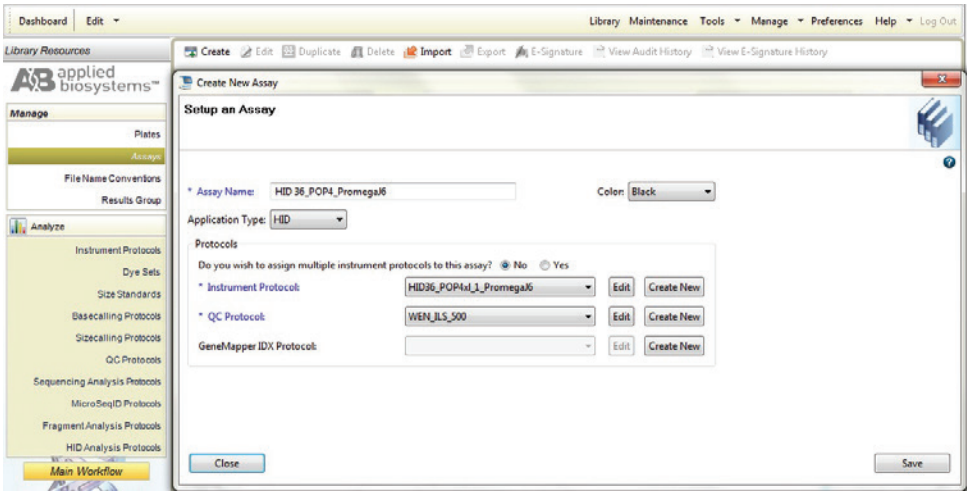


Figure 6. The Create New Assay window.

2.e. To create a new File Name Convention (Figure 7), navigate to the Library. Select “File Name Conventions”, and then select “Create”. Alternatively, a previously created File Name Convention may be used.

Select the File Name Attributes according to your laboratory practices, and save with a descriptive name.

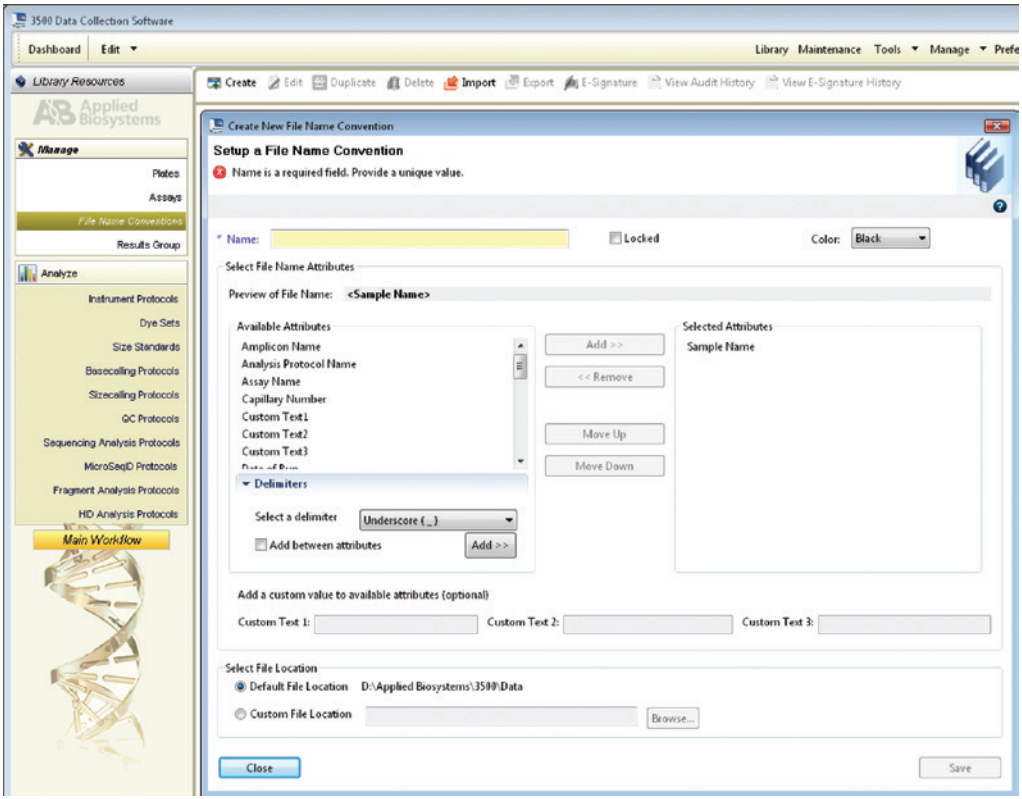


Figure 7. The Create New File Name Convention window.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

2.f. To create a new Results Group (Figure 8), navigate to the Library. Select "Results Group", and then select "Create". Alternatively, a previously created Results Group may be used.

Select the Results Group Attributes according to your laboratory practices. Save with a descriptive name.

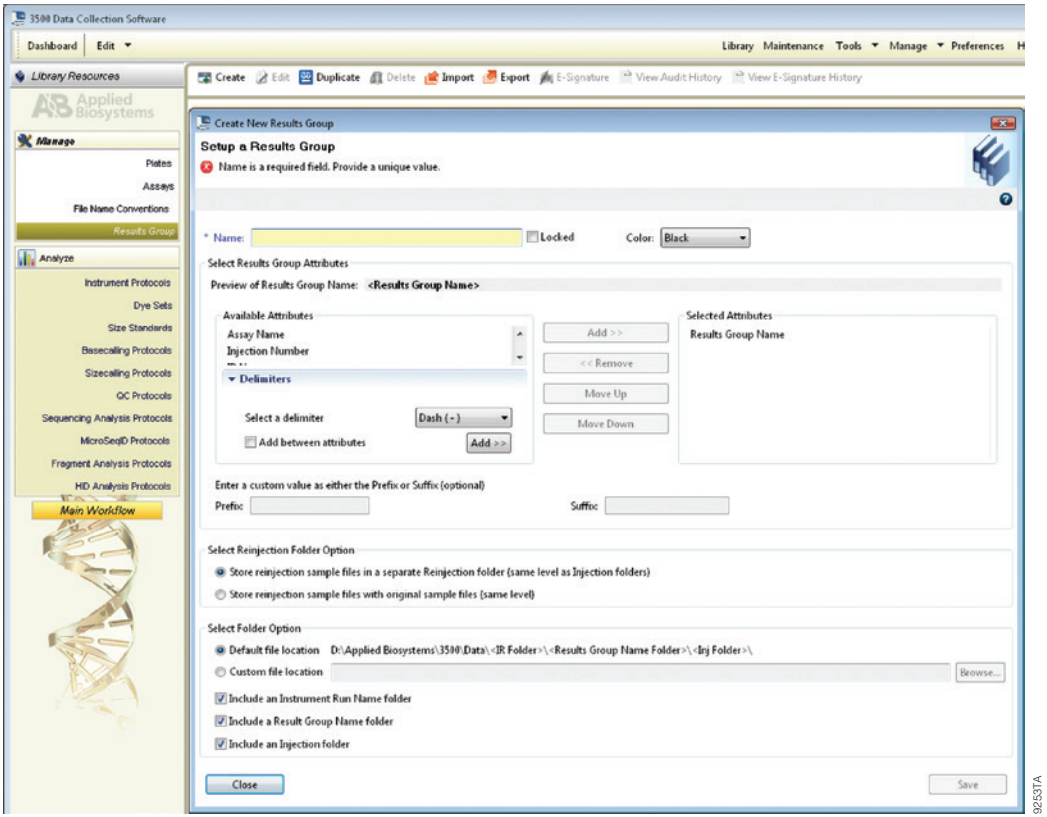


Figure 8. The Create New Results Group window.

3. To create a New Plate, navigate to the Library, and from the Manage menu, select “Plates”, and then “Create”.
4. Assign a descriptive plate name. Select the plate type “HID” from the drop-down menu (Figure 9).

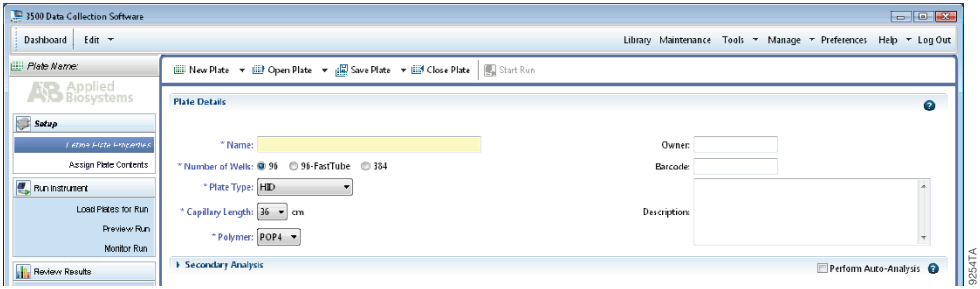


Figure 9. Defining plate properties.

5. Select “Assign Plate Contents” (Figure 10).

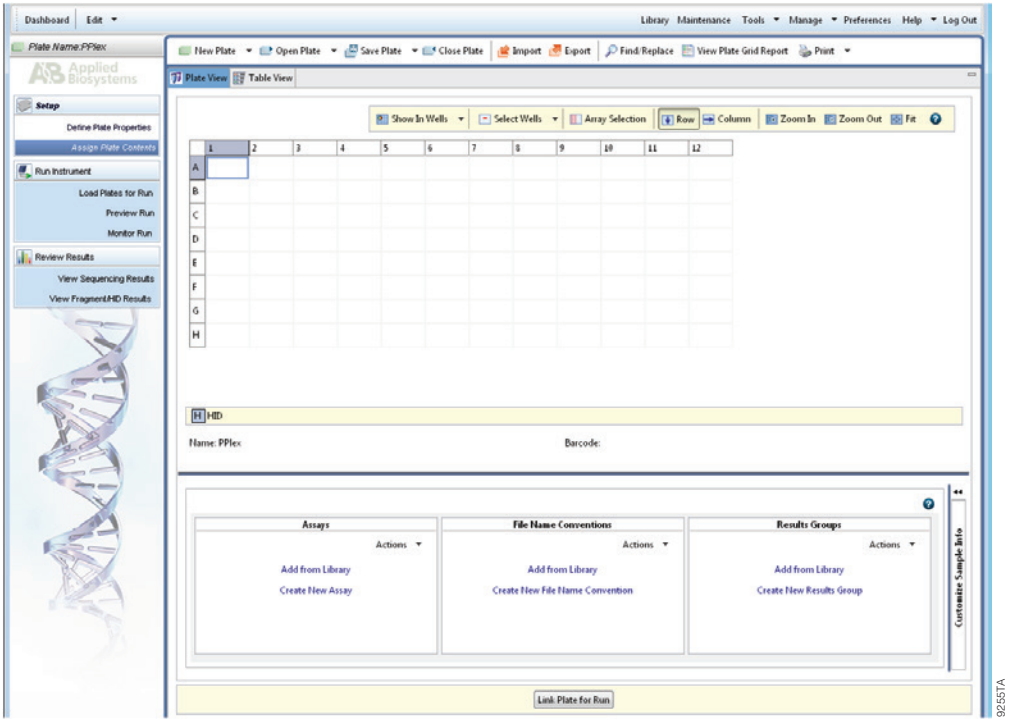


Figure 10. Assigning plate contents.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

6. Assign sample names to wells.
7. In the lower left portion of the screen, under "Assays", use the Add from Library option to select the Assay created in Step 2.d or one previously created. Click on the Add to Plate button, and close the window.
8. Under "File Name Conventions", use the Add from Library option to select the File Name Convention created in Step 2.e or one previously created. Click on the Add to Plate button, and close the window.
9. Under "Results Groups", use the Add from Library option to select the Results Group created in Step 2.f or one previously created. Click on the Add to Plate button, and close the window.
10. Highlight the sample wells, and then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.
11. Select "Link Plate for Run".
12. The Load Plate window will appear. Select "Yes".
13. In the Run Information window (Figure 11), assign a Run Name. Select "Start Run" (not shown).

    Each injection will take approximately 40 minutes.

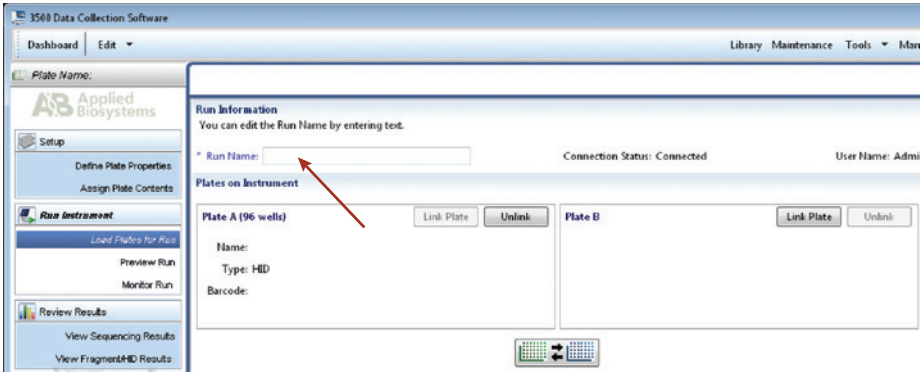


Figure 11. Assigning a run name.

6. Data Analysis Using GeneMapper® *ID*-X Software, Version 1.4

The instructions in this section were written using GeneMapper® *ID*-X software, version 1.4. Due to potential differences between individual software versions, some of the instructions may not apply to other software versions.

6.A. Importing VersaPlex™ 27PY Panels, Bins and Stutter Text Files into GeneMapper® *ID*-X Software, Version 1.4

To facilitate analysis of data generated with the VersaPlex™ 27PY System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID*-X software. We recommend that users receive training from Applied Biosystems on the GeneMapper® *ID*-X software to familiarize themselves with proper operation of the software.

Note: The panels, bins and stutter text files mentioned here are compatible with earlier versions of the GeneMapper® *ID*-X software.

Getting Started

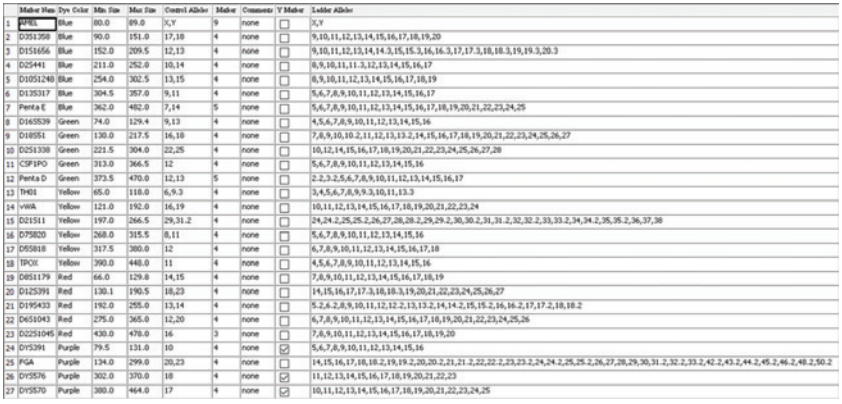
1. To obtain the proper panels, bins and stutter text files and WEN_ILS_500_IDX.xml file for the VersaPlex™ 27PY System go to:
   https://www.promega.com/resources/software-firmware/versaplex-27py-genemapper-id-x-software-panels-and-bin-sets/
2. Select the VersaPlex™ System that you are using, and select "GeneMapper ID-X". Enter your contact information, and select "Submit".
3. Save the VersaPlex_27PY_Panels_IDX_vX.x.txt, VersaPlex_27PY_Bins_IDX_vX.x.txt and VersaPlex_27PY_Stutter_IDX_vX.x.txt files, where "X.x" refers to the most recent version of the panels, bins and stutter text files, to a known location on your computer.
4. Save the WEN_ILS_500_IDX.xml file to a known location on your computer.

Importing Panels, Bins and Stutter Text Files

1. Open the GeneMapper® *ID*-X software.
2. Select "Tools" and then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select "File" and then "Import Panels".
5. Navigate to the panels text file downloaded in the Getting Started section. Select the file and then "Import".
6. In the navigation pane, highlight the VersaPlex™ 27PY panels folder that you just imported in Step 5.
7. Select "File" and then "Import Bin Set".
8. Navigate to the bins text file downloaded in the Getting Started section. Select the file and then "Import".
9. In the navigation pane, highlight the VersaPlex™ 27PY panels folder that you just imported in Step 5.

6.A. Importing VersaPlex™ 27PY Panels, Bins and Stutter Text Files into GeneMapper® *ID*-X Software, Version 1.4 (continued)

10. Select "File" and then "Import Marker Stutter". A warning box will appear asking if you want to overwrite current values. Select "Yes".
11. Navigate to the stutter text file imported in the Getting Started section. Select the file and then "Import".
12. In the Panel Manager, check the boxes to indicate DYS391, DYS576 and DYS570 are Y-markers. See Figure 12. This option is not available for older versions of the GeneMapper® *ID*-X software.
13. At the bottom of the Panel Manager window, select "OK". This will save the panels, bins and stutter text files and close the window.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	AMEL	Blue	80.0	89.0	X,Y	9	none	☐	X,Y
2	D351358	Blue	90.0	151.0	17,18	4	none	☐	9,10,11,12,13,14,15,16,17,18,19,20
3	D151656	Blue	152.0	209.5	12,13	4	none	☐	9,10,11,12,13,14,14.3,15,15.3,16,16.3,17,17.3,18,18.3,19,19.3,20.3
4	D25441	Blue	211.0	252.0	10,14	4	none	☐	8,9,10,11,11.3,12,13,14,15,16,17
5	D1051240	Blue	254.0	302.5	13,15	4	none	☐	8,9,10,11,12,13,14,15,16,17,18,19
6	D135317	Blue	304.5	357.0	9,11	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16,17
7	Penta E	Blue	362.0	482.0	7,14	5	none	☐	5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
8	D165539	Green	74.0	129.4	9,13	4	none	☐	4,5,6,7,8,9,10,11,12,13,14,15,16
9	D10551	Green	130.0	217.5	16,18	4	none	☐	7,8,9,10,10.2,11,12,13,13.2,14,15,16,17,18,19,20,21,22,23,24,25,26,27
10	D251338	Green	221.5	304.0	22,25	4	none	☐	10,12,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28
11	CSF1PO	Green	313.0	366.5	12	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16
12	Penta D	Green	373.5	470.0	12,13	5	none	☐	2.2,3.2,5,6,7,8,9,10,11,12,13,14,15,16,17
13	TH01	Yellow	65.0	118.0	6,9.3	4	none	☐	3,4,5,6,7,8,9,9.3,10,11,13.3
14	vWA	Yellow	121.0	192.0	16,19	4	none	☐	10,11,12,13,14,15,16,17,18,19,20,21,22,23,24
15	D21511	Yellow	197.0	266.5	29,31.2	4	none	☐	24,24.2,25,25.2,26,27,28,28.2,29,29.2,30,30.2,31,31.2,32,32.2,33,33.2,34,34.2,35,35.2,36,37,38
16	D75820	Yellow	268.0	315.5	8,11	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16
17	D5818	Yellow	317.5	360.0	12	4	none	☐	6,7,8,9,10,11,12,13,14,15,16,17,18
18	TPOX	Yellow	390.0	448.0	11	4	none	☐	4,5,6,7,8,9,10,11,12,13,14,15,16
19	D851179	Red	66.0	129.8	14,15	4	none	☐	7,8,9,10,11,12,13,14,15,16,17,18,19
20	D125391	Red	130.1	190.5	18,23	4	none	☐	14,15,16,17,17.3,18,18.3,19,20,21,22,23,24,25,26,27
21	D195433	Red	192.0	255.0	13,14	4	none	☐	5.2,6.2,8,9,10,11,12,12.2,13,13.2,14,14.2,15,15.2,16,16.2,17,17.2,18,18.2
22	D651043	Red	275.0	365.0	12,20	4	none	☐	6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26
23	D2251045	Red	430.0	478.0	16	3	none	☐	7,8,9,10,11,12,13,14,15,16,17,18,19,20
24	DYS391	Purple	79.5	131.0	10	4	none	☑	5,6,7,8,9,10,11,12,13,14,15,16
25	FGA	Purple	134.0	299.0	20,23	4	none	☐	14,15,16,17,18,18.2,19,19.2,20,20.2,21,21.2,22,22.2,23,23.2,24,24.2,25,25.2,26,27,28,29,30,31.2,32.2,33.2,42.2,43.2,44.2,45.2,46.2,48.2,50.2
26	DYS576	Purple	302.0	370.0	18	4	none	☑	11,12,13,14,15,16,17,18,19,20,21,22,23
27	DYS570	Purple	380.0	464.0	17	4	none	☑	10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25

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Figure 12. The GeneMapper® *ID*-X Software, Version 1.4, Y-Marker Check Box.

6.B. Importing the WEN ILS 500 IDX Size Standard into GeneMapper® *ID*-X Software, Version 1.4

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.C.

1. Select "Tools" and then "GeneMapper *ID*-X Manager".
2. Select the Size Standard tab.
3. Select "Import".
4. Navigate to the location of the WEN_ILS_500_IDX.xml file on your computer.
5. Highlight the file, and then select "Import".
6. Select "Done" to save changes and close the GeneMapper® *ID*-X Manager.

6.C. Creating a Size Standard with GeneMapper® *ID*-X Software, Version 1.4

1. Select "Tools" and then "GeneMapper *ID*-X Manager".
2. Select the Size Standard tab.
3. Select "New".
4. In the Size Standard Editor window (Figure 13), select "GeneMapper *ID*-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a detailed name, such as "WEN_ILS_500_IDX".
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). For more information about the WEN Internal Lane Standard 500, see Section 10.C, Figure 19.
8. Select "OK".

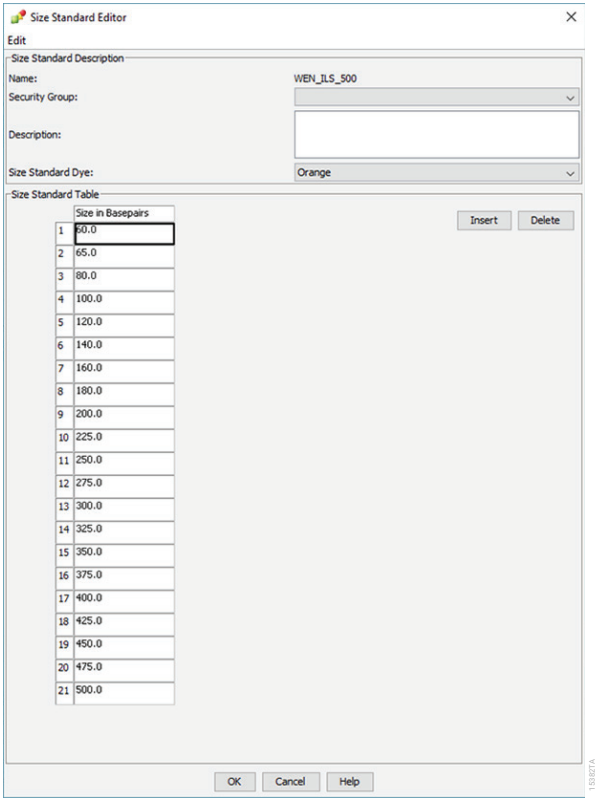

Figure 13. The GeneMapper® *ID*-X Software, Version 1.4, Size Standard Editor.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software, Version 1.4

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X software. They are not intended as a comprehensive guide for using GeneMapper® *ID*-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select "Tools" and then "GeneMapper *ID*-X Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select "GeneMapper *ID*-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "VersaPlex 27PY".
6. Select the Allele tab (Figure 14).
7. Select the bins text file that was imported in Section 6.A.
8. If you do not check the "Use marker-specific stutter ratio and distance if available" box, you will need to optimize these settings. In-house validation should be performed. If in-house validation is not performed, ensure that the "Use marker-specific stutter ratio and distance if available" box is checked. Doing this will assign locus-specific stutter filters and distances from the imported stutter file. We recommend the settings shown in Figure 14 for proper filtering of stutter peaks when using the VersaPlex™ 27PY System.

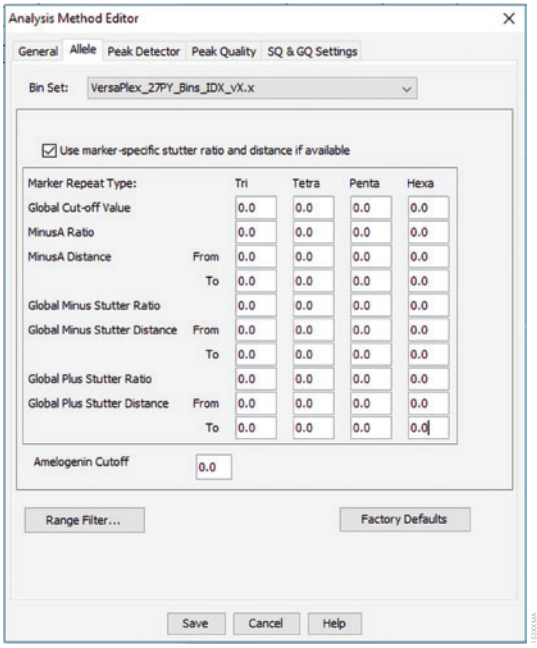

Figure 14. The GeneMapper® *ID*-X Software, Version 1.4, Allele tab.

9. Select the Peak Detector tab (Figure 15). You may need to optimize these settings. In-house validation should be performed.

   Notes:

   1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
   2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.
   3. If normalization was not used during data collection, make sure the normalization box is unchecked.

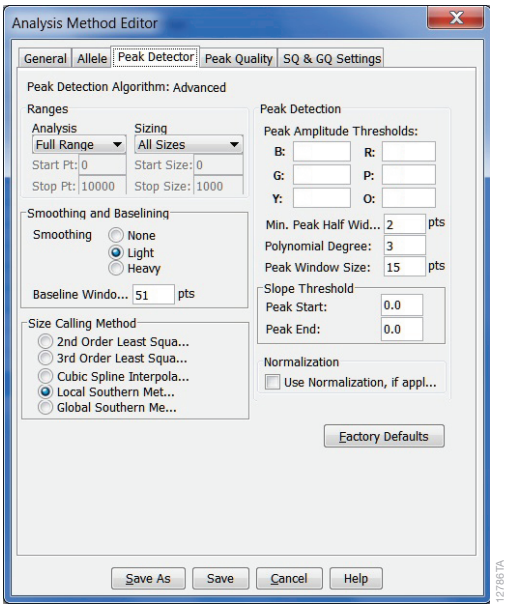

Figure 15. The GeneMapper® *ID*-X Software, Version 1.4, Peak Detector tab.

10. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 10 and 11, see the GeneMapper® *ID*-X user's manual for more information.

11. Select the SQ & GQ Settings tab. You may change these settings.

12. Select "Save" to save the new analysis method.

13. Select "Done" to exit the GeneMapper® *ID*-X Manager.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software, Version 1.4 (continued)

Processing Data for Casework Samples

1. Select "File" and then "New Project".
2. Select "Edit" and then "Add Samples to Project".
3. Browse to the location of the run files. Highlight desired files, and then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Allelic Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Allelic Ladder" in the Sample Type column for proper genotyping.
5. In the Analysis Method column, select the analysis method created previously in this section.
6. In the Panel column, select the panels text file that was imported in Section 6.A.
7. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.
8. Select "Analyze" (green arrow button) to start data analysis.

   Note: By default, the software is set to display the Analysis Requirements Summary window and Allelic Ladder Analysis Summary window if an issue is detected. After analysis is complete, the default setting is to show the Analysis Summary tab. If these default settings are changed, manual troubleshooting may be necessary.
9. If all analysis requirements are met, the Save Project window will open (Figure 16).

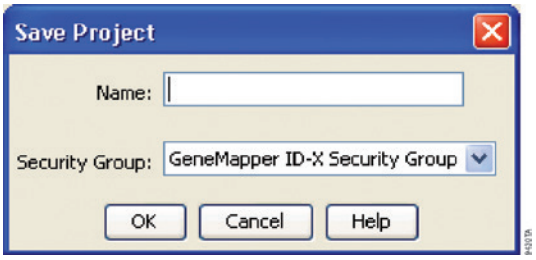

Figure 16. The Save Project window.

10. Enter the project name.
11. Choose the applicable security group from the drop-down menu, and then select "OK".

When the analysis is finished we recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory's data analysis protocols.

6.E. Controls in GeneMapper® *ID*-X Software

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.
2. Observe the results for the 2800M Control DNA. The expected 2800M DNA allele designations for each locus are listed in Table 7 (Section 10.A).

7. Results

Representative results of the VersaPlex™ 27PY System are shown in Figure 17. The VersaPlex™ 27PY Allelic Ladder Mix is shown in Figure 18.

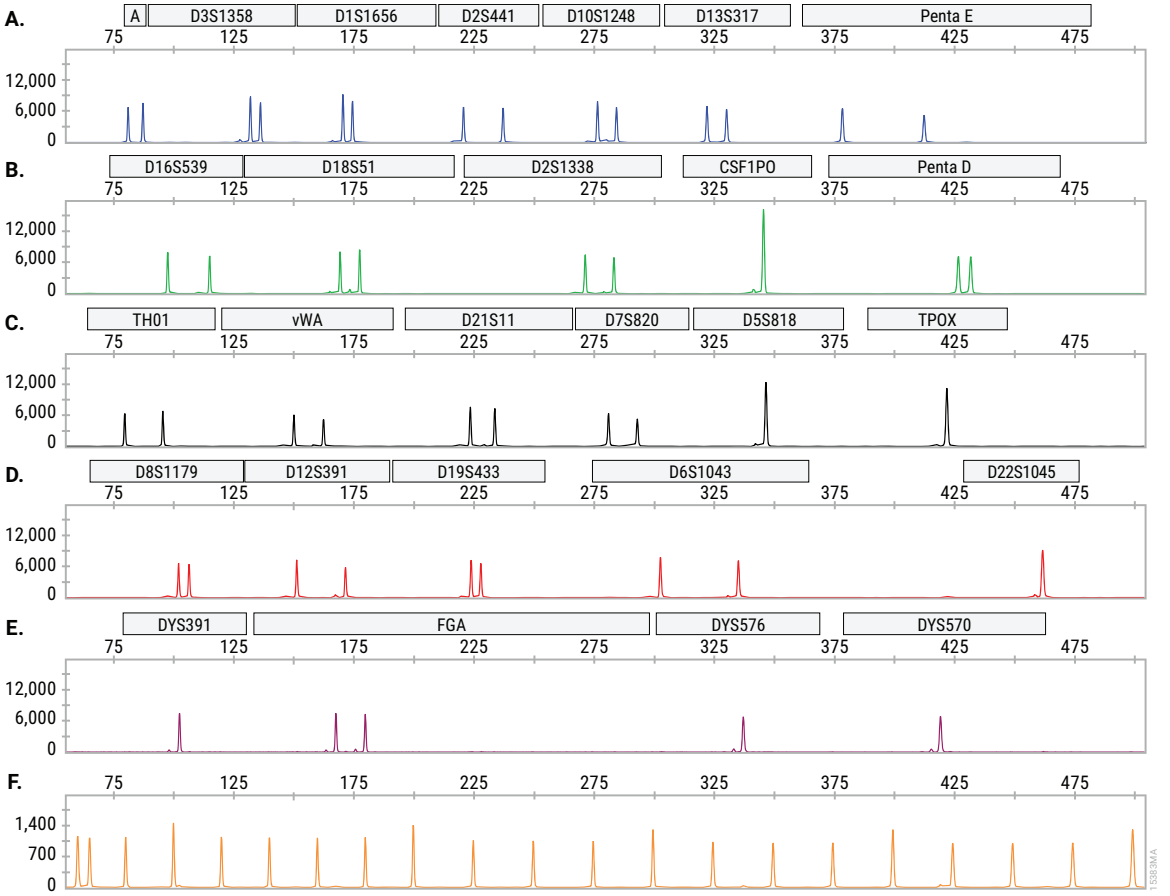

Figure 17. The VersaPlex™ 27PY System. The 2800M Control DNA (1.0ng) was amplified using the VersaPlex™ 27PY System and 29 cycles. Amplification products were mixed with WEN Internal Lane Standard 500 and analyzed using an Applied Biosystems® 3500xL Genetic Analyzer and a 1.2kV, 24-second injection. Results were analyzed using GeneMapper® *ID*-X software, version 1.4 and VersaPlex™ 27PY panels and bins text files. **Panel A**. An electropherogram showing the peaks of the FL-labeled loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D16S539, D18S51, D2S1338, CSF1PO and Penta D. **Panel C.** An electropherogram showing the peaks of the TMR-labeled loci: TH01, vWA, D21S11, D7S820, D5S818, and TPOX. **Panel D.** An electropherogram showing the peaks of the CXR-labeled loci: D8S1179, D12S391, D19S433, D6S1043 and D22S1045. **Panel E**. An electropherogram showing the TOM-labeled loci: DYS391, FGA, DYS576 and DYS570. **Panel F.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500.

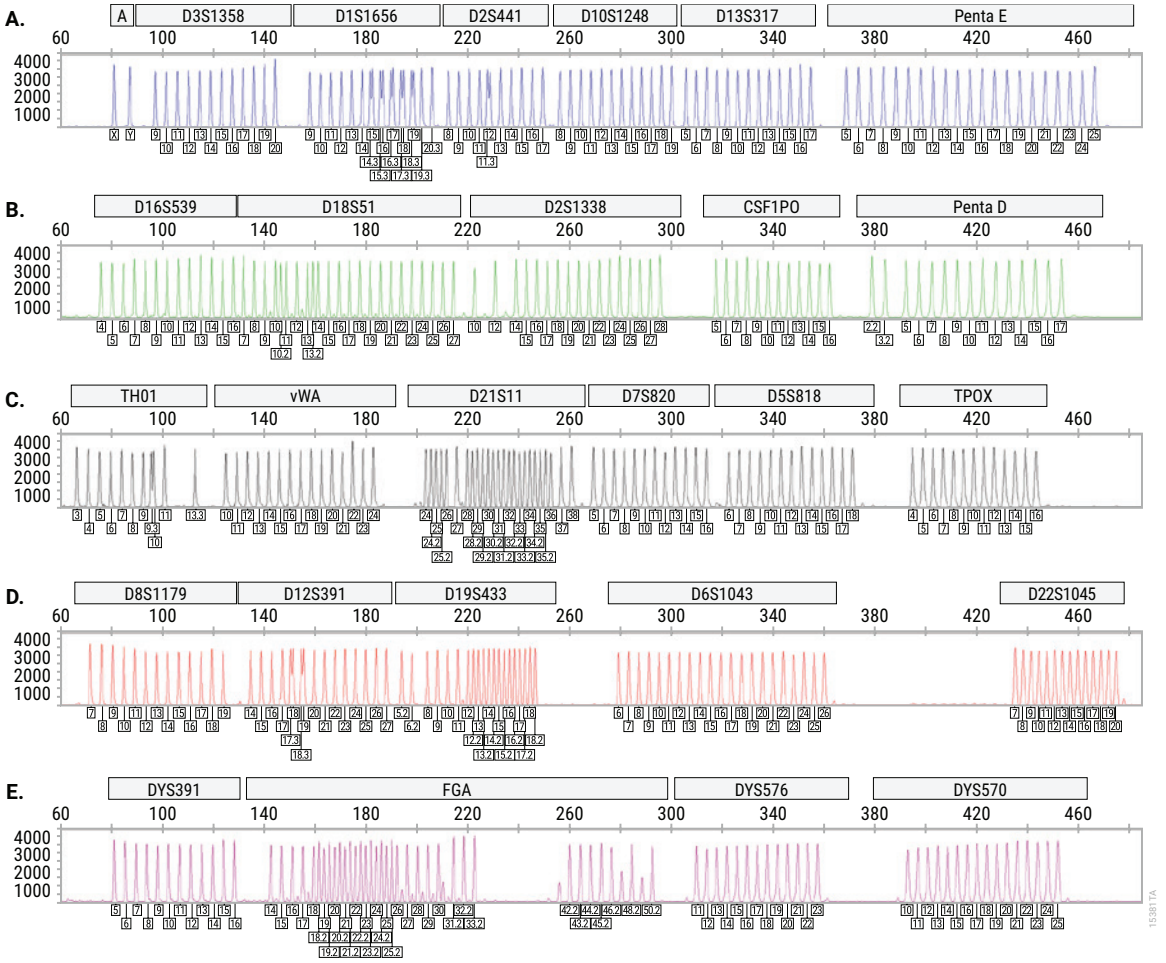

Figure 18. The VersaPlex™ 27PY Allelic Ladder Mix. The VersaPlex™ 27PY Allelic Ladder Mix was analyzed using an Applied Biosystems® 3500xL Genetic Analyzer and a 1.2kV, 24-second injection. The sample file was analyzed with the GeneMapper® *ID*-X software, version 1.4, and VersaPlex™ 27PY panels and bins text files. **Panel A.** The FL-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-labeled allelic ladder components and their allele designations. **Panel E.** The TOM-labeled allelic ladder components and their allele designations.

7. Results (continued)

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products often are observed one repeat unit smaller than the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percentage of stutter. A trinucleotide repeat locus, like D22S1045, will have more pronounced stutter in both n–3 and n+3 positions than a typical tetranucleotide repeat locus.

The mean plus three standard deviations at each locus is used for locus-specific stutter filtering for the VersaPlex™ 27PY System.

In addition to stutter peaks, DNA-dependent artifact peaks (Table 2) and DNA-independent artifact peaks (Table 3) can be observed at some VersaPlex™ 27PY System loci.

Table 2. DNA-Dependent Artifacts Observed with the VersaPlex™ 27PY System.

Locus	Artifact Size
Amelogenin	n–1
D1S1656	n–2
D13S317	n–2
D2S1338	low-level artifact 15–17bp below allele
vWA	n–2, elevated baseline in the locus
D7S820	n–2
D5S818	n–2
TPOX	low-level artifact 26–29bp below allele
D19S433	n–2
D22S1045	low-level artifact 22–25bp below allele
DYS391	n–1
FGA	n–2
DYS576	n–2

Table 3. DNA-Independent Artifacts Observed with the VersaPlex™ 27PY System.

Dye Label	Artifact Size[1]
FL	~65–79bp
	~113–124bp
	~137–148bp
JOE	~60–68bp
TMR	~57–62bp

[1]Artifact sizes may vary depending on CE instrumentation and environmental conditions in the laboratory.

Testing was performed with a variety of nonhuman DNA templates from bacteria, yeast, aves, mammals and primates to characterize known artifacts with the VersaPlex™ 27PY System. The artifacts listed in Table 4 were noted above the 175RFU threshold with 10ng of template DNA using an Applied Biosystems® 3500xL Genetic Analyzer. Partial profiles were obtained with all primate species tested, but these profiles can be distinguished from a human profile because most of the alleles were called as off-ladder or were outside the locus panels.

Table 4. Nonhuman DNA Cross-Reactivity.

DNA Source	Artifact Size	Dye Label
Fusobacterium nucleatum	~308bp	JOE
Chicken	~221bp	JOE
	~300bp	TMR
Mouse	~347bp	JOE
Pig	~259–260bp	FL
	~368–372bp	JOE
	~369–370bp	CXR

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8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

8.A. Amplification and Fragment Detection

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Because a small amount of template is used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample. Faint or absent peaks may be seen more often when using the maximum template volume or reduced amplification reaction volume.
	Insufficient template. Use the recommended amount of template DNA if available.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA), TE^{-4} buffer with 20µg/ml glycogen or nuclease-free water. Faint or absent peaks may be seen more often when using the maximum template volume or reduced amplification reaction volume.
	The VersaPlex™ 27PY 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 15 seconds before dispensing into the PCR amplification mix.
	An air bubble formed at the bottom of the reaction tube. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocol in Section 4. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the VersaPlex™ 27PY 5X Primer Pair Mix for 15 seconds before use.
	Poor capillary electrophoresis injection (WEN ILS 500 peaks also affected). Re-inject the sample. Check the laser power.

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	Samples were not denatured completely. Heat-denature samples for the recommended time, and then cool on crushed ice or a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, because this may lead to artifacts due to DNA re-annealing.
	Poor-quality formamide was used. Use only the recommended formamide when analyzing samples.
Faint or absent allele peaks for the positive control reaction	Improper storage of the 2800M Control DNA.
Extra peaks visible in one or all color channels	Artifacts of STR amplification. Amplification of excess amounts of purified DNA can result in a higher number of artifact peaks. Use the recommended amount of template DNA. See Section 7 for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Amplicon was diluted with amplification-grade water. It is recommended that quantitation of the extract be performed prior to amplification to optimize the target template. If dilution of amplicon is necessary, use a PCR buffer to do so, such as 1X Master Mix. The use of a PCR buffer introduces necessary buffering during the injection.
	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Samples were not denatured completely. Heat denature samples for the recommended time, and then cool on crushed ice or a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, because this may lead to artifacts due to DNA re-annealing.
	Do not store the PCR amplification mix for a prolonged period. Add the mix to the wells of the reaction plate as soon as the mix is prepared.
	Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of “shadow” peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distance as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing.

8.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform a 10-minute extension step at 60°C after thermal cycling (Section 4). • Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
	CE-related artifacts ("spikes"). Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples. • Perform a new spectral calibration, and re-run the samples. • Instrument sensitivities can vary. Optimize the injection conditions. See Section 5. • Reboot the Applied Biosystems® 3500 or 3500xL Genetic Analyzer and the instrument's computer. Repeat the spectral calibration. Do not allow borrowing when running the spectral calibration on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer.
	Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in artifacts.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
	Polymer-related artifacts. This system was developed using POP-4® polymer. To use other polymers, optimization and in-house validation are required. The use of POP-7® polymer can change the migration and sizing location of artifacts compared to that with POP-4® polymer.

Symptoms	Causes and Comments
Allelic ladder not running the same as samples	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Poor-quality formamide. Use only the recommended formamide when analyzing samples.
	Be sure the allelic ladder and samples are from the same instrument run.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes.
	Poor injection of allelic ladder. Include more than one ladder per instrument run.
	Internal size standard was not assigned correctly. Evaluate the sizing labels on the WEN ILS 500, and correct if necessary.
Peak height imbalance	Excessive amount of DNA. Amplification of >1.0ng of template in a 25µl reaction volume can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance. Imbalance may be seen more often when using the maximum template volume or a reduced amplification reaction volume.
	Miscellaneous balance problems. At the first use, thaw the 5X Primer Pair Mix and 5X Master Mix completely. Vortex the 5X Primer Pair Mix and 5X Master Mix for 15 seconds before use; do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.

8.B. GeneMapper® *ID*-X Software

Symptoms	Causes and Comments
Stutter peaks not filtered	Stutter text file was not imported into the Panel Manager when the panels and bins text files were imported.
	If the "Use marker-specific stutter ratio and distance if available" box is not checked, stutter distance must be defined in the Analysis Method Allele tab.
Samples in the project not analyzed	The Analysis Requirement Summary window was not active, and there was an analysis requirement that was not met. Turn on Analysis Requirement Summary in the Options menu, and correct the necessary analysis requirements to continue analysis.
Edits in label edit viewer cannot be viewed	To view edits made to a project, the project first must be saved. Close the plot view window, return to the main GeneMapper® *ID*-X page and save the project. Display the plot window again, and then view the label edit table.
Marker header bar for some loci are gray	When an edit is made to a locus, the quality flags and marker header bar automatically change to gray. To change the GQ and marker header bar for a locus to green, override the GQ in the plot window.
Alleles not called	To analyze samples with GeneMapper® *ID*-X software, at least one allelic ladder must be defined.
	An insufficient number of WEN ILS 500 fragments was defined. Be sure to define at least two WEN ILS 500 fragments smaller than the smallest sample peak and at least two WEN ILS 500 fragments larger than the largest sample peak. In this instance, the allelic ladder would have failed the allelic ladder quality check.
	Run was too short, and larger peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Symptoms	Causes and Comments
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID*-X software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section 6.D.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
	Incorrect polymer was used. Use of a polymer other than POP-4® polymer may change migration of the fragments. Alleles may migrate outside of the panel range established using POP-4® polymer.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.D. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration, and re-run the samples.
	Incorrect dye set was used.

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9. References

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10. Appendix

10.A. Advantages of Using the Loci in the VersaPlex™ 27PY System

A single VersaPlex™ 27PY System reaction amplifies all core loci required for US expanded CODIS and European databases (Tables 5 and 6). It also amplifies the D6S1043 locus, which is used in some regions of the world. The male-specific DYS391 locus is included to identify null Y results for Amelogenin. Additionally, two rapidly mutating Y-STR loci are included in the system. Table 7 lists the VersaPlex™ 27PY System alleles amplified from the 2800M Control DNA template.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage and terminal nucleotide addition (14,15). Repeat slippage, sometimes called “n–4 peaks”, “stutter” or “shadow peaks”, is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

Terminal nucleotide addition (16,17) occurs when a thermostable nonproofeading DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step at 60°C (18) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

10.A. Advantages of Using the Loci in the VersaPlex™ 27PY System (continued)

Table 5. The VersaPlex™ 27PY System Locus-Specific Information.

STR Locus	Label	Chromosomal Location[1]	Repeat Sequence[2] 5´→3´
Amelogenin[3]	FL	Xp22.1–22.3 and Y	NA
D3S1358	FL	3p21.31 (45.557Mb)	TCTA Complex
D1S1656	FL	1q42 (228.972Mb)	TAGA Complex
D2S441	FL	2p14 (68.214Mb)	TCTA
D10S1248	FL	10q26.3 (130.567Mb)	GGAA
D13S317	FL	13q31.1 (81.62Mb)	TATC
Penta E	FL	15q26.2 (95.175Mb)	AAAGA
D16S539	JOE	16q24.1 (84.944Mb)	GATA
D18S51	JOE	18q21.33 (59.1Mb)	AGAA (19)
D2S1338	JOE	2q35 (218.705Mb)	TGCC/TTCC
CSF1PO	JOE	5q33.1 (149.436Mb)	AGAT
Penta D	JOE	21q22.3 (43.88Mb)	AAAGA
TH01	TMR	11p15.5 (2.149Mb)	AATG (19)
vWA	TMR	12p13.31 (5.963Mb)	TCTA Complex (19)
D21S11	TMR	21q21.1 (19.476Mb)	TCTA Complex (19)
D7S820	TMR	7q21.11 (83.433Mb)	GATA
D5S818	TMR	5q23.2 (123.139Mb)	AGAT
TPOX	TMR	2p25.3 (1.472Mb)	AATG
D8S1179	CXR	8q24.13 (125.976Mb)	TCTA Complex (19)
D12S391	CXR	12p12 (12.341Mb)	AGAT/AGAC Complex
D19S433	CXR	19q12 (35.109Mb)	AAGG Complex
D6S1043	CXR	6q15 (92.449Mb)	AGAT
D22S1045	CXR	22q12.3 (35.779Mb)	ATT
DYS391	TOM	Y	TCTA
FGA	TOM	4q28 (155.866Mb)	TTTC Complex (19)
DYS576	TOM	Y	AAAG
DYS570	TOM	Y	TTTC

[1]Information about the chromosomal location of these loci can be found in references 20, 21 and 22 and at:

www.cstl.nist.gov/biotech/strbase/chrom.htm

[2]The August 1997 report (23,24) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[3]Amelogenin is not an STR.

NA = Not applicable

Table 6. The VersaPlex™ 27PY System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
Amelogenin	FL	89, 95	X, Y
D3S1358	FL	103–147	9–20
D1S1656	FL	161–208	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	FL	216–252	8–11, 11.3, 12–17
D10S1248	FL	259–303	8–19
D13S317	FL	308–358	5–17
Penta E	FL	371–471	5–25
D16S539	JOE	84–132	4–16
D18S51	JOE	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE	224–296	10, 12, 14–28
CSF1PO	JOE	318–362	5–16
Penta D	JOE	377–450	2.2, 3.2, 5–17
TH01	TMR	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR	127–183	10–24
D21S11	TMR	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
D7S820	TMR	269–313	5–16
D5S818	TMR	321–369	6–18
TPOX	TMR	393–441	4–16
D8S1179	CXR	76–124	7–19
D12S391	CXR	133–185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
D6S1043	CXR	276–356	6–26
D22S1045	CXR	431–470	7–20
DYS391	TOM	86–130	5–16
FGA	TOM	143–289	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
DYS576	TOM	308–356	11–23
DYS570	TOM	393–453	10–25

[1]The length of each allele in the allelic ladder has been confirmed by sequence analysis.
[2]When using an internal lane standard, such as the WEN Internal Lane Standard 500, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label and linker also affect migration of alleles.
[3]For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: **strbase.nist.gov/var_tab.htm**

10.A. Advantages of Using the Loci in the VersaPlex™ 27PY System (continued)

Table 7. The VersaPlex™ 27PY System Allele Determinations for 2800M Control DNA.

STR Locus	2800M
Amelogenin	X, Y
D3S1358	17, 18
D1S1656	12, 13
D2S441	10, 14
D10S1248	13, 15
D13S317	9, 11
Penta E	7, 14
D16S539	9, 13
D18S51	16, 18
D2S1338	22, 25
CSF1PO	12, 12
Penta D	12, 13
TH01	6, 9.3
vWA	16, 19
D21S11	29, 31.2
D7S820	8, 11
D5S818	12, 12
TPOX	11, 11
D8S1179	14, 15
D12S391	18, 23
D19S433	13, 14
D6S1043	12, 20
D22S1045	16, 16
DYS391	10
FGA	20, 23
DYS576	18
DYS570	17

10.B. DNA Extraction and Quantification Methods and Automation Support

Promega offers a wide variety of reagents and automated methods for sample preparation, DNA purification and DNA quantification prior to STR amplification.

For casework or samples that require DNA purification, we recommend the DNA IQ™ System (Cat.# DC6700), which is a DNA isolation system designed specifically for forensic samples (25). This system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ System eliminates PCR inhibitors and contaminants frequently encountered in casework samples. In addition, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials.

For applications requiring human-specific DNA quantification, the PowerQuant® System (Cat.# PQ5002, PQ5008) and the Plexor® HY System (Cat.# DC1000, DC1001) were developed (26,27). These qPCR-based methods provide total human and male-specific DNA quantification in one reaction. Additional ordering information is available in Section 10.E.

For information about automation of Promega chemistries on automated workstations using Identity Automation™ solutions, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/support/worldwide-contacts/**), or e-mail: **genetic@promega.com**

10.C. The WEN Internal Lane Standard 500

The WEN Internal Lane Standard 500 contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 19). Each fragment is labeled with WEN dye and can be detected separately (as a sixth color) in the presence of VersaPlex™ 27PY-amplified material. The WEN ILS 500 is designed for use in each CE injection to increase precision in analyses when using the VersaPlex™ 27PY System. Protocols to prepare and use this internal lane standard are provided in Section 5.

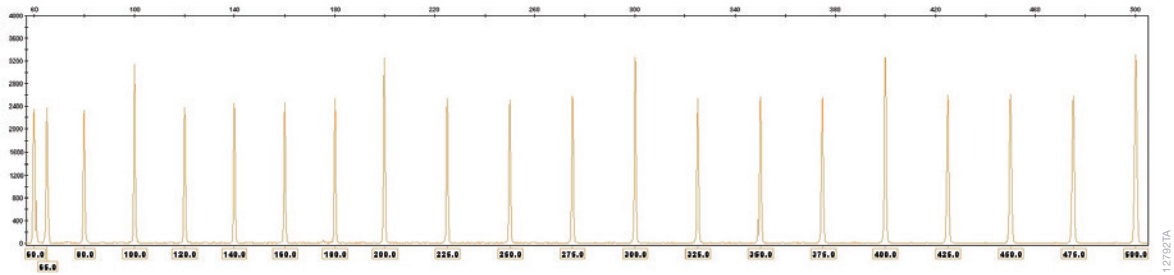

Figure 19. WEN Internal Lane Standard 500. An electropherogram showing the WEN Internal Lane Standard 500 fragments.

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10.D. Composition of Buffers and Solutions

TE^{-4} buffer (10mM Tris-HCl, 0.1mM EDTA [pH 8.0])

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Bring the final volume to 1 liter with deionized water.

TE^{-4} buffer with 20µg/ml glycogen

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)
20µg/ml	glycogen

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Add glycogen. Bring the final volume to 1 liter with deionized water.

10.E. Related Products

Accessory Components

Product	Size	Cat.#
VersaPlex™ 6C Matrix Standard	5 preps	DG4960
WEN Internal Lane Standard 500	200µl	DG5001
2800M Control DNA (10ng/µl)	25µl	DD7101
2800M Control DNA (0.25ng/µl)	500µl	DD7251
Water, Amplification Grade	6,250µl (5 × 1,250µl)	DW0991

Not for Medical Diagnostic Use.

Sample Preparation and DNA Quantification Systems

Product	Size	Cat.#
PowerQuant® System*	200 reactions	PQ5002
	800 reactions	PQ5008
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Plexor® HY System*	200 reactions	DC1001
	800 reactions	DC1000
Differex™ System*	50 samples	DC6801
	200 samples	DC6800l
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument*	1 each	AS4600
Maxwell® FSC DNA IQ™ Casework Kit*	48 preps	AS1550
Casework Extraction Kit*	100 preps	DC6745
Slicprep™ 96 Device*	10 pack	V1391

*Not for Medical Diagnostic Use.

[a]U.S. Pat. No. 9,139,868 and other patents pending.

[b]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

[c]Australian Pat. No. 724531, Canadian Pat. No. 2,251,793, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. Nos. 3602142 and 4034293, Chinese Pat. Nos. ZL99813729.4 and ZL97194967.0, European Pat. No. 0960207 and other patents pending.

[d]U.S. Pat. No. 6,238,863, Chinese Pat. No. ZL99802696.4, European Pat. No. 1058727, Japanese Pat. No. 4494630 and other patents pending.

[e]Allele sequences for one or more of the loci vWA, FGA, D8S1179, D21S11 and D18S51 in allelic ladder mixtures is licensed under U.S. Pat. Nos. 7,087,380 and 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

[f]TMR, CXR, TOM and WEN dyes are proprietary.