Promega

VersaPlex™ 27PY System for Use on the Spectrum Compact CE System Technical Manual

Instructions for Use of Product DC7020

TMD071 | 4/21

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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

STR (short tandem repeat) 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,b] 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, GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler, in addition to a protocol to separate amplified products and detect separated material on the Spectrum Compact CE System. 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. A protocol to operate the Spectrum Compact CE System is available separately. See the *Spectrum Compact CE System Operating Manual* #TMD058.

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

Product Components and Storage Conditions

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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°C to +10°C for **at least 24 hours** before use. After the first use, store the VersaPlex™ 27PY System components at +2°C to +10°C, where they are stable for 1 year; 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.

Available Separately

PRODUCT	SIZE	CAT.#
GeneMarker®HID Software for Spectrum CE Systems, Local	1 seat	CE3001
GeneMarker®HID Software for Spectrum CE Systems, Network	1 seat	CE3010
GeneMarker®HID Software for Spectrum CE Systems, Client	1 seat	CE3011

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

The proper panel .xml file for use with GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum) is available for download at: **www.promega.com/resources/software-firmware/str-analysis/versaplex-27py-system-software/**

The VersaPlex™ 6C Matrix Standard (Cat.# DG4960) is required for inital setup of the color separation matrix (see Section 3.2).

Before You Begin 3

3.1. 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, 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 optimization 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 (Master Mix, 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 (Allelic Ladder Mix and Internal Lane Standard). 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.

3.2. Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the Spectrum Compact CE System. A matrix must be generated for each individual instrument.

For protocol and additional information about matrix generation and spectral calibration, see the *VersaPlex™ 6C Matrix Standard for the Spectrum Compact CE System Technical Manual* #TMD072*.* This manual is available online at: **www.promega.com/protocols/**

Protocols for DNA Amplification Using the VersaPlex™ 27PY System

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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 gold-plated silver or 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. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. 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- 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 9.

4.1. Amplification of Extracted DNA in a 25µl Reaction Volume

The following protocol has been routinely used to amplify 1ng of template DNA in a 25µl reaction volume using the VersaPlex™ 27PY System.

Materials to be Supplied by the User

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

Amplification Setup

1. At 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°C to +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, as 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 it appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label them appropriately.

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

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 25µl	×		=	
VersaPlex™ 27PY 5X Master Mix	5µl	×		=	
VersaPlex™ 27PY 5X Primer Pair Mix	5µl	×		=	
template DNA (1ng)[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 for each sample to the respective well containing PCR amplification mix.

   Note: The VersaPlex™ 27PY System is optimized and balanced using 1ng 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 1ng in the desired template volume. Add 1ng of diluted DNA to a reaction well containing PCR amplification mix.

8. For the negative amplification control, pipet Water, Amplification Grade, 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 work well for 1ng of purified DNA templates. In-house validation should be performed.

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 gold-plated silver or 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

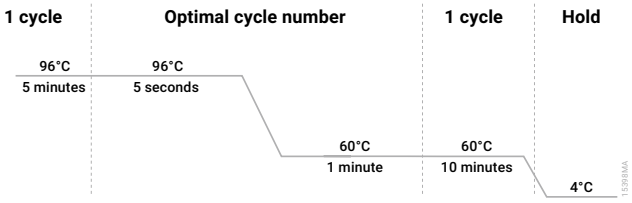

Figure 1. Thermal cycling protocol for the ProFlex® PCR System, GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler.

3. After completion of the thermal cycling protocol, proceed to 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.

PCR Optimization

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 sample types 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 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 for these sample types.

Detection of Amplified Fragments Using the Spectrum Compact CE System

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Instructions for detection of amplified fragments using the Spectrum Compact CE System can be used with the HITACHI DS3000 Compact CE Sequencer.

Materials to be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 8-tube strips
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer4 (Cat.# CE2304)
- Spectrum Compact Buffer (Cat.# CE2300)
- Spectrum Compact Cathode Septa Mat (Cat.# CE2301)
- Spectrum Compact Cathode Retainer (Cat.# CE2302)
- Spectrum Compact Strip Base & Retainer, 32-Well (Cat.# CE2332)
- MicroAmp® Optical 8-Tube Strip, 0.2ml (Applied Biosystems Cat.# 4316567)
- Strip Septa Mat, 8-Well (Cat.# CE2308)
- 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 the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Notes:

1. Only use MicroAmp™ Optical 8-Tube Strips (0.2ml) (Applied Biosystems Cat.# 4316567) as a source of 8-well strip tubes. Use of other 8-well strip tubes may affect performance or damage the Spectrum Compact CE System.
2. Wear gloves when handling consumables and sample cartridges.

Sample Layout

Samples prepared in 8-well strip tubes are assembled into the strip base and retainer (Figure 2) to form the sample cartridge (Figure 3) which can be loaded onto the Spectrum Compact CE System. Samples are injected in groups of four, such that one 8-well strip tube can be used for two injections. Each 8-well strip tube corresponds to one lane (A, B, C or D) of the sample cartridge. Samples are injected in groups of four across a lane (not by column). For example, samples in well positions A1 through A4 are injected together, followed by samples in well positions A5 through A8 for the second injection. This pattern is then repeated for samples in lanes B, C and D. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for information on changing the order of injections during run setup.

		Wells							
		1	2	3	4	5	6	7	8
Lane	A	Injection 1				Injection 2			
	B	Injection 3				Injection 4			
	C	Injection 5				Injection 6			
	D	Injection 7				Injection 8			

5.1. Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and Hi-Di™ formamide as follows: [(0.5µl ILS) × (# 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 of the 8-well strip tube(s).

4. Add 1µl of amplified sample (or 1µl of Allelic Ladder Mix) to each well. Cover wells with the Strip Septa Mat, 8-Well.

   Notes:

   1. To prevent cross-contamination, do not reuse Strip Septa mat, 8-Well. Always use a new Strip Septa Mat, 8-Well for each 8-well strip tube.
   2. 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, refer to the *Spectrum Compact CE System Operating Manual* #TMD058. 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.
   3. 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.
   4. Include an injection of allelic ladder every 4 injections (16 samples).

5. Centrifuge the 8-well strip tube(s) 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.

5.2. Assembling Sample Cartridge

1. Place the 8-well strip tube(s) with the Strip Septa Mat, 8-well, into the strip base (Figure 2). When using fewer than 4 strips in the run, you can place the strips in any lane.

   Note: Lane names A to D and well numbers 1 to 8 are embossed on the strip base. Be sure to check the lane name when placing a sample 8-well strip tube into the strip base to make certain that the correct 8-well strip tube is in the correct lane.

   Additionally, well numbers 1 to 8 are embossed on each 8-well strip tube. When placing a sample 8-well strip tube into the strip base, be sure that the well numbers on each sample 8-well strip tube match those on the strip base.

2. To complete the assembly, place the retainer over the strip(s) in the strip base, aligning the lane names A to D and well numbers 1 to 8 on the retainer to those on the strip base and pressing until the retainer clicks into the strip base (Figure 3).

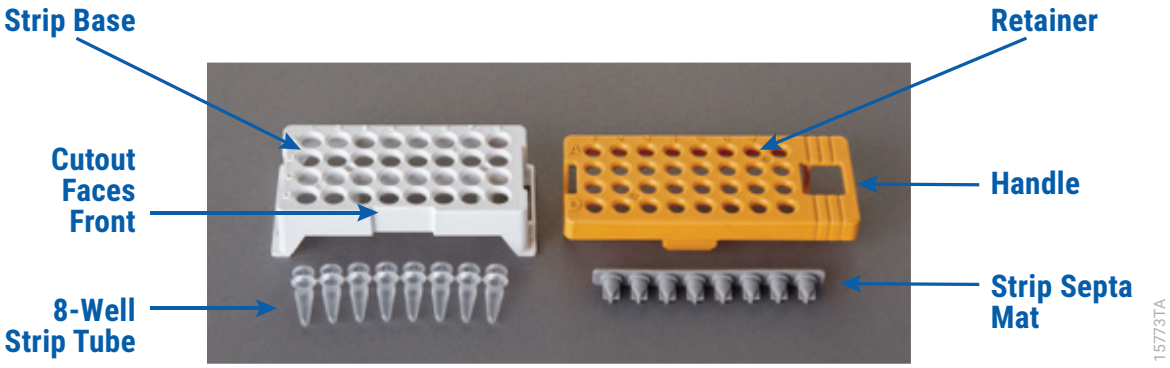

Figure 2. Assembling the Spectrum Compact Strip Base and Retainer.

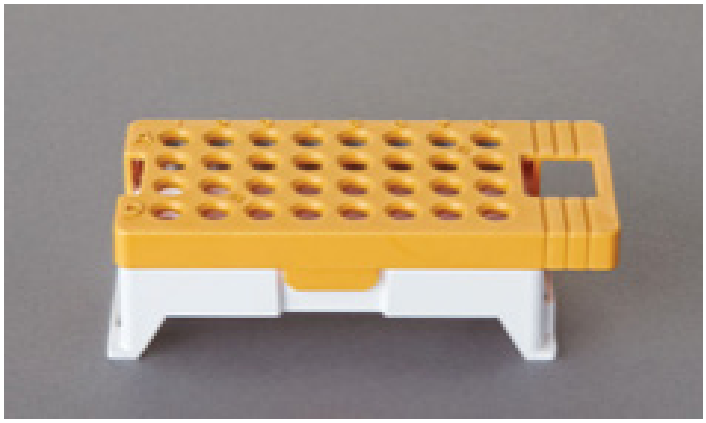

Figure 3. Assembled Spectrum Compact Sample Cartridge.

5.3. Instrument Preparation

These instructions are intended as a guide for running samples on the Spectrum Compact CE System (after successful spatial and spectral calibration with the VersaPlex™ 6C Matrix Standard). They are not intended as comprehensive instructions for using the Spectrum Compact CE System. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for instrument maintenance, instructions for installing consumables, performing a spatial calibration and managing protocols (Assay, Instrument, and Sizecalling protocols). Refer to *VersaPlex™ 6C Matrix Standard for the Spectrum Compact CE System Technical Manual* #TMD072 for instructions on performing a spectral calibration.

1. Before starting a run on the Spectrum Compact CE System, ensure all consumables are installed and in sufficient supply. For best quality results, use unexpired reagents that are within the recommended use range (Figure 5 and Table 2). Refer to the 'Consumables' screen to determine if any consumables need to be replaced. To access the 'Consumables' screen, select the **Consumables** icon in the Header on the 'Main Menu' screen (Figure 4). The 'Consumables' screen displays information for the four consumables on the instrument: Polymer, Capillary Cartridge and Anode and Cathode buffers.

Figure 4. Spectrum Compact CE System Software 'Main Menu' screen.

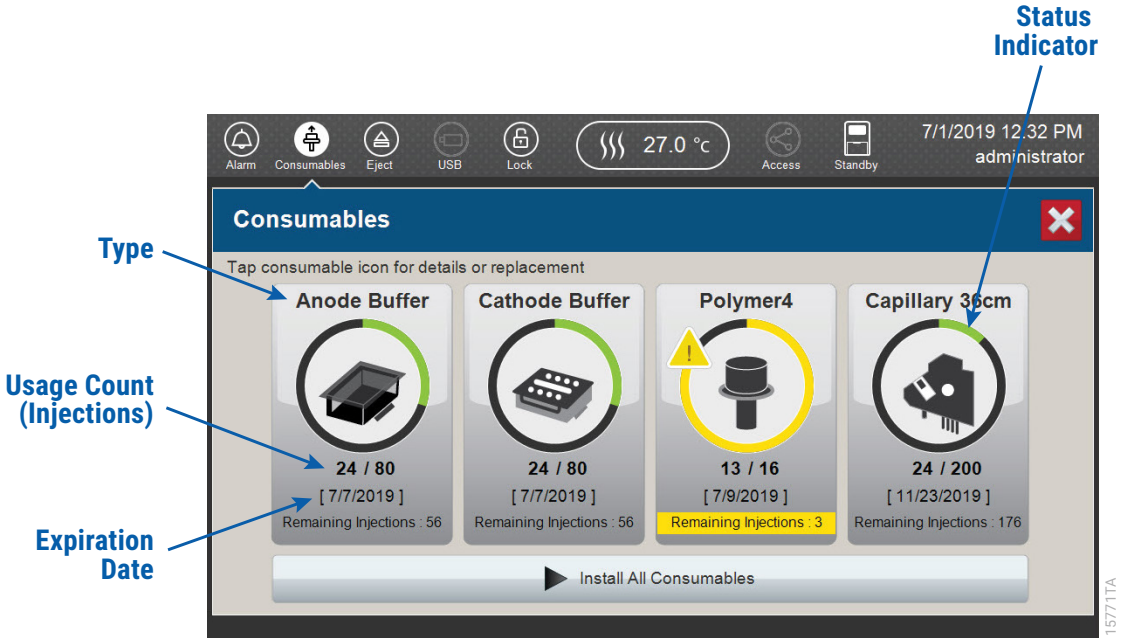

Figure 5. Spectrum Compact CE System 'Consumables' screen. Usage count (number of injections), on-instrument expiration date and remaining injections are displayed on the 'Consumables' screen for each consumable as well as polymer type.

A consumable status indicator will appear on the icons of consumables that need attention. There are three indicators (Table 2).

Table 2. Consumable Warning Symbols

Symbol	Description
	Reaching consumable expiration date, on-instrument expiration date or injection limit for consumable.
	Consumable expiration date, on-instrument expiration date or injection limit for consumable has passed.
	Cannot perform a run because maximum injection count was reached for the polymer.

2. Select the **Oven Temperature** icon in the Header on the 'Main Menu' screen as shown in Figure 6 to start preheating the oven temperature to 60°C. The temperature displayed will change as the temperature of the oven increases. When 60°C is reached a check mark will appear adjacent to the temperature.

   Note: We recommend you preheat the oven for at least 30 minutes prior to starting a run. The oven will automatically turn off after 2 hours if a run is not started.

Figure 6. Preheating Oven.

3. Select **Fragment Analysis** on the 'Main Menu' screen (Figure 7).

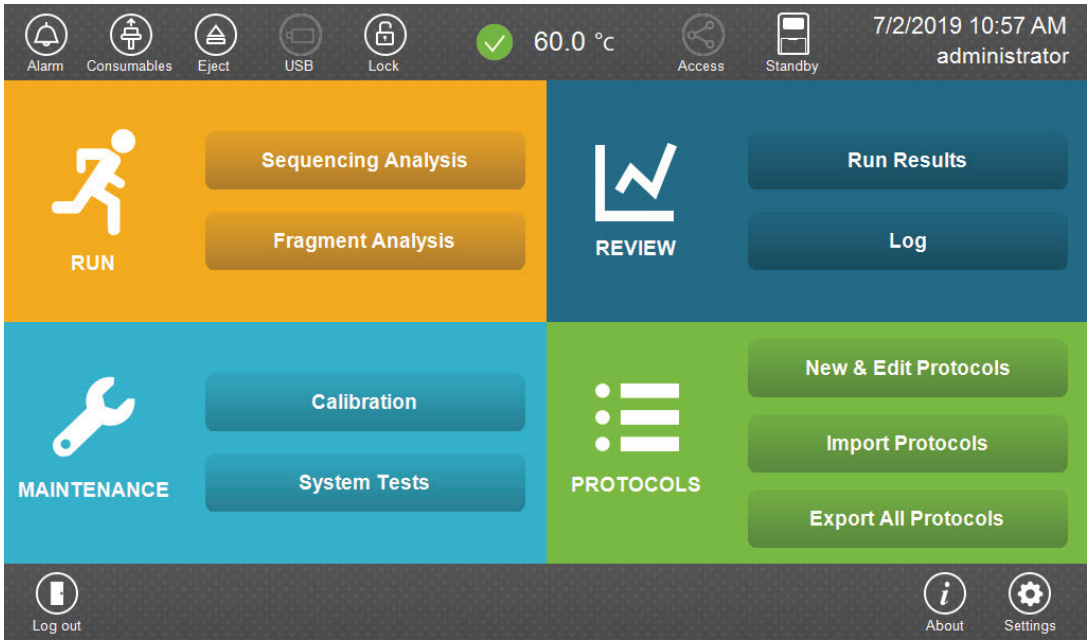

Figure 7. Spectrum Compact CE System Software 'Main Menu' screen.

4. Use the default name that includes date, time and 'Fragment' or enter a Run ID on the 'Set Run ID' screen. Select the **Run ID** box to open the 'Run ID' window and a keypad will become active on the touch screen. Alternatively, the Run ID can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System.

   Notes:

   1. The Run ID will be included in the file names for the samples.
   2. The following table lists rules for characters that can be used for a Run ID, Strip ID and Sample ID.

Acceptable Characters	1 to 30 characters
	Upper and lowercase alphabetic characters
	Numbers
	Symbols unless listed below
Unacceptable Characters	¥ / : , ; * ? ' < > |
	Spaces

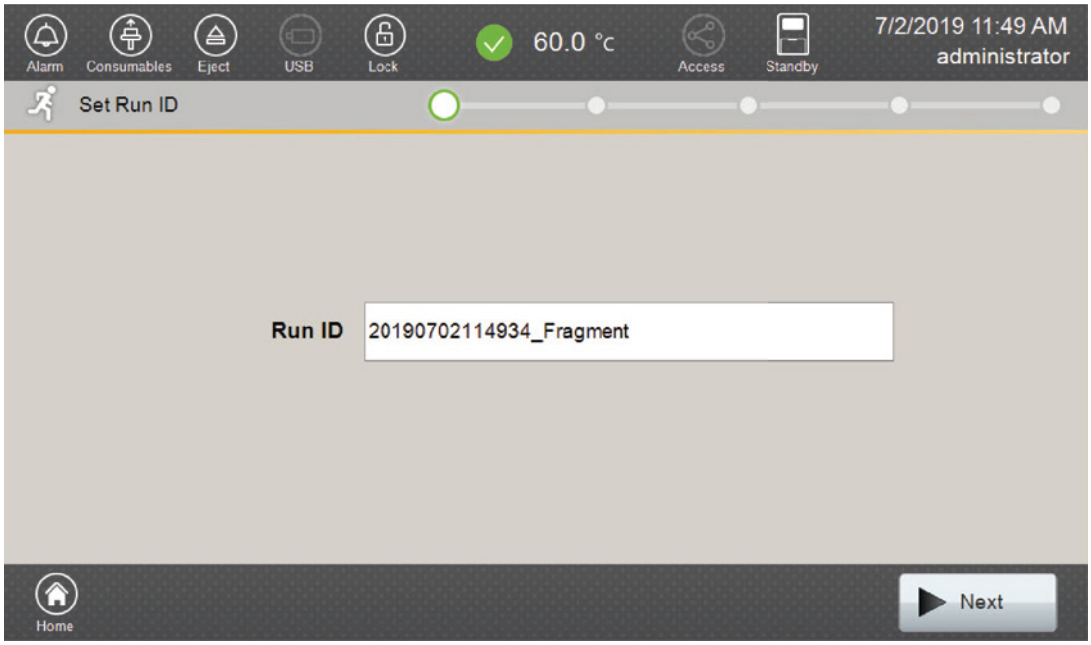

Figure 8. 'Set Run ID' screen.

5. Select **Next** to proceed to the message screen for placement of strips into the sample cartridge.
6. Follow the message screen for placement of strips into the sample cartridge (Figure 9).

   Note: Ensure that the correct strip tube is placed into the correct lane (A through D) on the strip base and that wells 1 to 8 of the strip tube are correctly aligned with well positions 1 to 8 on the strip base.

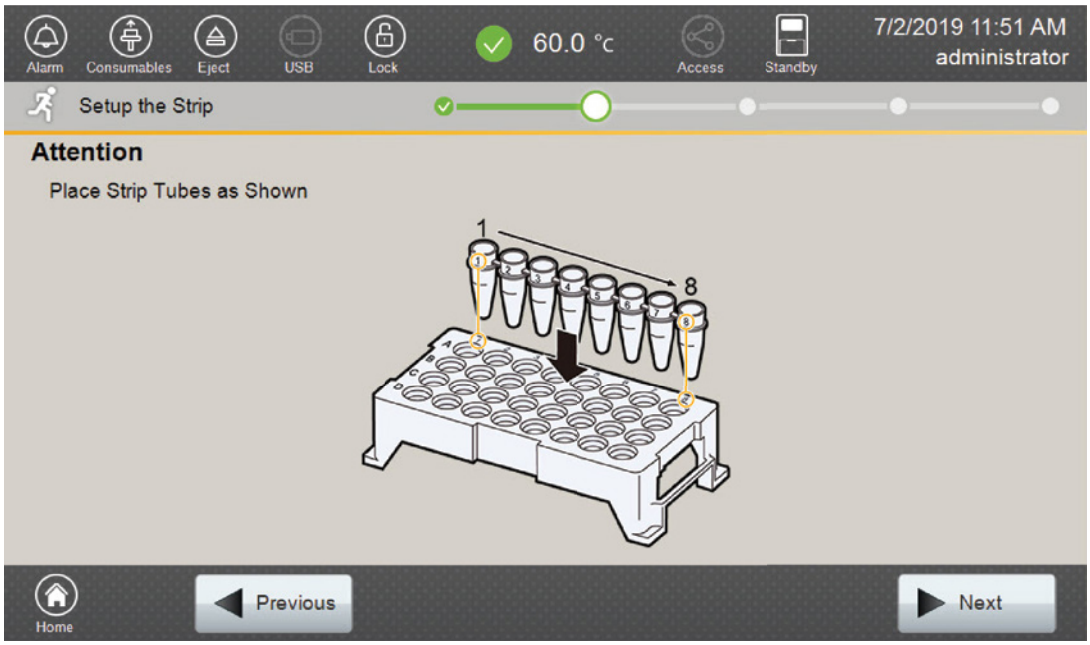

Figure 9. 'Setup the Strip' screen.

5.4. Assigning Run Information to Samples

There are four methods to assign run information to samples:

1. Creating new strip information,
2. Reusing run information from a list of completed runs,
3. Loading saved strip information and
4. Importing strip information.

The following instructions are for creating new strip information only. For details on the remaining three methods, please see the *Spectrum Compact CE System Operating Manual* #TMD058.

Note: The Spectrum Compact CE System includes software for remote access to the instrument. This software allows a user to create and setup sample strips by remotely accessing the Spectrum Compact CE System software using a PC connected directly or via a lab network to the instrument. This software also allows a user to edit protocols and assays, monitor runs and export data. Refer to the *Spectrum Compact CE System Remote Access Software Manual* #TMD064.

1. Select **Next** on the 'Setup the Strip' screen shown in Figure 9. This will take you to the 'Setup Strip Information' screen shown in Figure 10.

   Note: The 'Setup Strip Information' screen is divided into two sections: Sample information and Strip ID.

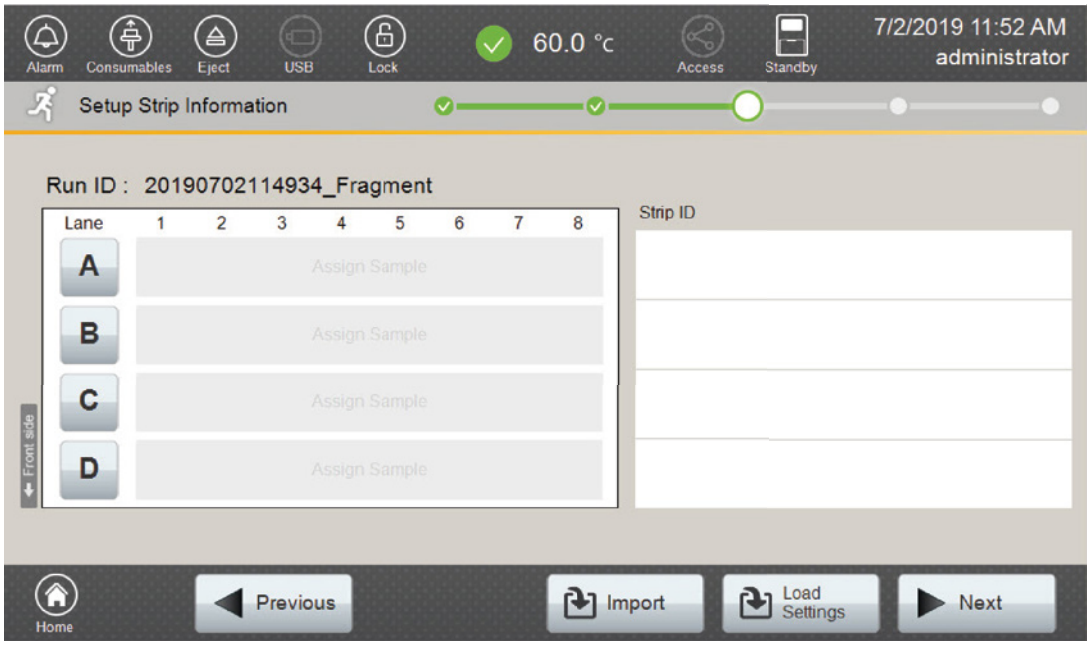

Figure 10. 'Setup Strip Information' screen.

2. Select a lane icon (A, B, C, or D) to enter information for that 8-well strip tube. This opens the 'Edit Strip Information' screen for that lane (Figure 11). This screen displays fields for defining the Strip ID, run assay, sample name and sample type for that lane. Each well in the strip is represented along the left side of the screen. The first injection set of the strip (Wells 1–4) is displayed on the screen. You can use the arrows on the right side of the screen to scroll to the second injection set (Wells 5–8).

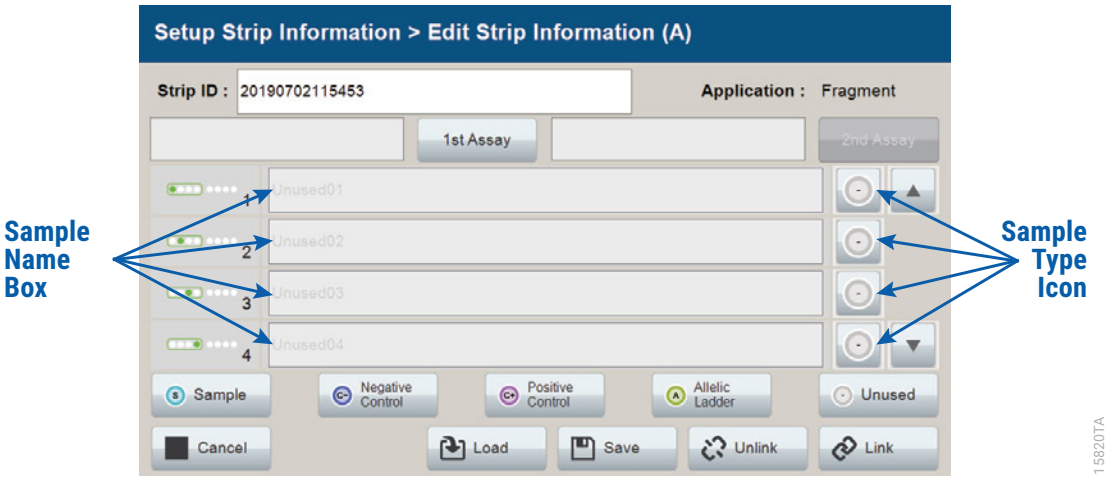

Figure 11. 'Edit Strip Information' screen.

3. The default Strip ID is date and time. It can be modified by selecting the Strip ID box. This opens the 'Set Strip ID' window and a keypad will become active on the touch screen (Figure 12). Alternatively, the Strip ID can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System. Enter the appropriate Strip ID, then select **OK** to exit and return to the 'Edit Strip Information' screen.

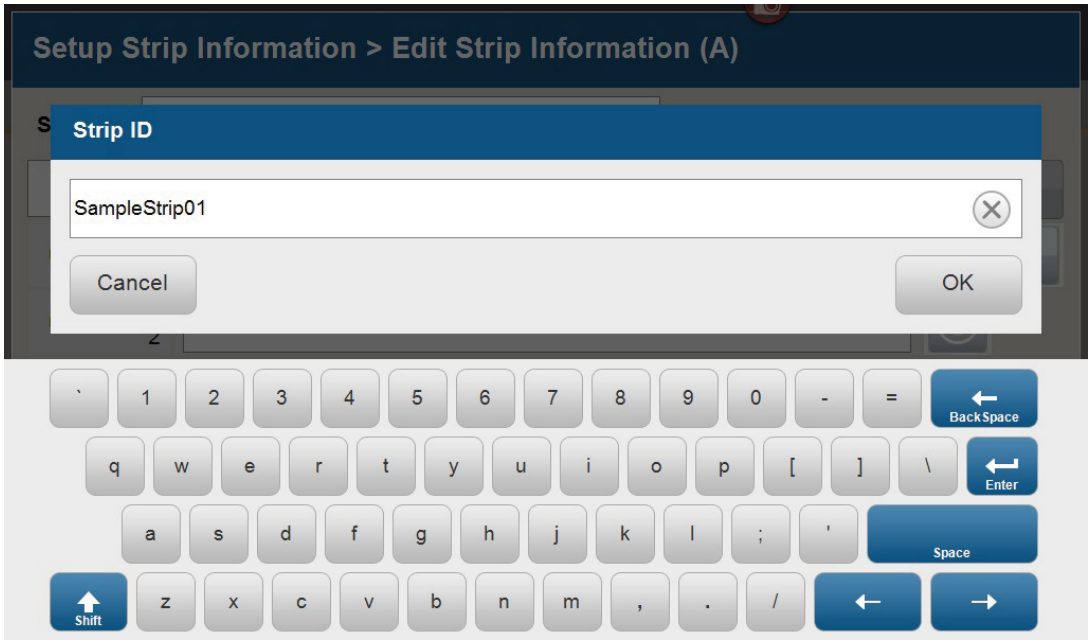

Figure 12. 'Set Strip ID' window.

4. Select a sample type on the 'Edit Strip Information' screen (Figure 13). Sample types must be selected for each well position before the Sample Name box becomes active for entry of sample name. Sample types are as follows.

Symbol	Sample Type
S	Sample
C-	Negative Control
C+	Positive Control
A	Allelic Ladder
-	Unused

5. To assign a sample type to a well, select the appropriate sample type along the bottom of the 'Edit Strip Information' screen (Figure 13), then select the **Sample Type** icon to the right of the sample name field for the desired well. This icon will then display the sample type selected for that well (Figure 14).

   Note: A sample type other than 'Unused' must be assigned to at least one well in each injection set. If all of the four wells in an injection set are assigned as 'Unused', the injection set will not be run. If all eight wells in a strip are assigned as 'Unused', a warning message will be displayed, and no strip information will be assigned. Unused wells for any set of four wells being injected should contain formamide alone. Do not leave uninjected wells empty.

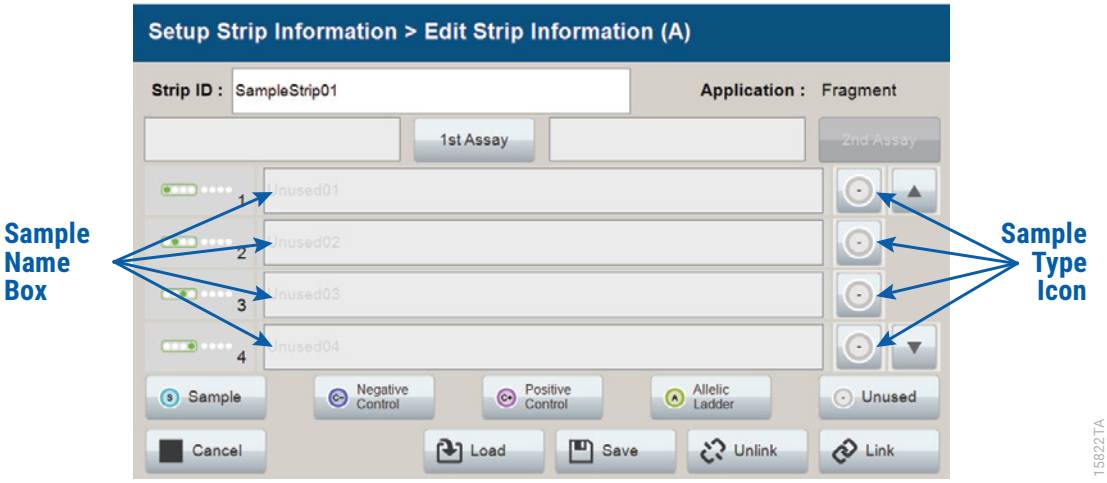

Figure 13. Sample Type and Sample Name on 'Edit Strip Information' screen.

6. Enter a sample name for each well position by selecting the **Sample Name** box adjacent to the well number on the 'Edit Strip Information' screen (Figure 14). This opens the 'Set Sample Name' window and a keypad will become active on the touch screen (Figure 15). Alternatively, the Sample Name can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System. Enter the appropriate sample name, then select **OK** to exit and return to the 'Edit Strip Information' screen.

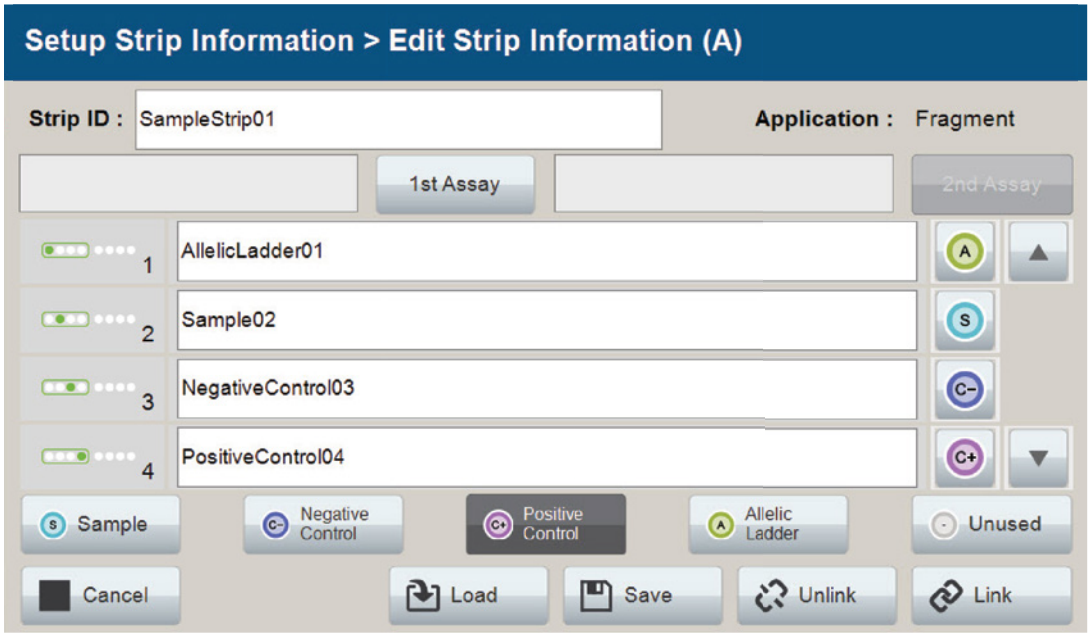

Figure 14. Sample Name Entry on 'Edit Strip Information' screen.

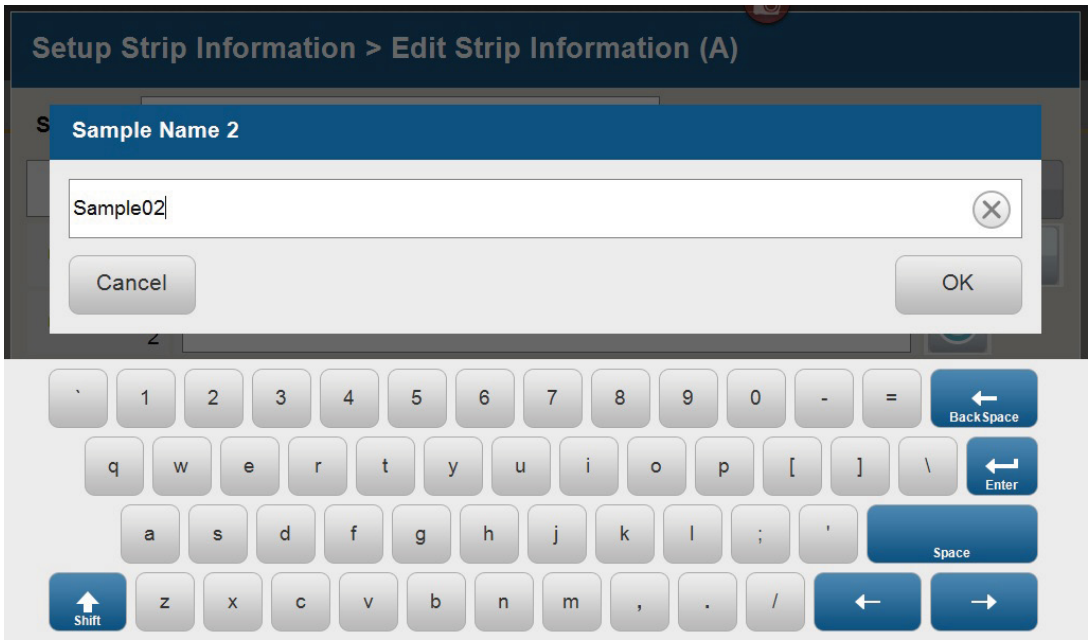

Figure 15. 'Set Sample Name' window.

7. The Spectrum Compact Control Software includes preloaded run assays for use with chemistries available from Promega. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for information on creating or modifying assays. To assign a run assay to an injection set, select **1st Assay** on the right side of the '1st Assay' field (Figure 14). This opens the 'Select Assay' window (Figure 16). Select 'Promega_6Dye_WENILS_36_P4' from the drop-down list (Figure 16) and then select **Apply**.

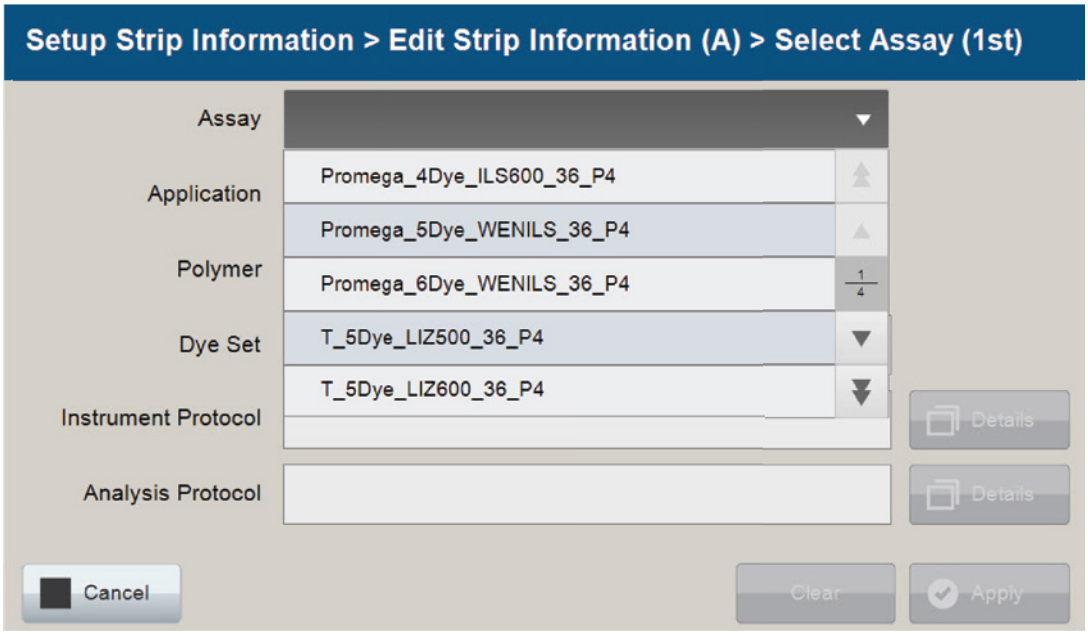

Figure 16. 'Select Assay' window.

8. You can verify the settings of the Instrument and Analysis Protocols associated with the assay chosen by selecting **Details** next to these fields (Figure 17). This will display a window showing the settings in these protocols but will not allow you to edit these settings. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 to edit the Instrument Protocol or Analysis Protocol. When the assay information is confirmed, select **Apply** to return to the 'Edit Strip Information' screen.

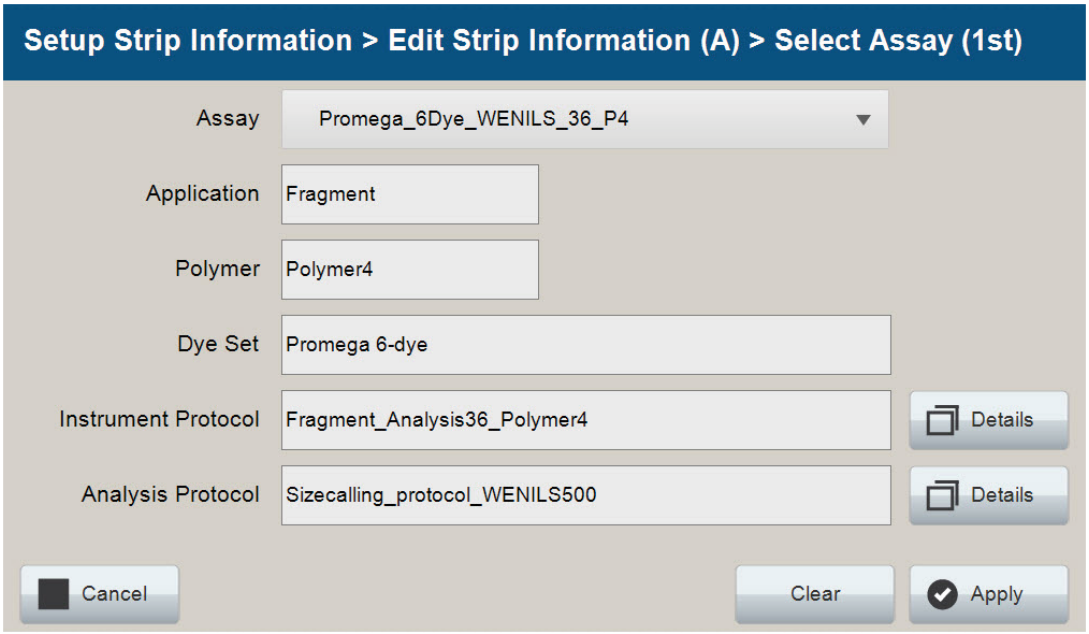

Figure 17. Accessing Instrument and Analysis Protocols on 'Select Assay' window.

9. Repeat these steps for the 2nd Assay field if a second assay will be run for the strip. A second assay might include a longer or shorter injection time.
10. Use the down arrow on the right side of the 'Edit Strip Information' screen to enter sample information and assign an assay for samples in wells 5–8 of the strip.
11. When all information is entered and verified for the strip, select **Link** on the lower right corner of the 'Edit Strip Information' screen (Figure 18). This will link the strip to the run. If you want to save the strip information to use in future runs, you need to save the information by selecting **Save** at the bottom of the 'Edit Strip Information' screen (Figure 18). This will save the strip information so that it can be loaded later into another run, as well as link the strip to the run. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for use of saved strip information.

    Notes:

    1. If you select **Unlink** without having previously selected **Save** at the bottom of the 'Edit Strip Information' screen, you will lose the strip information.
    2. If you forget to assign an assay in the 'Edit Strip Information' screen, a warning window stating "Invalid Data Entered" will appear. Close this window and assign an assay on the 'Edit Strip Information' screen before continuing.

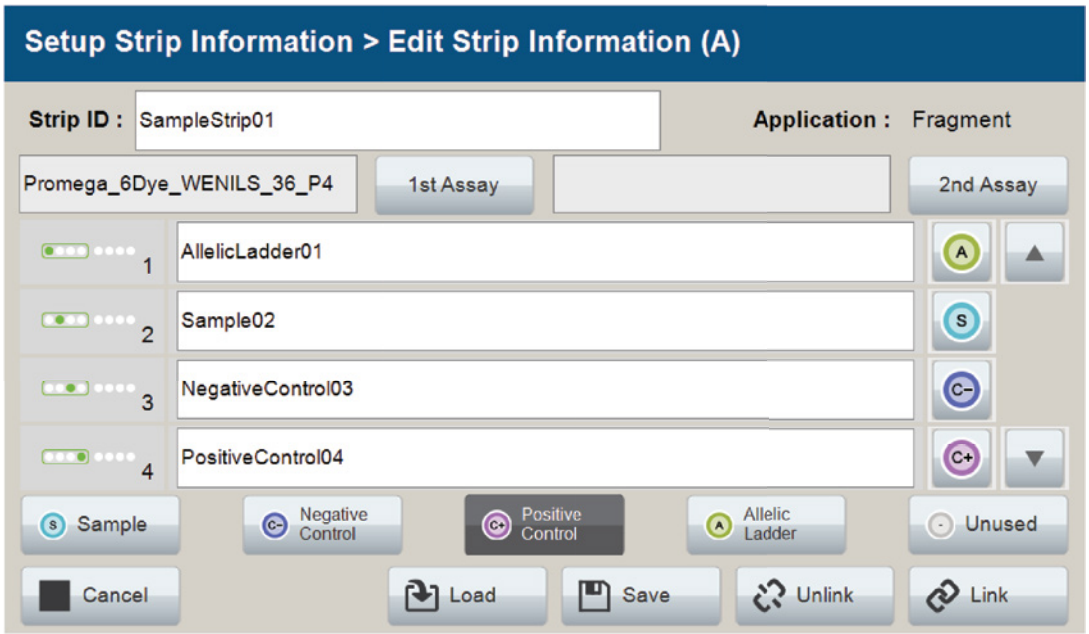

Figure 18. Completed 'Edit Strip Information' screen.

12. Repeat Steps 2–11 for additional lanes as necessary.
13. After all the required lanes have been set up, select **Next** on the 'Setup Strip Information' screen to load the sample cartridge (Figure 19).

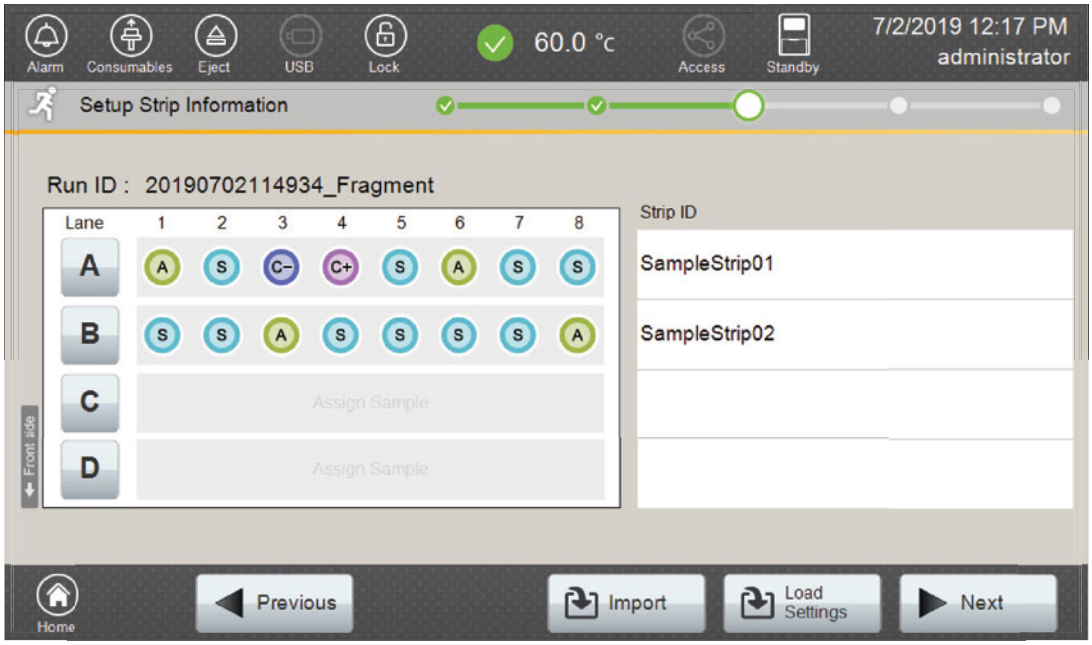

Figure 19. Completed 'Setup Strip Information' screen.

5.5. Loading the Sample Cartridge

1. After selecting **Next** on the 'Setup Strip Information' screen, a message window will open, indicating that the autosampler is moving, telling the user to not open the door. In addition, the status indicator flashes green while the autosampler is moving. After autosampler movement is complete, the message window closes, and the status indicator returns to a steady green.

   Note: Do not open the door while the autosampler is in motion.

2. Open the instrument door, then place the sample cartridge on the autosampler following the instructions displayed on the 'Install the Cartridge' screen (Figure 20). Press down on the yellow tab on the autosampler deck that locks the sample cartridge in place before placing sample cartridge into position. Release the tab to lock the sample cartridge in place on the autosampler deck.

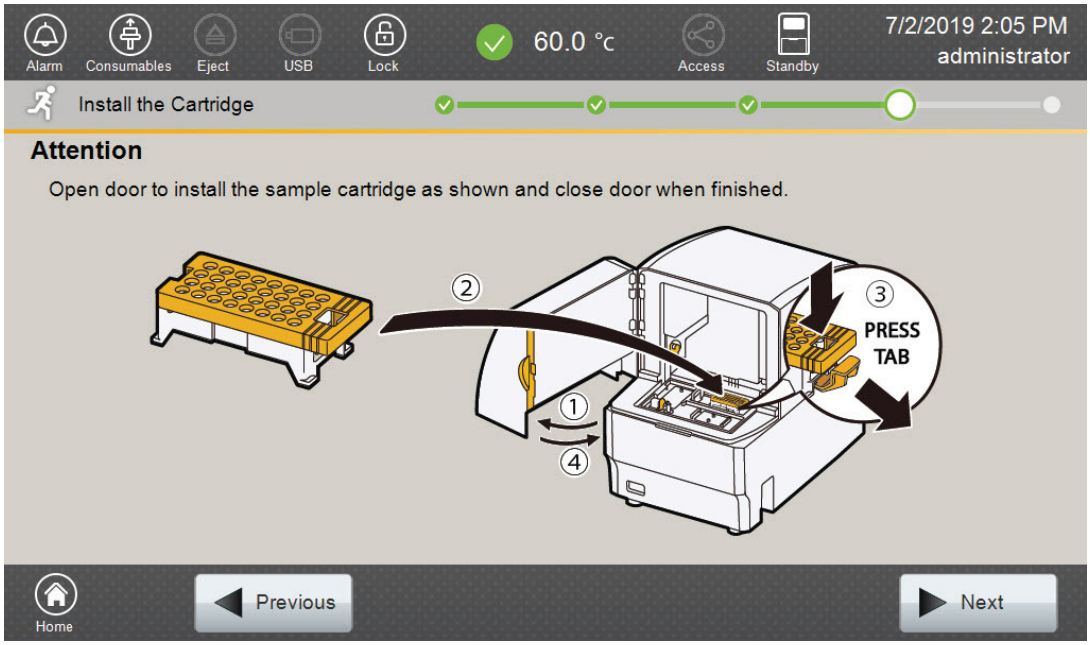

Figure 20. 'Install the Cartridge' screen.

3. When the sample cartridge is locked into place on the autosampler, close the instrument door and wait for the status indicator to stop flashing amber and turn steady green.

   Note: Do not open the door while the autosampler is in motion. Follow the instructions displayed on the screen.

4. After the autosampler has returned to its home position, the 'Edit Injection List' screen will be displayed (Figure 21).

5. Select **Run** to start the run.

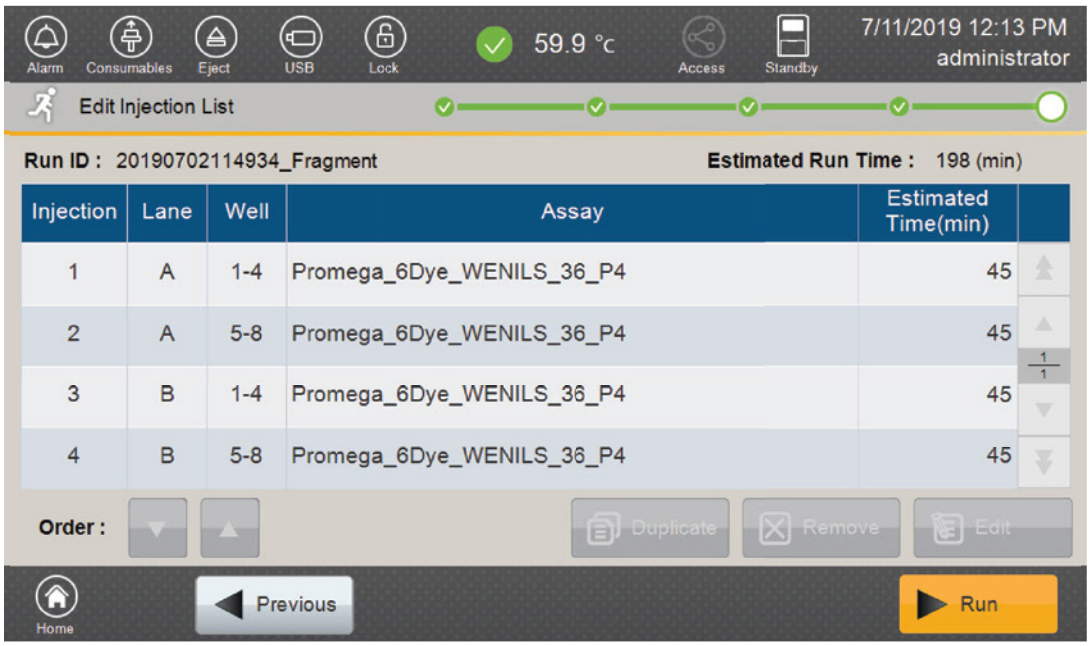

Figure 21. 'Edit Injection List' screen.

6. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for instructions on editing injection information, monitoring a run, viewing run results and exporting run data.

Data Analysis Using GeneMapper® *ID*-X Software

6

GeneMapper® *ID*-X Version 1.4 or greater is required for analysis of VersaPlex™ 27PY data from the Spectrum Compact CE System. Due to potential differences between software versions, some of the instructions may not apply to all software versions.

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

To facilitate analysis of data with the VersaPlex™ 27PY System, we have created panels, bins and stutter 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.

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: **www.promega.com/resources/software-firmware/versaplex-27py-genemapper-id-xsoftware-panels-and-bin-sets/**
2. Select the VersaPlex™ System that you are using, and then select **GeneMapper ID-X**. Enter your contact information, and then 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 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 above. Select the file, and then **Import.**
6. 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.
7. In the navigation pane, highlight the VersaPlex 27PY panels folder that you just imported in Step 5.
8. Select **File,** and then **Import Bin Set**.
9. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, and then **Import**.
10. In the navigation pane, highlight the VersaPlex 27PY panels folder that you just imported in Step 5.
11. Select **File**, and then **Import Marker Stutter**. A warning box will appear asking if you want to overwrite current values. Select **Yes**.
12. Navigate to the stutter text file downloaded in the Getting Started section above. Select the file, and then **Import**.
13. In the Panel Manager, check the boxes to indicate DYS391, DYS576 and DYS570 are Y-markers. See Figure 22. This option is not available for older versions of the GeneMapper® *ID*-X software (prior to version 1.4).
14. At the bottom of the Panel Manager window, select **OK**. This will save the panels, bins and stutter text files, and then close the window.

	Marker Nam	Dye Color	Min Size	Max Size	Control Allele	Marker	Comments	Y Marker	Ladder Allele
1	AMEL	Blue	80.0	89.0	X,Y	9	none	☐	X,Y
2	D3S1358	Blue	90.0	151.0	17,18	4	none	☐	9,10,11,12,13,14,15,16,17,18,19,20
3	D1S1656	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	D2S441	Blue	211.0	252.0	10,14	4	none	☐	8,9,10,11,11.3,12,13,14,15,16,17
5	D10S1248	Blue	254.0	302.5	13,15	4	none	☐	8,9,10,11,12,13,14,15,16,17,18,19
6	D13S317	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	D16S539	Green	74.0	129.4	9,13	4	none	☐	4,5,6,7,8,9,10,11,12,13,14,15,16
9	D18S51	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	D2S1338	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	D21S11	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	D7S820	Yellow	268.0	315.5	8,11	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16
17	D5S818	Yellow	317.5	380.0	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	D8S1179	Red	66.0	129.8	14,15	4	none	☐	7,8,9,10,11,12,13,14,15,16,17,18,19
20	D12S391	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	D19S433	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	D6S1043	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	D22S1045	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 22. The GeneMapper® *ID*-X, Version 1.4, Y-Marker check box.

6.2. Importing the WEN ILS 500 Size Standard into GeneMapper® *ID*-X Software

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.3.

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 downloaded in Section 6.1, Getting Started.
5. Highlight the file, and then select **Import**.
6. 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.
7. Select **Done** to save the changes and close the GeneMapper® *ID*-X Manager.

6.3. Creating a Size Standard with GeneMapper® *ID*-X Software

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 23), 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.

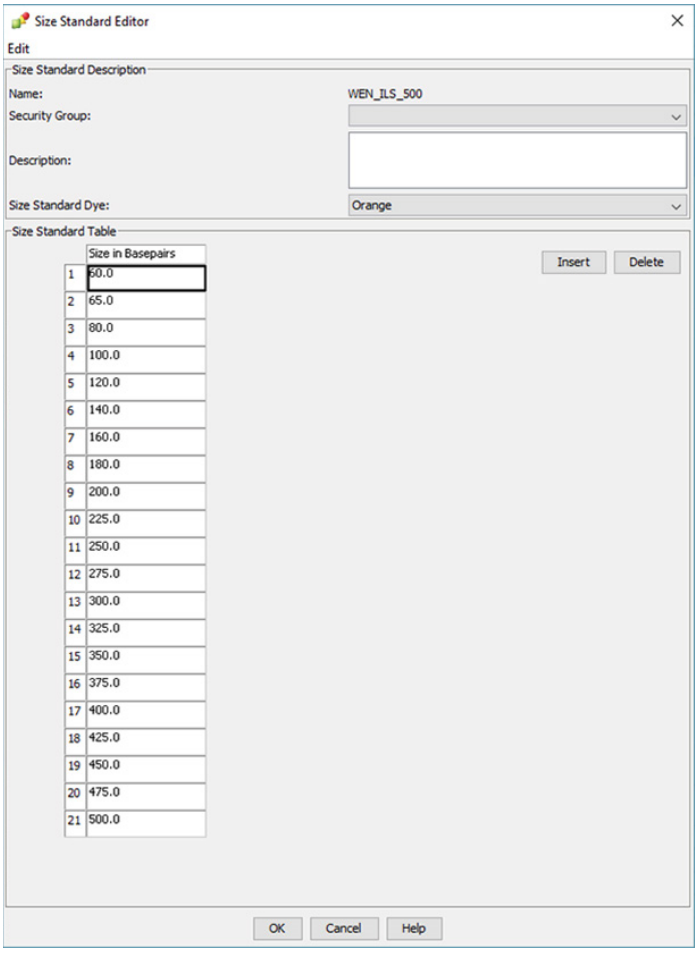

Figure 23. The GeneMapper® *ID*-X Size Standard Editor.

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). See Section 11.2, Figure 34.
8. Select **OK**.

6.4. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X Software. They are not intended as comprehensive instructions 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 24).

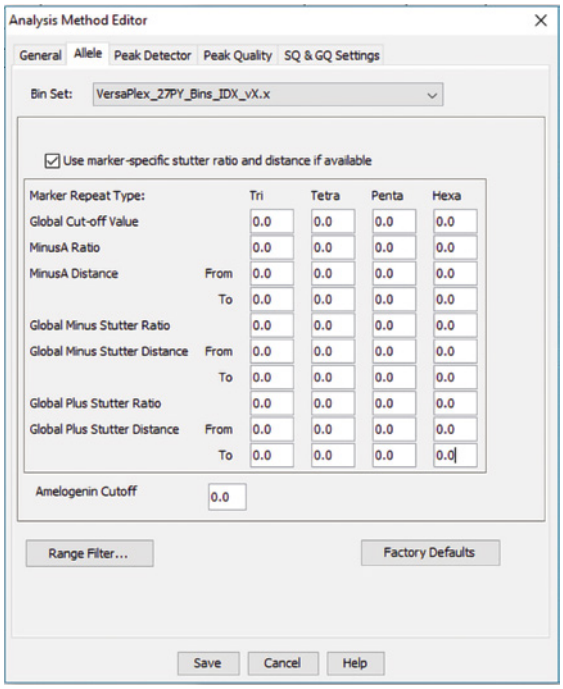

Figure 24. The GeneMapper® *ID*-X 'Allele' tab.

7. Select the bins text file that was imported in Section 6.1.
8. 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.

9. Select the 'Peak Detector' tab (Figure 25). You will 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. The normalization box can be checked or unchecked.

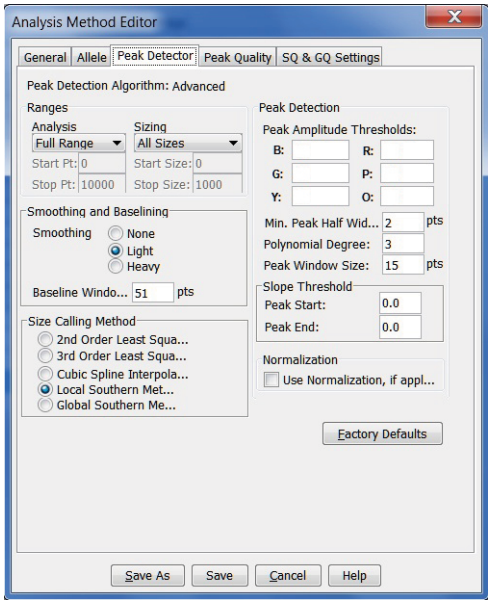

Figure 25. The GeneMapper® *ID*-X 'Peak Detector' tab.

10. Select the 'Peak Quality' tab. You can change the settings for peak quality.
11. Select the 'SQ & GQ Settings' tab. You can change these settings.

    Note: For Steps 10 and 11, see the GeneMapper® *ID*-X user's manual for more information. The settings in Steps 10 and 11 should be based on the results of your internal validation.

12. Select **Save** to save the new analysis method.
13. Select **Done** to exit the GeneMapper® *ID*-X Manager.

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. The sample types assigned in Section 5.4 will be included in the 'Sample Type' column. If needed, the drop-down menu in the 'Sample Type' column can be used to select **Allelic Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every run 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 6.1.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.2 or created in Section 6.3.
8. Select **Analyze** (green arrow button) to start data analysis.

   Note: By default, the software is set to display the 'Analysis Requirements Summary' window if an analysis requirement is not met.
9. If all analysis requirements are met, the 'Save Project' window will open (Figure 26).

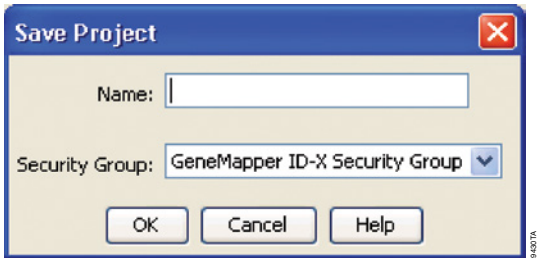

Figure 26. 'Save Project' window.

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, the 'Analysis Summary' screen will appear. 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.

6.5. 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 8 (Section 11.1).

Data Analysis Using GeneMarker®HID Software for Spectrum CE Systems

7

7.1. Installing the VersaPlex™ Panel File for the GeneMarker®HID Software for Spectrum CE Systems

1. To obtain the proper panel file for the VersaPlex™ 27PY System, go to: **www.promega.com/resources/software-firmware/str-analysis/versaplex-27py-system-software/**
2. Select the VersaPlex™ System that you are using, and choose **GeneMarker HID**. Enter your contact information and select **Submit**.
3. Download the VersaPlex® 27PY panel file and save the VersaPlex_27PY_Panel_vX.xml to a known location on your computer or in C:/Users/Public/Softgenetics/Panel folder.
4. Open GMHID-Spectrum.
5. If the panel file was not saved to the panel folder on the C:/ drive, perform the following steps:
    a. Select **Panel Editor** under to Tools.
    b. Select **Import Panels** under File.
    c. Navigate to the location of the panel file saved in step 3 then select **VersaPlex_27PY_Panel_vX.xml**, and then **Open**.
    d. Close the Panel Editor.

7.2. Creating an Analysis Method with GeneMarker®HID Software for Spectrum CE Systems

These instructions are intended as a guide to start analyzing data in GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum). They are not intended as comprehensive instructions for using GMHID-Spectrum. More detailed instructions can be found in the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555.

1. Open GMHID-Spectrum.
2. To access your data files, select **Open Data** in the Magic Wizard or under the File menu.

3. Select **Add**, then navigate to the directory containing your raw data files and select the desired files.
4. Select **Open**, and the selected files will appear in the Data File List (Figure 27).

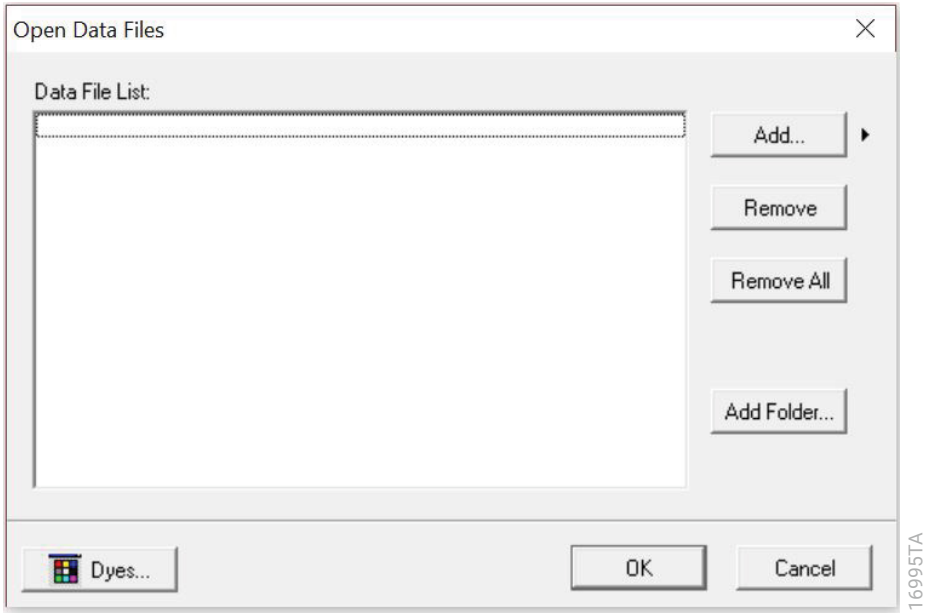

Figure 27. The GMHID-Spectrum Data File List.

5. Select **OK** in the 'Open Data Files' window, and the data will be uploaded into GMHID-Spectrum. In the Raw Data Tree, verify that the sample types (allelic ladder, positive control and negative control) are designated correctly. If sample types are not correct, designate sample types by right-clicking on the file name and selecting **Set Sample Type**.

   Note: Sample types are designated in the file name using preferences. In the View menu, select the 'Forensic' tab under Preferences, then enter sample name identifiers for ladder, positive and negative controls.

6. Use the Panel Editor in the Tools menu to select the VersaPlex_27PY panel from the Panel Template list. Click on the plus symbol to expand the list, right-click on a marker, and then select **Edit**. Enter laboratory-specified values for Min Homozygote Intensity, Homozygote Inconclusive Range, Min Heterozygote Intensity, Heterozygote Inconclusive Range, Min Heterozygote Imbalance and Max Heterozygote Imbalance (Figure 28). This will set values for peaks within the marker range. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

   Notes:

   1. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.
   2. These settings can be applied to all markers by checking the "Apply Homo/Hetero Settings to All Markers" check box or by dye channel by checking the "Apply Homo/Hetero Settings to Markers in this dye" check box.

7. Select **OK** and close the 'Panel Editor' window. Select **Save Changes** or **Save as New Panel** under the File menu then close the window.

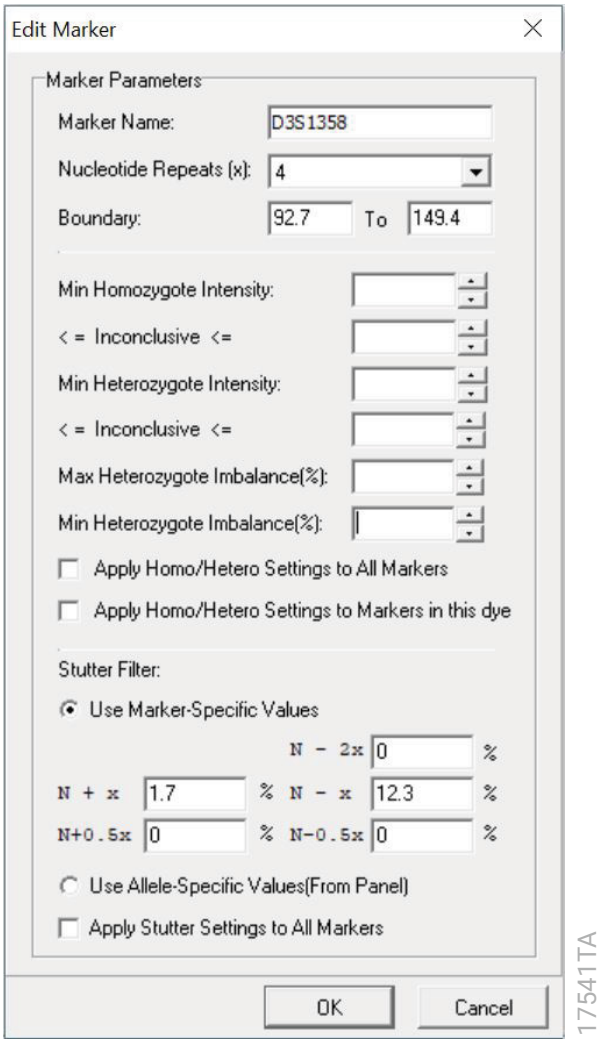

Figure 28. The 'Edit Marker' window for the D3S1358 marker.

8. Select **Run** in the Magic Wizard or choose the **Run Project** icon (green arrow) in the toolbar. The 'Template Selection' screen will appear (Figure 29).
   a. Make sure the button next to "Select an existing template or create one" is highlighted.
   b. Select the drop-down arrow next to the Panel field then select the VersaPlex 27PY panel file imported in Section 7.1, or updated and saved in Step 7.
   c. Select the drop-down arrow next to the Size Standard field then choose **ILS500**. Select **Orange** as the Standard Color.
   d. Enter a descriptive name in the Template Name field.
   e. Select **Save** to save the template.

   Note: Changes to the template can be saved at each step during the Run Project process: 'Template Selection', 'Data Process' and 'Additional Settings' screens.

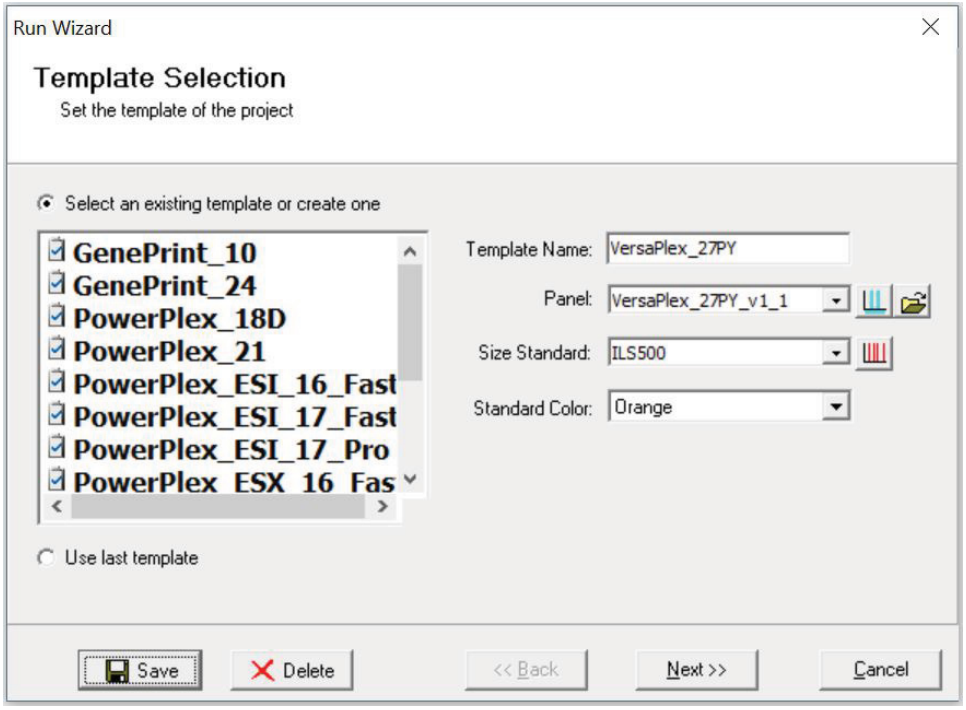

Figure 29. The 'Template Selection' window.

9. The ‘Data Process’ window will appear (Figure 30). For the Raw Data Analysis, we recommend using Auto Range, Smooth, Superior Baseline Subtraction and Local Southern for the Size Call.

   For the Allele Call, we recommend using Auto Range. Setting of the Max Intensity (peaks above which will be flagged) and the Min Intensity for Standard Color should be determined by your laboratory. For peaks outside of the panel range, the settings from the panel can be applied by checking the “Apply Nearest Marker Setting” box. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information on use of the Peak Detection Threshold. Select **Next**.

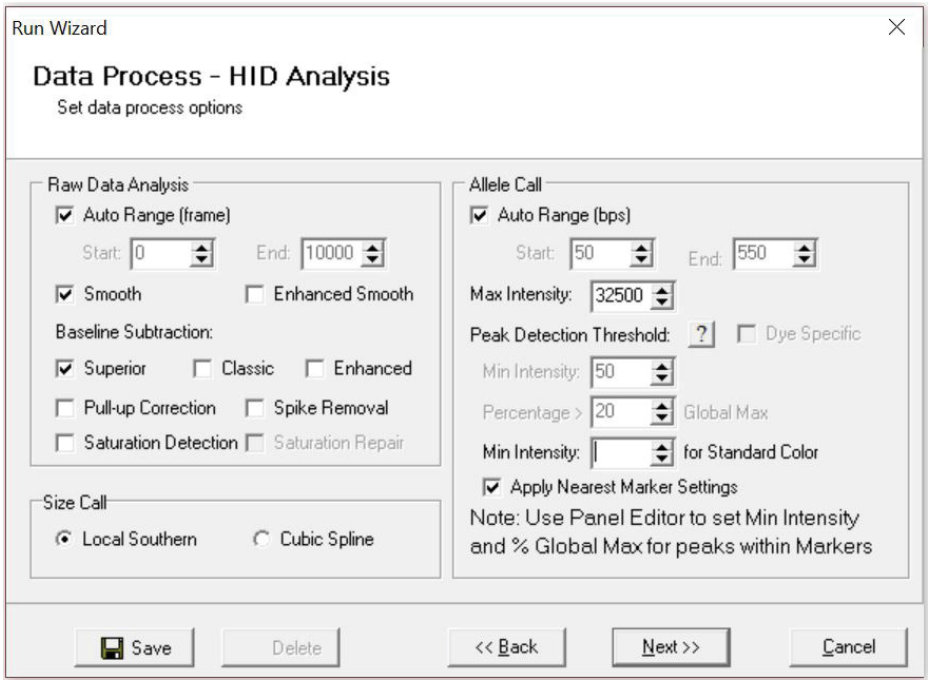

Figure 30. The ‘Data Process’ window for an analysis method.

10. The ‘Additional Settings’ window will appear (Figure 31). Verify that the boxes are checked for “Auto Select Best Ladder” and “Auto Panel Adjust”. The values displayed in the ‘Allele Evaluation’ dialogue box are defaults and will affect the quality values displayed in the plot settings. For more information on quality values, use of positive controls and mixture evaluation, refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555. Select **OK** to analyze the raw data.
11. Select **Save** to save changes made to the VersaPlex Template created in Step 8. This template can be used for future analyses.

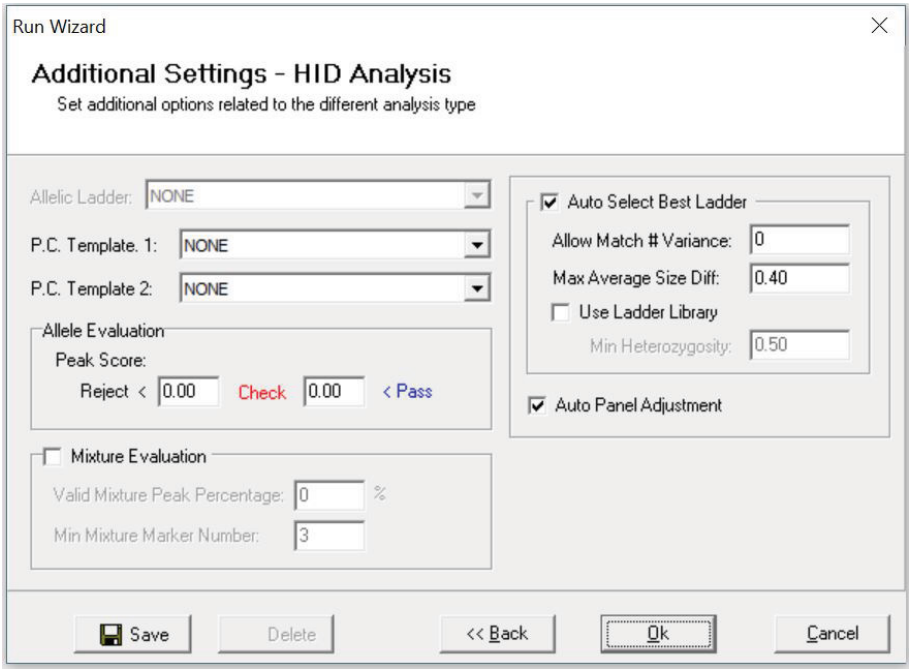

Figure 31. The 'Additional Settings' window.

12. When the analysis is complete, the 'Main Analysis' window will appear. We recommend that you review any yellow or red flagged markers and handle them according to your laboratory's standard operating procedures.

    Note: Quality and display settings are set using Preferences under the View menu. Refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

7.3. Controls in GeneMarker®HID Software for Spectrum CE Systems

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative controls should be devoid of amplification products.
2. Observe the results for the 2800M Control DNA. The expected 2800M allele designations for each locus are listed in Table 8 (Section 11.1).

Results

8

Representative results of the VersaPlex™ 27PY System are shown in Figure 32. The VersaPlex™ 27PY Allelic Ladder Mix is shown in Figure 33.

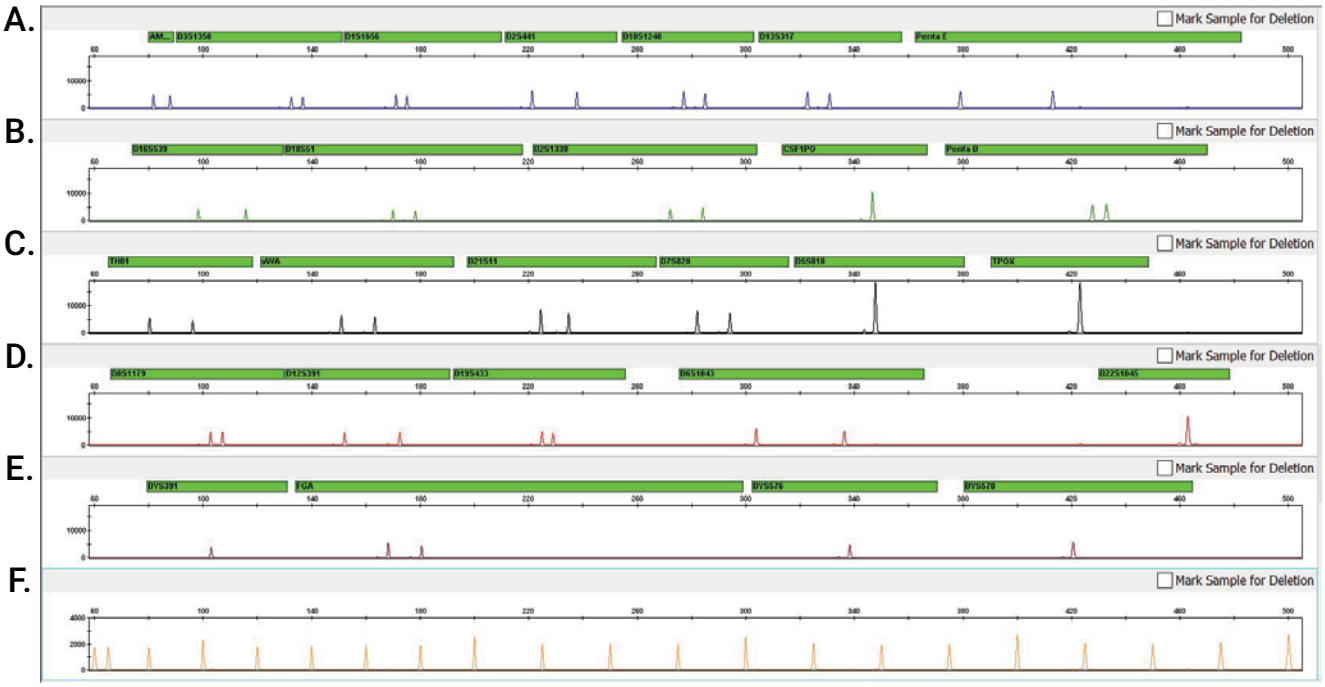

Figure 32. The VersaPlex™ 27PY System. The 2800M Control DNA (1.0ng) was amplified using the VersaPlex™ 27PY System and 29 cycles. Amplification products were mixed with WEN Internal Lane Standard 500 and analyzed using a Spectrum Compact CE System using a 1.6kV, 9-second injection. Results were analyzed using GeneMapper® *ID*-X software, version 1.6, and VersaPlex™ 27PY panels and bins text files. **Panel A.** An electropherogram showing the peaks of the FL-6C-labeled loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-6C-labeled loci: D16S539, D18S51, D2S1338, CSF1PO and Penta D. **Panel C.** An electropherogram showing the peaks of the TMR-6C-labeled loci: TH01, vWA, D21S11, D7S820, D5S818, and TPOX. **Panel D.** An electropherogram showing the peaks of the CXR-6C-labeled loci: D8S1179, D12S391, D19S433, D6S1043 and D22S1045. **Panel E.** An electropherogram showing the TOM-6C-labeled loci: DYS391, FGA, DYS576 and DYS570. **Panel F.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500.

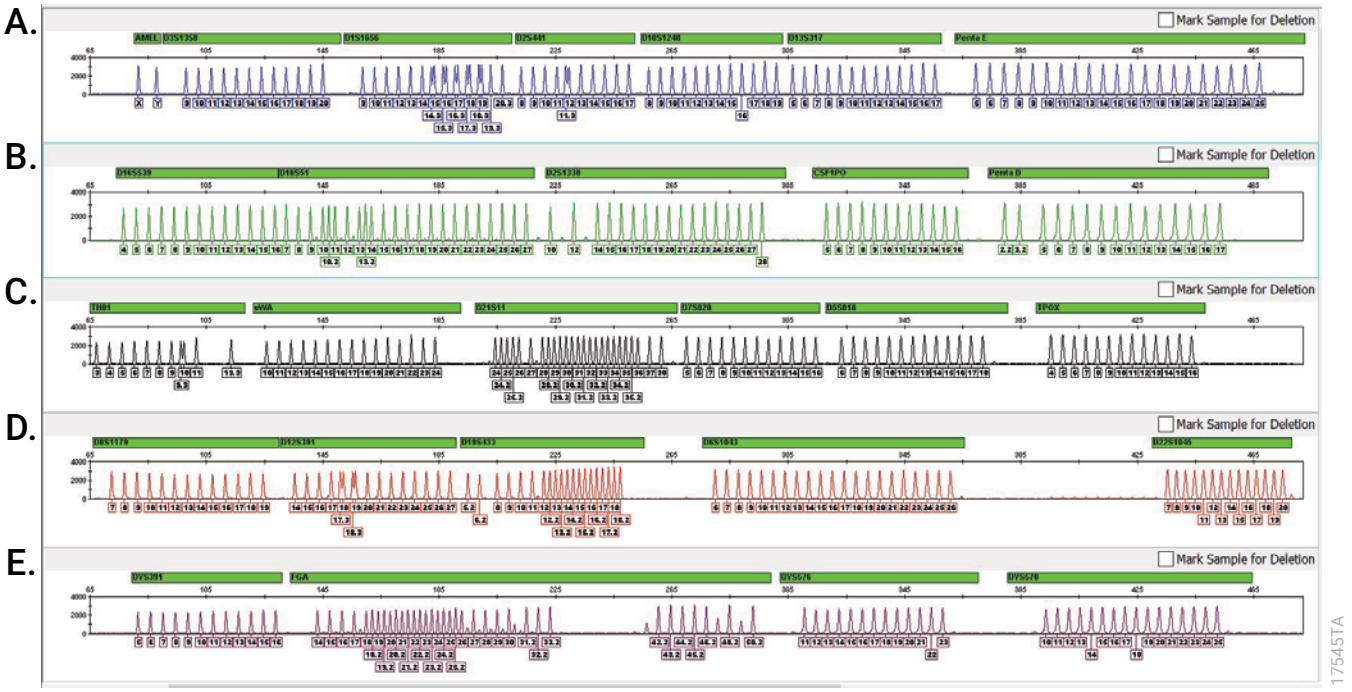

Figure 33. The VersaPlex™ 27PY Allelic Ladder Mix. The VersaPlex™ 27PY Allelic Ladder Mix was analyzed with a Spectrum Compact CE System Instrument using a 1.6kV, 9-second injection. The sample file was analyzed with the GeneMapper® *ID*-X software, version 1.6, and VersaPlex™ 27PY panels and bins text files. **Panel A.** The FL-6C-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-6C-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-6C-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-6C-labeled allelic ladder components and their allele designations. **Panel E.** The TOM-6C-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products are often observed one repeat unit below 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 percent 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 stutter plus three standard deviations observed at each locus is used in the VersaPlex™ 27PY panels text file for locus-specific filtering in GeneMapper® *ID*-X software and in the panel file imported into GMHID-Spectrum.

In addition to stutter peaks, you may observe the following low-level DNA-dependent artifact peaks (Table 3) and DNA-independent (with or without human genomic DNA) artifact peaks (Table 4) with the VersaPlex™ 27PY System.

Table 3. DNA-Dependent Artifacts Observed in Amplification Reactions with Human Genomic DNA.

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 4. DNA-Independent Artifacts Observed in Amplification Reactions with and without Human Genomic DNA.

Dye Label	Artifact Size[1]
FL-6C	~65–79 bases ~113–124 bases ~137–148 bases
JOE-6C	~60–68 bases
TMR-6C	~57–62 bases

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

As part of the developmental validation, 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 (14). The artifacts listed in Table 5 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 5. Nonhuman DNA Cross-Reactivity.

DNA Source	Artifact Size	Dye Label
Fusobacterium nucleatum	~308bp	JOE-6C
Chicken	~221bp	JOE-6C
	~300bp	TMR-6C
Mouse	~347bp	JOE-6C
Pig	~259–260bp	FL-6C
	~368–372bp	JOE-6C
	~369–370bp	CXR-6C

Troubleshooting

9

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**

Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for instrument troubleshooting. For troubleshooting GMHID-Spectrum, refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555.

9.1. Amplification and Fragment Detection

Symptoms	Causes and Comments
Faint or absent allele peaks	The Master Mix was not vortexed well before use. Vortex the Master Mix for 15 seconds before dispensing into the PCR amplification mix.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the Primer Pair Mix for 15 seconds before use.
	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 themal cycling.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocol. We have not tested reaction tubes, plates or thermal cyclers that are not listed. Calibrate the thermal cycler heating block if necessary.
	Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in loss of signal.
	Poor capillary electrophoresis injection (ILS peaks also affected). Re-inject the sample.
	Laser is starting to fail. Check laser power.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
Faint or absent allele peaks for the positive control reaction	Improper storage of the 2800M Control DNA. Store the 2800M Control DNA at 2–10°C. Make sure that the 2800M Control DNA is stored at 2–10°C for at least 24 hours before use. Vortex before use.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips and change gloves regularly.
	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 the DNA source as soon as possible to each well and follow immediately by thermal cycling.
	Samples were not denatured completely. Heat-denature samples for the recommended time, and 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, as this may lead to artifacts due to DNA re-annealing. 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.
	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 rerun the samples. • Confirm that the correct spectral was used for the sample run. • Instrument sensitivities can vary. Optimize the injection conditions. Refer to the instrument user manual.
	Dye blob artifacts. The signal strength of certain dye blob artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when left at 4°C for a few days. We recommend storing amplification products at –20°C.
	An incorrect internal lane standard was used. Use the size standard provided in the kit.

Symptoms	Causes and Comments
Allelic ladder not running the same as samples	Be sure the allelic ladder and samples are from the same instrument run.
	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature 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. Include one allelic ladder for every 16 samples.
	Internal size standard was not assigned correctly. Evaluate the sizing labels on the ILS and correct if necesary.
Peak height imbalance	Miscellaneous balance problems. At the first use, thaw the Primer Pair Mix and Master Mix completely. Vortex the Primer Pair Mix and Master Mix for 15 seconds before use; do not centrifuge the Primer Pair Mix or Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	PCR amplification mix was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.

9.2. Amplification of Extracted DNA

The following information is specific to amplification of purified DNA. For information about general amplification and detection, see Section 9.1.

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Depending on the DNA purification procedure used and sample source, inhibitors might be present in the DNA sample. This may be more of an issue as DNA sample volume increases as a percentage of the total amplification reaction volume.
	Insufficient template. Use the recommended amount of template DNA if available. Quantify template DNA before use if possible.
	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) or TE^{-4} buffer with 20µg/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl for extracted DNA. Decreasing the reaction volume may result in suboptimal performance.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Amplification of more than the recommended amount of purified DNA can result in a higher number of artifact peaks due to overamplification, resulting in saturating signal. Use the recommended amount of template DNA. See Results section for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.
	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 the recommended extension step at 60°C after thermal cycling. • 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.
Peak height imbalance	Amplification of greater than the recommended amount of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles. The amount of template will need to be optimized if you are using reduced reaction volumes.
	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.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.

9.3. 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.
	Be sure that the 'Use marker-specific stutter ratio and distance if available' box is checked. 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 'Project Options' menu under **File**, 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 changes 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 per folder of sample files being analyzed in the project.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS 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 the section 'Processing Data for Casework Samples' or 'Processing Data for Databasing or Paternity Samples.'
	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 Polymer4 may change migration of the fragments, causing alleles to migrate outside of the panel range.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen for the analysis in the GeneMapper® *ID*-X Peak Detector tab as described in sections 'Creating a Casework Analysis Method' or 'Creating a Databasing or Paternity Analysis Method.' Adjust the starting data point or 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**.
	An incorrect size standard was used.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS 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.
	An incorrect size standard was used.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration and re-run the samples.
	Confirm that the correct spectral was used to run the samples.

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Appendix 11

11.1. 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 (Table 6 and Table 7). 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 8 lists the VersaPlex™ 27PY System alleles amplified from the 2800M Control DNA.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage and terminal nucleotide addition (15,16). 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 (17,18) occurs when a thermostable nonproofreading 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 (19) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

Table 6. The VersaPlex™ 27PY System Locus-Specific Information.

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

[1]Information about chromosomal location of these loci can be found in references 21, 22 and 23 and at:

https://strbase.nist.gov//chrom.htm

[2]The August 1997 report (24,25) 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 7. 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[4]	FL-6C	89, 95	X, Y
D3S1358	FL-6C	103–147	9–20
D1S1656	FL-6C	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-6C	216–252	8–11, 11.3, 12–17
D10S1248	FL-6C	259–303	8–19
D13S317	FL-6C	308–358	5–17
Penta E	FL-6C	371–471	5–25
D16S539	JOE-6C	84–132	4–16
D18S51	JOE-6C	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE-6C	224–296	10, 12, 14–28
CSF1PO	JOE-6C	318–362	5–16
Penta D	JOE-6C	377–450	2.2, 3.2, 5–17
TH01	TMR-6C	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR-6C	127–183	10–24
D21S11	TMR-6C	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-6C	269–313	5–16
D5S818	TMR-6C	321–369	6–18
TPOX	TMR-6C	393–441	4–16
D8S1179	CXR-6C	76–124	7–19
D12S391	CXR-6C	133–185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR-6C	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-6C	276–356	6–26
D22S1045	CXR-6C	431–470	7–20
DYS391	TOM-6C	86–130	5–16
FGA	TOM-6C	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-6C	308–356	11–23
DYS570	TOM-6C	393–453	10–25

[1]The length of each allele in the allelic ladder has been confirmed by sequence analyses.

[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: **https://strbase.nist.gov/var_tab.htm**

[4]Amelogenin is not an STR.

Table 8. The VersaPlex™ 27PY System Allele Determinations for the 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

11.2. 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 34). 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.

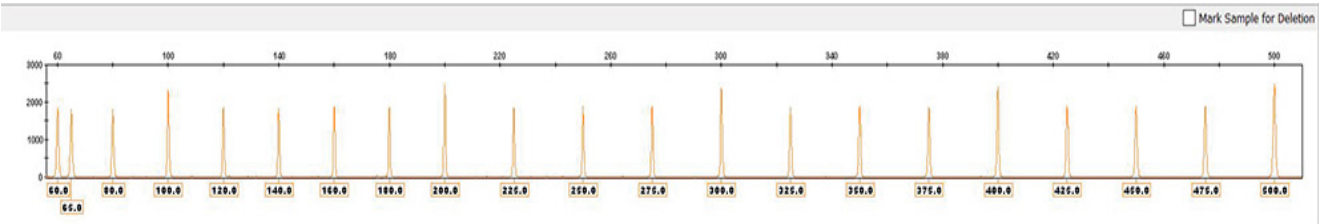

Figure 34. WEN Internal Lane Standard 500. An electropherogram showing the WEN Internal Lane Standard 500 fragments.

11.3. Composition of Buffers and Solutions

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

1.21g Tris base

0.037g EDTA ($Na_2EDTA \bullet 2H_2O$)

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 ($Na_2EDTA \bullet 2H_2O$)

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.

11.4. Related Products

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.

Spectrum Compact CE System Accessories and Consumables

PRODUCT	SIZE	CAT.#
Spectrum Compact Capillary Cartridge, 4-Capillary, 36cm	1 each	CE2340
Spectrum Compact Polymer4	4 × 64 wells	CE2304
Spectrum Compact Buffer	2 pairs	CE2300
Spectrum Compact Cathode Septa Mat	10 each	CE2301
Spectrum Compact Cathode Retainer	4 each	CE2302
Spectrum Compact Strip Base & Retainer, 32-Well	4 each	CE2332
Strip Septa Mat, 8-Well	24 each	CE2308

Not for Medical Diagnostic Use.

[a]U.S. Pat. No. 9,139,868, European Pat. No. 2972229, Japanese Pat. No. 6367307 and other patents pending.

[b]TMR-6C, CXR-6C, TOM-6C and WEN dyes are proprietary.

Class 1 Laser Product.