

# VersaPlex<sup>®</sup> 31P System for Use on the Spectrum Compact CE System Technical Manual

Instructions for Use of Product DC8020



# Table of Contents

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1	Description.....	4
2	Product Components and Storage Conditions.....	5
3	Before You Begin .....	7
	3.1 Precautions .....	7
	3.2 Spectral Calibration .....	8
4	Protocol for DNA Amplification Using the VersaPlex® 31P System...	9
	4.1 Amplifying Extracted DNA in a 25µl Reaction Volume .....	9
5	Detection of Amplified Fragments Using the Spectrum Compact CE System.....	13
	5.1 Sample Preparation .....	14
	5.2 Assembling Sample Cartridge .....	15
	5.3 Instrument Preparation .....	16
	5.4 Assigning Run Information to Samples .....	21
	5.5 Loading the Sample Cartridge .....	29

6	Data Analysis Using GeneMarker®HID Software .....	31
6.1	Creating an Analysis Method with GeneMarker®HID Software for Spectrum CE Systems .....	31
6.2	Controls in GeneMarker®HID Software for Spectrum CE Systems .....	37
6.3	Quality Indicators Small (QIS) and Large (QIL) .....	37
7	Results.....	38
8	Troubleshooting.....	42
8.1	Amplification and Fragment Detection .....	42
8.2	Amplifying Extracted DNA .....	45
8.3	GeneMarker®HID Software for Spectrum CE Systems .....	47
9	References.....	50
10	Appendix .....	52
10.1	Advantages of Using the Loci in the VersaPlex® 31P System .....	52
10.2	The CCO Internal Lane Standard 500 .....	57
10.3	Composition of Buffers and Solutions .....	57
10.4	Related Products .....	58

All technical literature is available at: **[www.promega.com/protocols/](http://www.promega.com/protocols/)**

Visit the website to verify that you are using the most current version of this Technical Manual.

Email Promega Technical Services if you have questions on use of this system: **[genetic@promega.com](mailto:genetic@promega.com)**

# 1 Description

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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 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® 31P System<sup>(a,b)</sup> is an 31-locus multiplex for human identification applications including forensic analysis, relationship testing and research use. This eight-color system provides co-amplification and fluorescent detection of 29 autosomal loci (D3S1358, D21S11, D7S820, D10S1248, D6S477, D16S539, D18S51, Penta E, D3S3045, TH01, vWA, D19S433, D5S818, D19S253, D2S441, D12S391, D13S317, Penta D, D2S1338, D6S1043, D10S1435, TPOX, D1S1656, D22S1045, CSF1PO, D15S659, D8S1179, FGA and D8S1132) as well as Amelogenin and DYS391 for gender determination. This panel of STR markers is specifically designed to include the 20 CODIS core loci along with Penta D, Penta E, and D6S1043. Six new autosomal loci (D6S477, D3S3045, D19S253, D10S1435, D15S659 and D8S1132) are also included for improved power of discrimination.

The VersaPlex® 31P 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® 31P 5X Master Mix.

This manual contains protocols for use of the VersaPlex® 31P System with the ProFlex® PCR System in addition to protocols to separate amplified products and detect separated material on the Spectrum Compact CE System. Amplification and detection instrumentation may vary. Protocols may need to be optimized for amount of template DNA amount, cycle number, loading volume and injection conditions 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](http://www.promega.com)**

## 2 Product Components and Storage Conditions

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PRODUCT	SIZE	CAT.#
VersaPlex® 31P System	200 reactions	DC8020

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

### Pre-Amplification Components Box

- 1,000µl VersaPlex® 31P 5X Master Mix
- 1,000µl VersaPlex® 31P 5X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 5 × 1,250µl Water, Amplification Grade

### Post-Amplification Components Box

- 100µl VersaPlex® 31P Allelic Ladder Mix
- 250µl CCO Internal Lane Standard 500



The VersaPlex® 31P 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 +2°C to +10°C. The VersaPlex® 31P 5X Primer Pair Mix, VersaPlex® 31P Allelic Ladder Mix and CCO Internal Lane Standard 500 (CCO 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.



Do not freeze reagents.

## 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 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-system-software](http://www.promega.com/resources/software-firmware/str-analysis/versaplex-system-software)**

The VersaPlex® 8C Matrix Standard (Cat.# DG5980), available separately, is required for initial setup of the color separation matrix (see Section 3.2).

# 3 Before You Begin

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## 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® Matrix Standards for Use on the Spectrum Compact CE System Technical Manual* #TMD072. This manual is available online at: **[www.promega.com/protocols/](http://www.promega.com/protocols/)**

# 4 Protocol for DNA Amplification Using the VersaPlex® 31P System

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The VersaPlex® 31P System is designed for amplifying extracted DNA in a 25µl reaction volume.

The VersaPlex® 31P System is compatible with the ProFlex® PCR System.

**Note:** You can use thermal cyclers other than the ones listed in this technical manual. Using thermal cyclers not listed here will require optimization of cycling conditions and in-house validation.

We recommend using gloves and aerosol-resistant pipette tips 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.



Take meticulous care to ensure successful amplification. See Section 8 for amplification troubleshooting.

## 4.1 Amplifying Extracted DNA in a 25µl Reaction Volume

### 4.1.1 Materials to be Supplied by the User

- ProFlex® PCR System (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

We routinely amplify 0.5ng of template DNA in a 25µl reaction volume using the following protocol.

## 4.1.2 Amplification Setup

1. Upon receipt, store the VersaPlex® 31P 5X Master Mix, VersaPlex® 31P 5X Primer Pair Mix and Water, Amplification Grade, at +2°C to +10°C.

**Note:** Centrifuge tubes briefly to move 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 can concentrate the reagents at the bottom of the tube.

2. Determine the number of reactions to be set up, including positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach consumes 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 new plate for reaction assembly, and label it appropriately. Alternatively, determine the number of new, 0.2ml reaction tubes required, and label them appropriately.
4. Add the final volume of each reagent listed in Table 1 to a new tube.

**Table 1. PCR Amplification Mix for Amplifying Extracted DNA.**

PCR Amplification Mix Component <sup>1</sup>	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25µl	×		=	
VersaPlex® 31P 5X Master Mix	5µl	×		=	
VersaPlex® 31P 5X Primer Pair Mix	5µl	×		=	
template DNA (0.5ng) <sup>2,3</sup>	up to 15µl				
<b>total reaction volume</b>	<b>25µl</b>				

<sup>1</sup>Add Water, Amplification Grade, to the tube first, and then add VersaPlex® 31P 5X Master Mix and VersaPlex® 31P 5X Primer Pair Mix. The template DNA will be added at Step 6.

<sup>2</sup>Store DNA templates in TE<sup>-4</sup> buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE<sup>-4</sup> buffer with 20µg/ml glycogen. If the DNA template is stored in TE buffer that is not at 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.

<sup>3</sup>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 (0.5ng) for each sample to the respective well containing PCR amplification mix.

**Note:** The VersaPlex® 31P System is optimized and balanced using 0.5ng 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 0.5ng in the desired template DNA volume. Add 0.5ng of diluted DNA to a reaction well containing PCR amplification mix.
8. For the negative amplification control, pipet Water, Amplification Grade, or TE<sup>-4</sup> 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 move contents to the bottom of the wells and remove any air bubbles.

### 4.1.3 Thermal Cycling on the ProFlex® PCR System

Amplification and detection instrumentation may vary. You may need to optimize protocols for the template DNA amount, cycle number, injection conditions and loading volume for your laboratory instrumentation. Tests at Promega shows that 30 cycles work well for 0.5ng of purified DNA templates. Perform in-house validation.

1. Place the reaction plate or tubes in the thermal cycler.
2. Select and run the recommended protocol provided below and in Figure 1. The total cycling time is approximately 75 minutes.

**Note:** Set the ramp speed to 6°C/s and the reaction volume to 25µl.

#### Thermal Cycling Protocol

96°C for 1 minute, then:

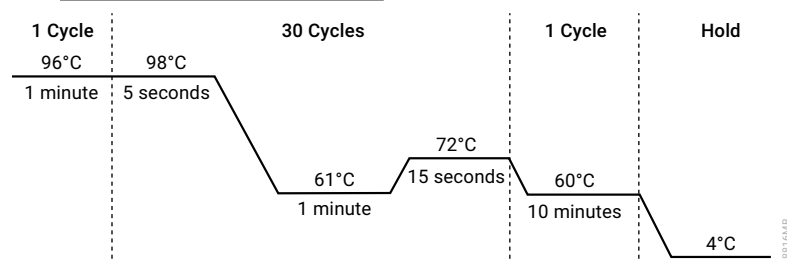
98°C for 5 seconds

61°C for 1 minute

72°C for 15 seconds for  
30 cycles, then:

60°C for 10 minutes

4°C soak



**Figure 1. The thermal cycling protocol for the ProFlex® PCR System.**

3. After completing 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.

# 5 Detection of Amplified Fragments Using the Spectrum Compact CE System

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Instructions for detecting 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, CE2404)
- 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)



Formamide quality 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 can 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:**

- a. 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.
- b. 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:  $[(0.5\mu\text{l ILS}) \times (\# \text{ samples})] + [(9.5\mu\text{l formamide}) \times (\# \text{ samples})]$   
**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 10 $\mu\text{l}$  of formamide/internal lane standard mix into each well of the 8-well strip tube(s).

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

**Notes:**

- a. 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.
  - b. 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.
  - c. Use an allelic ladder volume that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.
  - d. Include an allelic ladder injection 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.

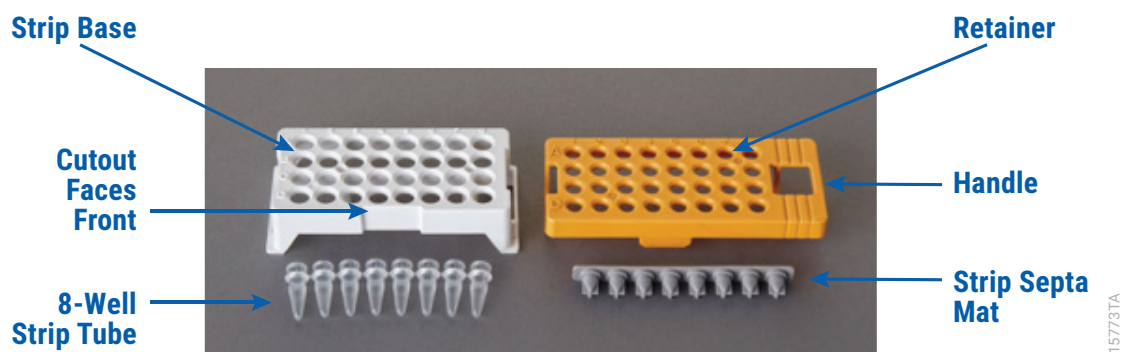
## 5.2 Assembling Sample Cartridge

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:** Lanes A–D and wells 1–8 are embossed on the strip base. Be sure to check the lane 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, wells 1–8 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 lanes A–D and wells 1–8 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.**

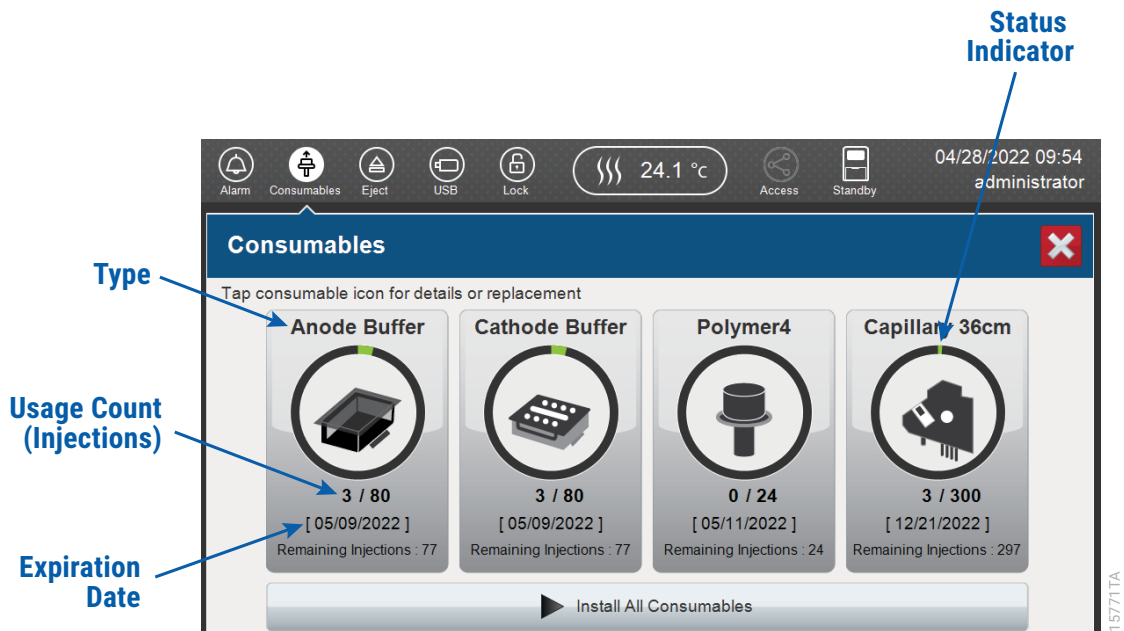


**Figure 3. Assembled Spectrum Compact Sample Cartridge.**

## 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® 8C 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® Matrix Standards 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 4 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 5). 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 '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.



**Figure 5. Spectrum Compact CE System Software 'Main Menu' screen.**

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 that 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.

**Oven Temperature Icon**



**Figure 6. Preheating Oven.**

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



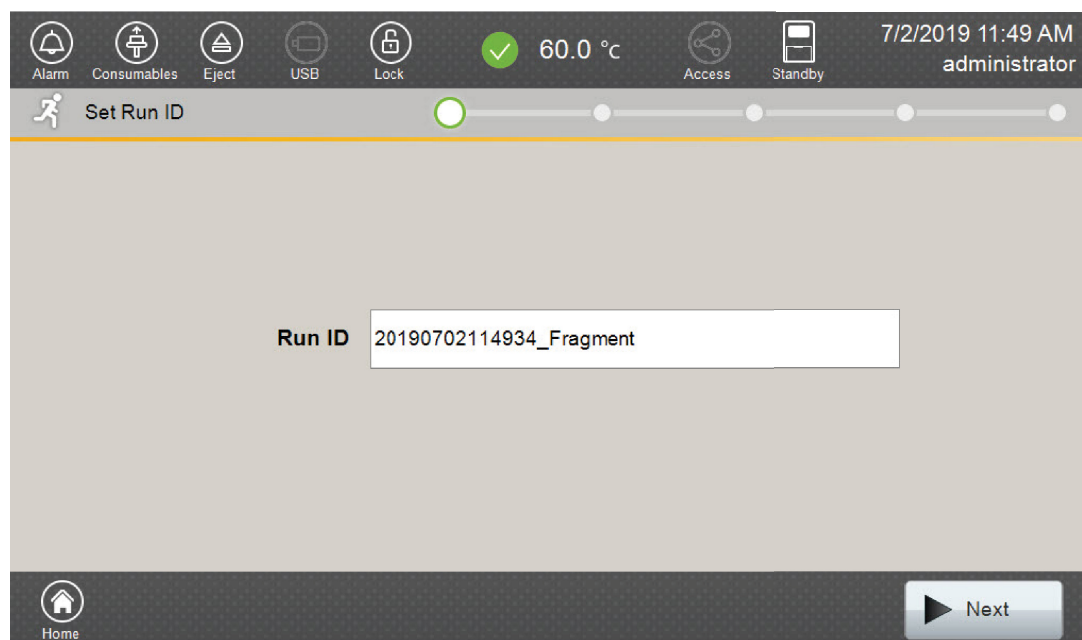
**Figure 7. Spectrum Compact CE System Software 'Main Menu' screen.**

4. Use the default name that includes date, time and "Fragment" or enter a Run ID on the 'Set Run ID' screen (Figure 8). 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:**

- a. The Run ID will be included in the file names for the samples.
- b. The following table lists rules for characters that can be used for a Run ID, Strip ID and sample name.

Acceptable Characters	1 to 30 characters for Strip IDs
	1 to 50 characters for Run IDs and sample names
	Upper and lowercase alphabetic characters
	Numbers
	Symbols unless listed below
Unacceptable Characters	#%&{} \<>*?/\$!'":@+`= and spaces

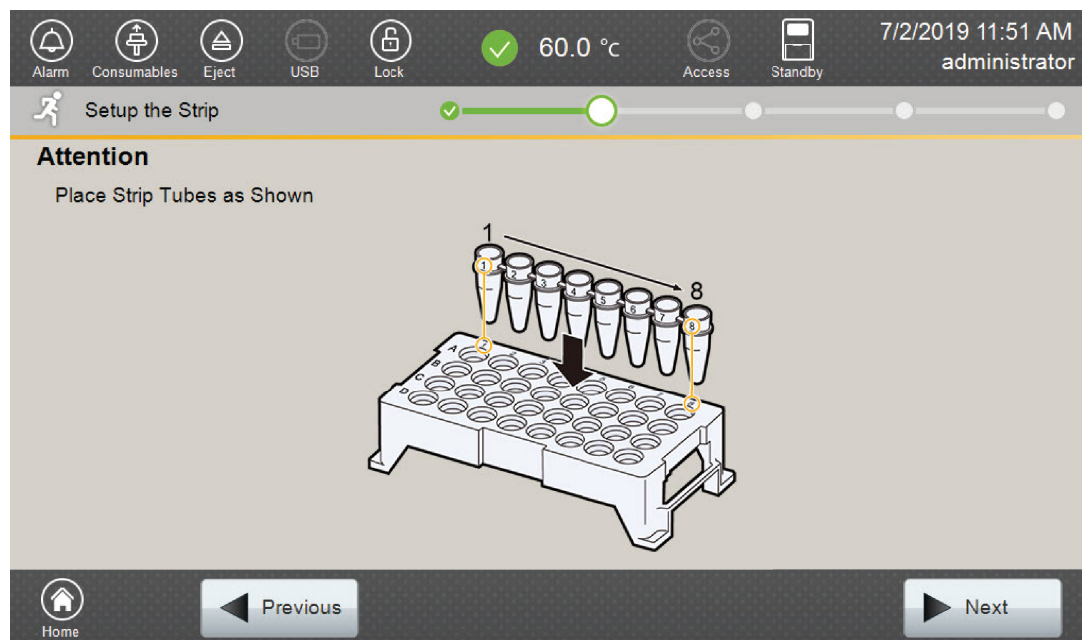


**Figure 8. 'Set Run ID' screen.**

5. Select **Next** to proceed to the message screen for placement of strips into the sample cartridge.

- 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–D) on the strip base and that wells 1–8 of the strip tube are correctly aligned with well positions 1–8 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:

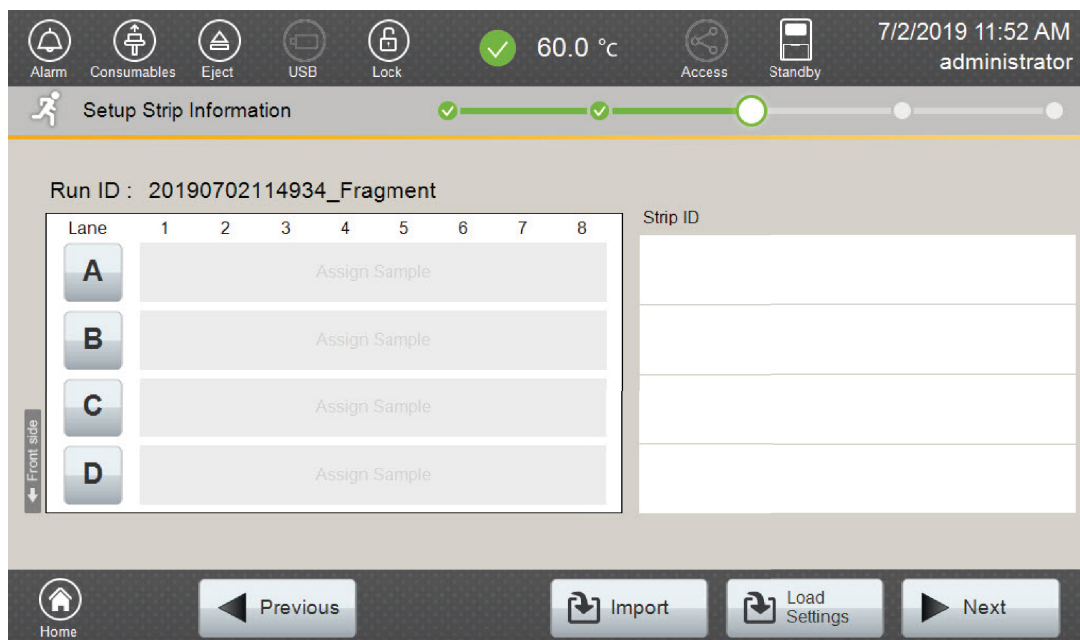
1. Creating new strip information,
2. Reusing run information from a list of completed runs,
3. Loading saved strip information and
4. Importing strip information.

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.

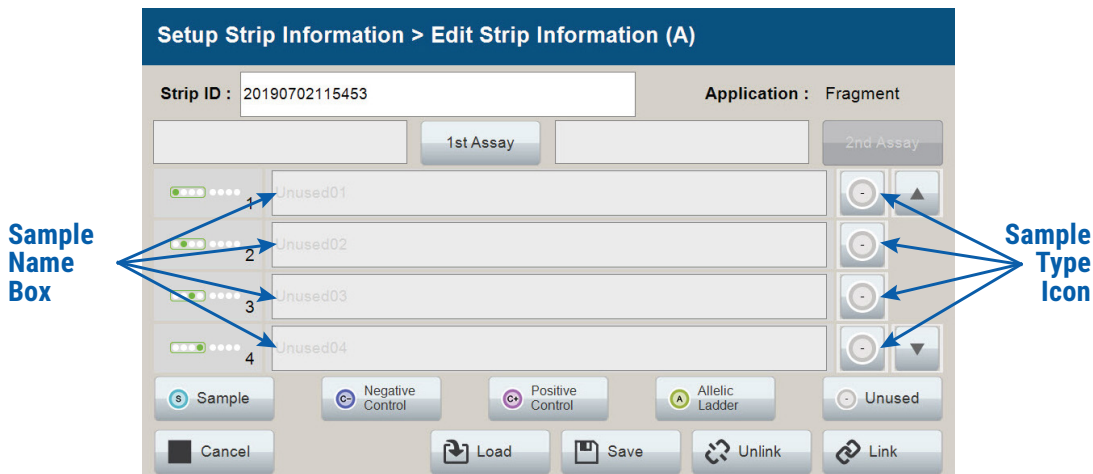
1. Select **Next** on the 'Setup the Strip' screen shown in Figure 9. This will take you to the 'Setup Strip Information' screen shown in Figure 10.

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



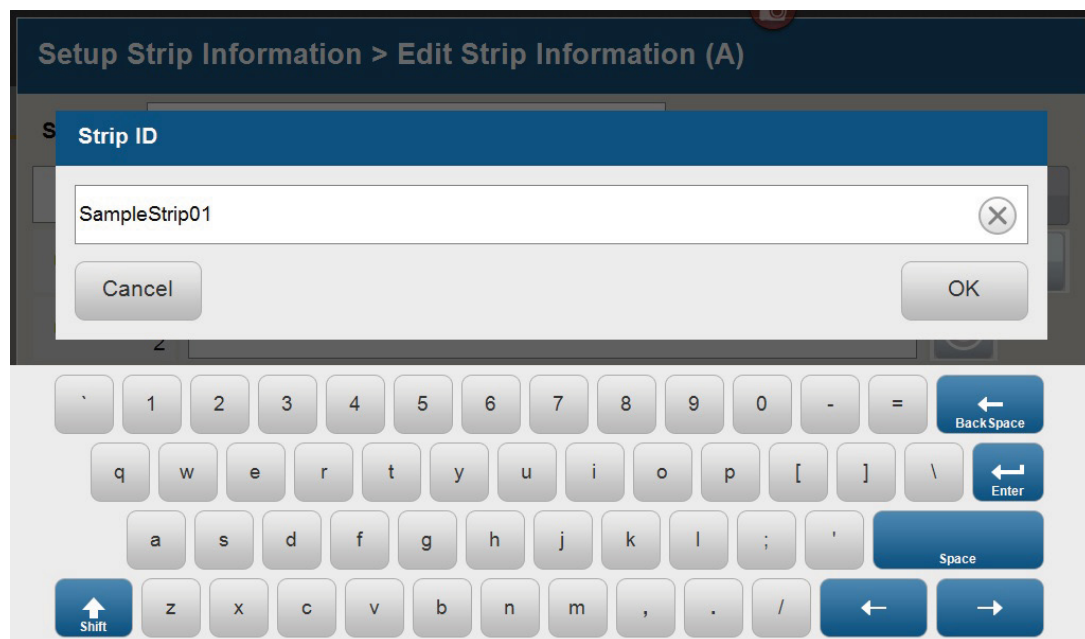
**Figure 10. 'Setup Strip Information' screen.**

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.**

- 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.



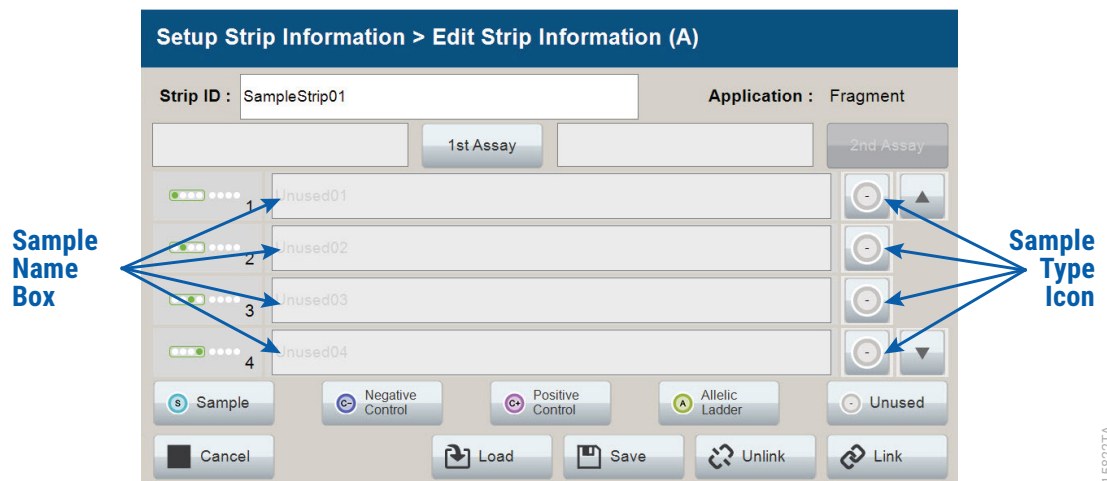
**Figure 12. 'Set Strip ID' window.**

- 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
	Sample
	Negative Control
	Positive Control
	Allelic Ladder
	Unused

- 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.

**Setup Strip Information > Edit Strip Information (A)**

Strip ID : SampleStrip01      Application : Fragment

	1st Assay	2nd Assay
1	AllelicLadder01	A
2	Sample02	S
3	NegativeControl03	C-
4	PositiveControl04	C+

Buttons: Sample, Negative Control, Positive Control, Allelic Ladder, Unused, Cancel, Load, Save, Unlink, Link

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

**Setup Strip Information > Edit Strip Information (A)**

**Sample Name 2**

Sample02

Buttons: Cancel, OK

Virtual Keyboard: BackSpace, Enter, Space, Shift, Arrow keys

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\_8Dye\_CCOILS\_36\_P4** from the drop-down list (Figure 16) and then select **Apply**.

**Setup Strip Information > Edit Strip Information (A) > Select Assay (1st)**

Assay	<div>▼</div>	
Application	Promega_4Dye_ILS600_36_P4	▲
	Promega_5Dye_WENILS_36_P4	▲
Polymer	Promega_6Dye_WENILS_36_P4	$\frac{1}{3}$
Dye Set	Promega_8Dye_CCOILS_36_P4	▼
	T_5Dye_O500(75-500)_36_P4	▼
Instrument Protocol		
Analysis Protocol		

Figure 16. 'Select Assay' window.

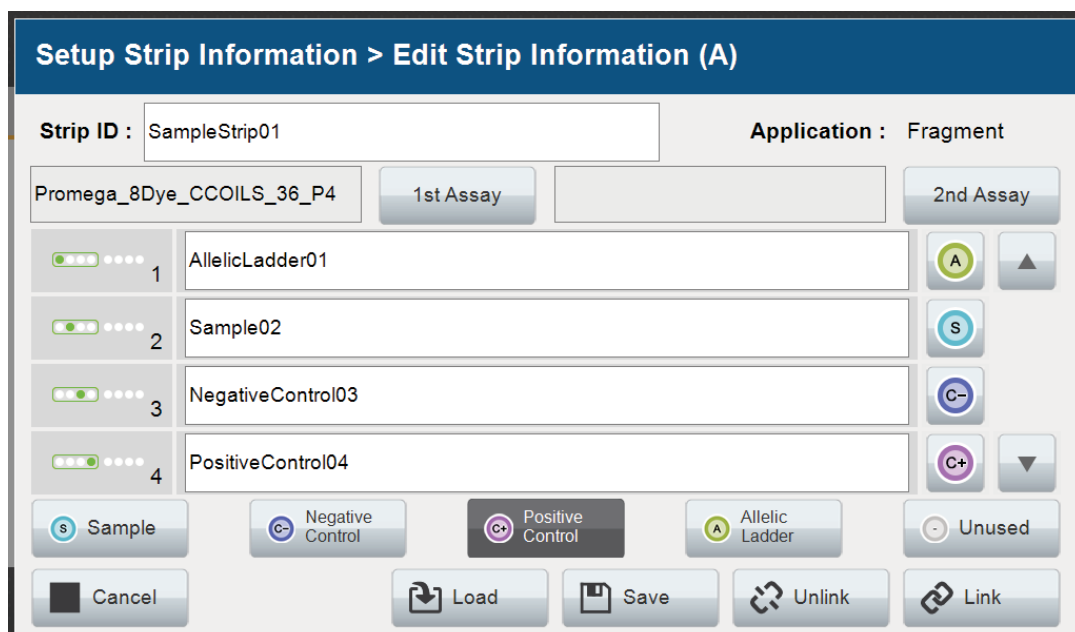
8. You can verify the settings of the Instrument and Analysis Protocols associated with the assay chosen by selecting **Details** next to these fields (Figure 17). This will display a window showing the settings in these protocols but will not allow you to edit these settings. Refer to the *Spectrum 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.

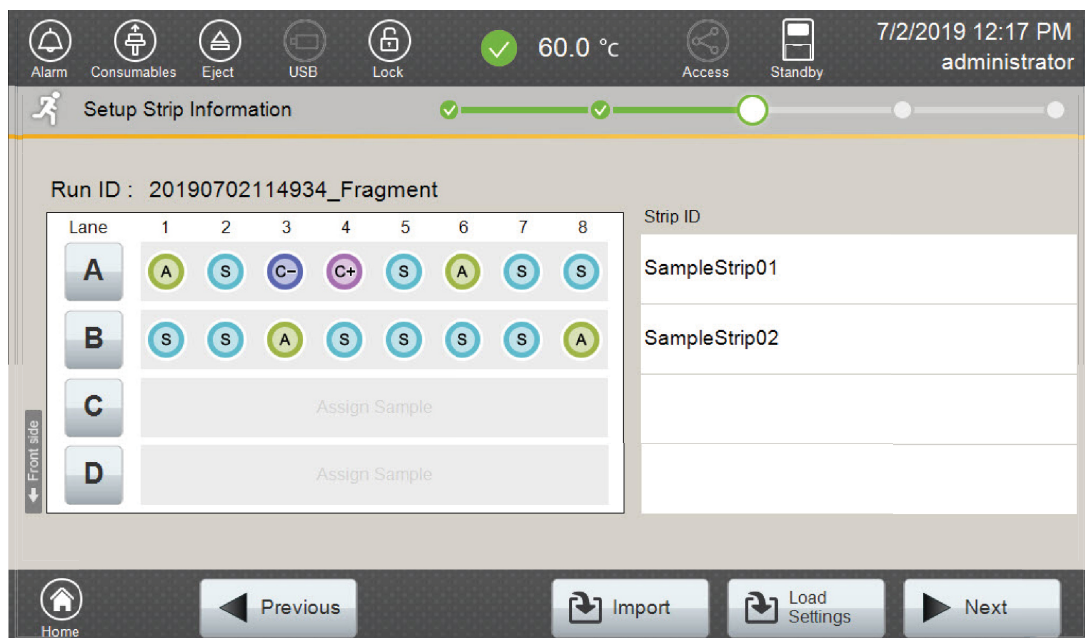
**Notes:**

- If you select **Unlink** without having previously selected **Save** at the bottom of the 'Edit Strip Information' screen, you will lose the strip information.
- 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.**

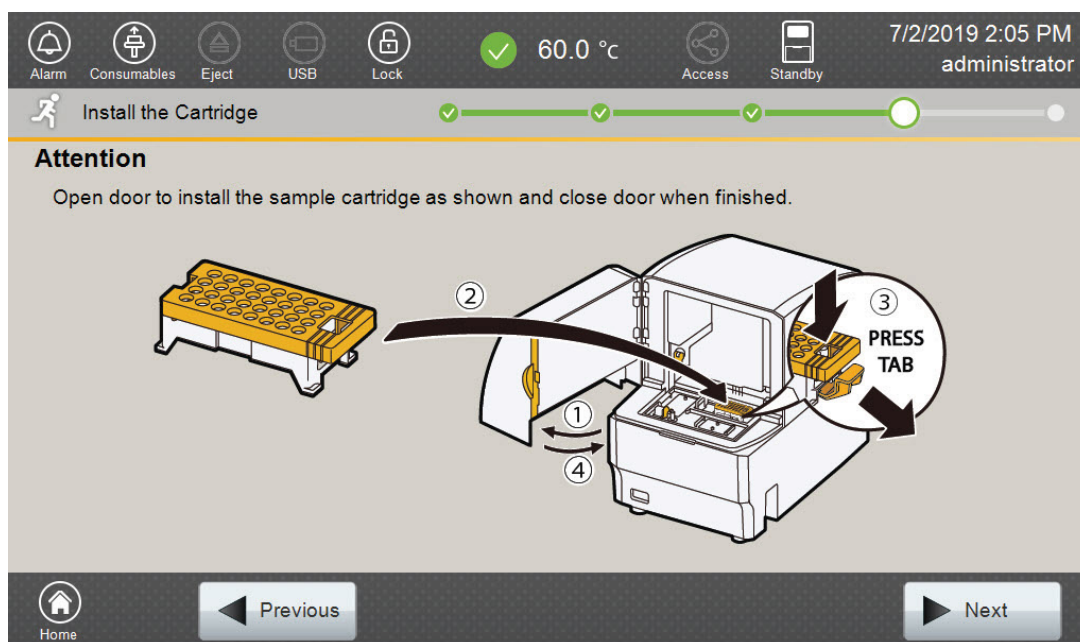
## 5.5 Loading the Sample Cartridge

1. After selecting **Next** on the 'Setup Strip Information' screen, a message window will open, indicating that the autosampler is moving and 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.

Alarm Consumables Eject USB Lock 59.9 °C Access Standby 08/24/2023 14:31 administrator

Edit Injection List

Run ID : 20230824143012\_Fragment Estimated Run Time : 198 (min)

Injection	Lane	Well	Assay	Estimated Time(min)
1	A	1-4	Promega_8Dye_CCOILS_36_P4	45
2	A	5-8	Promega_8Dye_CCOILS_36_P4	45
3	B	1-4	Promega_8Dye_CCOILS_36_P4	45
4	B	5-8	Promega_8Dye_CCOILS_36_P4	45

Order : [Up] [Down] [Duplicate] [Remove] [Edit]

Home Previous Run

**Figure 21. 'Edit Injection List' screen.**

- 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.

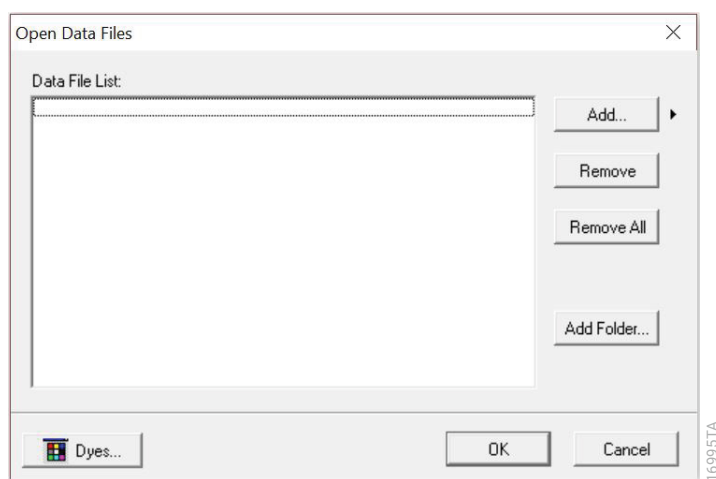
# 6 Data Analysis Using GeneMarker®HID Software

## 6.1 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*.

The current VersaPlex.xml panel files are preloaded in the software or available at: **[www.promega.com/resources/software-firmware/str-analysis/versaplex-system-software](http://www.promega.com/resources/software-firmware/str-analysis/versaplex-system-software)**

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 .fsa files.
4. Select **Open**, and the selected files will appear in the Data File List (Figure 22).



**Figure 22. The GMHID-Spectrum Data File List.**

5. Select **OK** in the 'Open Data Files' window, and the data will be imported into GMHID-Spectrum. In the 'Raw Data' folder in the File Navigator, verify that the sample types (ladder sample, positive control and negative control) assigned in Section 5 are designated correctly. If sample types are not correct, designate sample types by right-clicking on the file name and selecting **Set Sample Type**.
6. Use the Panel Editor in the Tools menu to import the VersaPlex\_31P panel by selecting **File** and then **Import Panels**. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555* for more information (Section 8.D.3).
7. Select the VersaPlex\_31P panel from the Panel Template list. Click on the plus symbol to expand the list, select a marker name, then right-click and 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 23). This will set values for peaks within the marker range. 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.

There are two options for setting stutter filters: marker-specific and allele-specific. To use marker-specific stutter filters, select **Use Marker-Specific Values**. Marker-specific stutter filters are preloaded in the VersaPlex\_31P panel file. Stutter filters for one marker can be applied to all markers in a kit by checking the box "Apply Stutter Settings to All Markers". To use allele-specific values, select the radio button next to "Use Allele-Specific Values (From Panel)" in the 'Edit Marker' window, then enter the appropriate stutter filters in the Panel Table of the 'Panel Editor' window. For more information, refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555*.

**Note:** Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

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

Edit Marker ✕

Marker Parameters:

Marker Name:

Nucleotide Repeats (x):

Boundary:  To

Min Homozygote Intensity:

< = Inconclusive <=

Min Heterozygote Intensity:

< = Inconclusive <=

Max Heterozygote Imbalance(%):

Min Heterozygote Imbalance(%):

☐ Apply Homo/Hetero Settings to All Markers

☐ Apply Homo/Hetero Settings to Markers in this dye

Stutter Filter:

☒ Use Marker-Specific Values

N - 2x  %

N + x  % N - x  %

N+0.5x  % N-0.5x  %

☐ Use Allele-Specific Values(From Panel)

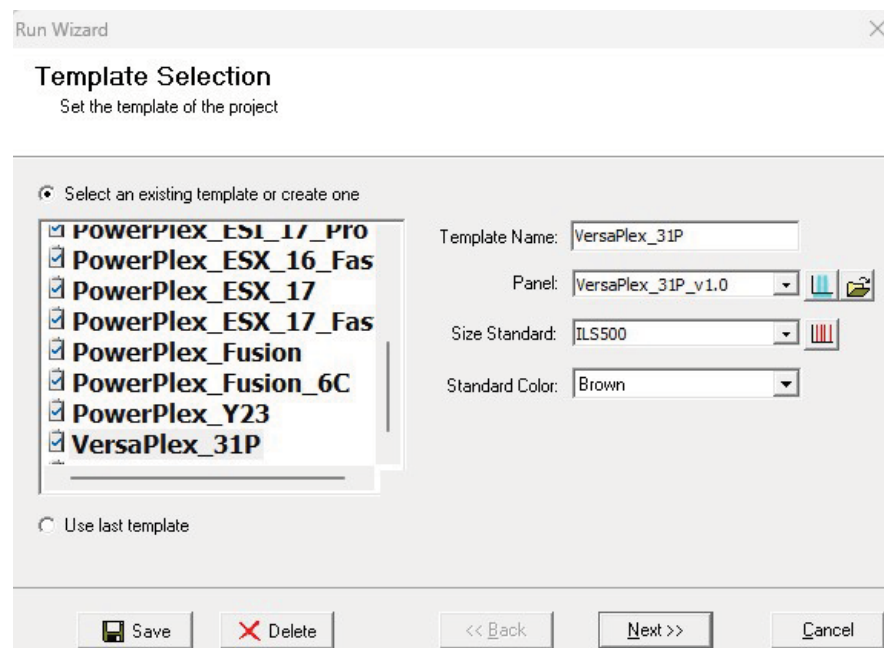
☐ Apply Stutter Settings to All Markers

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Figure 23. The 'Edit Marker' window for the D3S1358 marker.

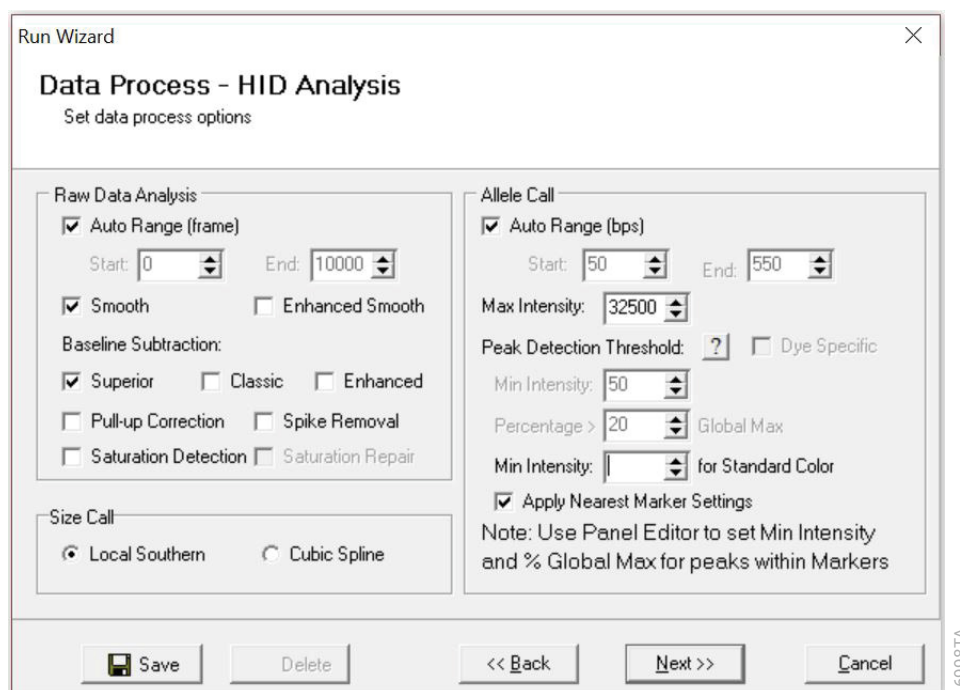
8. Choose **Run** in the Magic Wizard or select the **Run Project** icon (green arrow) in the toolbar. The 'Template Selection' screen will appear (Figure 24). Select the VersaPlex\_31P template and the settings shown in Figure 24. If the VersaPlex\_31P template is not available, check the "Select an existing template or create one" box and provide a new name. Select the VersaPlex\_31P panel that you created in Steps 6–8 from the panel drop-down field. The Size Standard must be ILS500 and the Standard Color must be Brown. Select **Save** to ensure this template will be stored among the other analysis run templates. Select **Next**.

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



**Figure 24.** The 'Template Selection' window.

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**Figure 25. The 'Data Process' window for an analysis method.**

9. The 'Data Process – HID Analysis' window will appear (Figure 25). 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 26. The 'Additional Settings' window for an analysis method.**

10. The 'Additional Settings – HID Analysis' window will appear (Figure 26). Select **31P\_2800M** as the P.C. Template 1 and verify that the boxes are checked for "Auto Select Best Ladder", "Auto Panel Adjustment" and "Sample Quality Peak(s)". If the VersaPlex 31P positive control template is not available, consult the *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555* and use the Positive Control Template Editor to set up a new template. Ensure that "QIS:QIS" is selected for the Small Allele and "QIL:QIL" for the Large Allele in the Sample Quality Peak(s) Section. 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 a second positive control and mixture evaluation, refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555*. Select **OK** to analyze the raw data.
11. When the analysis is complete, the 'Main Analysis' window will appear, displaying the processed data. We recommend that you review any yellow or red flagged peaks 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 *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555* for more information.

## 6.2 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 except for QIS and QIL.
2. Observe the results for the 2800M Control DNA. The expected 2800M allele designations for each locus are listed in Table 11 (Section 10.1).

## 6.3 Quality Indicators Small (QIS) and Large (QIL)

The VersaPlex® 31P System includes PCR primers and synthetic DNA templates used to amplify two Quality Indicators, assisting in the analysis and interpretation of STR data. The Quality Indicator Small (QIS) migrates at approximately 75bp while the Quality Indicator Large (QIL) migrates at approximately 435bp. QIS and QIL cover the lower and higher base pair range of the amplification product. While the presence or absence of QIS and QIL can be used to assess the overall success of the PCR reaction, their relative peak heights are also useful in determining sample inhibition or degradation. Table 3 provides additional information to assist with the interpretation of QIS and QIL data.

**Table 3. Interpretation of QIS and QIL Peaks.**

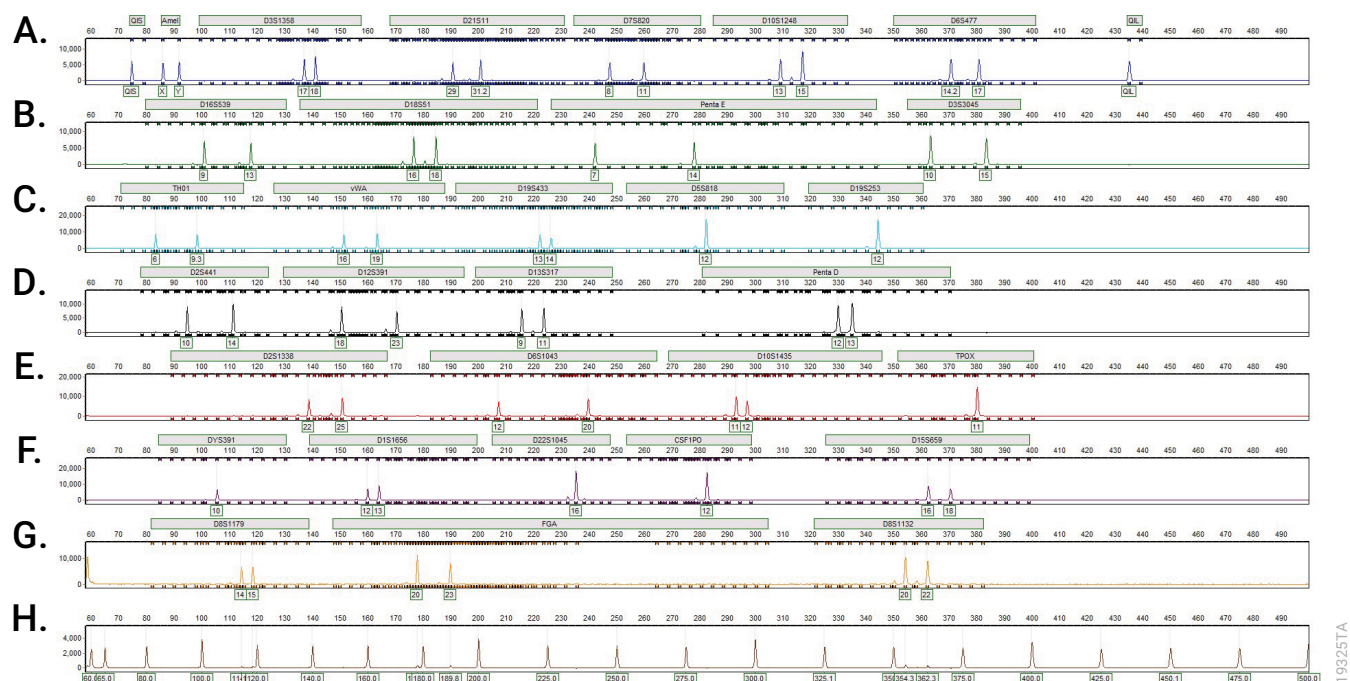
Allele Peaks	QIS Peak	QIL Peak	Amplification Interpretation
High	Present	Present	PCR successful
Absent	Present	Present	No or low DNA
Absent	Absent	Absent	PCR failure
Absent	Present	Absent	PCR inhibition
Ski-Slope	Present	Absent	PCR inhibition
Ski-Slope	Present	Present	DNA degradation

GeneMarker®HID Software for Spectrum CE Systems can automatically flag samples if the ratio of QIL/QIS falls below a user-defined threshold. This threshold can be set using the "Quality Indicator Flag 'Q'" in the 'Sample Quality' tab of the 'Preferences' window. Perform in-house validation to define the QIL/QIS threshold for each laboratory. Refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555* for more information.

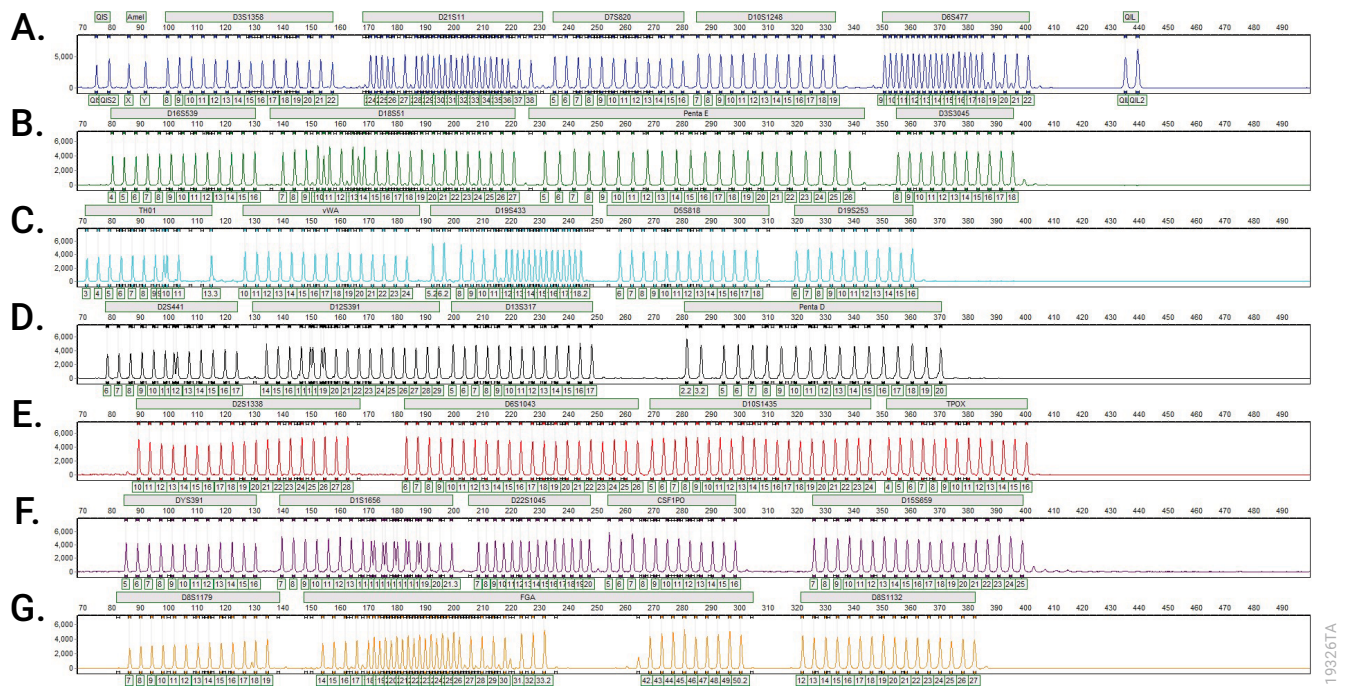
**Note:** Only one peak should be present for QIS and QIL in successful amplification reactions, even though the allelic ladder shows the presence of two alleles each. GeneMarker®HID Software for Spectrum CE Systems requires the presence of two alleles per marker in the allelic ladder for successful analysis.

# 7 Results

Representative results of the VersaPlex® 31P System are shown in Figure 27. The VersaPlex® 31P Allelic Ladder Mix is shown in Figure 28.



**Figure 27. The VersaPlex® 31P System.** The 2800M Control DNA (0.5ng) was amplified using the VersaPlex® 31P System. Amplification products were mixed with CCO Internal Lane Standard 500 and analyzed with a Spectrum Compact CE System and a 1.6kV, 9-second injection. Results were analyzed using GeneMarker®HID Software for Spectrum CE Systems, version 3.2.0 and VersaPlex® 31P panel file. **Panel A.** The peaks of the FL-8C-labeled loci: Q1S, Amelogenin, D3S1358, D21S11, D7S820, D10S1248, D6S477 and Q1L. **Panel B.** The peaks of the JOE-8C-labeled loci: D16S539, D18S51, Penta E and D3S3045. **Panel C.** The peaks of the AQA-8C-labeled loci: TH01, vWA, D19S433 D5S818 and D19S253. **Panel D.** The peaks of the TMR-8C-labeled loci: D2S441, D12S391, D13S317 and Penta D. **Panel E.** The peaks of the CXR-8C-labeled loci: D2S1338, D6S1043, D10S1435 and TPOX. **Panel F.** The peaks of the TOM-8C-labeled loci: DYS391, D1S1656, D22S1045, CSF1PO and D15S659. **Panel G.** The peaks of the WEN-8C-labeled loci: D8S1179, FGA and D8S1132. **Panel H.** The 60bp to 500bp fragments of the CCO Internal Lane Standard 500.



**Figure 28. The VersaPlex® 31P Allelic Ladder Mix.** The VersaPlex® 31P Allelic Ladder Mix was analyzed with a Spectrum CE Compact System and a 1.6kV, 9-second injection. The sample file was analyzed with the GeneMarker®HID Software for Spectrum CE Systems, version 3.2.0, and VersaPlex® 31P panel file. **Panel A.** The FL-8C-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-8C-labeled allelic ladder components and their allele designations. **Panel C.** The AQA-8C-labeled allelic ladder components and their allele designations. **Panel D.** The TMR-8C-labeled allelic ladder components and their allele designations. **Panel E.** The CXR-8C-labeled allelic ladder components and their allele designations. **Panel F.** The TOM-8C-labeled allelic ladder components and their allele designations. **Panel G.** The WEN-8C-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. Trinucleotide repeat loci, like D22S1045, will have more pronounced stutter in both  $n-3$  and  $n+3$  positions than a typical tetranucleotide repeat locus. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

The mean stutter plus three standard deviations observed at each locus is used in the VersaPlex\_31P panel file for marker-specific filtering.

In addition to stutter peaks, you may observe the following low-level DNA-dependent artifact peaks (Table 4) and DNA-independent (with or without human genomic DNA) artifact peaks (Table 5) with the VersaPlex® 31P System.

**Table 4. DNA-Dependent Artifacts Observed in Amplification Reactions with Human Genomic DNA.**

Locus	Artifact Size
Q1S/Q1L	$n-1$ , $n+1$
Amelogenin	$n-1$ , $n+1$
D18S51	$n-2$
vWA	$n-1$
D19S433	$n-2$
D2S441	$n-2$
D1S1656	$n-2$
FGA	$n-2$

**Table 5. DNA-Independent Artifacts Observed in Amplification Reactions with and without Human Genomic DNA.**

Dye Label	Artifact Size <sup>1</sup>
FL-8C	~58–74 bases
JOE-8C	~70–76 bases
AQA-8C	~58–65 bases ~360–370 bases <sup>2</sup>
TMR-8C	~58–70 bases ~59–61 bases <sup>2</sup>
CXR-8C	~418–421 bases <sup>2</sup>
TOM-8C	~58–78 bases
WEN-8C	~58–83 bases ~86–88 bases

<sup>1</sup>Artifact sizes may vary, depending on CE instrumentation and environmental conditions in the laboratory.

<sup>2</sup>These artifacts can be observed during amplifications where the annealing step occurs below the recommended temperature (61°C).

A variety of nonhuman DNA templates from bacteria, yeast and mammals were tested to characterize known artifacts with the VersaPlex® 31P System. The artifacts listed in Table 6 were noted above a 150RFU threshold with 5ng of template DNA using the Spectrum CE System.

**Table 6. Nonhuman DNA Cross-Reactivity.**

DNA Source	Artifact Size	Dye Label
Pig	~370–372 bases	JOE-8C
	~372–374 bases	TMR-8C
Skin Microbiome Genomic Mix (ATCC)	~290–292 bases	CXR-8C
Horse	~460–462 bases	TOM-8C
Mouse	~87–89 bases	CXR-8C

# 8 Troubleshooting

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

## 8.1 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 thermal 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.
	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°C to +10°C. Vortex before use.

Symptoms	Causes and Comments
Faint or absent allele peaks for the positive control reaction (continued)	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). Reinject the sample.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
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.
	Some artifacts noted in Table 5 (Section 7, Results) may occur when the annealing temperature is below optimal temperature. Ensure the thermal cycler is calibrated and the proper cycling protocol is used.
	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 reannealing.
	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. Reinject samples to confirm. Ensure no urea crystals are present in the polymer cartridge before installing on the CE.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	<p>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.</p> <ul style="list-style-type: none"> <li>Perform a new spectral calibration and rerun the samples.</li> <li>Confirm that the correct spectral was used for the sample run.</li> <li>Instrument sensitivities can vary. Optimize the injection conditions. Refer to the instrument user manual.</li> </ul>
	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.
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 necessary.
Peak height imbalance	<p>Miscellaneous balance problems. 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.</p>
	PCR amplification mix was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.

## 8.2 Amplifying Extracted DNA

The following information is specific to amplifying purified DNA. For information about general amplification and detection, see Section 8.1.

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 20 $\mu$ g/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25 $\mu$ l 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	<p>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 Section 7, Results, for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.</p>
	<p>Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete adenylation.</p> <ul style="list-style-type: none"> <li>• Be sure to perform the recommended extension step at 60°C after thermal cycling.</li> <li>• Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation.</li> <li>• Decrease cycle number.</li> <li>• Increase the final extension time.</li> </ul>
Peak height imbalance	<p>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.</p>
	<p>Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.</p>
	<p>Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.</p>
	<p>Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.</p>
	<p>Imbalance may be seen more often when using the maximum template volume or a reduced amplification reaction volume.</p>
	<p>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.</p>

## 8.3 GeneMarker®HID Software for Spectrum CE Systems

Symptoms	Causes and Comments
Stutter peaks not filtered	Be sure the 'Use Marker-Specific Values' box in the 'Panel Editor' is checked. Alternatively, select the 'Use Allele-Specific Values (From Panel)' box in the 'Edit Marker' window and enter the appropriate stutter filters in the Panel Table of the 'Panel Editor' window.
Alleles not called	To analyze samples with GMHID-Spectrum, at least one allelic ladder must be present in the data set. Ensure that the allelic ladder is designated as such in Section 5.3 or 6.1.
	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. <ul style="list-style-type: none"> <li>Create a new size standard using the internal lane standard fragments present in the sample.</li> <li>Rerun samples using a longer run time.</li> </ul>
	A low-quality allelic ladder was used during analysis. Ensure that only high- quality allelic ladders are used for analysis. See the 'Off-ladder or off-bin alleles' column below for more information.
Off-ladder or off-bin 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 panel file selected for analysis was incorrect for the STR system used. Assign a correct panel file that corresponds to the STR system used for amplification.
	The allelic ladder was not designated as an allelic ladder in Section 5.3 or 6.1.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.

Symptoms	Causes and Comments
Off-ladder or off-bin alleles (continued)	<p>A low-quality allelic ladder was used during analysis. Ensure that only high- quality allelic ladders are used for analysis.</p> <p>Allelic ladders are flagged for Analysis Quality when:</p> <ul style="list-style-type: none"> <li>• There is an error in bin shifting.</li> <li>• Peaks in virtual bins have greater than expected peak heights.</li> <li>• The expected peaks cannot be identified in a marker.</li> </ul> <p>When expected peaks cannot be identified in a marker, the marker bar will be shaded dark green, indicating the marker failed analysis.</p> <p>When errors in bin shifting occur or when peaks in virtual bins have higher than expected peak heights, the marker bars will be outlined in red. Any peaks that cause an 'Analysis Quality' flag are indicated in the electropherogram and Peak Table.</p> <p>When Flag Variant Alleles in Ladder is selected in the 'Display' tab of the 'Preferences' window, peaks in virtual bins with higher than expected peak heights are indicated by yellow vertical bars and green allele labels. You can set your preferences to automatically delete these peaks by selecting Auto-Delete Alleles in Virtual Bins in Allelic Ladder in the 'Forensics' tab of the 'Preferences' window.</p> <p>Peaks that are not present in the expected bins are marked with red vertical bars and red allele labels.</p>
Size standard not called or poor quality	<p>If a partial range was chosen for the Allele Call analysis in the Data Process - HID Analysis window of the Run Wizard, the chosen starting or end point was incorrect. Adjust the starting point or end point or use the Auto Range setting for the analysis.</p> <p>The incorrect Standard Color was selected in the Template Selection window of the Run Wizard. The Standard Color should be Brown.</p>

Symptoms	Causes and Comments
Size standard not called or poor quality (continued)	<p>If the quality of the size standard peaks is not sufficient (and the 'Low ILS Quality Flag 'SQ' section in the 'Sample Quality' tab of the 'Preferences' window is selected), the size standard is marked with a red 'SQ' flag in the Sample File Tree of the 'Main Analysis' window and in the upper left corner of the sample electropherogram. There are three criteria that determine whether a size standard fails the size quality requirements:</p> <ul style="list-style-type: none"> <li>The sample size standard does not contain all peaks that are marked as "Enabled" in the chosen size standard template.</li> <li>Not all of the expected size standard peaks are called in the 'Main Analysis' window. If the expected size standard peaks are present but are not labeled, open the Calibration Charts and select the affected sample from the Sample List. Edit the size calls of the sample size standard to remove the extra peak(s) and define the correct peak(s). Right-click anywhere in the Sample Size Standard Chart and select <b>Update Calibration</b>.</li> <li>Not all of the expected size standard peaks are of high quality such that the absolute difference in size between the actual size standard peaks and the expected, Enabled size standard peaks is less than 0.2bp.</li> </ul>
	An incorrect size standard was used.
	<p>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.</p> <ul style="list-style-type: none"> <li>Create a new size standard using the internal lane standard fragments present in the sample.</li> <li>Rerun samples using a longer run time.</li> </ul>
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 rerun the samples.
	Confirm that the correct spectral was used to run the samples.

# 9

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# 10 Appendix

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## 10.1 Advantages of Using the Loci in the VersaPlex® 31P System

A single VersaPlex® 31P System reaction amplifies all the 20 CODIS core loci, Penta D, Penta E, and D6S1043 and six novel autosomal loci (D6S477, D3S3045, D19S253, D10S1435, D15S659, and D8S1132) (Table 7 and Table 8). The male-specific DYS391 locus is included to identify null Y results for Amelogenin. Table 9 lists the VersaPlex® 31P System alleles amplified from the 2800M standard 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 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 (18) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

**Table 7. The VersaPlex® 31P System Locus-Specific Information.**

<b>STR Locus</b>	<b>Label</b>	<b>Chromosomal Location<sup>1</sup></b>	<b>Repeat Sequence<sup>2</sup> 5'→3'</b>
Amelogenin <sup>3</sup>	FL-8C	Xp22.1–22.3 and Y	NA
D3S1358	FL-8C	3p21.31 (45.557Mb)	TCTA Complex
D21S11	FL-8C	21q21.1 (19.476Mb)	TCTA Complex (20)
D7S820	FL-8C	7q21.11 (83.433Mb)	GATA
D10S1248	FL-8C	10q26.3 (130.567Mb)	GGAA
D6S477	FL-8C	6p25.1 (6.14Mb)	TCTA Complex
D16S539	JOE-8C	16q24.1 (84.944Mb)	GATA
D18S51	JOE-8C	18q21.33 (59.1Mb)	AGAA (20)
Penta E	JOE-8C	15q26.2 (95.175Mb)	AAAGA
D3S3045	JOE-8C	3q13.12 (107.27Mb)	AGAT Complex
TH01	AQA-8C	11p15.5 (2.149Mb)	AATG (20)
vWA	AQA-8C	12p13.31 (5.963Mb)	TCTA Complex (20)
D19S433	AQA-8C	19q12 (35.109Mb)	AAGG Complex
D5S818	AQA-8C	5q23.2 (123.139Mb)	AGAT
D19S253	AQA-8C	19p13.12 (15.62Mb)	ATCT
D2S441	TMR-8C	2p14 (68.214Mb)	TCTA
D12S391	TMR-8C	12p12 (12.341Mb)	AGAT/AGAC Complex
D13S317	TMR-8C	13q31.1 (81.62Mb)	TATC
Penta D	TMR-8C	21q22.3 (43.88Mb)	AAAGA
D2S1338	CXR-8C	2q35 (218.705Mb)	TGCC/TTCC
D6S1043	CXR-8C	6q15 (92.449Mb)	AGAT
D10S1435	CXR-8C	10p15.3 (2.20Mb)	TATC
TPOX	CXR-8C	2p25.3 (1.472Mb)	AATG
DYS391	TOM-8C	Y	TCTA
D1S1656	TOM-8C	1q42 (228.972Mb)	TAGA Complex
D22S1045	TOM-8C	22q12.3 (35.779Mb)	ATT
CSF1PO	TOM-8C	5q33.1 (149.436Mb)	AGAT
D15S659	TOM-8C	15q21.1 (46.08Mb)	TATC

STR Locus	Label	Chromosomal Location <sup>1</sup>	Repeat Sequence <sup>2</sup> 5'→3'
D8S1179	WEN-8C	8q24.13 (125.976Mb)	TCTA Complex (20)
FGA	WEN-8C	4q28 (155.866Mb)	TTTC Complex (20)
D8S1132	WEN-8C	8q23.1 (106.32Mb)	TCTA Complex

<sup>1</sup>Find information about chromosomal location of these loci in references 19, 20 and 21 and at:

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

<sup>2</sup>The August 1997 report (22,23) 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."

<sup>3</sup>Amelogenin is not an STR.

NA = Not Applicable

**Table 8. The VersaPlex® 31P System Allelic Ladder Information.**

STR Locus	Label	Size Range of Allelic Ladder Components <sup>1,2</sup> (bases)	Repeat Numbers of Allelic Ladder Components <sup>3</sup>
QIS	FL-8C	75, 79	QIS, QIS2
Amelogenin	FL-8C	86, 92	X, Y
D3S1358	FL-8C	100–158	8–22
D21S11	FL-8C	171–227	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	FL-8C	236–281	5–16
D10S1248	FL-8C	286–333	7–19
D6S477	FL-8C	351–401	9.2, 10, 10.2, 11, 11.2, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18–22
QIL	FL-8C	436, 440	QIL, QIL2
D16S539	JOE-8C	81–131	4–16
D18S51	JOE-8C	141–222	7–10, 10.2, 11–13, 13.2, 14–27
Penta E	JOE-8C	232–339	5–26
D3S3045	JOE-8C	356–396	8–18
TH01	AQA-8C	72–115	3–9, 9.3, 10–11, 13.3
vWA	AQA-8C	127–184	10–24
D19S433	AQA-8C	193–244	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
D5S818	AQA-8C	258–306	6–18
D19S253	AQA-8C	320–361	6–16
D2S441	TMR-8C	79–124	6–11, 11.3, 12–17

STR Locus	Label	Size Range of Allelic Ladder Components <sup>1,2</sup> (bases)	Repeat Numbers of Allelic Ladder Components <sup>3</sup>
D12S391	TMR-8C	134–195	14–17, 17.3, 18, 18.3, 19–29
D13S317	TMR-8C	200–248	5–17
Penta D	TMR-8C	282–371	2.2, 3.2, 5–20
D2S1338	CXR-8C	90–163	10–28
D6S1043	CXR-8C	183–265	6–26
D10S1435	CXR-8C	269–346	5–24
TPOX	CXR-8C	352–400	4–16
DYS391	TOM-8C	86–131	5–16
D1S1656	TOM-8C	140–200	7–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3, 21.3
D22S1045	TOM-8C	209–248	7–20
CSF1PO	TOM-8C	254–298	5–16
D15S659	TOM-8C	327–400	7–25
D8S1179	WEN-8C	86–135	7–19
FGA	WEN-8C	154–301	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, 47.2, 48.2, 49.2, 50.2
D8S1132	WEN-8C	322–382	12–27

<sup>1</sup>The length of each allele in the allelic ladder has been confirmed by sequence analyses.

<sup>2</sup>When using an internal lane standard, such as the CCO 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.

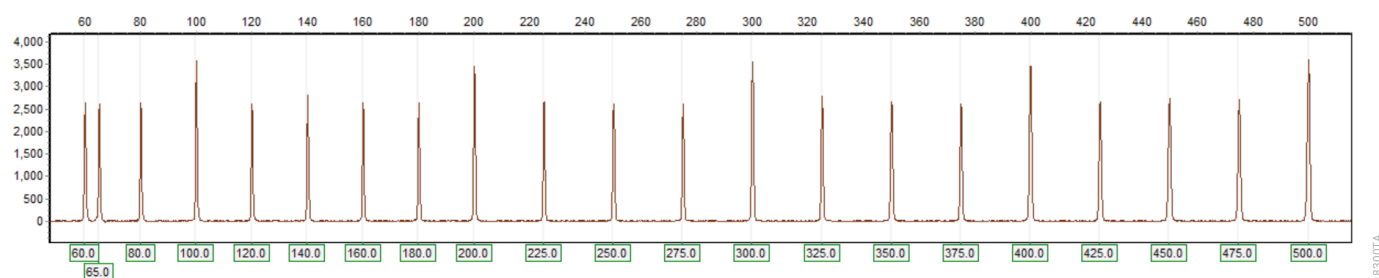
<sup>3</sup>For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: [https://strbase.nist.gov/var\\_tab.htm](https://strbase.nist.gov/var_tab.htm)

**Table 9. The VersaPlex® 31P System Allele Determinations for the 2800M Control DNA.**

<b>STR Locus</b>	<b>2800M</b>
Amelogenin	X, Y
D3S1358	17, 18
D21S11	29, 31.2
D7S820	8, 11
D10S1248	13, 15
D6S477	14.2, 17
D16S539	9, 13
D18S51	16, 18
Penta E	7, 14
D3S3045	10, 15
TH01	6, 9.3
vWA	16, 19
D19S433	13, 14
D5S818	12
D19S253	12
D2S441	10, 14
D12S391	18, 23
D13S317	9, 11
Penta D	12, 13
D2S1338	22, 25
D6S1043	12, 20
D10S1435	11, 12
TPOX	11
DYS391	10
D1S1656	12, 13
D22S1045	16
CSF1PO	12
D15S659	16, 18
D8S1179	14, 15
FGA	20, 23
D8S1132	20, 22

## 10.2 The CCO Internal Lane Standard 500

The CCO 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 29). Each fragment is labeled with CCO-8C dye and can be detected separately (as an eighth color) in the presence of VersaPlex® 31P-amplified material. The CCO ILS 500 is designed for use in each CE injection to increase precision in analysis when using the VersaPlex® 31P System. Section 5 provides protocols to prepare and use this internal lane standard.



**Figure 29. CCO Internal Lane Standard 500.** An electropherogram showing the CCO Internal Lane Standard 500 fragments.

## 10.3 Composition of Buffers and Solutions

### TE<sup>-4</sup> buffer (10mM Tris-HCl, 0.1mM EDTA [pH 8.0])

1.21g      Tris base  
0.037g      EDTA (Na<sub>2</sub>EDTA • 2H<sub>2</sub>O)

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<sup>-4</sup> buffer with 20µg/ml glycogen

1.21g      Tris base  
0.037g      EDTA (Na<sub>2</sub>EDTA • 2H<sub>2</sub>O)  
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.

## 10.4 Related Products

PRODUCT	SIZE	CAT.#
VersaPlex® 8C Matrix Standard	5 preps	DG5980
CCO Internal Lane Standard 500	250µl	DG8571
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
	4 × 96 wells	CE2404
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.

<sup>(a)</sup>U.S. Pat. No. 9,139,868, European Pat. No. 2972229, Japanese Pat. No. 6367307 and other patents pending.

<sup>(b)</sup>AQA-8C, TMR-8C, CXR-8C, TOM-8C, WEN-8C and CCO-8C dyes are proprietary.

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