VersaPlex® 31P System for Use on the Spectrum CE System Technical Manual

Instructions for Use of Product DC8020

TMD083 | 7/25

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All technical literature is available at: **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**

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 can 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[a,b] is a 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 CE System. Amplification and detection instrumentation may vary. Protocols may need to be optimized for template DNA amount, cycle number, loading volume and injection conditions for your laboratory instrumentation. Perform in-house validation. A protocol to operate the Spectrum CE System is available separately. See the *Spectrum CE System Operating Manual* #TMD052.

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

2 Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
VersaPlex® 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

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

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 (e.g., 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 CE System. Generate a matrix 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 CE System Technical Manual* #TMD082. This manual is available online at: **www.promega.com/protocols/**

4 DNA Amplification Protocol Using the VersaPlex® 31P System

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: Thermal cyclers other than the ones listed in this technical manual can be used. Using thermal cyclers not listed here requires 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 there is 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 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® 31P 5X Master Mix	5µl	×		=	
VersaPlex® 31P 5X Primer Pair Mix	5µl	×		=	
template DNA (0.5ng)[2,3]	up to 15µl				
total reaction volume	**25µl**				

[1]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.

[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 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.

[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 immediately start 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 DNA template amount used should be based on internal validation results and may differ.

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^{-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 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 can vary. You may need to optimize protocols for the template DNA amount, cycle number, injection conditions and loading volume for your laboratory instrumentation. Our tests have shown 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 protocol provided below and in Figure 1. The total cycling time is approximately 75 minutes.

   Note: Set the ramp speed to 6°C/second 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 hold

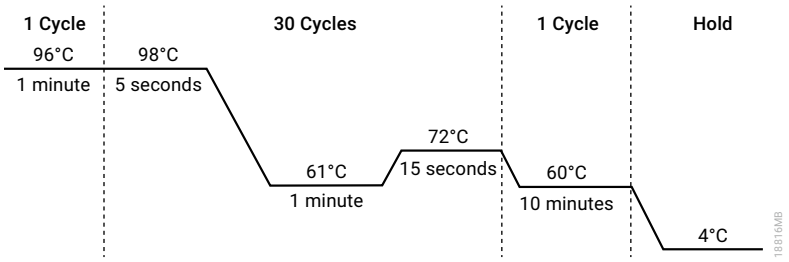

Figure 1. The thermal cycling protocol for the ProFlex® PCR System.

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 Detecting Amplified Fragments Using the Spectrum CE System

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 96-well plates
- aerosol-resistant pipette tips
- Spectrum Capillary Array, 8-capillary (Cat.# CE2008)
- Spectrum Polymer4, 384 Wells (Cat.# CE2048) or Spectrum Polymer4, 960 Wells (Cat.# CE2040)
- Spectrum Buffer (Cat.# CE2001)
- Spectrum Cathode Septa Mat (Cat.# CE2002)
- Septa Mat, 96-Well (Cat.# CE2696) or equivalent Applied Biosystems septa mat
- Spectrum Plate Base & Retainer, 96-Well (Cat.# CE5004)
- MicroAmp® optical 96-well plate (or equivalent; Applied Biosystems)
- 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 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.

Note: Wear gloves when handling consumables.

5.1 Preparing Samples

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: Adjust the internal lane standard volume in the loading cocktail to change the size standard peak intensity 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 96-well plate.
4. Add 1µl of amplified sample (or 1µl of Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

   Notes:

   a. Instrument detection limits vary so you may need to increase or decrease the injection time or the sample amount mixed with the loading cocktail. To modify the injection time, refer to the *Spectrum CE System Operating Manual* #TMD052. 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.

   b. 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.

   c. Include an injection of allelic ladder every 2 injections (16 samples).

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

5.2 Preparing the Instrument

Refer to the *Spectrum CE System Operating Manual* #TMD052 for the instrument maintenance schedule and instructions to install the capillary array, anode buffer, cathode buffer and polymer bottle. Perform and activate the appropriate spectral calibration on the instrument before using it for samples. For instructions on performing a spectral calibration, see the *VersaPlex® Matrix Standards for Use on the Spectrum CE System Technical Manual* #TMD082.

Open the Spectrum Control Software (SCS). The 'Home' screen appears upon launch (Figure 2), providing access to four workflow menus (Home, Review Plates, Maintenance and Settings; see Figure 3) and status indicators. The screen is divided into three sections: Header, Task Pane and Status Bar.

Figure 2. Spectrum Control Software 'Home' screen.

The Header (Figure 3) is fixed and remains available to the user throughout all navigation processes. This area contains Status Indicators, Message Center, Start Button, Run Time Indicator and Workflow Menu. The Header also shows the status of the plate positions within the Plate Drawer.

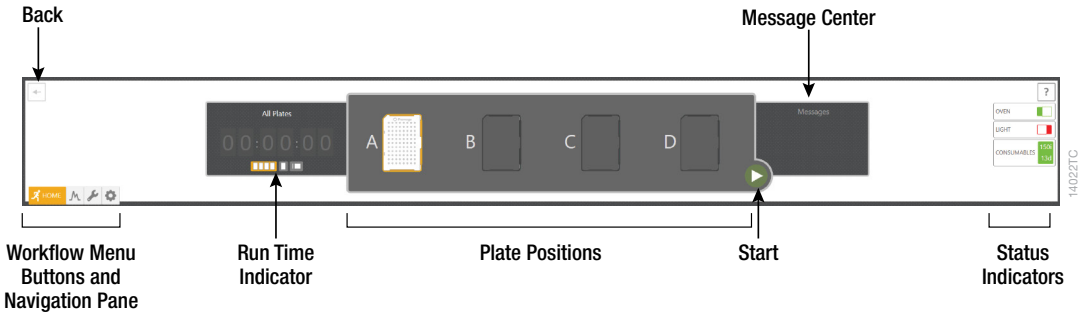

Figure 3. Header.

Each button in the Workflow Menu provides access to a list of workflow-specific functions. Workflow Buttons and the Navigation Pane are highlighted to indicate the user's current location within the SCS.

Icon	Button Name	Function
	Back	Navigates to the previous screen
	Start/stop	Starts or stops all currently scheduled plates (enabled when plates are linked for run and sufficient consumables are available)
	Help	Opens PDF of Spectrum CE System Operating Manual
	Home	Displays Home Menu
	Review Plates	Displays Review Plates Menu
	Maintenance	Displays Maintenance Menu
	Settings	Displays Settings Menu

The SCS contains several indicators in the header. Each indicator provides information about a specific function or component. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information.

Before starting a run, ensure all consumables are installed and in sufficient supply. For best results, use unexpired reagents that are within the recommended use range. Refer to the 'Consumables' screen (Figure 4) to determine if any consumables need to be replaced. To access the 'Consumables' screen, select the Consumables status indicator in the Header on the SCS screen (Figure 3). The 'Consumables' screen (Figure 4) displays information for the four RFID-tagged consumables on the instrument: Polymer, Capillary Array and Anode and Cathode Buffers.

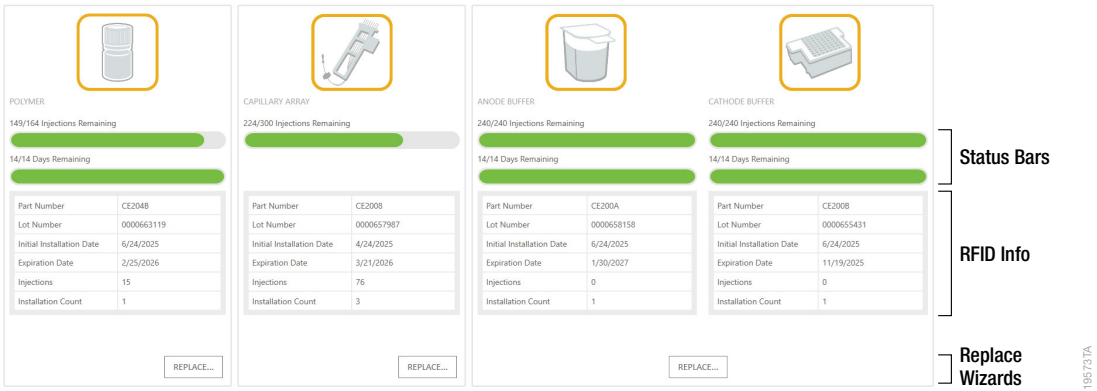

Figure 4. 'Consumables' screen.

The following information is tracked through these tags:

- Part Number
- Lot Number
- Initial Installation Date
- Expiration Date
- Injections
- Installation Count

Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details and information on installing consumables.

Select **Oven Heater Indicator** in the Header (Figure 3) to start preheating the oven to 60°C. The oven temperature will be displayed in the status bar at the bottom of the screen and the indicator will change from red to green when the oven preheating is complete.

Note: We recommend preheating 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. Inspect the fluid block, tubing, polymer syringe and polymer bottle for bubbles, and perform a bubble purge if necessary.

5.3 Creating a Plate Record

On the 'Home' screen, there are three options displayed for plate setup: New Plate, Import Plate and Draft Plates (Figure 2).

Create a plate record for each plate run. This record defines the Sample IDs of each sample on a plate, the protocol to apply to each injection and other user-defined items. There are four main methods for preparing a new plate record:

- Manually entering a new plate record
- Manually importing a plate record document
- Automatically importing a plate record document through the barcoding process
- Duplicating information from a completed plate

The following instructions are for manually entering a new plate record. For the other methods, refer to the *Spectrum CE System Operating Manual* #TMD052.

1. Select **New Plate** from the 'Home' screen. This will open a blank 'Plate' screen that is divided into three sections: Plate Information, Sample Information and Injection Information (Figure 5).

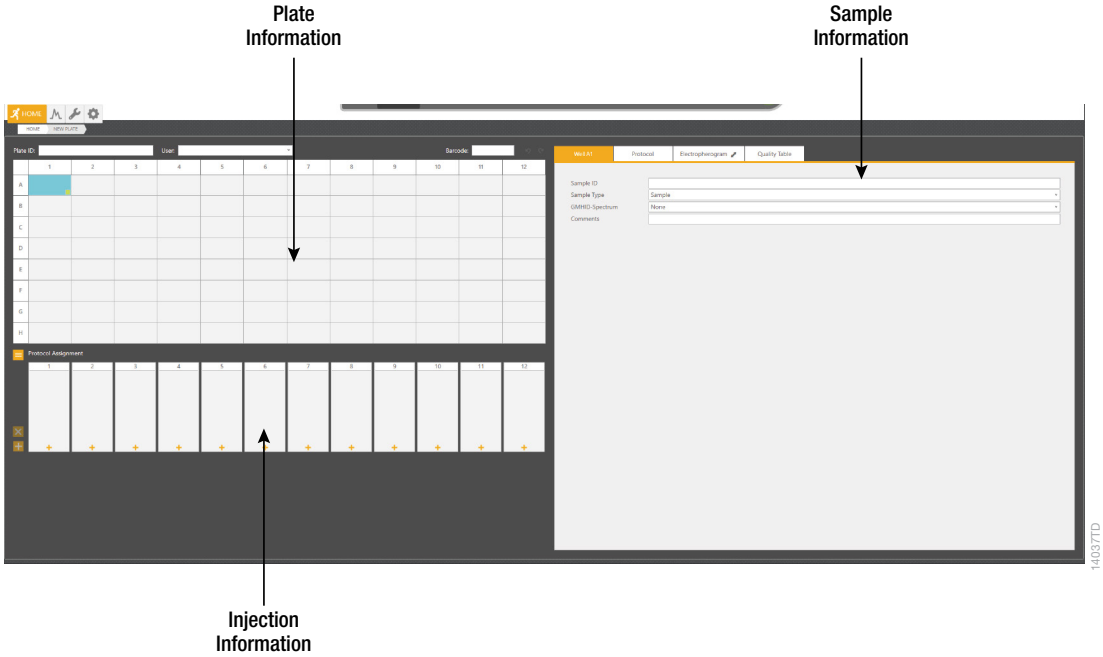

Figure 5. 'Plate' screen.

2. Use the default Plate ID that includes date and time or enter information into the 'Plate ID' field (Figure 6).
3. If desired, enter user information into the 'User' field (Figure 6).

   Note: The 'User' field is a user-defined section that can be used to search or filter previously created or completed plates (refer to the *Spectrum CE System Operating Manual* #TMD052 for information on reviewing plate records). Previously entered user information is available via the drop-down option on the right side of the field (Figure 6).

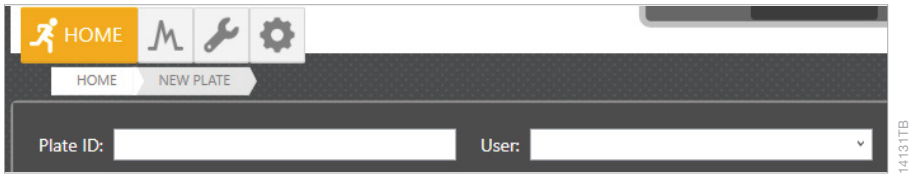

Figure 6. 'Plate ID' and 'User' fields of the plate screen.

4. Enter Sample IDs (sample names) for each sample on the plate. Enter a Sample ID directly in the cells of the plate map area of the Plate Information section by selecting the appropriate well(s) and entering the ID information. Right clicking a sample well provides editing options: Cut, Copy, Paste and Clear. There are also Undo and Redo shortcut buttons to the right of the bar code field.

   Alternatively, enter Sample IDs in the 'Sample ID' field of the Sample Information section (Figure 5).

   The green box in the lower right corner of a sample well (Figure 7) can be used to fill other wells with the same information by left clicking and dragging the green box to the other wells.

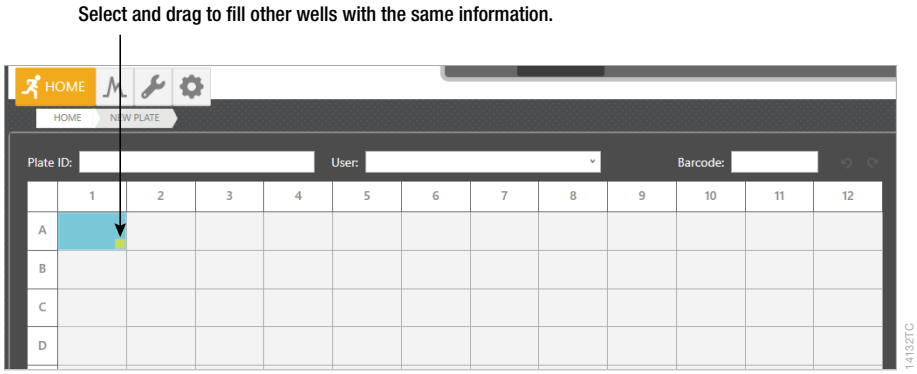

Figure 7. Filling information across multiple sample wells.

5. Select sample types for each sample or group of samples on the plate. Select the sample(s) then use the drop-down menu (Figure 8) to designate the well(s) as Sample, Ladder, Positive Control or Negative Control. These designations will be included in the output file for downstream analysis.

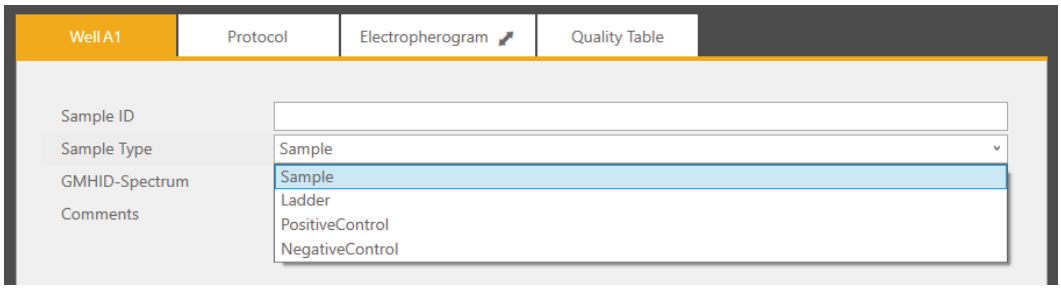

Figure 8. Drop-down options for sample type.

6. **Optional:** Select **VersaPlex_31P** in the GMHID-Spectrum drop-down menu (Figure 9).

   Notes:

   a. The appropriate GMHID-Spectrum Run Wizard template must be selected for use of the Quality Table. These templates are either preloaded or can be edited within the Spectrum CE System workstation's copy of GMHID-Spectrum. Select the appropriate sample type for each well to ensure complete GMHID-Spectrum analysis functionality. Refer to the *Spectrum CE System Operating Manual* #TMD052 and *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

   b. Selecting the GMHID-Spectrum template does not affect downstream analysis.

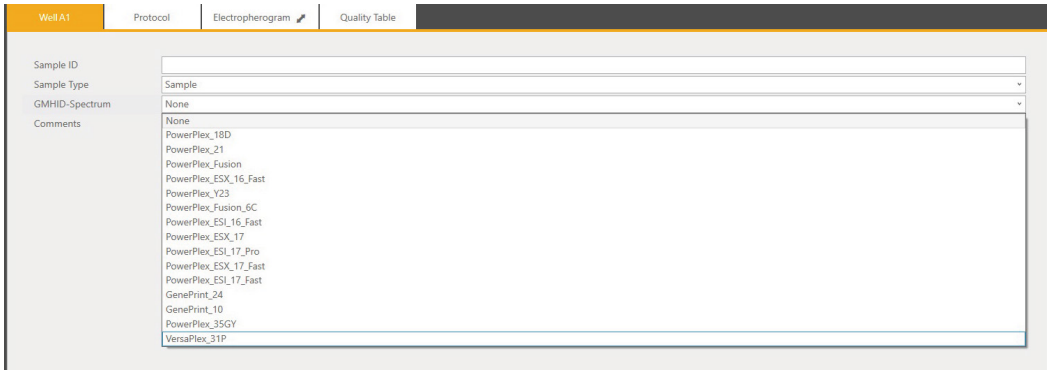

Figure 9. Drop-down options for GeneMarker®HID Software for Spectrum CE Systems run wizard templates.

7. When a run protocol has been assigned to an injection set, an injection number will be assigned and displayed in a circle within the injection set box. There are three methods to assign run protocol(s) to samples:

   - To add a run protocol to selected samples, highlight the desired wells (Ctrl + right-click or drag with the mouse). Then right-click, mouse over **Add Injection To Selected Wells** then select the **Promega 8-Dye (8C)** protocol from the pop-out menu.
   - To add a run protocol to individual injections, select the **+** button under the box for that injection set then select the **Promega 8-Dye (8C)** protocol from the pop-out menu.
   - To add a run protocol to multiple injections, highlight the desired injections in the plate map (click-drag, Ctrl + left-click or click the upper left cell). Then select the **+** button on the left side of the injection information section and select **Promega 8-Dye (8C)** (Figure 10) from the pop-out menu.

Notes:

a. A sample ID must be present in at least one well of an injection set for a run protocol to be assigned to the set.

b. The Spectrum Control Software includes preloaded run protocols for use with Promega chemistries. Refer to the *Spectrum CE System Operating Manual* #TMD052 for instructions on creating a new protocol or modifying an existing protocol.

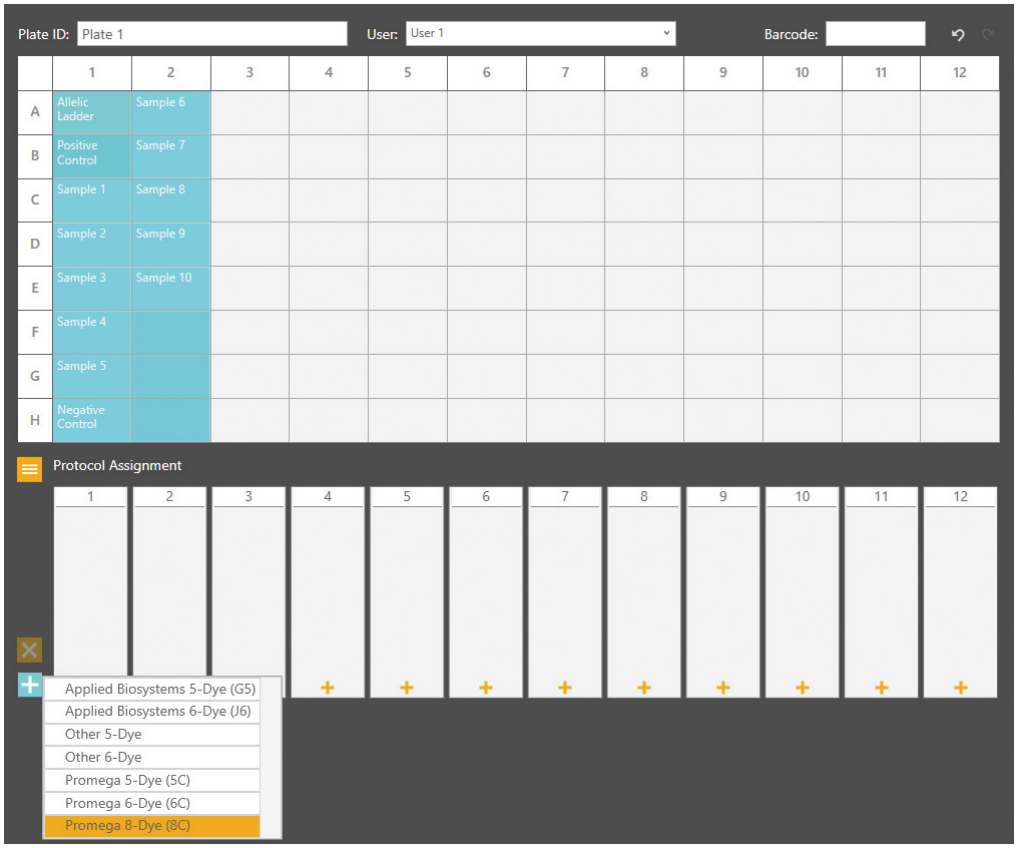

Figure 10. Assigning run protocol to multiple injections.

8. Verify the assigned run protocol(s).

   To verify the parameters of the assigned run protocol(s), select the injection number or a sample well in the plate map then select the 'Protocol' tab of the Sample Information section (Figure 11), which now displays the name of the assigned protocol. The information in this tab is read-only.

   Note: Refer to the *Spectrum CE System Operating Manual* #TMD052 for instructions on creating or modifying protocols.

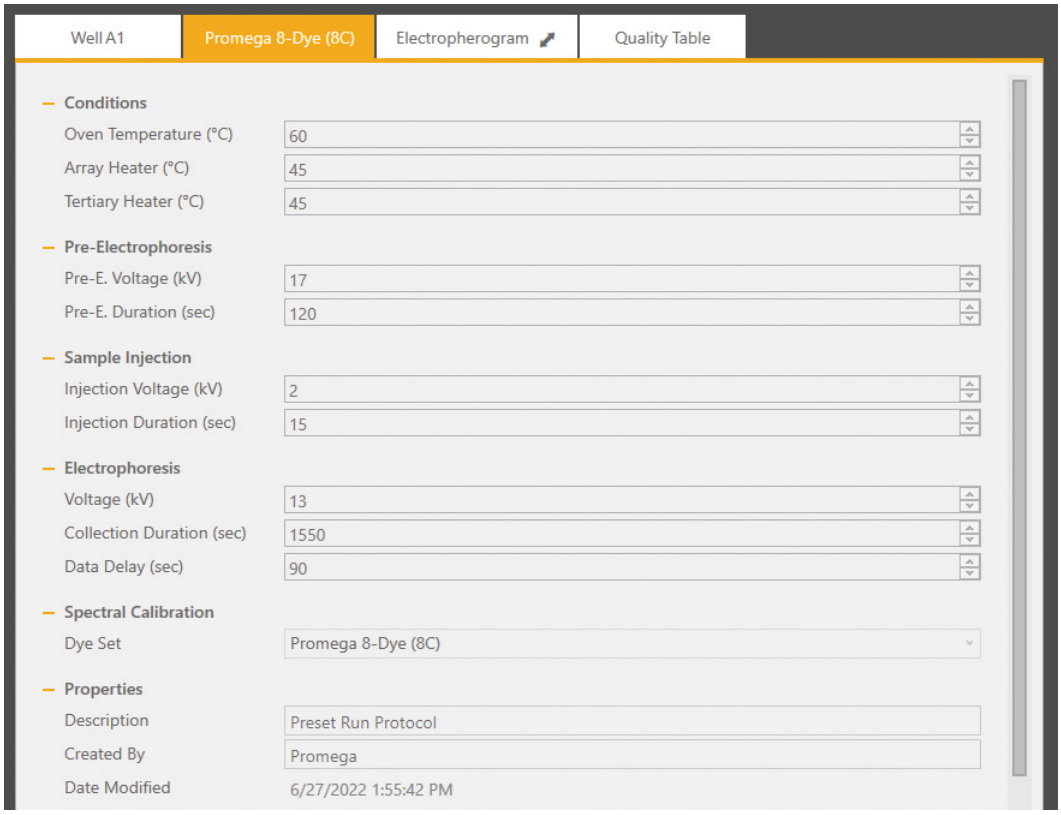

Figure 11. The 'Protocol' tab of the Sample Information section.

9. The plate record is automatically saved and in Draft status until it is linked to a plate position. To access a list of all plates in the Draft status, select the **Draft Plates** button from the 'Home' screen.

5.4 Preparing and Loading the Plate Assembly

1. Place the 96-well plate created in Section 5.1 into the Spectrum plate base, lining up the notch above well A12 with the notch on the base.
2. To complete the plate assembly, place the Spectrum plate retainer over the plate/base assembly, lining up the notch on the retainer with the notch on the plate and base. Verify that the retainer is locked in place on both sides of the plate, sitting evenly on top of the base (Figure 12).

Figure 12. The Spectrum plate assembly.

3. Confirm the drawer handle light is illuminated, indicating that the drawer is unlocked and ready for plate loading. Alternatively, check the Message Center to verify that the drawer is unlocked. The Drawer Status indicator is in the Message Center located in the Header (Figure 3).
4. Check the Plate Position Status indicator to note which of the four plate positions (A, B, C or D) is open and does not already contain a plate.
5. Open the plate drawer and place the plate assembly in an open plate position, verify that the notch on the plate base retainer aligns with the notch in the plate drawer then close the drawer.

   Notes:

   a. When the plate is seated properly, the Plate Positions indicator will indicate that there is a plate present in the corresponding plate position.

   b. An error message will be displayed if the plate position is occupied by a plate that is being processed.

5.5 Linking a Plate for a Run

1. A plate record must be linked to the plate before adding it to the run queue.
2. Open the draft plate record, then select **Link** in the Plate Position indicator under the position where the plate was placed. Once the plate is successfully linked, the text under the position will change status to **Unlink** and the Plate ID will appear above the position (Figure 13).
3. Select the **Start** button to begin the run.

   Notes:

   a. If a plate is linked to a plate position while the instrument is processing another plate, the newly linked plate will be entered automatically into the run queue.

   b. The status of all linked plates will update in the Plate Positions indicator throughout the run.

   c. To access the plate screen for linked plates, select the plate image in the Plate Positions indicator.

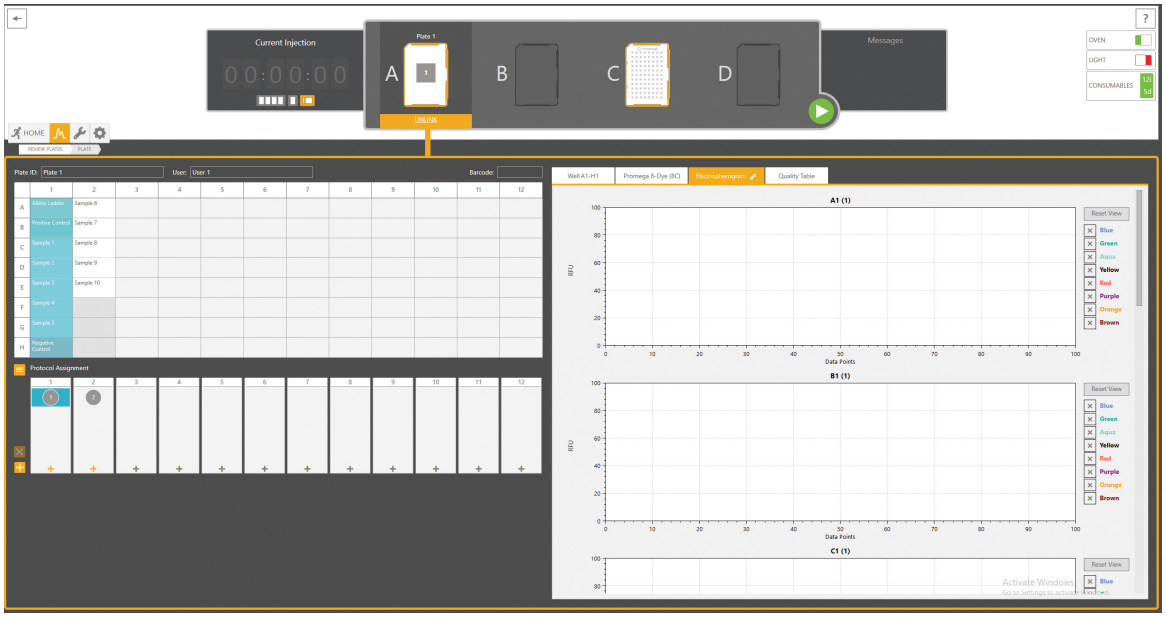

Figure 13. Linked plate in the Spectrum Control Software.

5.6 Monitoring a Run

1. The Plate Status is indicated by the icon in the Plate Positions indicator section of the Header.
2. To access the injection status and view data, select the plate image in the Header.
   a. The injection status will be displayed in the Injection Information section of the plate record.
   b. To view electropherograms, select a well or column and then select the 'Electropherogram' tab in the Sample Information section.
3. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information on plate and injection status indicators as well as reordering, adding or removing injections and plates in the run queue.

5.7 Exporting Data

Data from completed injections can be automatically exported as .promega files into a defined location on the instrument hard drive. Please confirm that the 'Activate Auto Export' check box has been selected in the 'Data Export' tab of the Preferences section. Exported .promega files can be analyzed using GeneMarker®HID Software for Spectrum CE Systems.

Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information on file location and file naming conventions.

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 available at: **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 .promega files.
4. Select **Open**, and the selected files will appear in the Data File List (Figure 14).

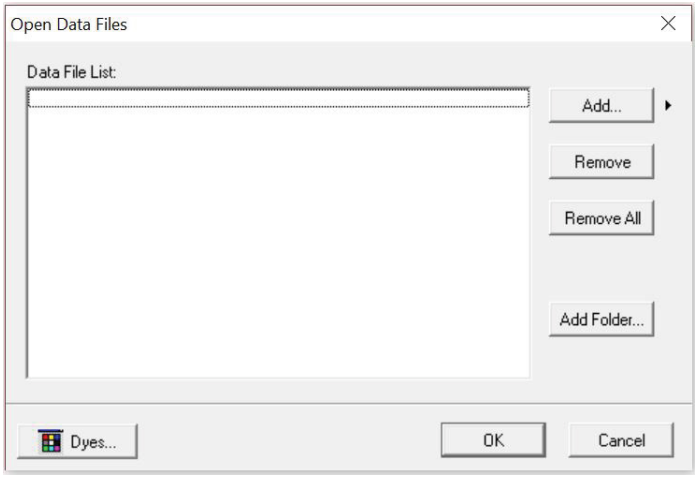

Figure 14. The GMHID-Spectrum Data File List.

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 15). 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.

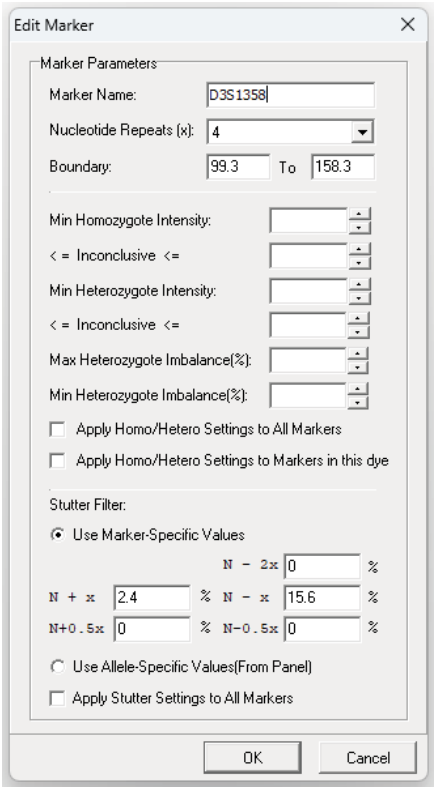

Figure 15. The 'Edit Marker' window for the D3S1358 marker.

8. Select **Run** in the Magic Wizard or click the **Run Project** icon (green arrow) in the toolbar. The 'Template Selection' screen will appear (Figure 16). Select the VersaPlex_31P template and the settings shown in Figure 16. 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.

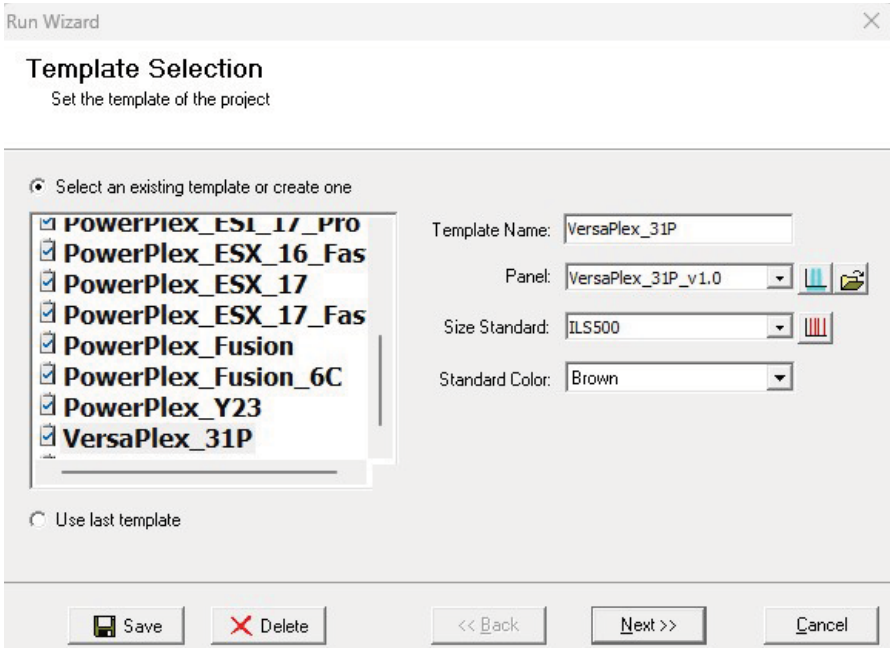

Figure 16. The 'Template Selection' window.

9. The 'Data Process – HID Analysis' window will appear (Figure 17). 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**.

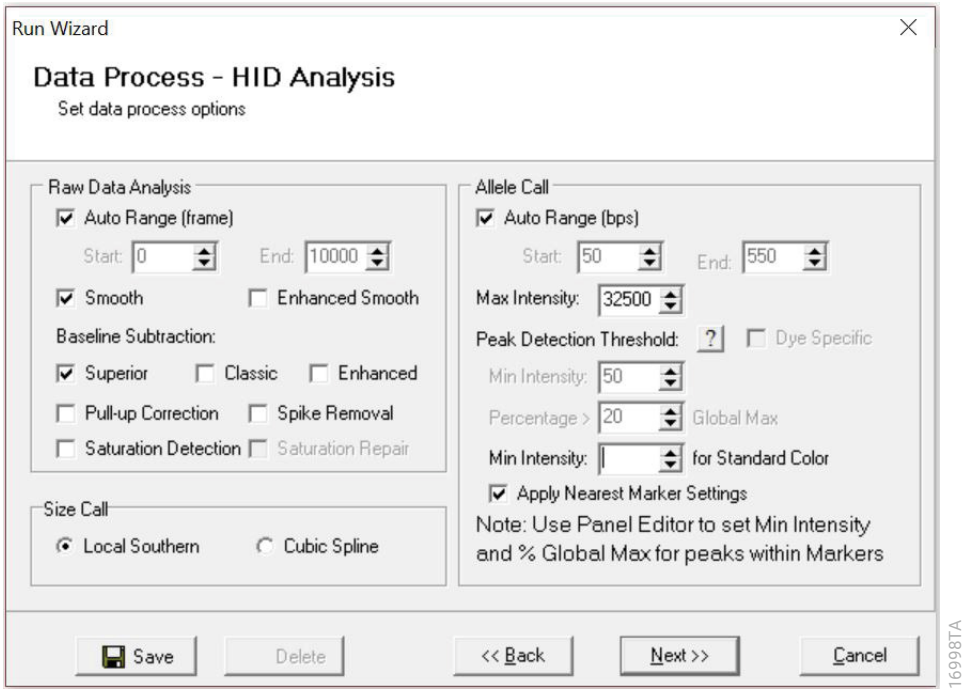

Figure 17. The 'Data Process' window for an analysis method.

10. The 'Additional Settings – HID Analysis' window will appear (Figure 18). 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.

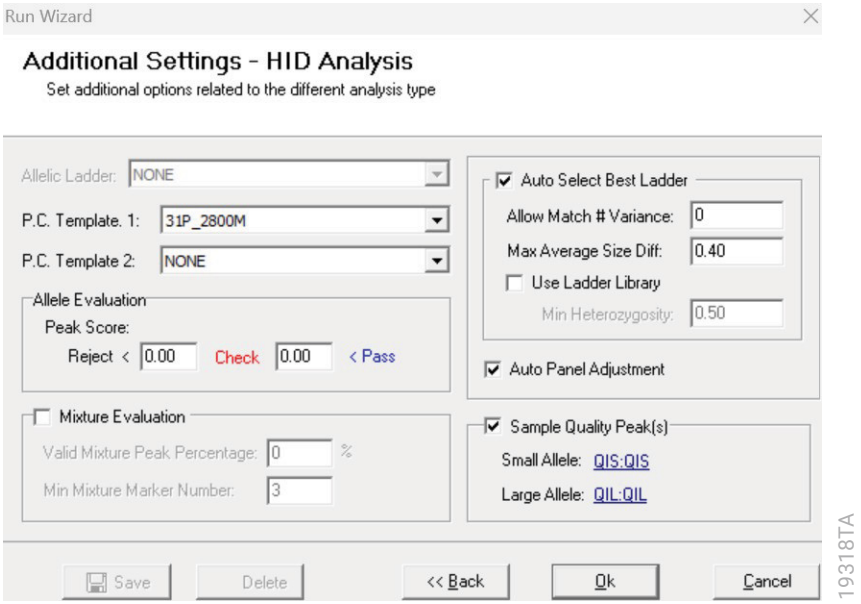

Figure 18. The 'Additional Settings' window for an analysis method.

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 10 (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 STR data analysis and interpretation. 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. The presence or absence of QIS and QIL can be used to assess the overall success of the PCR amplification while their relative peak heights are also useful in determining sample inhibition or degradation. Table 2 provides additional information to assist with the interpretation of QIS and QIL data.

Table 2. 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 *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 19. The VersaPlex® 31P Allelic Ladder Mix is shown in Figure 20.

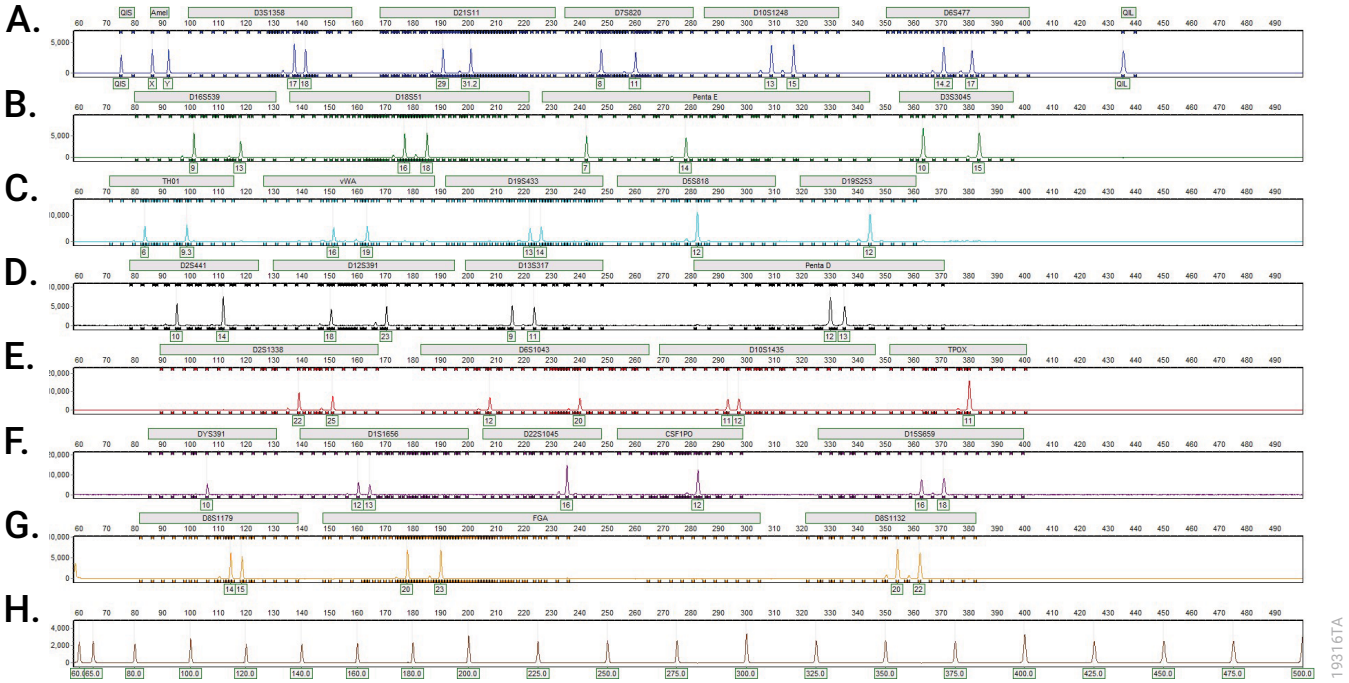

Figure 19. An electropherogram of 2800M Control DNA amplified by the VersaPlex® 31P System. The 2800M Control DNA (0.5ng) was amplified using the VersaPlex® 31P System. Amplification products were mixed with CCO Internal Lane Standard 500 and analyzed with a Spectrum CE System and a 2kV, 15-second injection. Results were analyzed using GeneMarker®HID Software for Spectrum CE Systems, version 3.2 and VersaPlex® 31P panel file. **Panel A.** The peaks of the FL-8C-labeled loci: QIS, Amelogenin, D3S1358, D21S11, D7S820, D10S1248, D6S477 and QIL. **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.

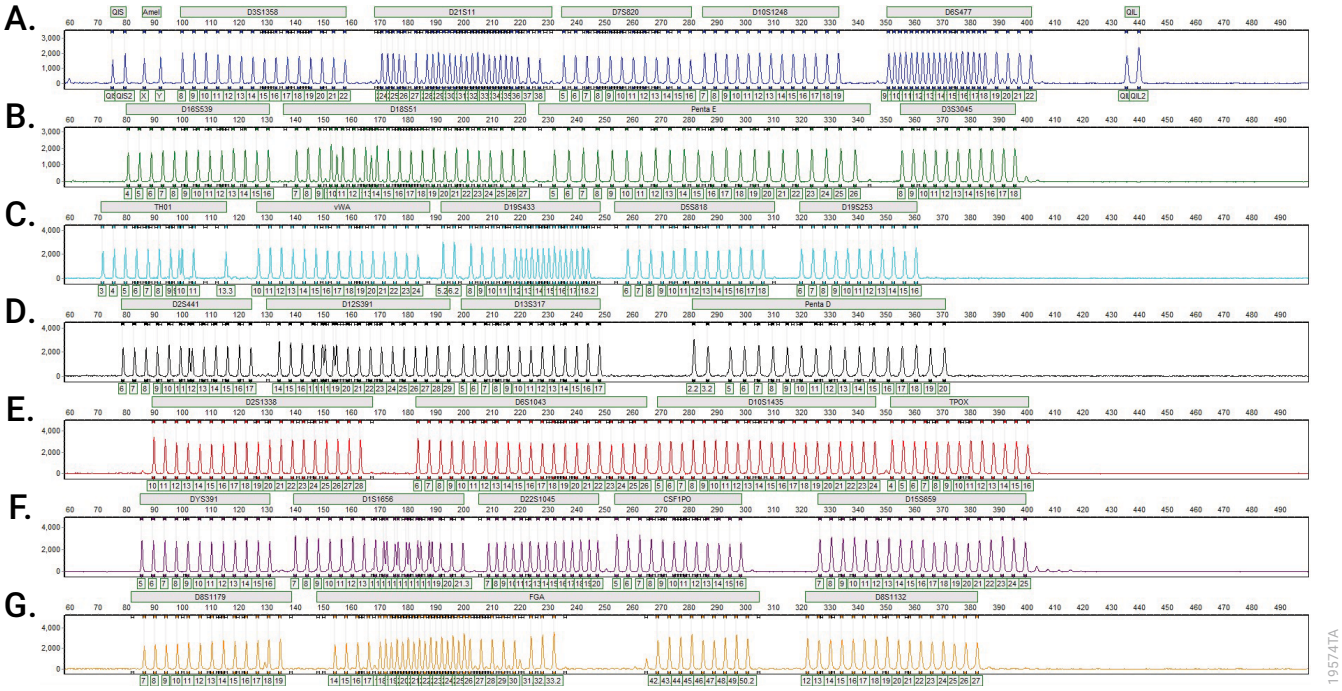

Figure 20. The VersaPlex® 31P Allelic Ladder Mix. The VersaPlex® 31P Allelic Ladder Mix was analyzed with a Spectrum CE System and a 2kV, 15-second injection. The sample file was analyzed with the GeneMarker®HID Software for Spectrum CE Systems, version 3.2.0, and VersaPlex® 31P panel file. **Panel A.** 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. Alleles with a greater number of repeat units will frequently 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 and is preloaded in 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® 31P System.

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

Locus	Artifact Size
QIS/QIL	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 4. DNA-Independent Artifacts Observed in Amplification Reactions with and without Human Genomic DNA.

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

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

[2]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 5 were noted above a 150RFU threshold with 5ng of template DNA using the Spectrum CE System.

Table 5. 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

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 CE System Operating Manual* #TMD052 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 4 (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 reannealing. 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 bottle before installing on the CE.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	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.
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	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.
	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 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	Amplifying 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.
	STR amplification artifacts. Amplifying STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete adenylation. • 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	Amplifying 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.

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. • Create a new size standard using the internal lane standard fragments present in the sample. • Rerun 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. 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)	A low-quality allelic ladder was used during analysis. Ensure that only high- quality allelic ladders are used for analysis. Allelic ladders are flagged for Analysis Quality when: • There is an error in bin shifting • Peaks in virtual bins have greater than expected peak heights • The expected peaks cannot be identified in a marker. When expected peaks cannot be identified in a marker, the marker bar will be shaded dark green, indicating the marker failed analysis. 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. 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. Peaks that are not present in the expected bins are marked with red vertical bars and red allele labels.
Size standard not called or poor quality	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. The incorrect Standard Color was selected in the 'Template Selection' window of the Run Wizard. The Standard Color should be Brown.

Symptoms	Causes and Comments
Size standard not called or poor quality (continued)	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: • The sample size standard does not contain all peaks that are marked as "Enabled" in the chosen size standard template. • 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 **Update Calibration**. • 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.
	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. • Rerun 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 rerun the samples.
	Confirm that the correct spectral was used to run the samples.

9 References

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10 Appendix

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 6 and Table 7). The male-specific DYS391 locus is included to identify null Y results for Amelogenin. Table 8 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 the recommended amounts of template DNA are used.

Table 6. The VersaPlex® 31P System Locus-Specific Information.

STR Locus	Label	Chromosomal Location[1]	Repeat Sequence[2] 5´→3´
Amelogenin[3]	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[1]	Repeat Sequence[2] 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

[1]Find information about chromosomal location of these loci in references 19, 20 and 21 and at:
https://strbase.nist.gov//chrom.htm

[2]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."

[3]Amelogenin is not an STR.

NA = Not Applicable

Table 7. The VersaPlex® 31P System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
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

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
D2S441	TMR-8C	79–124	6–11, 11.3, 12–17
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

[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 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.

[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**

Table 8. The VersaPlex® 31P System Allele Determinations for the 2800M Control DNA.

STR Locus	2800M
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 21). 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.

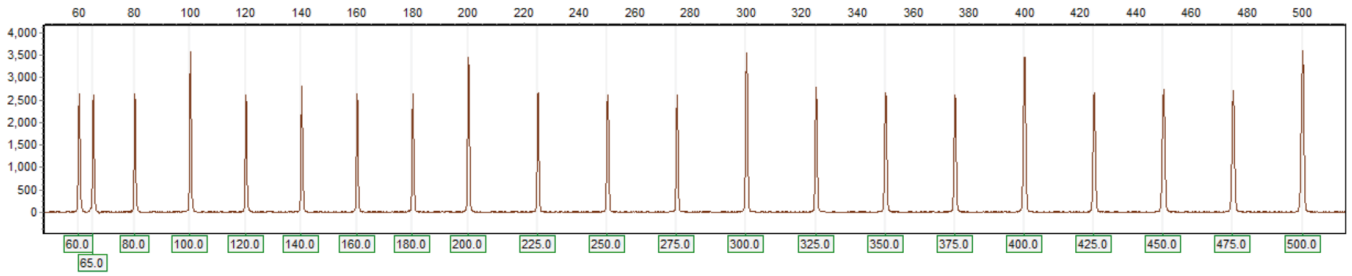

Figure 21. CCO Internal Lane Standard 500. An electropherogram showing the CCO Internal Lane Standard 500 fragments.

10.3 Composition of Buffers and Solutions

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

1.21g Tris base

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

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

TE^{-4} buffer with 20µg/ml glycogen

1.21g Tris base

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

20µg/ml glycogen

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

10.4 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 CE System Accessories and Consumables

PRODUCT	SIZE	CAT.#
Spectrum Capillary Array, 8-Capillary	1 each	CE2008
Spectrum Polymer4	384 wells	CE2048
	960 wells	CE2040
Spectrum Buffer	2 pair	CE2001
Spectrum Cathode Septa Mat	10 each	CE2002
Septa Mat, 96-Well	10 each	CE2696
Spectrum Plate Base & Retainer, 96-Well	4 each	CE5004
Spectrum Wash Solution	1 each	CE2099

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]AQA-8C, TMR-8C, CXR-8C, TOM-8C, WEN-8C and CCO-8C dyes are proprietary.

Class 1 Laser Product.