GenePrint® 24 System for Use on the Spectrum Compact CE System Technical Manual

Instructions for use of Products B1870 and B1874

TM525 | 7/20

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All technical literature is available at: **www.promega.com/protocols/**

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

E-mail Promega Technical Services if you have questions on use of this system: **genetic@promega.com**

1 Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using 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 GenePrint® 24 System[a,b] is a 24-locus multiplex system designed to generate a multi-locus human DNA profile from a variety of human-derived biological sources. This five-color system allows co-amplification and fluorescent detection of the following autosomal STR loci: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D10S1248, D22S1045, D2S441, D1S1656, D12S391, D2S1338, D19S433, Penta D and Penta E plus Amelogenin for gender determination. In addition, the male-specific DYS391 locus is included to identify null Y allele results for Amelogenin.

The *GenePrint*® 24 System provides all necessary materials to amplify the STR loci listed above from human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the *GenePrint*® 24 Master Mix. This manual contains protocols for use of the *GenePrint*® 24 System with the GeneAmp® PCR System 9700 and Veriti® 96-Well standard thermal cyclers, in addition to a protocol to separate amplified products and detect separated material on the Spectrum Compact CE System. A protocol to operate the Spectrum CE System is available separately. See the *Spectrum Compact CE System Operating Manual* #TMD058.

We have tested the GenePrint® 24 System with GeneMapper® Software version 5.0 and GeneMarker® HID Software for Spectrum Systems (GMHID-Spectrum). Other software versions and packages may be available for use; however, the options available in other versions and packages may differ slightly from the options listed in this technical manual.

Amplification and detection instrumentation may vary. You may need to optimize protocols, including amount of template DNA, cycle number and injection conditions for your laboratory instrumentation. In-house optimization should be performed.

The *GenePrint*® 24 System is intended for research use only and is not intended for use in diagnostic, forensic or paternity procedures.

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

Product Components and Storage Conditions

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PRODUCT	SIZE	CAT.#
GenePrint® 24 System	100 reactions	B1870

This product is intended for research use only and is not intended for use in diagnostic, forensic or paternity procedures. This system contains sufficient reagents for 100 reactions of 12.5µl each. Includes:

Pre-Amplification Components Box

- 250µl *GenePrint*® 24 5X Master Mix
- 250µl *GenePrint*® 24 5X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 3 × 1,250µl Water, Amplification Grade

Post-Amplification Components Box

- 50µl *GenePrint*® 24 Allelic Ladder Mix
- 200µl WEN Internal Lane Standard 500

PRODUCT	SIZE	CAT.#
GenePrint® 24 System	400 reactions	B1874

This product is intended for research use only and is not intended for use in diagnostic, forensic or paternity procedures. This system contains sufficient reagents for 400 reactions of 12.5µl each. Includes:

Pre-Amplification Components Box

- 4 × 250µl *GenePrint*® 24 5X Master Mix
- 4 × 250µl *GenePrint*® 24 5X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 5 × 1,250µl Water, Amplification Grade

Post-Amplification Components Box

- 2 × 50µl *GenePrint*® 24 Allelic Ladder Mix
- 2 × 200µl WEN Internal Lane Standard 500

Storage Conditions

Upon receipt, store all components except the 2800M Control DNA at –30°C to –10°C in a nonfrost-free freezer. Store the 2800M Control DNA at 2–10°C. Make sure that the 2800M Control DNA is stored at 2–10°C for **at least 24 hours** before use. After the first use, store the *GenePrint*® 24 System components at 2–10°C, where they will be stable for 1 year. Do not refreeze.

The *GenePrint*® 24 5X Primer Pair Mix, *GenePrint*® 24 Allelic Ladder Mix and WEN Internal Lane Standard 500 (WEN ILS 500) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Available Separately

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

The proper panels and bins text files with stutter ratios and size standard .xml file for use with GeneMapper® software are available for download at:
www.promega.com/GenePrintPanels/

The proper panel .xml file for use with GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum) is preloaded in GMHID-Spectrum or downloadable at:
www.promega.com/GenePrintPanels/

Before You Begin 3

3.1. Precautions

The quality of purified DNA, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and 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. The matrix standards are provided separately.

For protocol and additional information about matrix generation and spectral calibration on the Spectrum Compact CE System, see the *GenePrint® Matrix Standards for the Spectrum Compact CE System Technical Manual* #TM594. This manual is available online at: **www.promega.com/protocols/**

Protocol for DNA Amplification Using the *GenePrint*® 24 System

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The *GenePrint*® 24 System was developed to amplify extracted DNA from human-derived biological samples. Slight protocol variations may be required for optimal performance of each template source.

The *GenePrint*® 24 System is optimized for the GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block and the Veriti® 96-Well Thermal Cycler.

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

Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 9.

Materials to be Supplied by the User

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

We routinely amplify 2.5–5.0ng of template DNA in a 12.5µl reaction volume using 26 cycles and the protocol detailed below.

Amplification Setup

1. At first use, thaw the *GenePrint*® 24 5X Master Mix, *GenePrint*® 24 5X Primer Pair Mix and Water, Amplification Grade, completely. After the first use, store the reagents at 2–10°C. Do not refreeze.

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

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.
3. Use a clean MicroAmp® plate for reaction assembly, and label it appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label them appropriately.
4. Add the final volume of each reagent listed in Table 1 to a clean tube.

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

PCR Amplification Mix Component[1]	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 12.5µl	×		=	
GenePrint® 24 5X Master Mix	2.5µl	×		=	
GenePrint® 24 5X Primer Pair Mix	2.5µl	×		=	
template DNA (2.5–5.0ng)[2,3,4]	up to 7.5µl				
total reaction volume	**12.5µl**				

[1]Add Water, Amplification Grade, to the tube first, and then add *GenePrint*® 24 5X Master Mix and *GenePrint*® 24 5X Primer Pair Mix. The template DNA will be added at Step 6.

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

[3]Apparent DNA concentrations can differ, depending on the DNA quantification method used (10). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

[4]The *GenePrint*® 24 System protocol was optimized for 2.5–5.0ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your laboratory's optimization and may be different than the amount suggested in this protocol.

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

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

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

6. Add the template DNA for each sample to the respective well containing PCR amplification mix.
7. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 1.0ng/µl. Add 2.5ng or 5.0ng 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 reactions to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

You may need to optimize cycle number. Amplification using the optimal cycle number should yield DNA profiles with no allele drop-out and few or no off-scale allele peaks.

1. Place the reaction plate or tubes in the thermal cycler.
2. Select and run the recommended protocol, which is provided below and in Figure 1.

Notes:

1. When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%. Do not use 9600 emulation mode.
2. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

Thermal Cycling Protocol
96°C for 1 minute, then: 94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 26 cycles, then: 60°C for 10 minutes 4°C soak

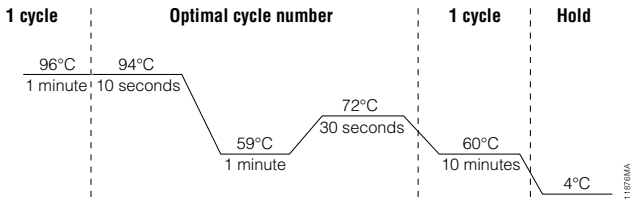

Figure 1. The thermal cycling protocol for the GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler.

3. After completion of the thermal cycling protocol, proceed to fragment analysis or store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts. For best results, do not leave the amplified products in the thermal cycler at 4°C overnight.

Detection of Amplified Fragments Using the Spectrum Compact CE System

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

Materials to be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 8-tube strips
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer7 (Cat.# CE2307)
- 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)
- Strip Septa Mat, 8-Well (Cat.# CE2308)
- MicroAmp® Optical 8-Tube Strip, 0.2ml (Applied Biosystems Cat.# 4316567)
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Notes:

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

Sample Layout

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

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

5.1. Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and Hi-Di™ formamide as follows: [(0.5µl ILS) × (# samples)] + [(9.5µl formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be adjusted to change the intensity of the size standard peaks based on laboratory preferences.
2. Vortex for 10–15 seconds to mix.
3. Pipet 10µl of formamide/internal lane standard mix into each well of the 8-well strip tube(s).

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

   Notes:

   1. To prevent cross-contamination, do not reuse Strip Septa Mat, 8-Well. Always use a new Strip Septa Mat, 8-Well, for each 8-well strip tube.
   2. Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time, refer to the *Spectrum Compact CE System Operating Manual* #TMD058. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.
   3. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your laboratory's optimization.
   4. Include an injection of allelic ladder every 4 injections (16 samples).

5. Centrifuge the 8-well strip tube(s) briefly to remove air bubbles from the wells.
6. Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

5.2. Assembling Sample Cartridge

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

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

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

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

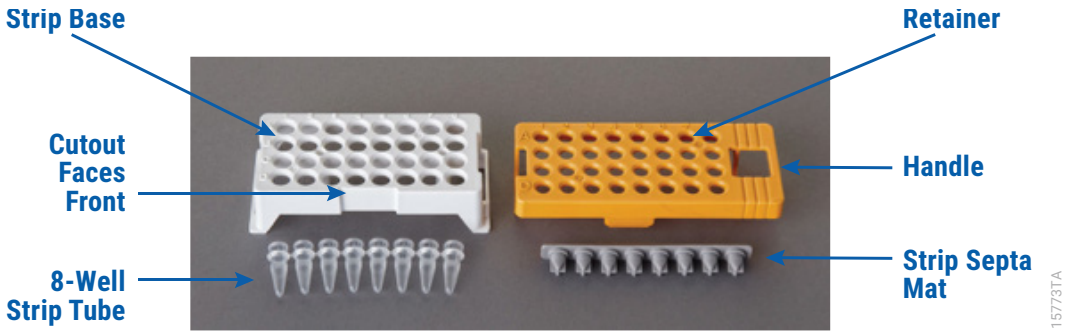

Figure 2. Assembling the Spectrum Compact Strip Base and Retainer.

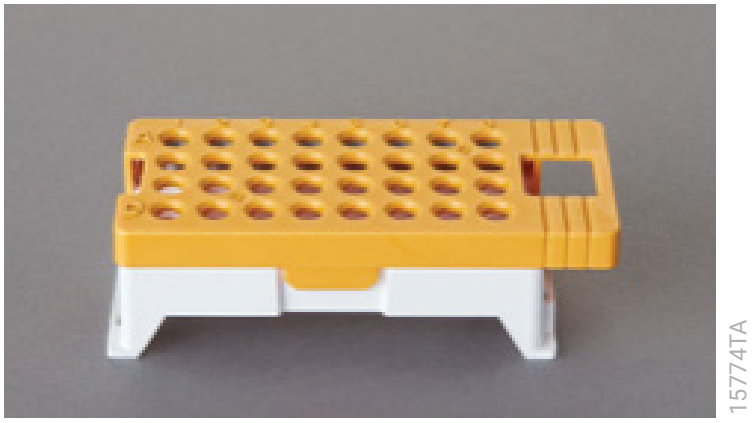

Figure 3. Assembled Spectrum Compact Sample Cartridge.

5.3. Instrument Preparation

These instructions are intended as a guide for running samples on the Spectrum Compact CE System (after successful spatial and spectral calibration with the *GenePrint*® 5C 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 the *GenePrint*® *Matrix Standards for the Spectrum Compact CE System Technical Manual* #TM594 for instructions on performing a spectral calibration.

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

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

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

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

Table 2. Consumable Warning Symbols.

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

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

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

Figure 6. Preheating Oven.

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

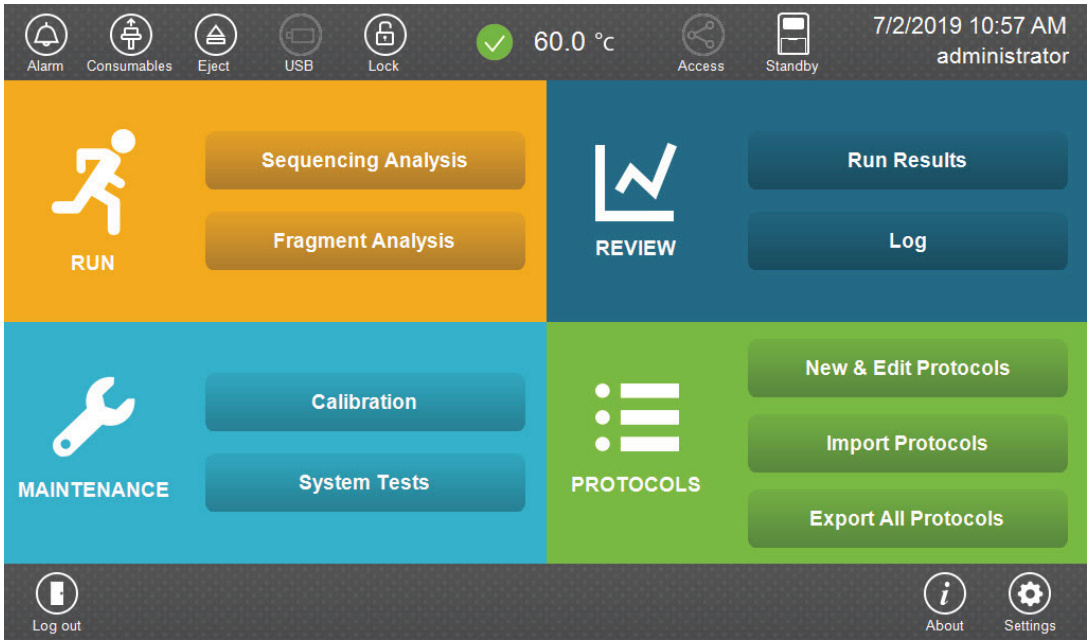

Figure 7. Spectrum Compact CE System Software 'Main Menu' screen.

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

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

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

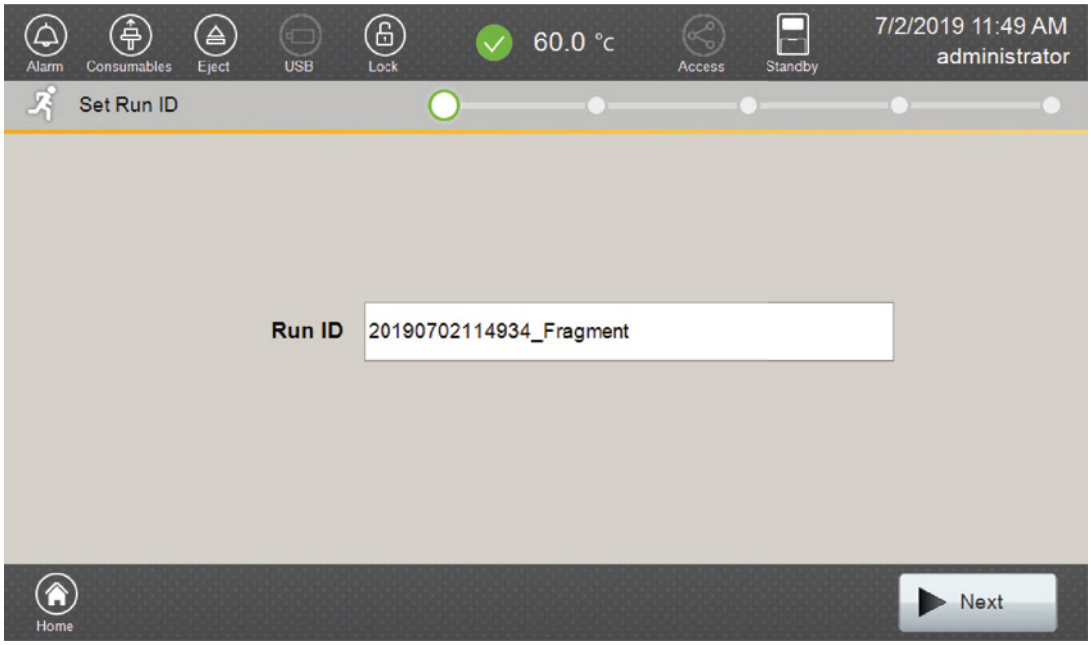

Figure 8. 'Set Run ID' screen.

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

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

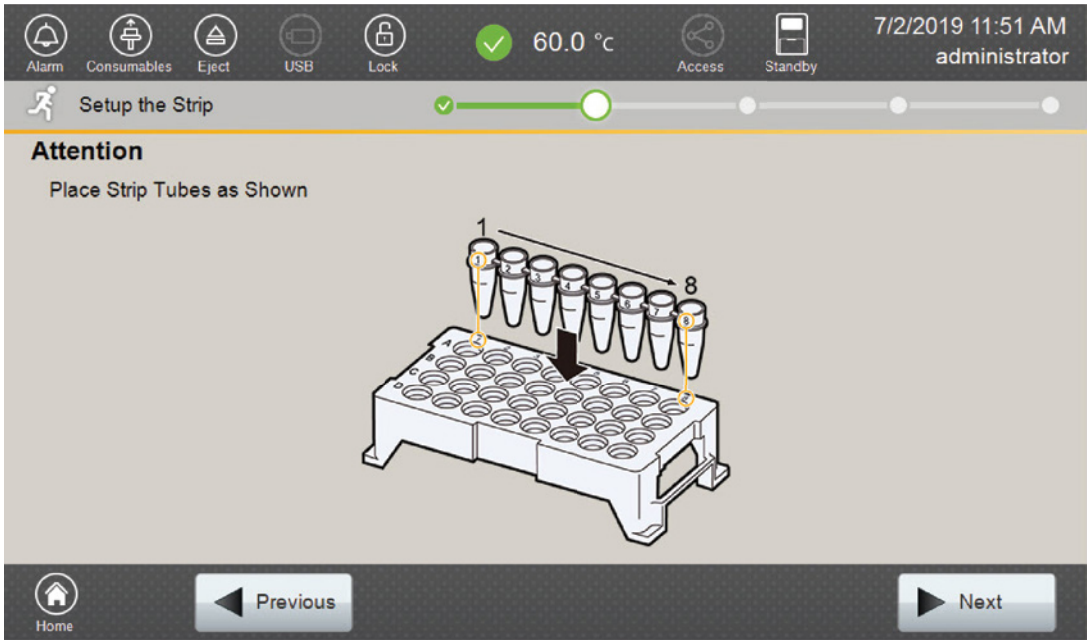

Figure 9. 'Setup the Strip' screen.

5.4. Assigning Run Information to Samples

There are four methods to assign run information to samples:

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

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

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

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

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

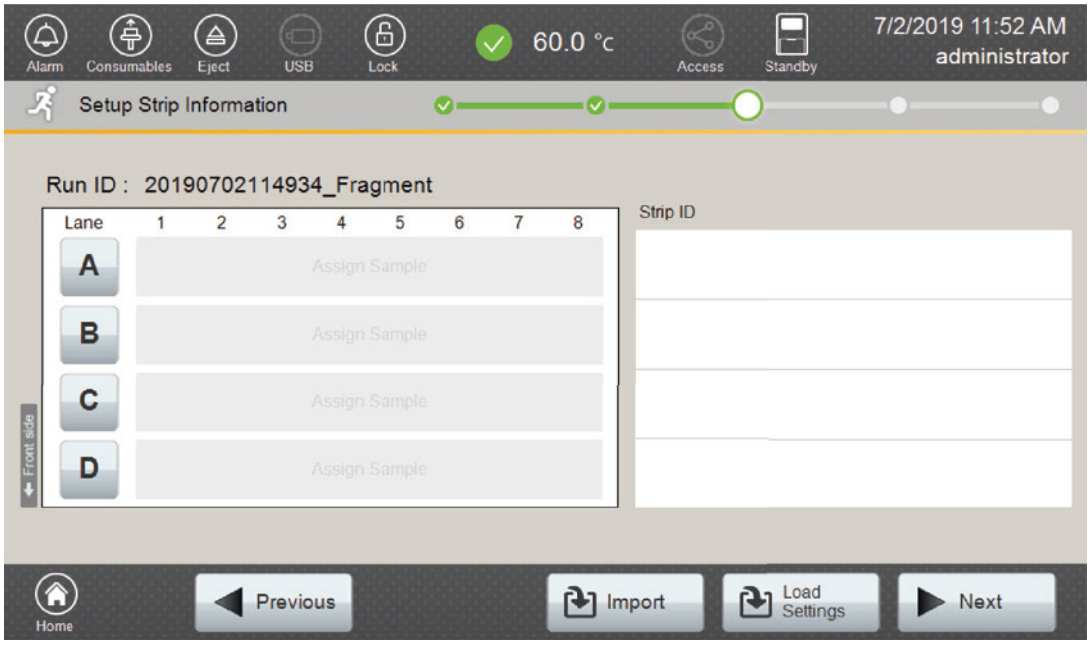

Figure 10. 'Setup Strip Information' screen.

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

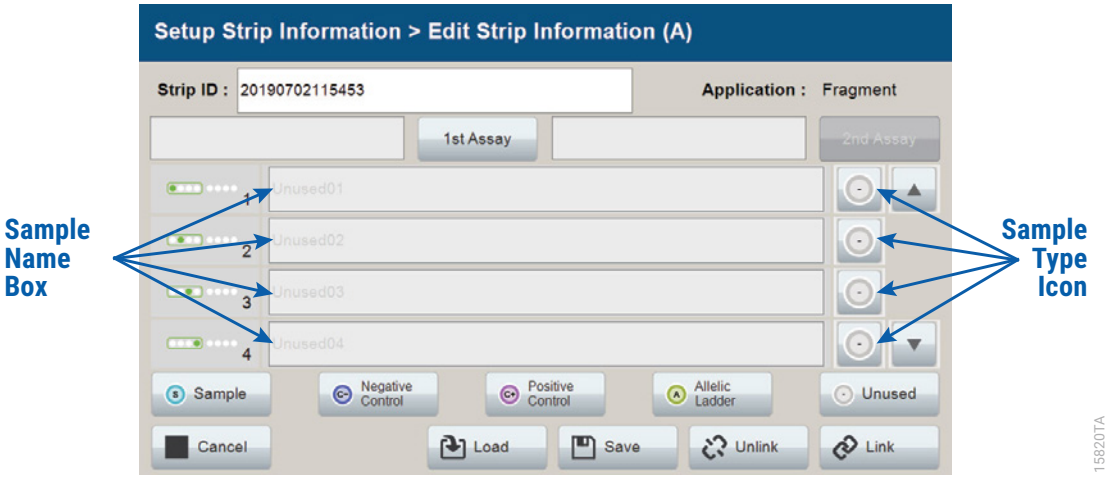

Figure 11. 'Edit Strip Information' screen.

3. The default Strip ID is date and time. It can be modified by selecting the **Strip ID** field. 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, and then select **OK** to exit and return to the 'Edit Strip Information' screen.

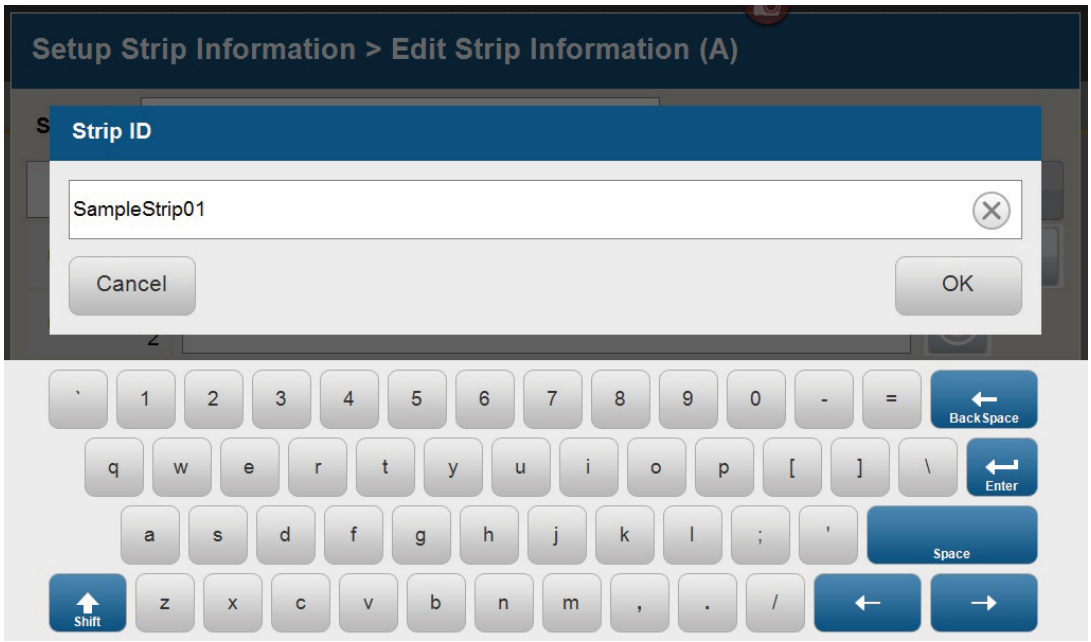

Figure 12. 'Set Strip ID' window.

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

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

5. To assign a sample type to a well, select the appropriate sample type along the bottom of the 'Edit Strip Information' screen (Figure 13), and 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.

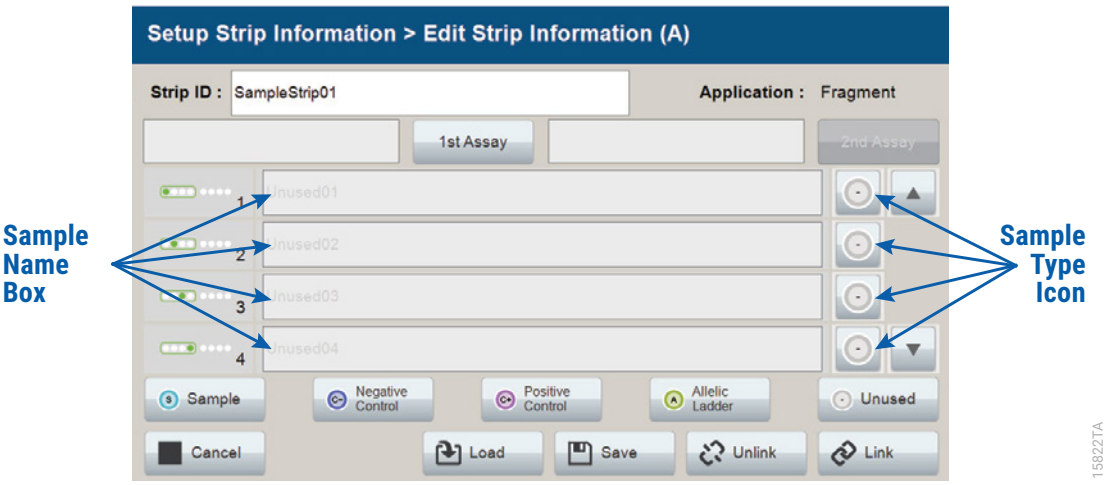

Figure 13. Sample Type and Sample Name on 'Edit Strip Information' screen.

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, and then select **OK** to exit and return to the 'Edit Strip Information' screen.

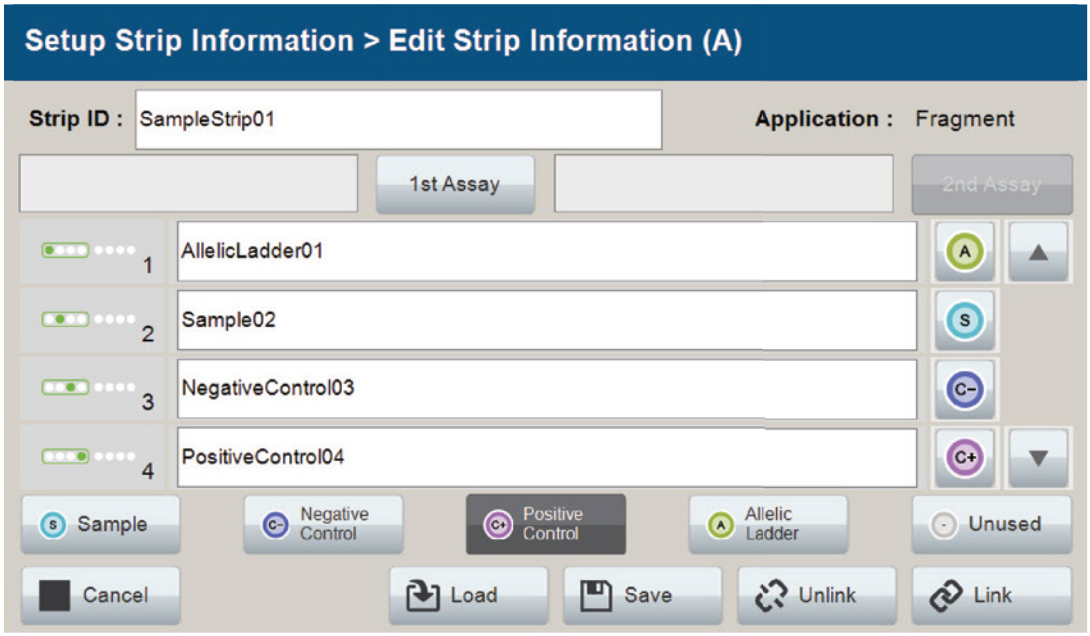

Figure 14. Sample Name Entry on 'Edit Strip Information' screen.

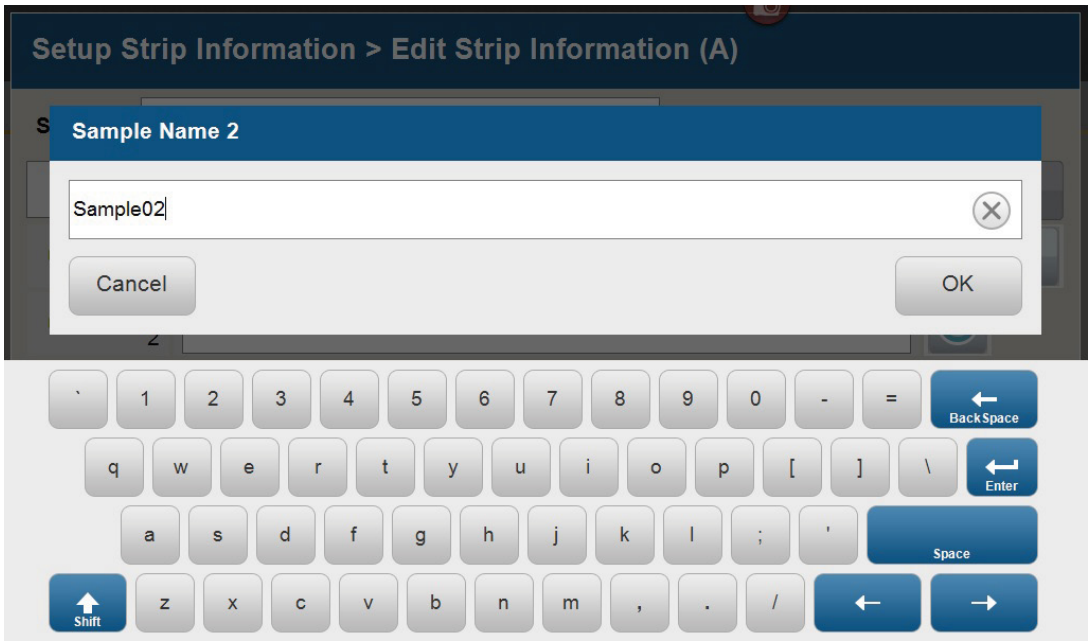

Figure 15. 'Set Sample Name' window.

7. The Spectrum Compact Control Software includes pre-loaded 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 the **1st Assay** button on the right side of the '1st Assay' field (Figure 14). This opens the 'Select Assay' window (Figure 16). Select 'Promega_5Dye_WENILS_36_P7' from the drop-down list (Figure 16) and then select **Apply**.

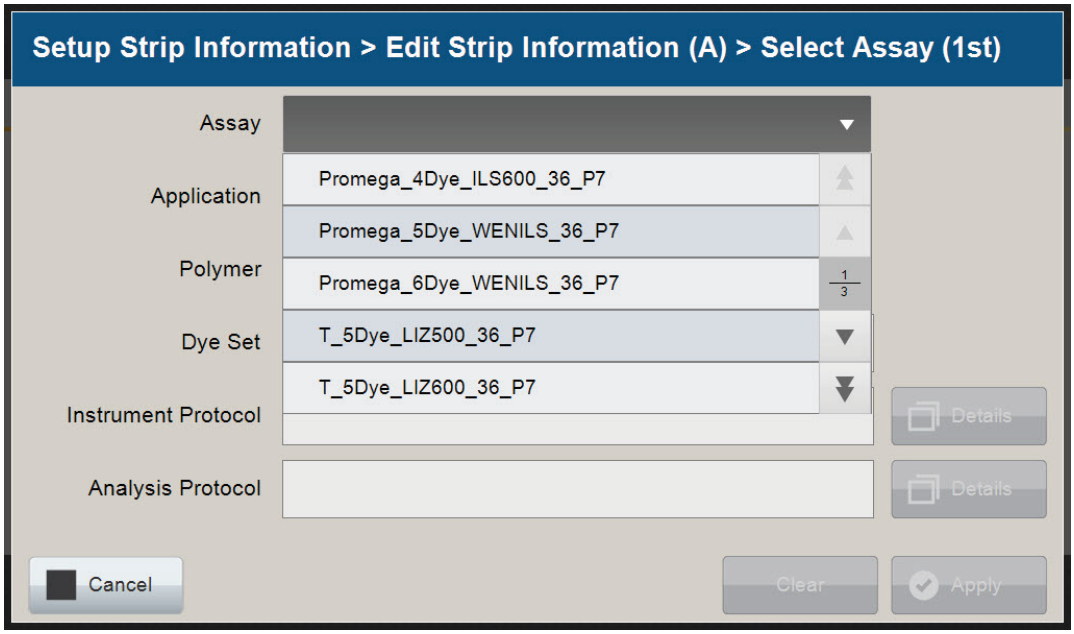

Figure 16. 'Select Assay' window.

8. You can verify the settings of the Instrument Protocol and Analysis Protocol 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.

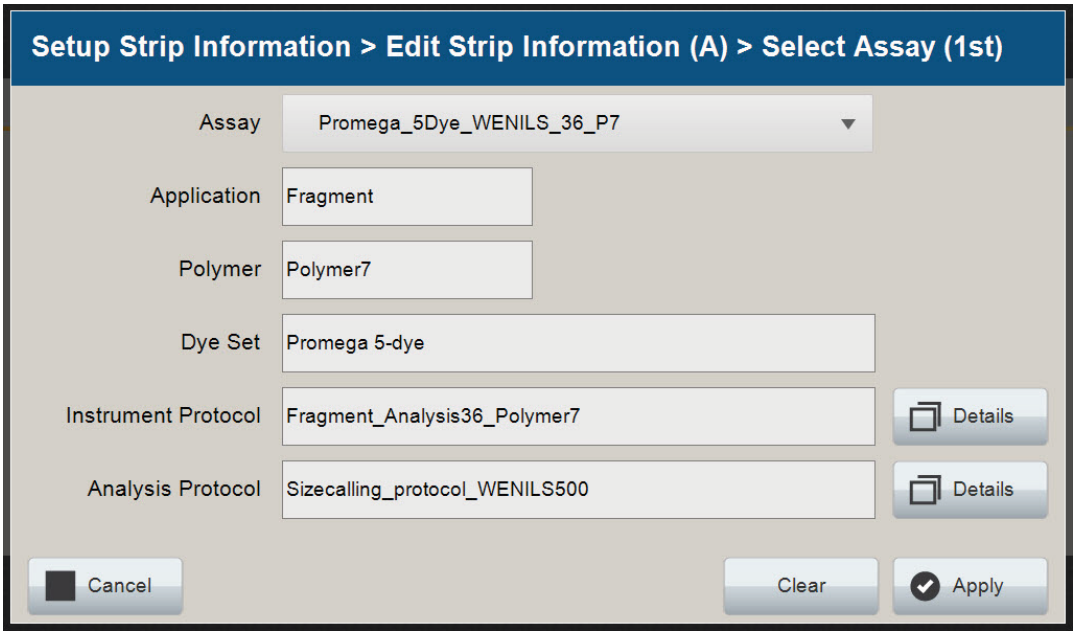

Figure 17. Accessing Instrument and Analysis Protocols on 'Select Assay' window.

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, 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 save the strip to the run. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for use of saved strip information.

    Notes:

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

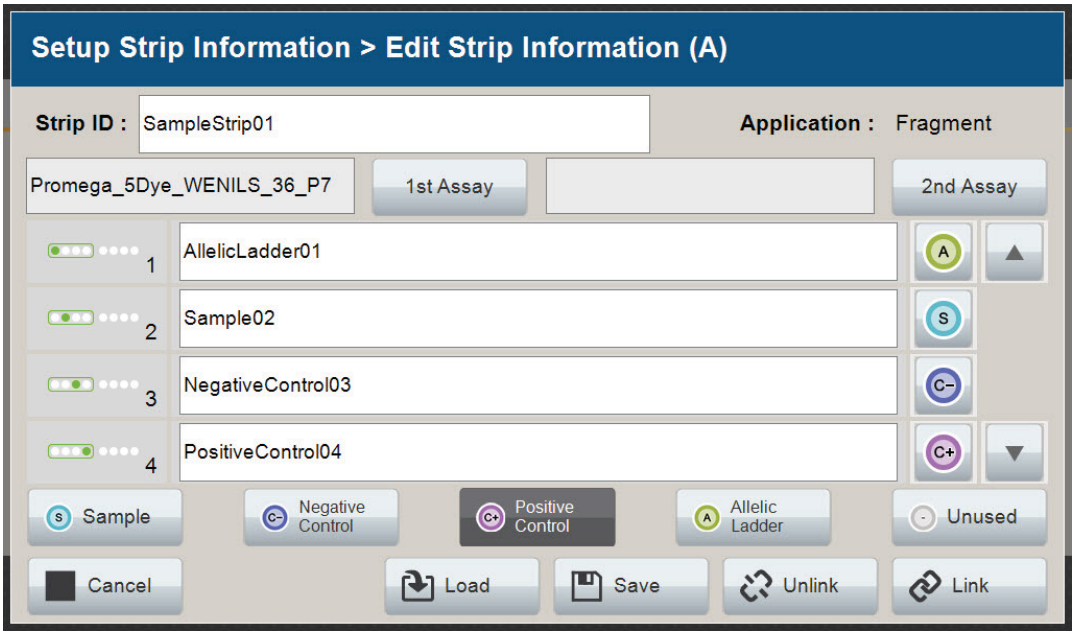

Figure 18. Completed 'Edit Strip Information' screen.

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

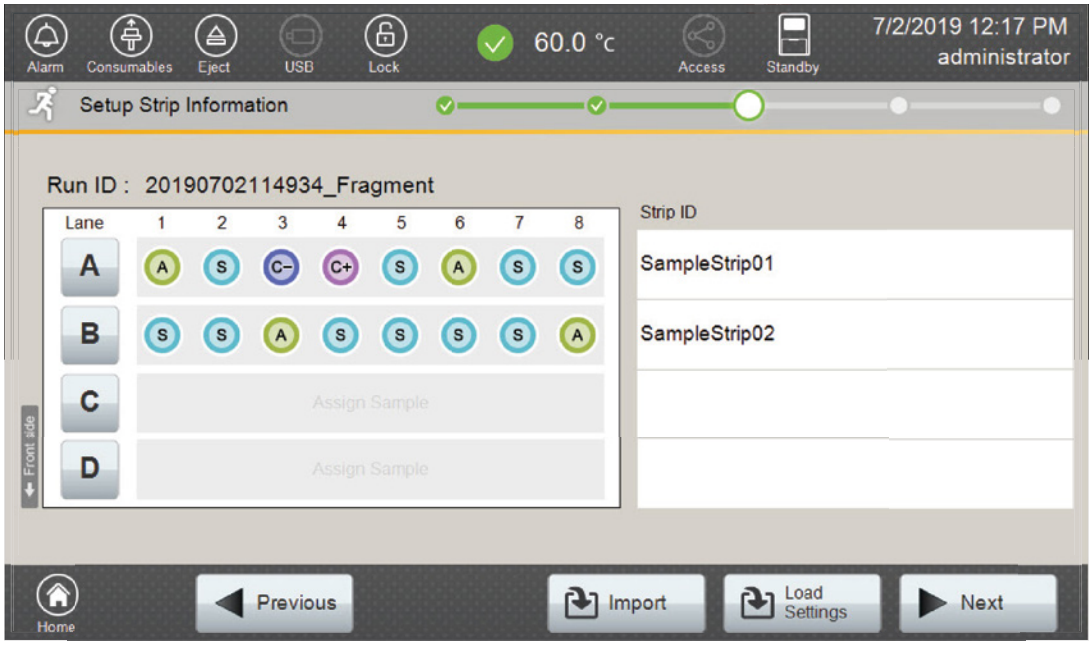

Figure 19. Completed 'Setup Strip Information' Screen.

5.5. Loading the Sample Cartridge

1. After selecting **Next** on the 'Setup Strip Information' screen, a message window will open, indicating that the autosampler is moving 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, and then place the sample cartridge on the autosampler, following the instructions displayed on the 'Install the Cartridge' screen (Figure 20). Press down on the yellow tab on the autosampler deck that locks the sample cartridge in place before placing sample cartridge into position. Release the tab to lock the sample cartridge in place on the autosampler deck.

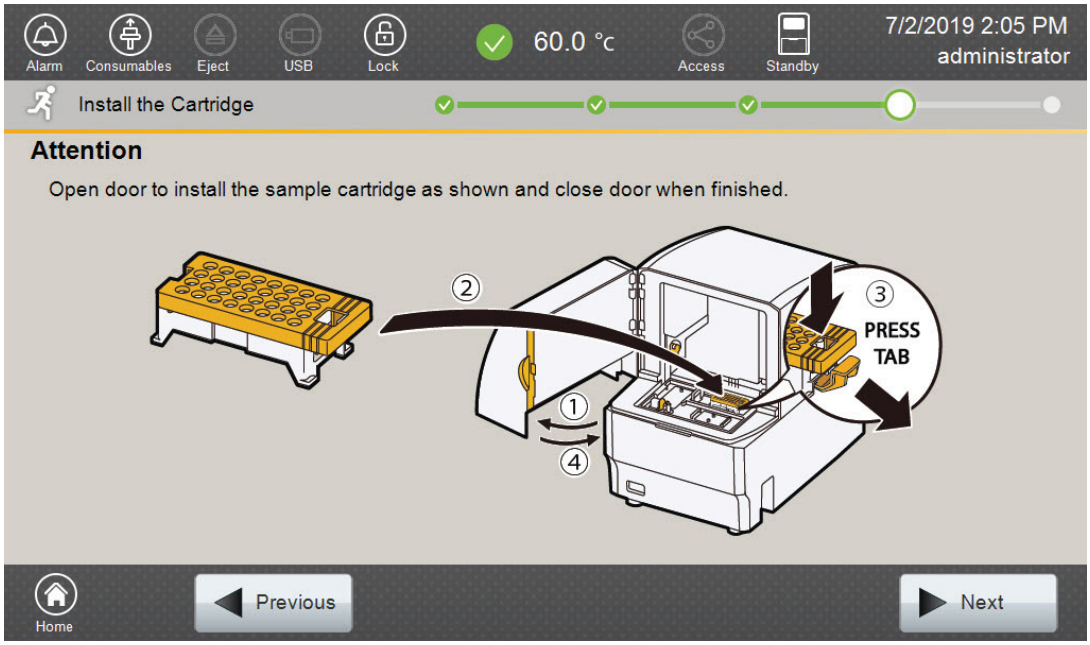

Figure 20. 'Install the Cartridge' Screen.

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

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

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

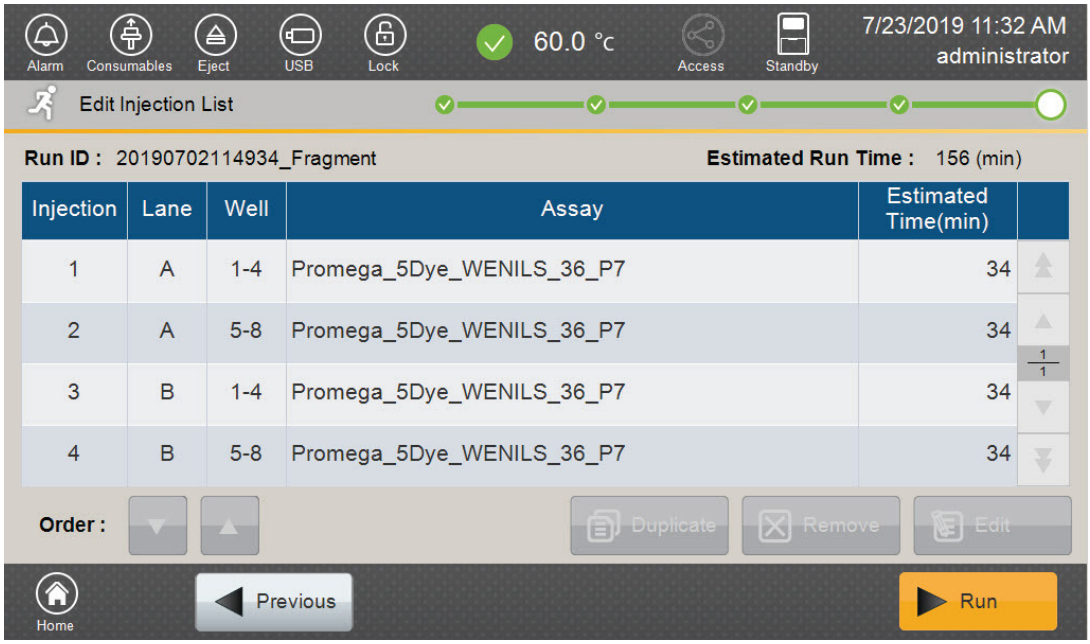

Figure 21. 'Edit Injection List' Screen.

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

Data Analysis Using GeneMapper® Software

6

GeneMapper® version 4.1 or greater is required for analysis of *GenePrint*® 24 data from the Spectrum Compact CE System.

6.1. Importing *GenePrint*® 24 Panels and Bins Text Files into GeneMapper® Software

To analyze data generated with the *GenePrint*® 24 System, you will need panels and bins text files, an ILS/size standard file and an analysis method .xml file. We recommend that users receive training from Applied Biosystems on the GeneMapper® software to familiarize themselves with proper operation of the software.

Getting Started

1. To obtain the analysis files for the *GenePrint*® 24 System, go to: **www.promega.com/GenePrintPanels/**
2. Select the *GenePrint*® 24 System, GeneMapper as the software and Spectrum Compact for the instrument. Enter your contact information, and then select **Submit**. An e-mail will be sent to the e-mail address provided with a link for the file download. There will be six files included in the download: a WEN ILS/size standard file, a *GenePrint*® 24 panels file, a *GenePrint*® 24 bins file, a Table Settings file, a Plot Settings file and the *GenePrint*® 24 Analysis Method file.
3. Save all files to a known location on your computer.

Importing Panels and Bins Files

1. Open the GeneMapper® software.
2. Select **Tools,** and then **Panel Manager**.
3. Highlight the **Panel Manager** icon in the upper left navigation pane.
4. Select **File,** and then **Import Panels**.
5. Navigate to the panels text file downloaded in the Getting Started section above. Select the file, and then **Import.**
6. In the navigation pane, highlight the *GenePrint*® 24 panels folder that you just imported in Step 5.
7. Select **File,** and then **Import Bin Set**.
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, and then **Import**.
9. In the navigation pane, highlight the *GenePrint*® 24 panels folder that you just imported in Step 5 to view the marker information if desired.
10. At the bottom of the Panel Manager window, select **Apply**. This will save the panels and bins files. Select **OK** to close the window.

6.2. Importing the WEN ILS 500 Size Standard into GeneMapper® Software

There are two options when creating an internal lane standard/size standard. Use this protocol or the alternative protocol in Section 6.3.

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **Import**.
4. Navigate to the location of the WEN_ILS_500.xml file downloaded in Section 6.1, Getting Started.
5. Highlight the file, and then select **Import**.
6. Select **Done** to save the changes and close the GeneMapper® Manager.

6.3. Creating a Size Standard with GeneMapper® Software

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **New**.
4. In the 'Size Standard Editor' window (Figure 22), select **Basic or Advanced** and then **OK**.

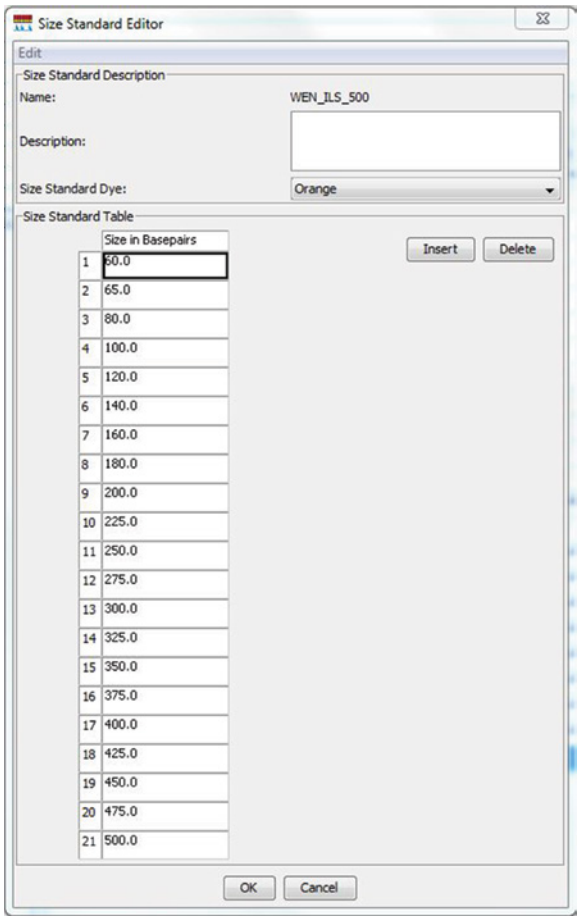

Figure 22. The GeneMapper® Software, Version 5.0, 'Size Standard Editor' window.

5. Enter a detailed name, such as "WEN_ILS_500."
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Figure 22.
8. Select **OK**.

6.4 Importing Table and Plot Settings Files

1. Select **Tools** and then **GeneMapper Manager**.
2. Select the 'Table Settings' tab.
3. Select **Import**, and then navigate to the location of the *GenePrint*® 24 Table Settings file that you downloaded. Import the file.
4. Select the 'Plot Settings' tab.
5. Select **Import**, and then navigate to the location of the *GenePrint*® 24 Plot Settings file that you downloaded. Import the file.

These files will now be available to choose in the drop-down menus in the Samples Tab view (for Table Setting, Figure 23) and in the Samples Plot view (for Plot Settings, Figure 24).

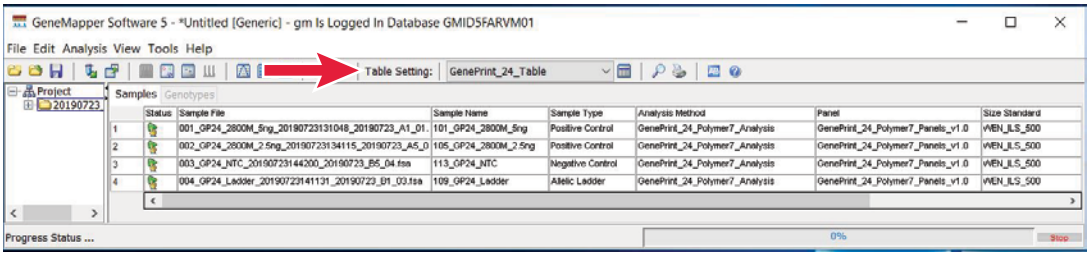

Figure 23. Drop-down menu location for the *GenePrint*® 24 Table Setting in the Samples Tab view.

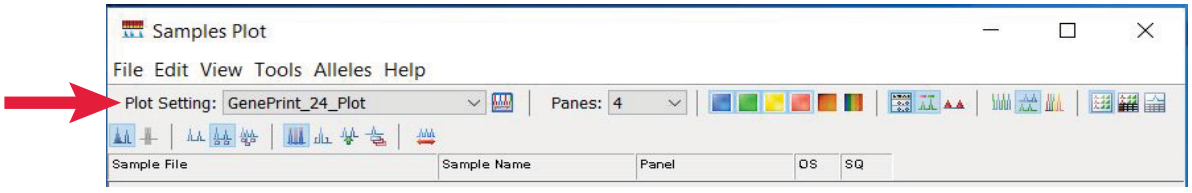

Figure 24. Drop-down menu location for the *GenePrint*® 24 Plot Setting in the Samples Plot view.

6.5 Importing the *GenePrint*® 24 Analysis Method

1. Select **Tools** and then **GeneMapper Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **Import**, and then navigate to the location of the *GenePrint*® 24 Analysis Method file that you downloaded in Section 6.1. Import the file.
4. Note that this method will have "HID" as the analysis type (Figure 25).

Only "HID" type methods can use the allelic ladder files for correct genotyping in GeneMapper® software. An "HID" type analysis method cannot be originally created within the software.

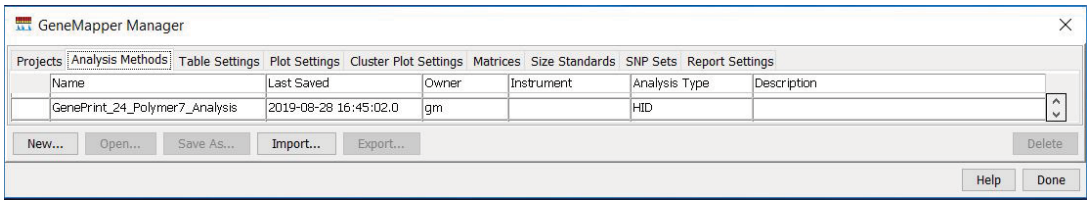

Figure 25. The GenePrint 24 Analysis Method will have "HID" as the analysis type.

5. To verify the correct bin settings, locate the new *GenePrint*® 24 Analysis Method at the bottom of the list of methods. Select the analysis method, and then choose **Open**. Select the Allele tab, and choose 'GenePrint_24_Polymer7_bins' from the drop-down menu at the top, if it does not already appear. Verify that all settings appear as shown in Figure 26.

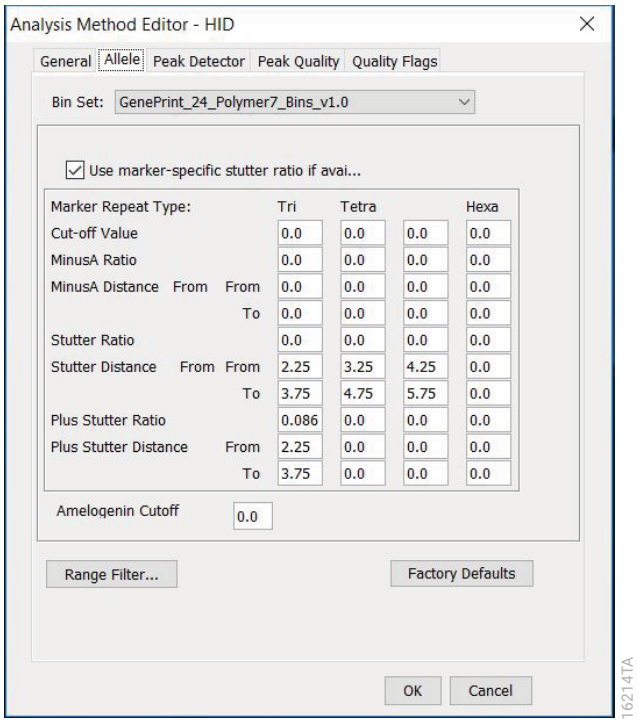

Figure 26. 'Allele' Tab settings.

6. To verify or set the correct peak detection settings, choose the 'Peak Detector' tab. Enter the peak amplitude threshold values for your laboratory, and leave all other settings as shown in Figure 27.

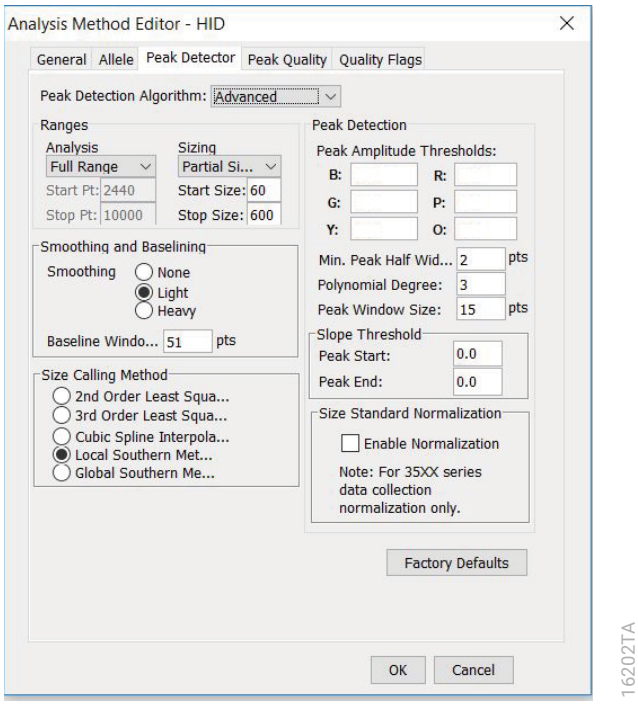

Figure 27. 'Peak Detector' tab settings.

6.6 Analysis of *GenePrint*® 24 Data in GeneMapper® Software

Once all analysis files are imported into GeneMapper® Software, data analysis can be performed. To start a new project for analysis, perform the following steps:

1. In the **File** menu, choose **New Project – Generic**. Be sure to choose "Generic" and not "Microsatellite".

   Note: If you choose a "Microsatellite" project, you will not be able to select your HID analysis method when analyzing the data, and correct genotyping across all loci will not occur.
2. In the top center of the window, choose **GenePrint_24_Table** from the drop-down menu.
3. Import your sample files by choosing **File** and **Add Samples to Project**. Navigate to the folder that contains the data files, select the folder and choose **Add to List**. Once all files are selected, choose **Add** to bring all files into the project.

   Note: Every folder of data must have at least one acceptable allelic ladder file for correct genotyping of the data.
4. The sample types assigned in Section 5.4 will be included in the 'Sample Type' column. If needed, the drop-down menu in the 'Sample Type' column can be used to select **Allelic Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample.
5. In the 'Analysis Method' column, choose **GenePrint_24_Polymer7_Analysis** from the drop-down menu.
6. In the 'Panel' column, select **GenePrint_24_Polymer7_Panels** by opening the folder and double-clicking on the file.
7. In the 'Size Standard' column, select **WEN_ILS_500**.
8. Repeat these choices for all samples or fill down the columns.
9. Once all of these parameters are entered, click on the green arrow in the menu bar to analyze the data.
10. A 'Save Project' window will open, providing a space where the project can be named. Name the project, and then select **OK**.

The GeneMapper® software will proceed with analysis by first analyzing the allelic ladder files. Analysis will pause for a few seconds about every 10 samples to save the analyzed data to the database. The data are now ready for review. See Figure 28.

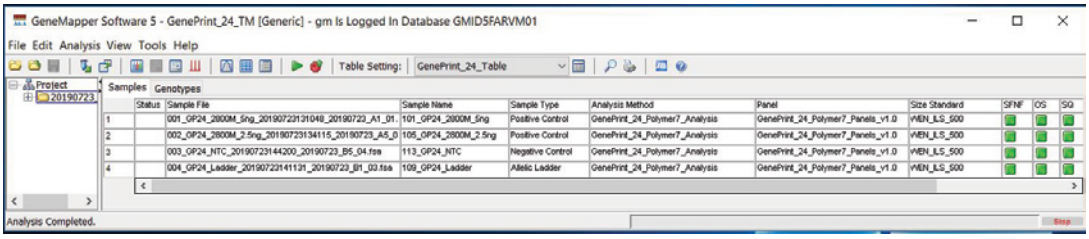

Figure 28. The analyzed project.

Data Analysis Using GeneMarker®HID Software for Spectrum CE Systems

7

7.1. 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 for Spectrum CE Systems User Manual* #TM555.

The current GenePrint.xml panel files are preloaded in the software or available at: **www.promega.com/GenePrintPanels/**

1. Open GMHID-Spectrum.
2. To access your data files, select **Open Data** in the Magic Wizard or under the **File** menu.
3. Select **Add**, then navigate to the directory containing your raw data files and select the desired files.
4. Select **Open**, and the selected files will appear in the 'Data File List' (Figure 29).

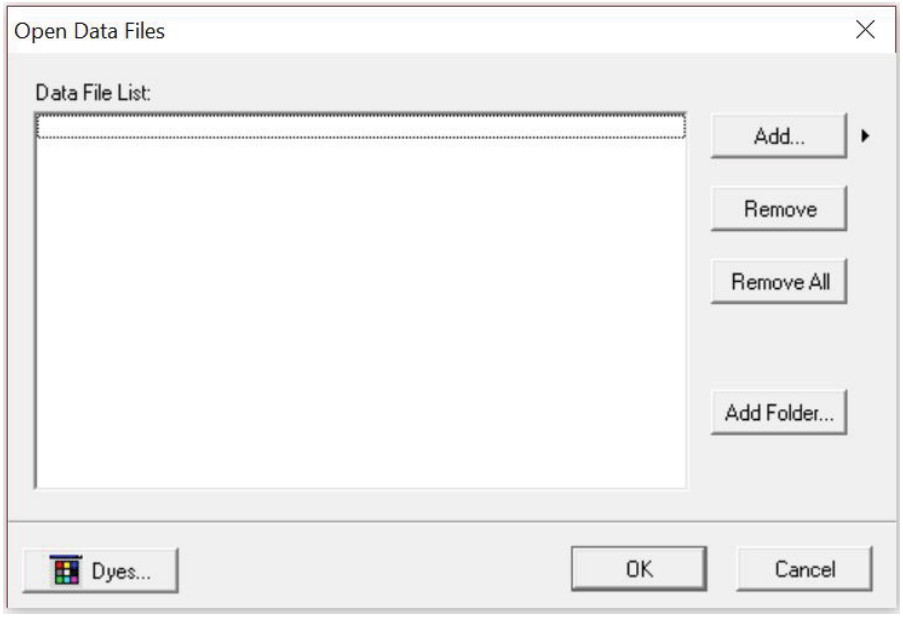

Figure 29. The GMHID-Spectrum 'Data File List.'

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

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

6. Use the **Panel Editor** in the **Tools** menu to select the GenePrint_24_Polymer7 panel from the **Panel Template** list. Click on the plus symbol to expand the list, right-click on a marker, and then select **Edit**. Enter laboratory-specified values for Min Homozygote Intensity, Homozygote Inconclusive Range, Min Heterozygote Intensity, Heterozygote Inconclusive Range, Min Heterozygote Imbalance and Max Heterozygote Imbalance (Figure 30). This will set values for peaks within the marker range. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

   Notes:

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

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

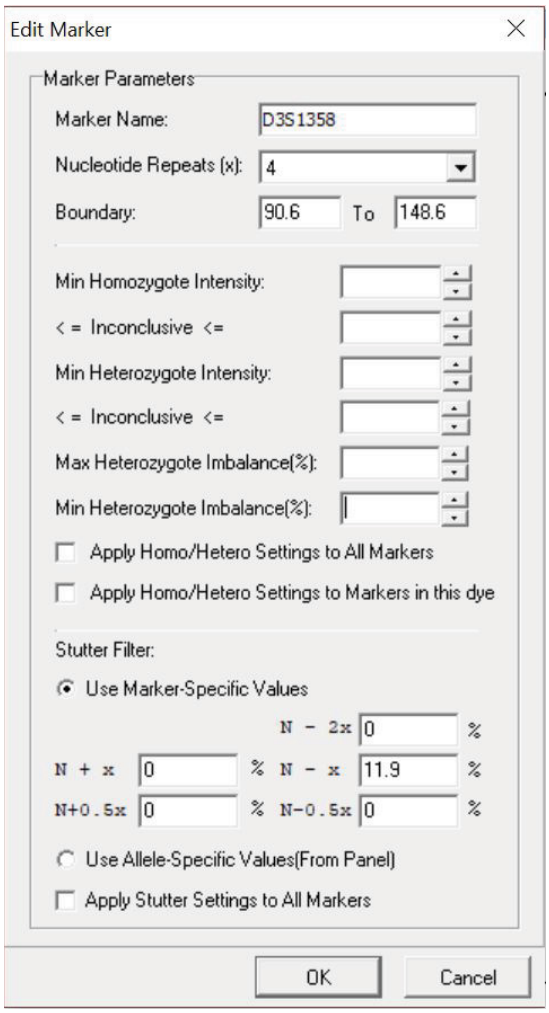

Figure 30. The 'Edit Marker' window for the D3S1358 marker.

8. Click on **Run** in the Magic Wizard or click the **Run Project** icon (green arrow) in the toolbar. The 'Template Selection' screen will appear (Figure 31). Select the GenePrint_24 template and the settings shown in Figure 31. Verify the panel that you created in Step 6 is selected in the Panel drop-down field. The Size Standard must be ILS500 and the Standard Color must be Orange. Select **Next**.

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

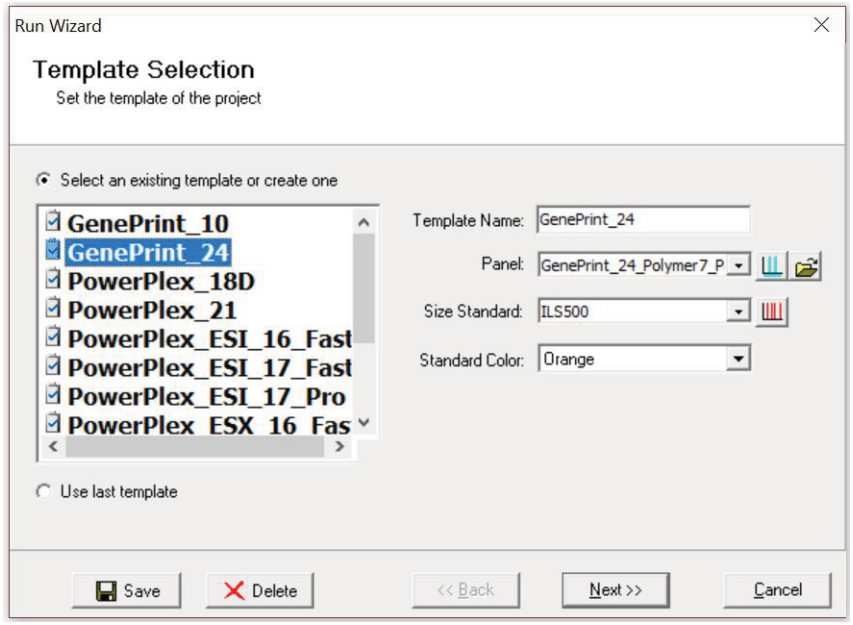

Figure 31. The 'Template Selection' window.

9. The 'Data Process' window will appear (Figure 32). 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 for Spectrum CE Systems User Manual* #TM555 for more information on use of the Peak Detection Threshold. Select **Next**.

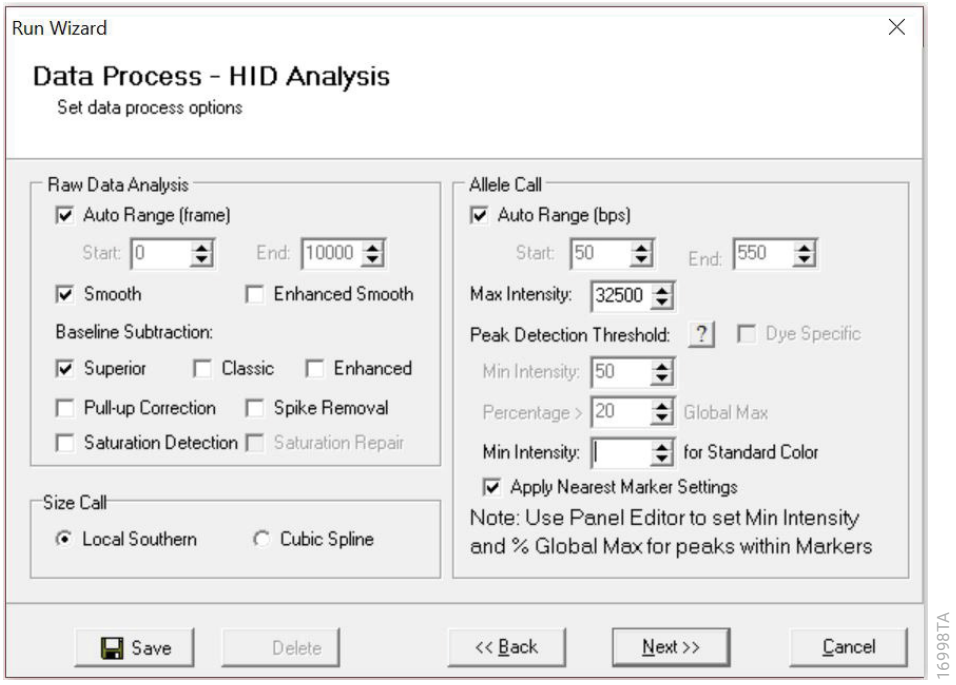

Figure 32. The 'Data Process' window for an analysis method.

10. The 'Additional Settings' window will appear (Figure 33). Select **GenePrint_24_2800M** as the P.C. Template 1 and verify that the boxes are checked for 'Auto Select Best Ladder' and 'Auto Panel Adjust'. The values displayed in the 'Allele Evaluation' dialogue box are defaults and will affect the quality values displayed in the plot settings. For more information on quality values, use of 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.

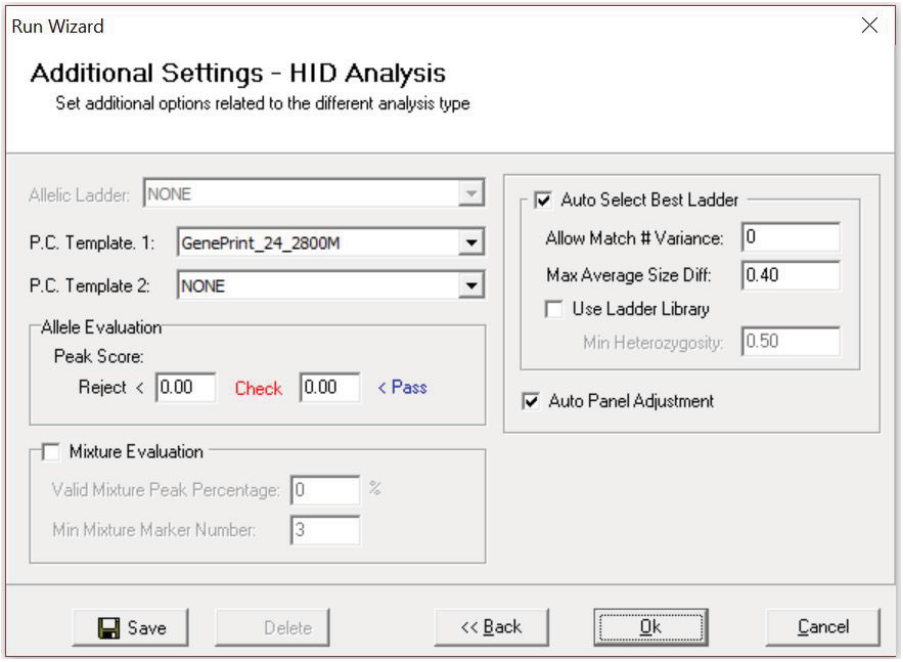

Figure 33. The 'Additional Settings' window.

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

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

7.2. Controls in the GMHID-Spectrum Software

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative controls should be devoid of amplification products.
2. Observe the results for the 2800M Control DNA. The expected 2800M allele designations for each locus are listed in Table 7 (Section 11.1).

Results

8

Representative results of the *GenePrint*® 24 System are shown in Figure 34. The *GenePrint*® 24 Allelic Ladder Mix is shown in Figure 35.

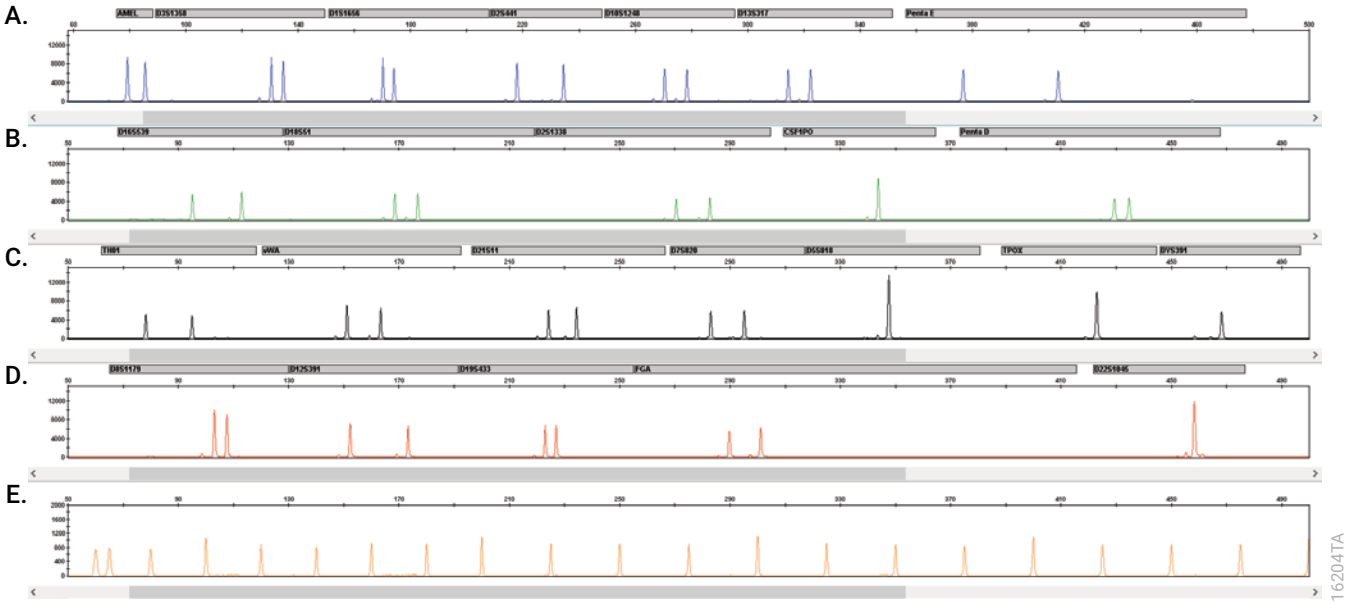

Figure 34. The GenePrint® 24 System. The 2800M Control DNA (2.5ng) was amplified using the GenePrint® 24 System, 26 cycles and GeneAmp® PCR System 9700 with a silver block. Amplification products were mixed with WEN Internal Lane Standard 500 and and analyzed with a Spectrum Compact CE System using a 1.6kV, 9-second injection. Results were analyzed using GeneMapper® software, version 5.0, and *GenePrint*® 24 panels and bins text files. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D16S539, D18S51, D2S1338, CSF1PO and Penta D. **Panel C**. An electropherogram showing the peaks of the TMR-ET-labeled loci: TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D8S1179, D12S391, D19S433, FGA and D22S1045. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500.

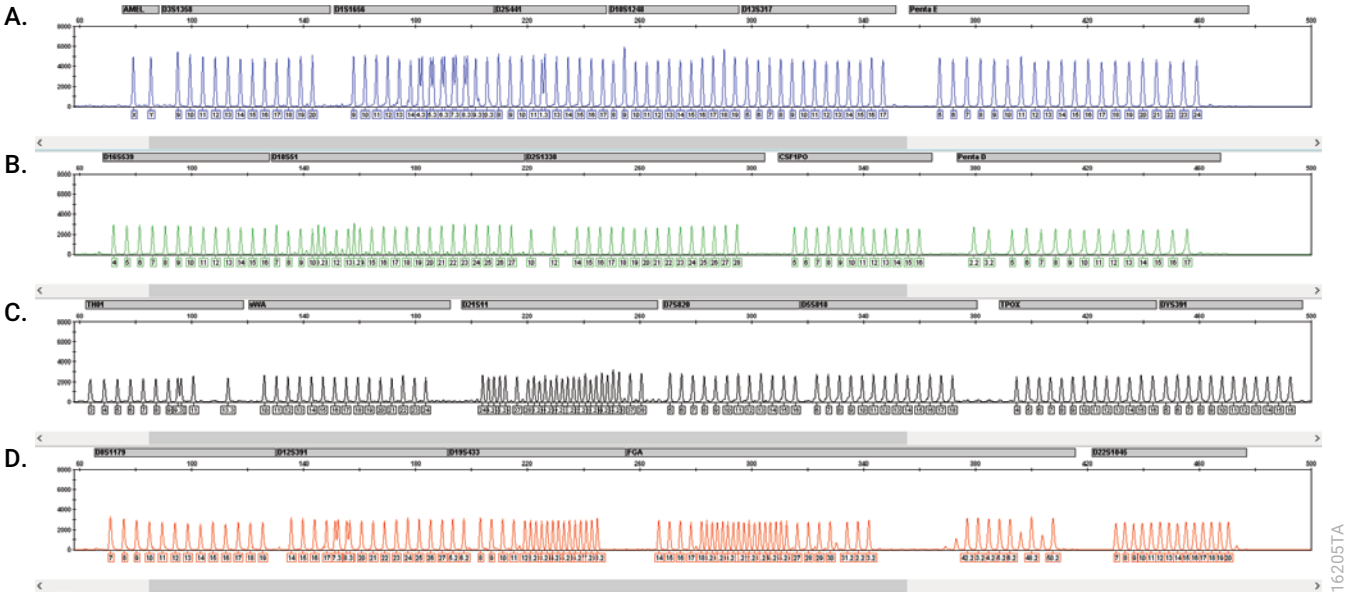

Figure 35. The *GenePrint*® 24 Allelic Ladder Mix. The *GenePrint*® 24 Allelic Ladder Mix was analyzed with a Spectrum Compact CE System and a 1.6kV, 9-second injection. The sample file was analyzed with the GeneMapper® software, version 5.0, and *GenePrint*® 24 panels and bins text files. **Panel A.** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-ET-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. A trinucleotide repeat locus, like D22S1045, will have more pronounced stutter in both n–3 and n+3 positions than a typical tetranucleotide repeat locus. The 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 *GenePrint*® 24 stutter text file for locus-specific filtering in GeneMapper® software and in the panel file included 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 *GenePrint*® 24 System.

Table 3. DNA-Dependent Artifacts Observed with or without Human Genomic DNA.

Locus or Dye Label	Artifact Size
CXR	~175–183 bases
Fluorescein	~88–112[1]
JOE	~77–80 bases[2]
	~217 bases[2]
	~250 bases[2]
TMR	~64–67 bases[2]
	~69–72 bases[2]
Amelogenin	n–1
D1S1656	n–2, n+2[3]
D2S441	n–1, n–2, n+2[3]
D10S1248	n–2, n+2[3]
D13S317	n–2, n+2[3]
Penta E	n–2, n+2[3], ~n–10 to n–11[4]
D16S539	n–2, n+2[3]
D18S51	n–2, n+2[3]
D21S11	n–2, n+2[3]
D7S820	n–2, n+2[3]
D5S818	n–2, n+2[3], ~n–6 to n–7[5], ~n–8 to n–9[5]
D12S391	n–2, n+2, n–3[3]
D19S433	n–2, n+2[3]

[1]For artifacts in this size range, RFU approximately 1.5% or less of the main peaks may be observed at the D3S1358 locus.

[2]These are typically below common minimum thresholds.

[3]Number of bases below (n–) or above (n+), the true allele peak, respectively.

[4]Low-intensity peaks (50–100RFU) that migrate approximately 10–11 bases in front of the main allele may represent DNA secondary structure.

[5]Low intensity peaks (50–200RFU) that migrate approximately 6–7 bases and 8–9 bases in front of the main allele may represent DNA secondary structure.

Table 4. DNA-Independent Artifacts Observed with or without Human Genomic DNA.

Dye Label	Artifact Size[1]
Fluorescein	~55–68 bases
	~70–77 bases
	~80–88 bases[2]
JOE	~70–77 bases[2]
	~80–88 bases[2]
TMR	~50–58 bases

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

[2]The signal strength of these artifacts increases with storage of the amplification plate at 4°C, most commonly when plates are left at 4°C for a few days. We recommend storing amplification products at –20°C.

Troubleshooting 9

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

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

9.1. Amplification and Fragment Detection

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

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips and change gloves regularly.
	Do not store the PCR amplification mix for a prolonged period. Add the mix to the wells of the reaction plate as soon as the mix is prepared. Add the DNA source as soon as possible to each well and follow immediately by thermal cycling.
	Samples were not denatured completely. Heat-denature samples for the recommended time, and cool on crushed ice or a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of 'shadow' peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distance as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing.
	CE-related artifacts ('spikes'). Minor voltage changes or urea crystals passing by the laser can cause 'spikes' or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples. • Perform a new spectral calibration and rerun the samples. • Confirm that the correct spectral was used for the sample run. • Instrument sensitivities can vary. Optimize the injection conditions. Refer to the instrument user manual.
	Dye blob artifacts. The signal strength of certain dye blob artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when left at 4°C for a few days. We recommend storing amplification products at –20°C.
	An incorrect internal lane standard was used. Use the size standard provided in the kit.

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

9.2. Amplification of Extracted DNA

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

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Depending on the DNA purification procedure used and sample source, inhibitors might be present in the DNA sample. This may be more of an issue as DNA sample volume increases as a percentage of the total amplification reaction volume.
	Insufficient template. Use the recommended amount of template DNA if available. Quantify template DNA before use if possible.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl for extracted DNA. Decreasing the reaction volume may result in suboptimal performance.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Amplification of more than the recommended amount of purified DNA can result in a higher number of artifact peaks due to overamplification, resulting in saturating signal. Use the recommended amount of template DNA. See Results section for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Amplification of greater than the recommended amount of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.
	Imbalance may be seen more often when using the maximum template volume or a reduced amplification reaction volume.
	The reaction volume was too low. This system is optimized for a final reaction volume of 12.5µl. Decreasing the reaction volume may result in suboptimal performance.

9.3. GeneMapper® Software

Symptoms	Causes and Comments
Alleles not called	To analyze samples with GeneMapper® Software, the project analysis type must be “Generic”, the analysis method must be “HID” (imported in Section 6.5) and the size standard must have “Basic or Advanced” as the analysis type.
	To analyze samples with GeneMapper® software, at least one allelic ladder must be defined per folder of sample files being analyzed in the project.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the ‘Sample Type’ column.
	To analyze samples with GeneMapper® Software, the project analysis type must be “Generic”, the analysis method must be “HID” (imported in Section 6.5) and the size standard must have “Basic or Advanced” as the analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
	Incorrect polymer was used. Use of a polymer other than Polymer7 may change migration of the fragments. Alleles may migrate outside of the panel range.

Symptoms	Causes and Comments
Size standard not called correctly	Starting data point was incorrect for the partial range chosen for the analysis. Adjust the starting data point or use a full range for the analysis. Alternatively, use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto-Adjust Sizes**.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration and re-run the samples.
	Confirm that the correct spectral was used to run the samples.
No alleles called, but no error message appears	Panels text file or size standard not selected for the sample in the project. Be sure to select the appropriate panels file and size standard for all samples in the project.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger peaks to be missing. This will cause sizing quality to be flagged as 'red', and no allele sized will be called.
Error message: 'Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit'	The bins text file assigned in the analysis method does not match the bins selected in the project. Review the analysis method in the GeneMapper Manager. Confirm the correct bins text file is selected.
Error message after attempting to import Panels and Bins text files: 'Unable to save Panel data: java. SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated'	There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order.

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Appendix 11

11.1. *GenePrint*® 24 System Locus Information

The loci amplified using the *GenePrint*® 24 System are shown in Table 5 and Table 6. Table 7 lists the *GenePrint*® 24 System alleles amplified from 2800M Control DNA.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage (11,12) and terminal nucleotide addition (13,14). 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 (13,14) occurs when a thermostable nonproofeading DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step at 60°C (15) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

Table 5. The *GenePrint*® 24 System Locus-Specific Information.

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

[1]Information about chromosomal location of these loci can be found in references 17, 18 and 19 and at: **https://strbase.nist.gov//chrom.htm**

[2]The August 1997 report (20,21) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used."

[3]Amelogenin is not an STR.

NA = Not Applicable

Table 6. The *GenePrint*® 24 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
Amelogenin[4]	Fluorescein	89, 95	X, Y
D3S1358	Fluorescein	103–147	9–20
D1S1656	Fluorescein	161–208	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	Fluorescein	214–250	8–11, 11.3, 12–17
D10S1248	Fluorescein	255–299	8–19
D13S317	Fluorescein	302–350	5–17
Penta E	Fluorescein	371–466	5–24
D16S539	JOE	84–132	4–16
D18S51	JOE	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE	224–296	10, 12, 14–28
CSF1PO	JOE	318–362	5–16
Penta D	JOE	377–450	2.2, 3.2, 5–17
TH01	TMR-ET	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR-ET	127–183	10–24
D21S11	TMR-ET	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
D7S820	TMR-ET	269–313	5–16
D5S818	TMR-ET	321–369	6–18
TPOX	TMR-ET	393–441	4–16
DYS391	TMR-ET	422–486	5–16
D8S1179	CXR-ET	76–124	7–19
D12S391	CXR-ET	133-185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
FGA	CXR-ET	265–411	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	CXR-ET	425–464	7–20

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

[2]When using an internal lane standard, such as the WEN Internal Lane Standard 500, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label and linker also affect migration of alleles.

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

[4]Amelogenin is not an STR.

Table 7. The *GenePrint*® 24 System Allele Determinations for the 2800M Control DNA.

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

11.2. The WEN Internal Lane Standard 500

The WEN Internal Lane Standard 500 contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 36). Each fragment is labeled with WEN dye and can be detected separately (as a fifth color) in the presence of *GenePrint*® 24-amplified material. The WEN ILS 500 is designed for use in each CE injection to increase precision in analyses when using the *GenePrint*® 24 System. Protocols to prepare and use this internal lane standard are provided in Section 5.

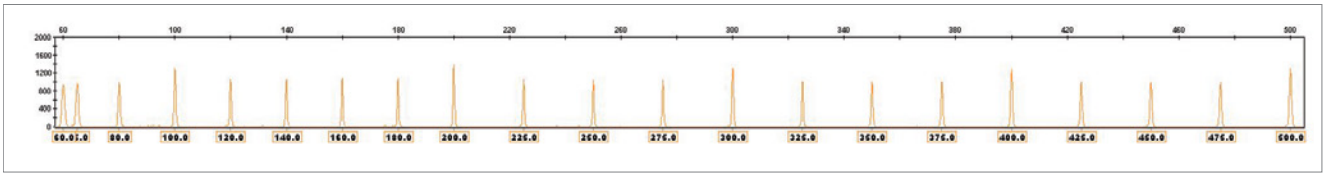

Figure 36. WEN Internal Lane Standard 500. An electropherogram showing the WEN Internal Lane Standard 500 fragments.

11.3. Composition of Buffers and Solutions

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

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

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

TE^{-4} buffer with 20µg/ml glycogen

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

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

11.4. Related Products

PRODUCT	SIZE	CAT.#
GenePrint® 5C Matrix Standard[1]	50µl	B1930
2800M Control DNA (10ng/µl)[2]	25µl	DD7101
2800M Control DNA (0.25ng/µl)[2]	500µl	DD7251
Water, Amplification Grade[2]	6.25ml (5 × 1.25ml)	DW0991

[1]For In Vitro Research Use Only. Not for Diagnostic Procedures.

[2]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 Polymer7	4 × 64 wells	CE2307
Spectrum Compact Buffer	2 pairs	CE2300
Spectrum Compact Cathode Septa Mat	10 each	CE2301
Spectrum Compact Cathode Retainer	4 each	CE2302
Spectrum Compact Strip Base & Retainer, 32-Well	4 each	CE2332
Strip Septa Mat, 8-Well	24 each	CE2308

Not for Medical Diagnostic Use.

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

[b]TMR-ET, CXR-ET and WEN dyes are proprietary.

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Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Class 1 Laser Product.