



GenePrint® 10 System for Use on the Spectrum Compact CE System Technical Manual

Instructions for Use of Product B9510



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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

Description

Cell line misidentification is an important concern for researchers. In some cases, laboratories have invested substantial time and effort researching cell lines that were later revealed to be misidentified (1). This has prompted the National Institutes of Health to issue a notice to researchers strongly recommending authentication procedures when using cultured cells (2). Genetic profiling can be used for human cell line authentication using short tandem repeat (STR) loci (3–6). Capes-Davis *et al.* published a list of misidentified or contaminated human cell lines (7); the updated list can be found at: iclac.org/databases/cross-contaminations/

STR loci consist of short, repetitive sequence elements 3–7 base pairs in length. These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction. 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 American Tissue Culture Collection Standards Development Organization Workgroup recently issued standard ASN-0002 (8), which recommends the use of at least eight STR loci (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317 and D5S818), plus Amelogenin for gender identification, for human cell line authentication. The International Cell Line Authentication Committee (ICLAC) has posted a worksheet that can be used to calculate the match criteria for human cell lines. The worksheet can be found via the References link on their web site at: iclac.org/resources/match-criteria-worksheet/

The *GenePrint*® 10 System allows co-amplification and three-color detection of nine human loci, including the ASN-0002 loci (TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317 and D5S818) as well as D21S11. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92×10^9 .

The *GenePrint*[®] 10 System contains all materials necessary to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the *GenePrint*[®] 10 5X Master Mix. An internal lane standard (ILS) and allelic ladder are provided for sizing and genotyping of amplified fragments, and the 2800M Control DNA is supplied as a positive control. The ILS is added to every sample after amplification and used within each capillary electrophoresis run to determine the size of each amplified product. The allelic ladder consists of the most common alleles at a particular locus and is used as a standard to positively identify each allele. *GenePrint*[®] 10 Allelic Ladder Mix information, including the size range and repeat numbers for each allele, can be found in Section 11.1. The 2800M Control DNA has a known genotype and can be used to verify genotyping accuracy.

This manual contains a protocol for resolving alleles of the *GenePrint*[®] 10 System using the Spectrum Compact CE System. Instructions for operating the Spectrum Compact CE System are available in the *Spectrum Compact CE System Operating Manual* #TMD058. Amplification and detection instrumentation may vary. You may need to optimize protocols including cycle number and injection conditions (or loading volume) for each laboratory instrument.

The proper panels and bin text files with stutter ratios and size standard .xml file for use with GeneMapper[®] software can be downloaded 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/**

Product Components and Storage Conditions

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PRODUCT	SIZE	CAT.#
<i>GenePrint</i> [®] 10 System	50 reactions	B9510

Not for Medical Diagnostic Use. Cat.# B9510 contains sufficient reagents for 50 reactions of 25µl each.
Includes:

Pre-Amplification Components Box

- 250µl *GenePrint*[®] 10 5X Master Mix
- 250µl *GenePrint*[®] 10 5X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 1.25ml Water, Amplification Grade

Post-Amplification Components Box

- 50µl *GenePrint*[®] 10 Allelic Ladder Mix
- 150µl Internal Lane Standard 600

Storage Conditions:

For long-term storage, 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. For daily use, the *GenePrint*[®] 10 System components can be stored for up to 1 week at 2–10°C. Make sure that the 2800M Control DNA is stored at 2–10°C for **at least 24 hours** before use.

The *GenePrint*[®] 10 5X Primer Pair Mix, *GenePrint*[®] 10 Allelic Ladder Mix and Internal Lane Standard 600 (ILS 600) 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
PunchSolution™ Kit	100 preps	DC9271

The PowerPlex® 4C Matrix Standard (Cat.# DG4800) is required for initial setup of the color separation matrix (See Section 3.2).

Before You Begin

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*[®] 10 System

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The *GenePrint*[®] 10 System is optimized for the GeneAmp[®] PCR System 9700 with a gold-plated silver or silver sample block.

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 9.1.

Materials to be Supplied by the User

- GeneAmp[®] PCR System 9700 with a gold-plated silver or silver sample block (Applied Biosystems)
- microcentrifuge
- MicroAmp[®] optical 96-well reaction plates (Applied Biosystems)
- aerosol-resistant pipette tips

The *GenePrint*[®] 10 System is optimized to amplify 10ng of template DNA in a 25 μ l reaction using the protocol detailed below. Preferential amplification of smaller loci can occur. Expect to see high peak heights for smaller loci and relatively lower peak heights for larger loci if more than the recommended amount of template is used. Reduce the amount of template DNA or number of cycles to correct this.

Amplification Setup

1. Thaw the *GenePrint*[®] 10 5X Master Mix and *GenePrint*[®] 10 5X Primer Pair Mix completely.
Note: Centrifuge tubes briefly to bring contents to the bottom, 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.
4. Add the final volume of each reagent listed in Table 1 to a clean tube. Add Water, Amplification Grade, to the tube first, then add *GenePrint*[®] 10 5X Master Mix and *GenePrint*[®] 10 5X Primer Pair Mix. The template DNA will be added at Step 6.

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

PCR Amplification Mix Component ¹	Volume per Reaction	×	Number of Reactions	=	Final Volume
	to a final volume of				
Water, Amplification Grade	25µl	×		=	
<i>GenePrint</i> [®] 10 5X Master Mix	5µl	×		=	
<i>GenePrint</i> [®] 10 5X Primer Pair Mix	5µl	×		=	
template DNA (10ng) ^{1,2}	up to 15µl				
total reaction volume	25µl				

¹Store 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.

²Apparent DNA concentrations can differ, depending on the DNA quantification method used (9). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

- Vortex the PCR amplification mix for 5–10 seconds, 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.

- Add template DNA (10ng) for each sample to the respective well containing PCR amplification mix.
- For the positive amplification control, vortex the tube of 2800M Control DNA, then add 1µl of 2800M Control DNA to a reaction well containing PCR amplification mix.
- 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.
- Seal the plate.

Optional: Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

This manual contains a protocol for use of the *GenePrint*[®] 10 System with the GeneAmp[®] PCR System 9700. We have not tested other thermal cyclers. For information about other thermal cyclers, please contact Promega Technical Services by e-mail: genetic@promega.com

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 28 cycles work well for 10ng of purified DNA template.

- Place the MicroAmp[®] plate in the thermal cycler.
- Select and run the recommended protocol. Be sure that “Max” mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp[®] PCR System 9700 is provided below. The estimated total cycling time is 1.5 hours.

Note: 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 28 cycles, then:

60°C for 10 minutes

4°C soak

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.


Detection of Amplified Fragments Using the Spectrum Compact CE System


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

Materials to be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 8-tube strips
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer4 (Cat.# CE2304)
- 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 1) to form the sample cartridge (Figure 2), which can be loaded onto the Spectrum Compact CE System. Samples are injected in groups of four, such that one 8-well strip tube can be used for two injections. Each 8-well strip tube corresponds to one lane (A, B, C or D) of the sample cartridge. Samples are injected in groups of four across a lane (not by column). For example, samples in well positions A1 through A4 are injected together, followed by samples in well positions A5 through A8 for the second injection. This pattern is then repeated for samples in lanes B, C and D. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for information on changing the order of injections during run setup.

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

5.1. Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and Hi-Di™ formamide as follows: $[(0.5\mu\text{l ILS}) \times (\# \text{ samples})] + [(9.5\mu\text{l formamide}) \times (\# \text{ samples})]$
Note: The volume of internal lane standard used in the loading cocktail can be adjusted to change the intensity of the size standard peaks based on laboratory preferences.
2. Vortex for 10–15 seconds to mix.
3. Pipet 10µl of formamide/internal lane standard mix into each well of the 8-well strip tube(s).

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

Notes:

1. To prevent cross-contamination, do not reuse Strip Septa Mat, 8-Well. Always use a new Strip Septa Mat, 8-Well, for each 8-well strip tube.
2. Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time, refer to the *Spectrum Compact CE System Operating Manual #TMD058*. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.
3. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.
4. Include an injection of allelic ladder every 4 injections (16 samples).
5. Centrifuge the 8-well strip tube(s) briefly to remove air bubbles from the wells.
6. Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

5.2. Assembling Sample Cartridge

1. Place the 8-well strip tube(s) with the Strip Septa Mat, 8-well, into the strip base (Figure 1). 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 2).

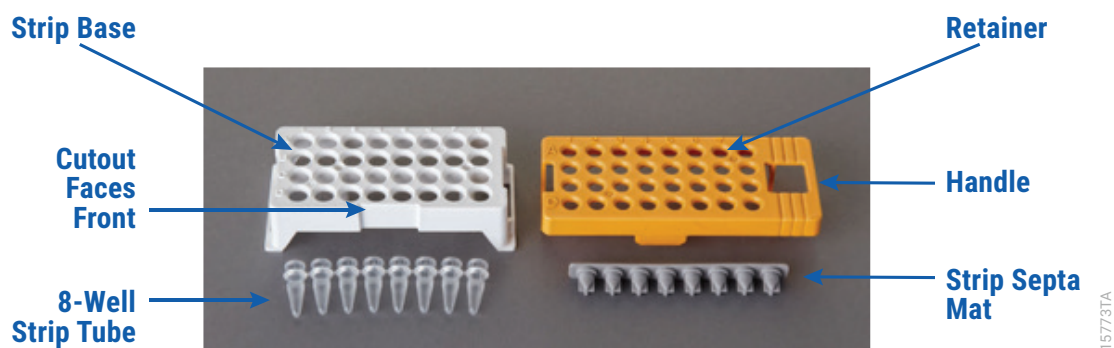


Figure 1. Assembling the Spectrum Compact Strip Base and Retainer.



Figure 2. 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 PowerPlex® 4C 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 *PowerPlex® Matrix Standards for the Spectrum Compact CE System Technical Manual #TMD062* 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 4 and Table 2). Refer to the 'Consumables' screen to determine if any consumables need to be replaced. To access the 'Consumables' screen, select the **Consumables** icon in the Header on the 'Main Menu' screen (Figure 3). The 'Consumables' screen displays information for the four consumables on the instrument: Polymer, Capillary Cartridge and Anode and Cathode Buffers.



Figure 3. Spectrum Compact CE System Software 'Main Menu' screen.

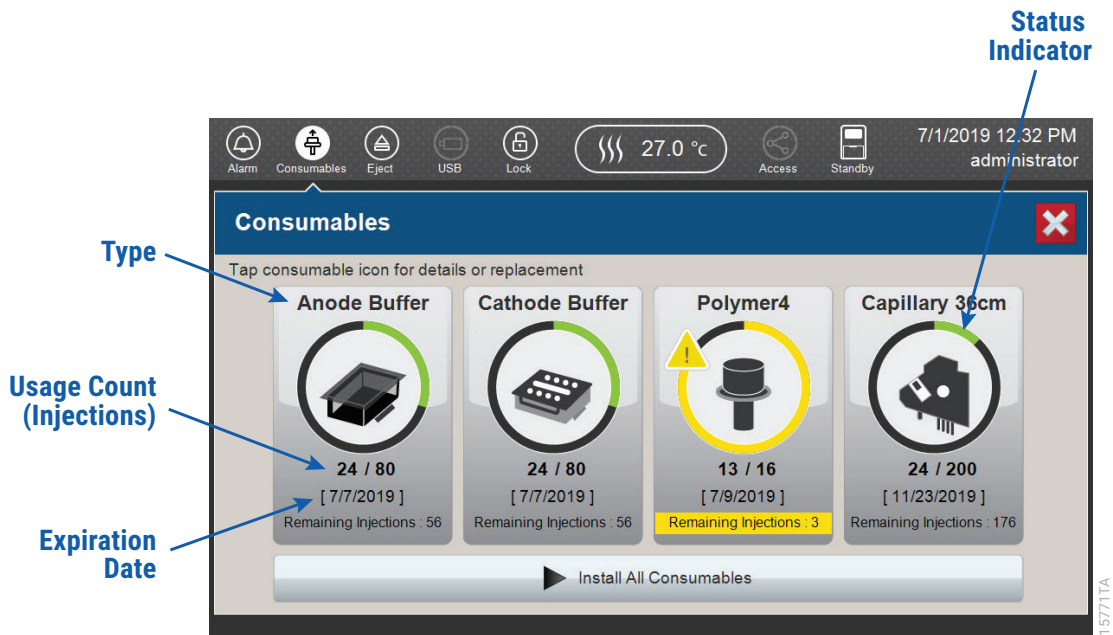





Figure 4. Spectrum Compact CE System 'Consumables' screen. Usage count (number of injections), on-instrument expiration date and remaining injections are displayed on the 'Consumables' screen for each consumable, as well as polymer type.

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 5 to start preheating the oven temperature to 60°C. The temperature displayed will change as the temperature of the oven increases. When 60°C is reached a check mark will appear adjacent to the temperature.

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

Oven Temperature Icon

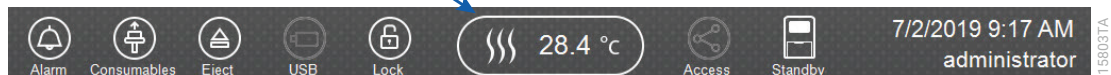


Figure 5. Preheating Oven.

3. Select **Fragment Analysis** on the 'Main Menu' screen (Figure 6).



Figure 6. Spectrum Compact CE System Software 'Main Menu' screen.

- Use the default name that includes date, time and 'Fragment' or enter a Run ID on the 'Set ID' screen (Figure 7). 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:

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

Acceptable Characters	1 to 30 characters for Strip IDs
	1 to 50 characters for Run IDs and sample names
	Upper and lowercase alphabetic characters
	Numbers
	Symbols unless listed below
Unacceptable Characters	¥ / : , ; * ? ' < >
	Spaces

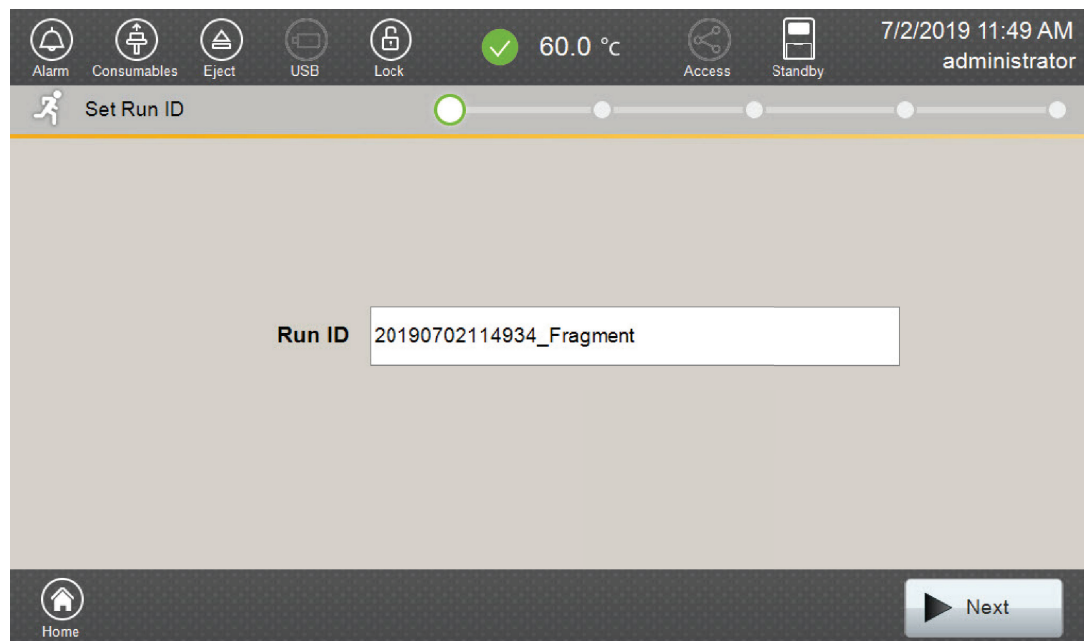


Figure 7. 'Set Run ID' screen.

- Select **Next** to proceed to the message screen for placement of strips into the sample cartridge.
- Follow the message screen for placement of strips into the sample cartridge (Figure 8).

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.

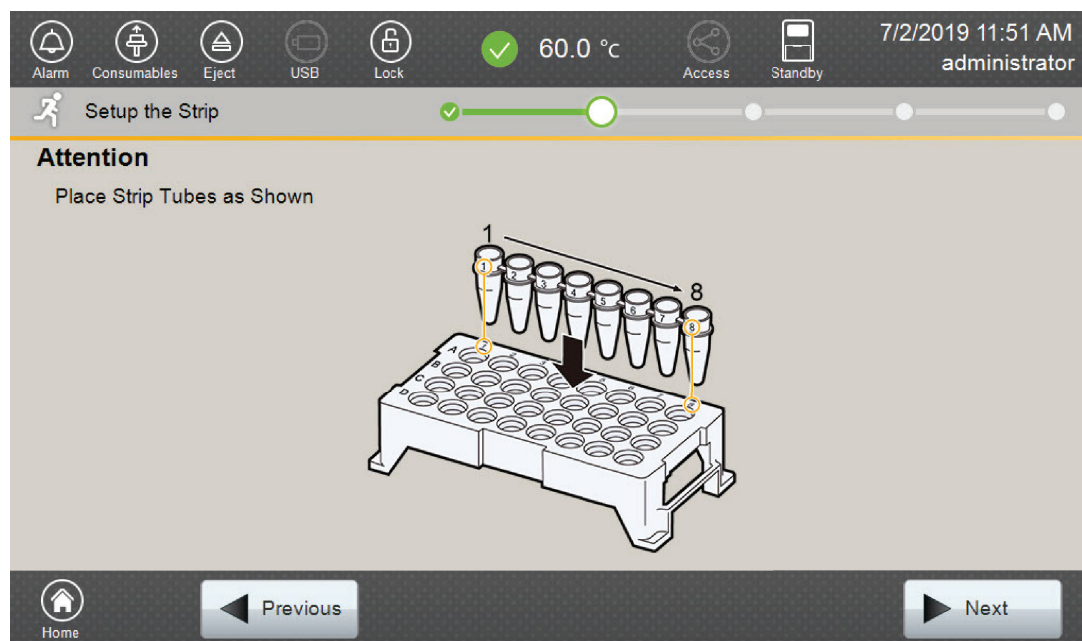


Figure 8. 'Setup the Strip' screen.

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 8. This will take you to the 'Setup Strip Information' screen shown in Figure 9.

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

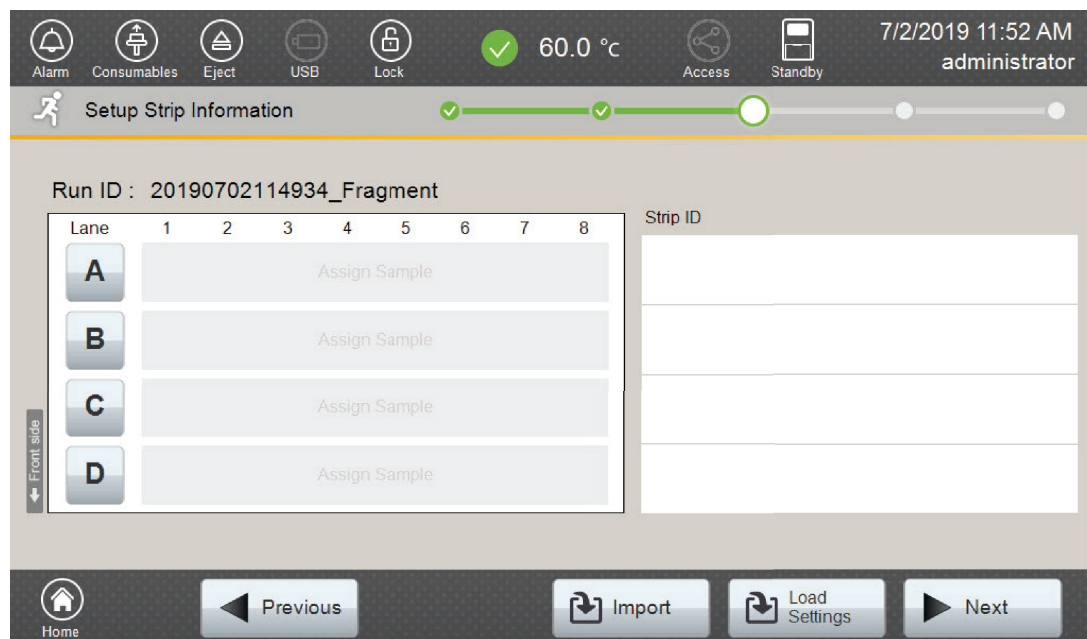


Figure 9. 'Setup Strip Information' screen.

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 10). 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).

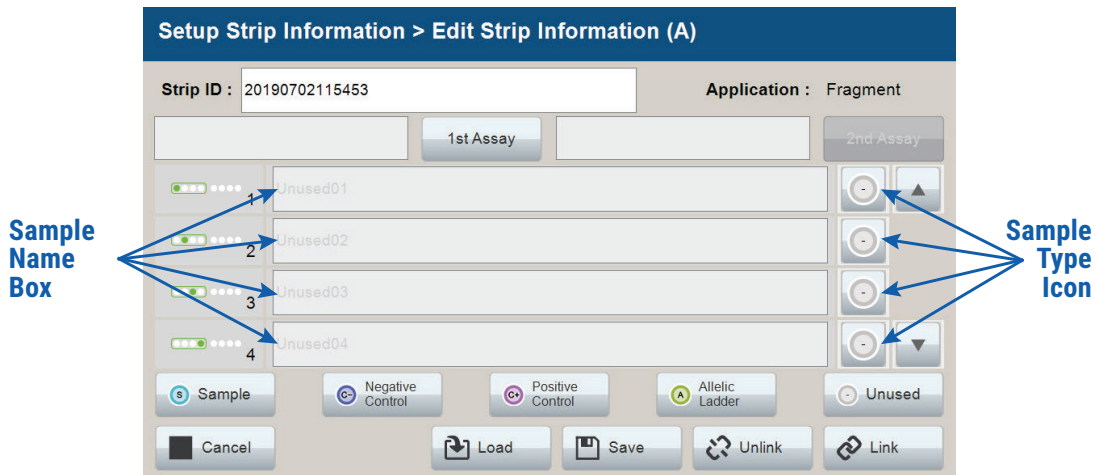


Figure 10. 'Edit Strip Information' screen.

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 11). 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.

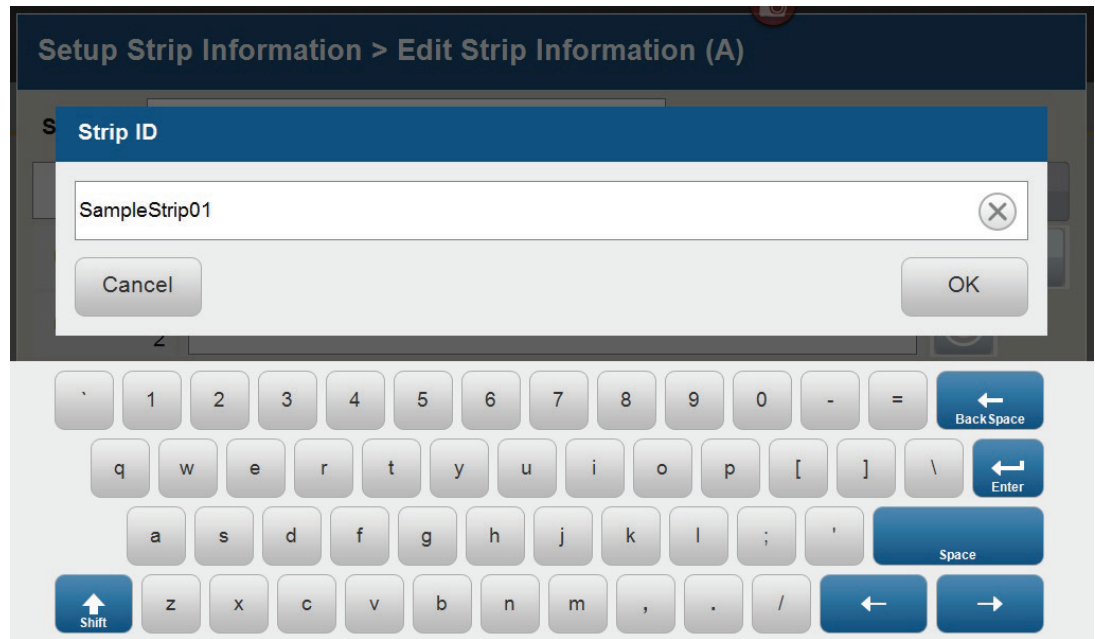







Figure 11. 'Set Strip ID' window.

4. Select a sample type on the 'Edit Strip Information' screen (Figure 12). Sample types must be selected for each well position before the Sample Name box becomes active for entry of sample name. Sample types are as follows.

Symbol	Sample Type
	Sample
	Negative Control
	Positive Control
	Allelic Ladder
	Unused

5. To assign a sample type to a well, select the appropriate sample type along the bottom of the 'Edit Strip Information' screen (Figure 12), 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 13).

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.

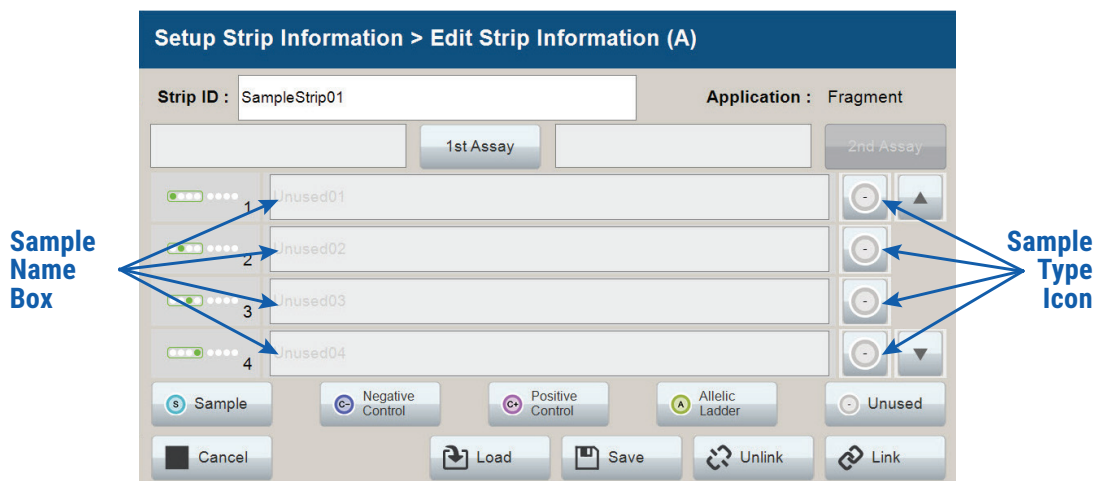


Figure 12. Sample Type and Sample Name on 'Edit Strip Information' screen.

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 13). This opens the 'Set Sample Name' window and a keypad will become active on the touch screen (Figure 14). 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.

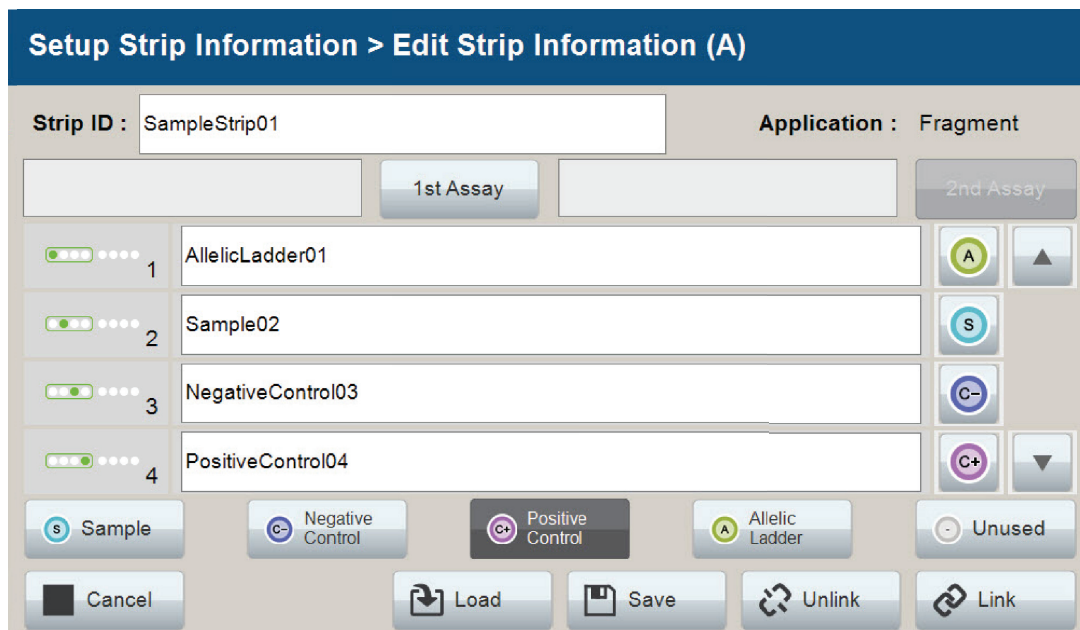


Figure 13. Sample Name Entry on 'Edit Strip Information' screen.

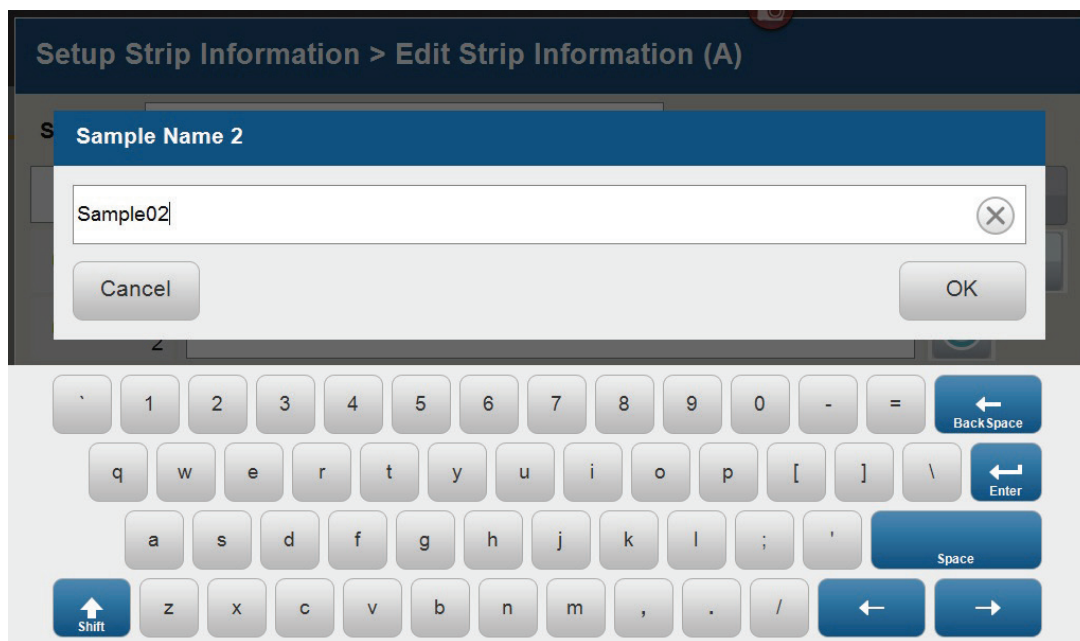


Figure 14. 'Set Sample Name' window.

7. The Spectrum Compact Control Software includes preloaded run assays for use with chemistries available from Promega. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for information on creating or modifying assays. To assign a run assay to an injection set, select **1st Assay** on the right side of the 1st Assay field (Figure 13). This opens the 'Select Assay' window (Figure 15). Select "Promega_4Dye_ILS600_36_P4" from the drop-down list (Figure 15) and then select **Apply**.

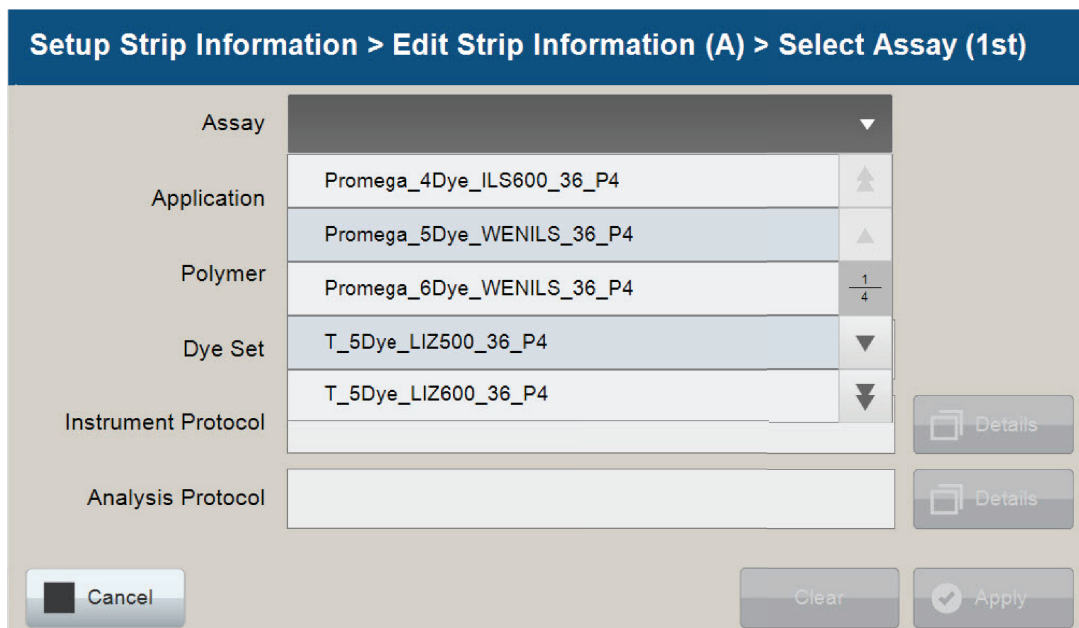


Figure 15. 'Select Assay' window.

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 16). 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.

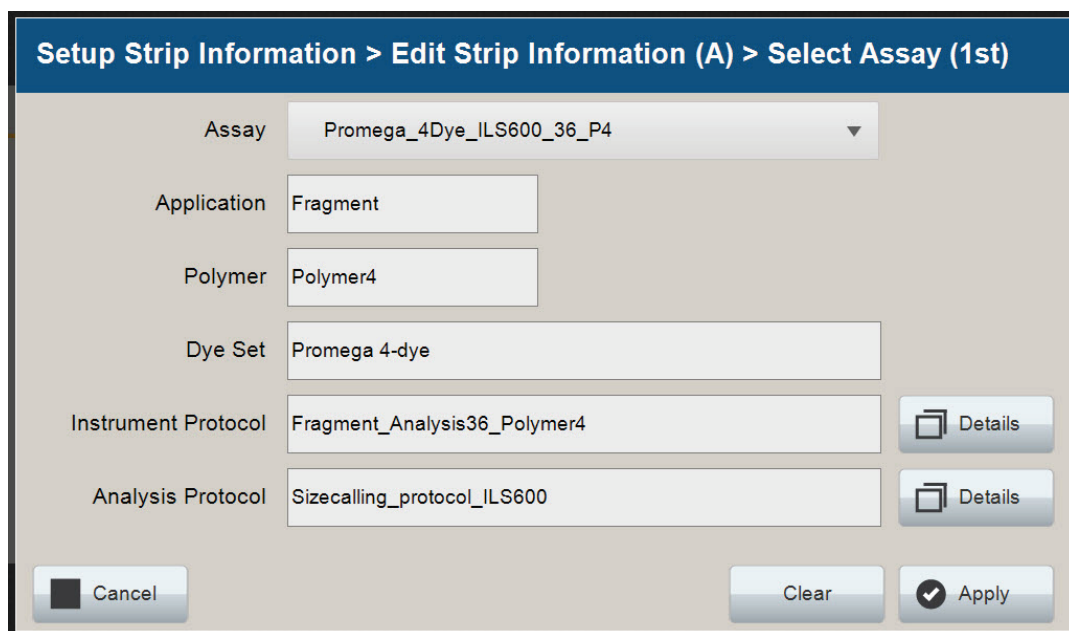


Figure 16. Accessing Instrument and Analysis Protocols on 'Select Assay' window.

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 17). 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 17). This will save the strip information so that it can be loaded later into another run as well as link the strip to the run. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for use of saved strip information.

Notes:

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

Setup Strip Information > Edit Strip Information (A)

Strip ID : SampleStrip01 Application : Fragment

Promega_4Dye_ILS600_36_P4 1st Assay 2nd Assay

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

Sample Negative Control Positive Control Allelic Ladder Unused

Cancel Load Save Unlink Link

Figure 17. 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 18).

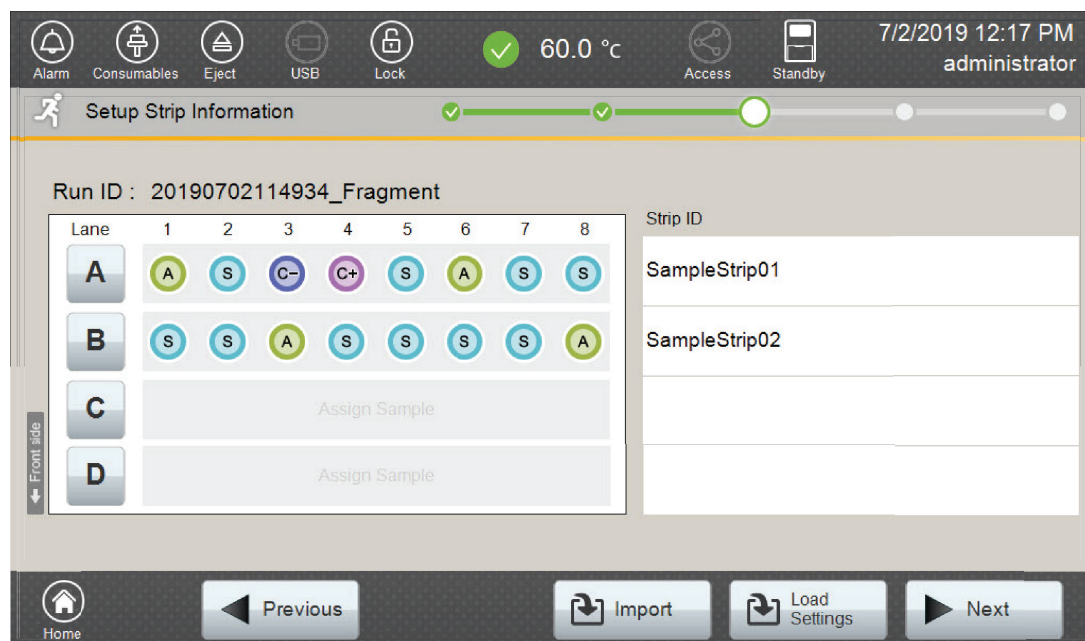


Figure 18. Completed 'Setup Strip Information' screen.

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 19). 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.

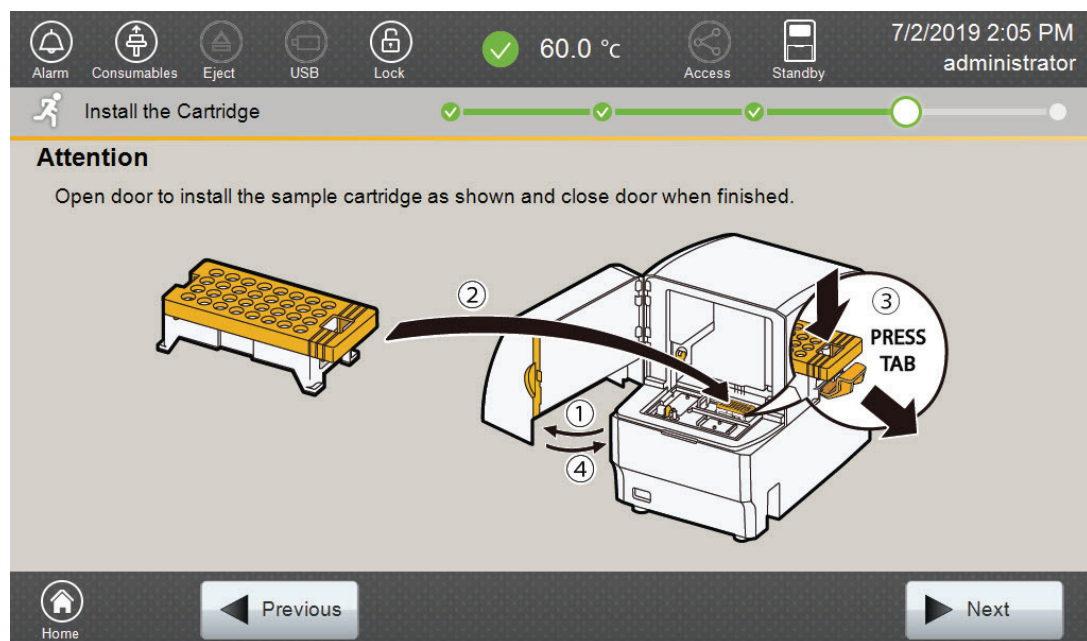


Figure 19. 'Install the Cartridge' screen.

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 20).
5. Select **Run** to start the run.

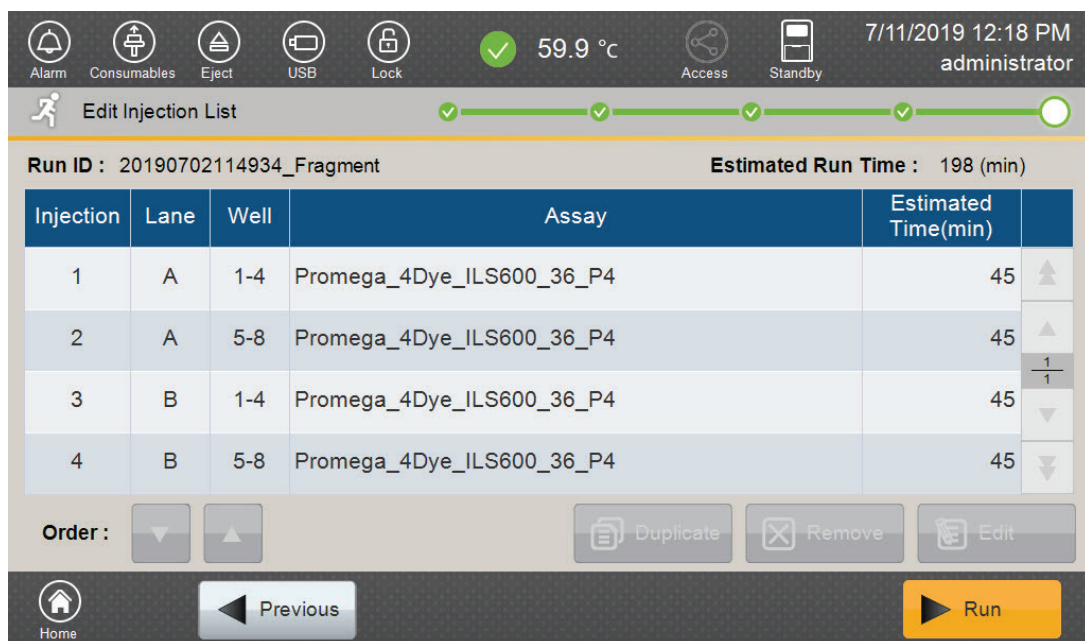


Figure 20. 'Edit Injection List' screen.

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*® 10 data from the Spectrum Compact CE System.

6.1. Importing Files for *GenePrint*® 10 Data Analysis into GeneMapper® Software

To facilitate analysis of data with the *GenePrint*® 10 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.

Note: The analysis method contains a 20% main peak filter.

Getting Started

1. To obtain the analysis files for the *GenePrint*® 10 System, go to:
www.promega.com/GenePrintPanels/
2. Select the *GenePrint*® 10 System, the appropriate GeneMapper 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: an ILS 600/size standard file, a *GenePrint*® 10 panels file, a *GenePrint*® 10 bins file, a Table Settings file, a Plot Settings file and the *GenePrint*® 10 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*® 10 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*® 10 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 ILS 600 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 ILS_600.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**. In the 'Size Standard Editor' window (Figure 21), select **Basic or Advanced** and then **OK**.
4. Enter a detailed name, such as 'ILS_600.'

The screenshot shows the 'Size Standard Editor' window. The 'Name' field is filled with 'ILS_600'. The 'Description' field is empty. The 'Size Standard Dye' dropdown menu is set to 'Red'. The 'Size Standard Table' contains the following data:

	Size in Basepairs
1	60.0
2	80.0
3	100.0
4	120.0
5	140.0
6	160.0
7	180.0
8	200.0
9	225.0
10	250.0
11	275.0
12	300.0
13	325.0
14	350.0
15	375.0
16	400.0
17	425.0
18	450.0
19	475.0
20	500.0
21	550.0
22	600.0

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Figure 21. The GeneMapper® Software, Version 5.0, Size Standard Editor.

5. Choose 'Red' for the Size Standard Dye.
6. Enter the sizes of the internal lane standard fragments (60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases). See Figure 21.
7. 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*® 10 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*® 10 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 22) and Samples Plot view (for Plot Settings, Figure 23).

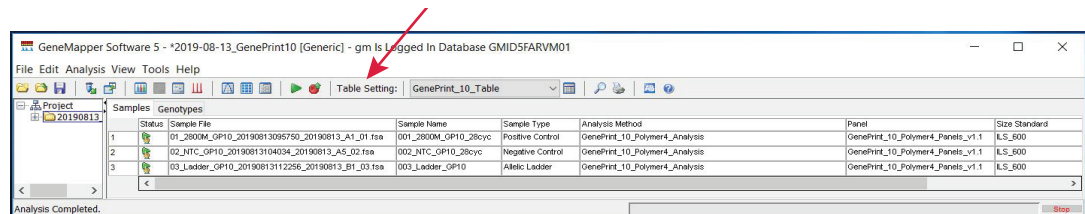


Figure 22. Drop-down menu location for the *GenePrint*® 10 Table Setting in the Samples Tab view.

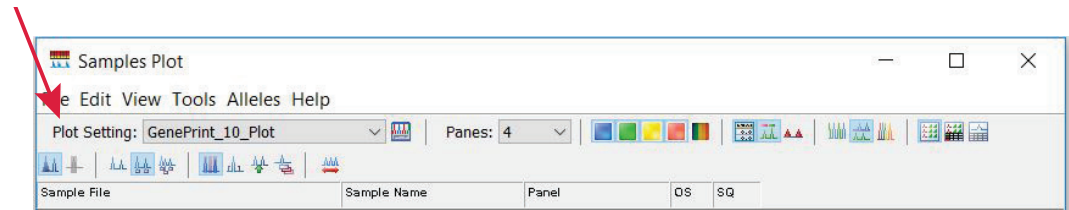



Figure 23. Drop-down menu location for the *GenePrint*® 10 Plot Setting in the Samples Plot view.

6.5. Importing the *GenePrint*® 10 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*® 10 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 24).

 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.

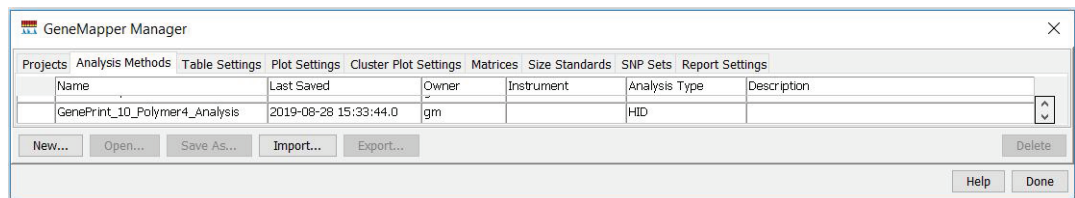


Figure 24. The GenePrint 10 Analysis Method will have 'HID' as the analysis type.

5. To verify the correct bin settings, locate the new *GenePrint*® 10 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_10_Polymer4_bins' from the drop-down menu at the top, if it does not already appear. Verify that all settings appear as shown in Figure 25.

Note: The Cut-off Value in the 'Allele' tab is set at 0.2 for a 20% main peak filter.

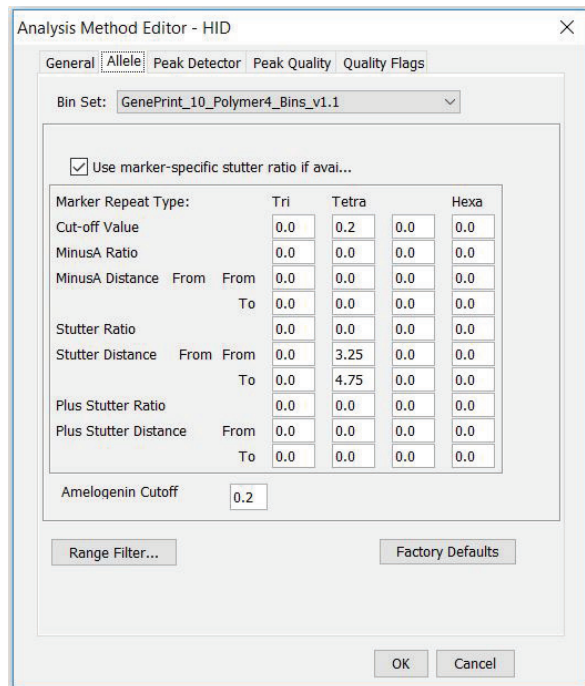


Figure 25. 'Allele Tab' settings.

- 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 26.

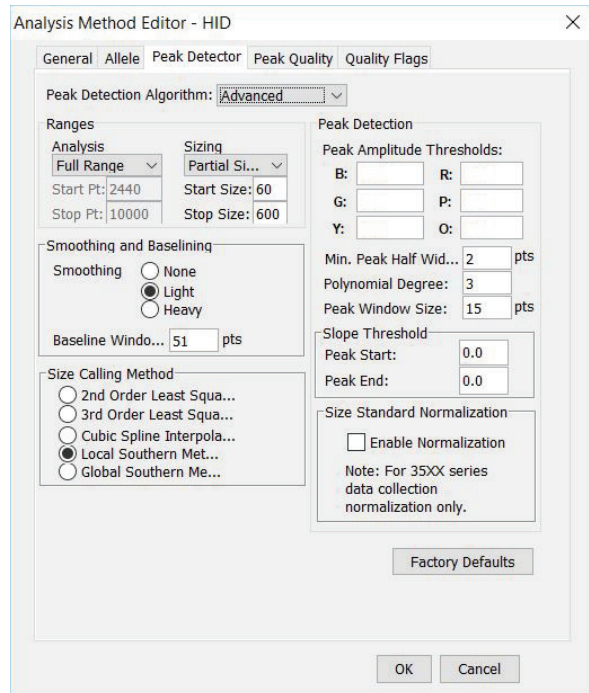


Figure 26. 'Peak Detector' tab settings.

6.6. Analysis of *GenePrint*® 10 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:

- 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.

- In the top center of the window, choose **GenePrint_10_Table** from the drop-down menu.
- 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_10_Polymer4_Analysis** from the drop-down menu.
6. In the 'Panel' column, select **GenePrint_10_Polymer4_Panels** by opening the folder and double-clicking on the file.
7. In the 'Size Standard' column, select **ILS_600**.
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 27.

Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	SFNF	OS	SQ
1	01_2800M_GP10_20190813095750_20190813_A1_01.fsa	001_2800M_GP10_28cyc	Positive Control	GenePrint_10_Polymer4_Analysis	GenePrint_10_Polymer4_Panels_v1.1	ILS_600	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
2	02_NTC_GP10_20190813104034_20190813_AS_02.fsa	002_NTC_GP10_28cyc	Negative Control	GenePrint_10_Polymer4_Analysis	GenePrint_10_Polymer4_Panels_v1.1	ILS_600	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
3	03_Ladder_GP10_20190813112256_20190813_B1_03.fsa	003_Ladder_GP10	Allelic Ladder	GenePrint_10_Polymer4_Analysis	GenePrint_10_Polymer4_Panels_v1.1	ILS_600	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Figure 27. The analyzed project.

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 Software 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 28).

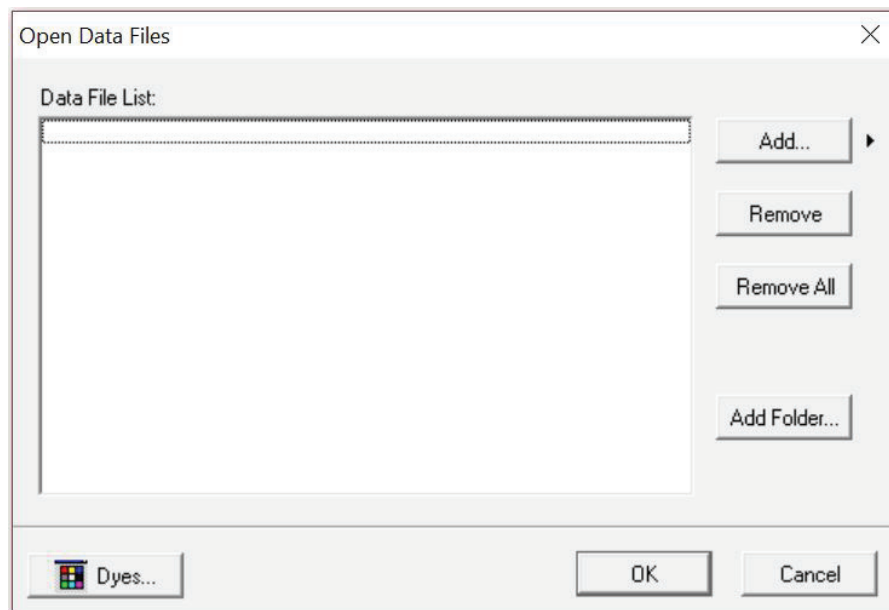


Figure 28. The GMHID-Spectrum Data File List.

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_10 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 29). 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" check box or by dye channel by checking the "Apply Homo/Hetero Settings to Markers in this dye" check box.
7. Select **OK** and close the 'Panel Editor' window. Select **Save Changes** or **Save as New Panel** under the File menu then close the window.

Edit Marker [X]

Marker Parameters:

Marker Name: TH01

Nucleotide Repeats (x): 4

Boundary: 151.7 To 191.7

Min Homozygote Intensity: []

< = Inconclusive <= []

Min Heterozygote Intensity: []

< = Inconclusive <= []

Max Heterozygote Imbalance(%): []

Min Heterozygote Imbalance(%): 20

Apply Homo/Hetero Settings to All Markers

Apply Homo/Hetero Settings to Markers in this dye

Stutter Filter:

Use Marker-Specific Values

N - 2x [0] %

N + x [0] % N - x [6] %

N+0.5x [0] % N-0.5x [0] %

Use Allele-Specific Values(From Panel)

Apply Stutter Settings to All Markers

OK Cancel

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Figure 29. The 'Edit Marker' window for the TH01 marker.

- 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 30). Select the GenePrint_10 template and the settings shown in Figure 30. Verify the panel that you created in Step 6 is selected in the Panel drop-down field. The Size Standard must be ILS600 and the Standard Color must be Red. 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.

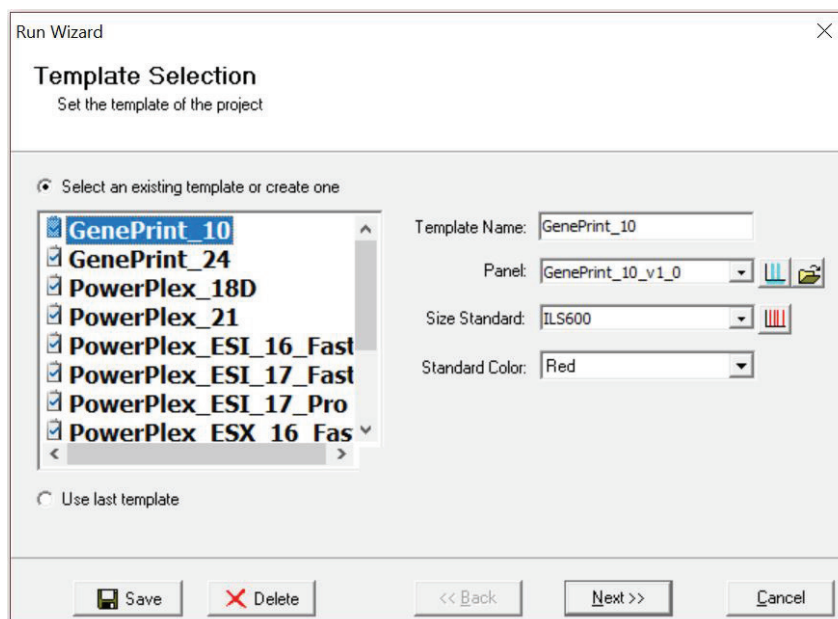


Figure 30. The 'Template Selection' window.

9. The 'Data Process' window will appear (Figure 31). 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**.

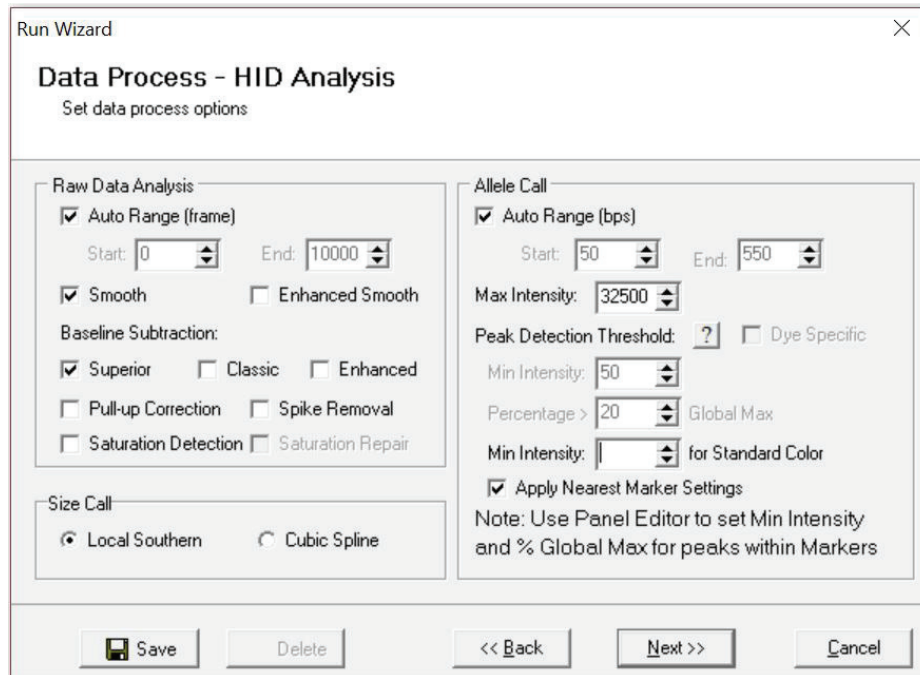


Figure 31. The 'Data Process' window for an analysis method.

- The 'Additional Settings' window will appear (Figure 32). Select **GenePrint_10_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.

Run Wizard

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: GenePrint_10_2800M

P.C. Template 2: NONE

Allele Evaluation
Peak Score:
Reject < 0.00 Check 0.00 < Pass

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.40
 Use Ladder Library
Min Heterozygosity: 0.50
 Auto Panel Adjustment

Save Delete << Back Ok Cancel

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Figure 32. The 'Additional Settings' window.

- 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.

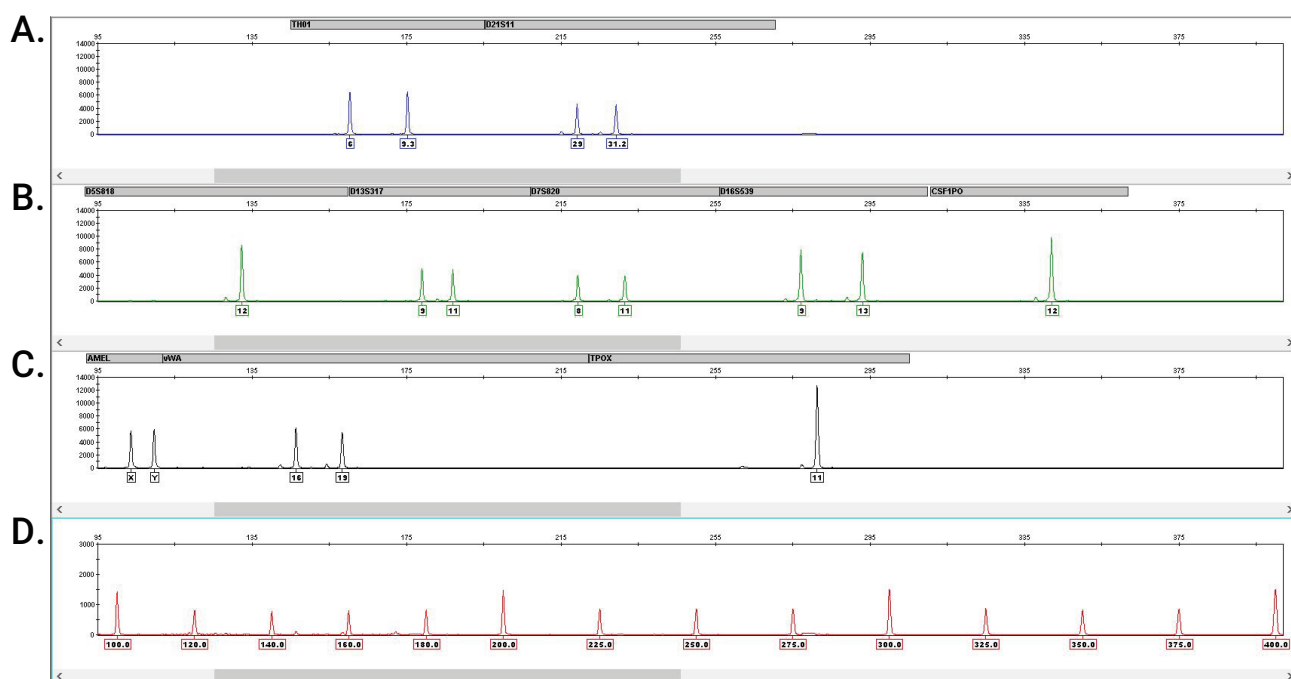
Note: Quality and display settings are set using Preferences under the View menu. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual #TM555* for more information.

7.2. Controls in the GMHID-Spectrum Software

- Observe the results for the negative control. Using the protocols defined in this manual, the negative controls should be devoid of amplification products.
- Observe the results for the 2800M Control DNA. The expected 2800M allele designations for each locus are listed in Table 10 (Section 11.1).

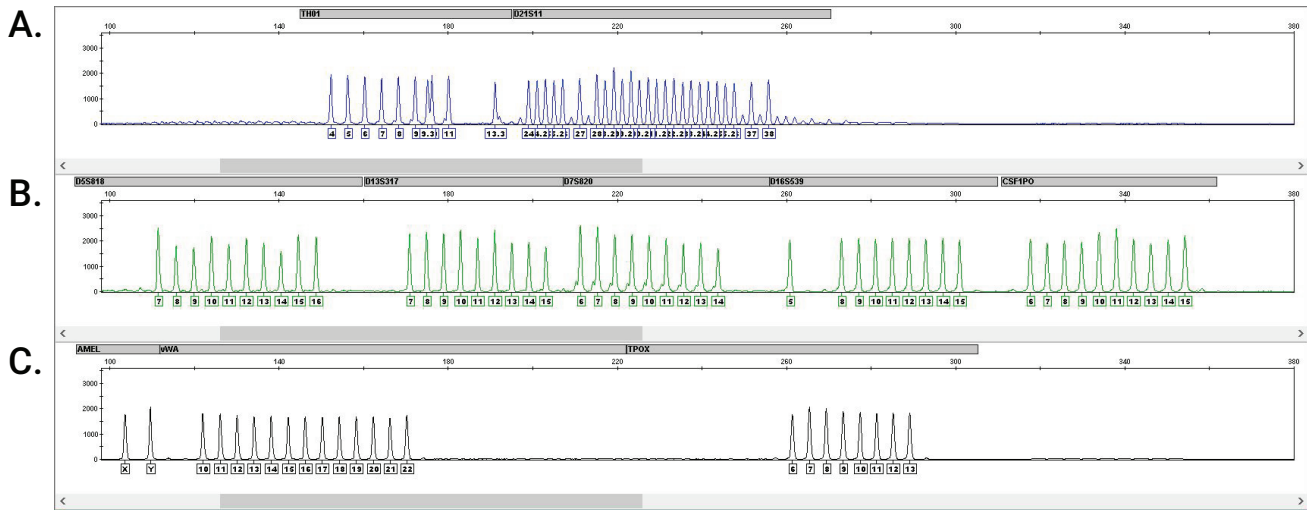
Results

Representative results of *GenePrint*[®] 10 System are shown in Figure 33. The *GenePrint*[®] 10 Allelic Ladder Mix is shown in Figure 34.



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Figure 33. Representative data for the *GenePrint*[®] 10 System. The 2800M Control DNA (10ng) was amplified using the *GenePrint*[®] 10 System. Amplification products were mixed with Internal Lane Standard 600 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 the *GenePrint*[®] 10 panels and bins files. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: TH01 and D21S11. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D5S818, D13S317, D7S820, D16S539 and CSF1PO. **Panel C.** An electropherogram showing the peaks of the TMR-labeled loci: Amelogenin, vWA and TPOX. **Panel D.** An electropherogram showing the 100 to 400 base fragments of the Internal Lane Standard 600.



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Figure 34. The GenePrint® 10 Allelic Ladder. The GenePrint® 10 Allelic Ladder Mix was analyzed with a Spectrum Compact CE System using a 1.6kV, 9-second injection. The sample file was analyzed with the GeneMapper® Software, version 5.0, and the GenePrint® 10 panels and bins 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-labeled allelic ladder components and their allele designations.



Locus-to-locus peak height imbalance will likely occur with cell line DNA. Normal genomic DNA has equal copies of each locus, and amplification will result in relatively even locus-to-locus balance. Cell line DNA can have mutations that affect the locus-to-locus allele peak height balance. Additionally, cell lines occasionally have tri-allelic patterns at a locus. For example, K562 DNA has a tri-allelic pattern at the D21S11 locus. The STR genotype of a cell line can evolve over multiple passages. Users can genotype cell line DNA regularly with the GenePrint® 10 System to monitor any change in the STR genotype.

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. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

In addition to stutter peaks, other artifact peaks can be observed at some *GenePrint*[®] 10 System loci. Low-level products can be seen in the $n-2$ and $n+2$ positions (two bases below and above the true allele peak, respectively) with some loci, such as D21S11. Samples may show low-level artifacts in the noncalling regions between the D7S820 and D16S539 allele ranges. Occasionally an off-ladder artifact can be observed in the 270–271bp position in the JOE dye channel. One or more extra peaks that are not directly related to amplification may be observed at positions 8–26 bases smaller than TPOX alleles. These extra peaks occur when the amplified peaks are particularly intense (high signal level or template amount); the formamide, polymer or capillary was of poor quality; or denaturation was ineffective. Samples with high signal or template amount may show peaks in the $n-1$ position for Amelogenin as well as low level artifact peaks smaller than the allele peaks in D13S317 and vWA.

We have carefully selected STR loci and primers to avoid or minimize artifacts, including those associated with thermostable DNA polymerases, such as repeat slippage and terminal nucleotide addition. Repeat slippage (10,11), sometimes called “ $n-4$ peaks”, “stutter” or “shadow bands”, is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA sample material or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

Terminal nucleotide addition (12,13) occurs when a nonproofreading thermostable 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 of 60°C for 10 minutes (14) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (15,16). D21S11 displays numerous, relatively common microvariants.

CE-related artifacts are occasionally seen in one or all color channels. 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 the samples to confirm.

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 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 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.
	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. <ul style="list-style-type: none"> • 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 necessary.
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.
	<p>Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3' A residue.</p> <ul style="list-style-type: none"> • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Amplification of greater than the recommended amount of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.
	Imbalance may be seen more often when using the maximum template volume or a reduced amplification reaction volume.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.

9.3. Direct Amplification of DNA from Storage Card Punches

The following information is specific to direct amplification of DNA from storage card punches. For additional information about general amplification and detection, see Section 9.1.

Symptoms	Causes and Comments
Faint or absent allele peaks	For direct amplification reactions that require the addition of AmpSolution™ Reagent, its omission can result in inhibition of the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.
	DNA was not accessible on nonlytic material. Pretreat nonlytic materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Poor sample transfer to storage card or variable sampling from storage card. Take punches from a different portion of the card. Increasing cycle number can increase peak heights.
	Too much sample in the reaction can result in inhibition, decreasing intensity of peaks (especially larger amplicons). Be sure to use the recommended number of punches. Follow the manufacturer's recommendations when depositing sample onto the storage card.
	Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.
	The reaction volume was too low. Decreasing the reaction volume from that recommended in the protocol may result in suboptimal performance. Use the recommended number of punches for the reaction volume used.
	Active PunchSolution™ Reagent carried over into amplification reactions with nonlytic card punches. Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. We have not tested longer incubation times.
	Inactive PunchSolution™ Reagent was used to pretreat nonlytic punches. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.

Symptoms	Causes and Comments
Faint or absent allele peaks in the positive control reaction	<p>If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle number, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. Use the recommended amount of 2800M Control DNA per amplification reaction. This mass of DNA should be reduced if cycle number is increased and increased if the cycle number is decreased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively. We do not recommend including blank punches in the 2800M Control DNA reaction.</p>
Extra peaks visible in one or all color channels	<p>Punch contaminated with DNA from another sample. Perform punches on a blank card between samples to minimize potential for carryover between samples.</p>
	<p>Amplification of processed punches with high amounts of DNA can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. Be sure to use the recommended number of punches. Use of a larger punch size or a smaller reaction volume may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number.</p>
	<p>Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain the DNA in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as 'shadow' peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two 'shadow' peaks that differ in size from one another by approximately the same distance as the single-stranded alleles.</p>
	<p>Artifacts of STR amplification. Direct amplification of high amounts of template can result in a higher number of artifact peaks. Use the recommended punch size and number of punches. Optimize the cycle number. See Results section for additional information on stutter and artifacts.</p>
	<p>Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3' A residue.</p> <ul style="list-style-type: none"> • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Decrease cycle number. • Increase the final extension time.

Symptoms	Causes and Comments
Peak height imbalance	<p>Excessive amount of DNA. Amplification of high amounts of template can result in an imbalance, with smaller loci showing more product than larger loci.</p> <ul style="list-style-type: none"> • Be sure to use the recommended number of punches. Follow the manufacturer's recommendations when depositing sample onto the card. • Decrease cycle number.
	<p>The cycle number was too high. Decrease the cycle number by one cycle and repeat the amplification.</p>
	<p>For direct amplification reactions that require the addition of AmpSolution™ Reagent, its omission can result in inhibition of the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.</p>
	<p>Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.</p>
	<p>The reaction volume was too low. Decreasing the reaction volume can result in suboptimal performance due to inhibitors present in lytic cards and PunchSolution™ Reagent.</p>
	<p>DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat nonlytic materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.</p>
	<p>Active PunchSolution™ Reagent carried over into amplification reactions with nonlytic card punches. Larger loci are most susceptible to carryover and will drop out before the smaller loci.</p> <ul style="list-style-type: none"> • Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. • We recommend treating one 1.2mm nonlytic card punch with 10µl of PunchSolution™ Reagent and using one punch per 12.5µl or 25µl amplification reaction. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.
	<p>Inactive PunchSolution™ Reagent was used to pretreat nonlytic punches. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.</p>

Symptoms	Causes and Comments
Extreme variability in sample-to-sample peak heights	There can be significant individual-to-individual variability in the number of cells on a card, resulting in peak height variability between samples. The PunchSolution™ Kit maximizes the recovery of amplifiable DNA from nonlytic punches but does not normalize the amount of DNA present.

9.4. 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.
	<p>Run was too short, and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run.</p> <ul style="list-style-type: none"> • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Symptoms	Causes and Comments
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® 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 Polymer4 may change migration of the fragments. Alleles may migrate outside of the panel range.
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. <ul style="list-style-type: none"> • 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.

Symptoms	Causes and Comments
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. SQLException: 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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11.1. Additional STR Locus Information

Additional information about the human STR loci amplified by the *GenePrint*[®] 10 System can be found in Table 3. *GenePrint*[®] 10 System allelic ladder information can be found in Table 4.

Table 3. The GenePrint® 10 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence¹ 5'→3'
TH01	FL	11p15.5	HUMTH01, human tyrosine hydroxylase gene	AATG (17)
D21S11	FL	21q11–21q21	HUMD21LOC	TCTA Complex (17)
D5S818	JOE	5q23.3–32	NA	AGAT
D13S317	JOE	13q22–q31	NA	TATC
D7S820	JOE	7q11.21–22	NA	GATA
D16S539	JOE	16q24–qter	NA	GATA
CSF1PO	JOE	5q33.3–34	HUMCSF1PO, human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
Amelogenin ²	TMR	Xp22.1–22.3 and Y	HUMAMEL, human Y chromosomal gene for Amelogenin-like protein	NA
vWA	TMR	12p13.31	HUMVWFA31, human von Willebrand factor gene	TCTA Complex (17)
TPOX	TMR	2p23–2pter	HUMTPOX, human thyroid peroxidase gene	AATG

¹The August 1997 report (18,19) 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.'

²Amelogenin is not an STR.

TMR = carboxy-tetramethylrhodamine

FL = fluorescein

JOE = 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

NA = not applicable

Table 4. The GenePrint® 10 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components^{1,2} (bases)	Repeat Numbers of Allelic Ladder Components³
TH01	FL	156–195	4–9, 9.3, 10–11, 13.3
D21S11	FL	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
D5S818	JOE	119–155	7–16
D13S317	JOE	176–208	7–15
D7S820	JOE	215–247	6–14 ³
D16S539	JOE	264–304	5, 8–15
CSF1PO	JOE	321–357	6–15
Amelogenin	TMR	106, 112	X, Y
vWA	TMR	123–171	10–22
TPOX	TMR	262–290	6–13

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

²When using an internal lane standard, such as the Internal Lane Standard 600, 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 also affect migration of alleles.

³HeLa cells have a microvariant allele 13.3 at the D13S317 locus. This will appear as an off-ladder allele (see https://strbase.nist.gov/var_D13S317.htm#Tri).

Table 5. The GenePrint® 10 System Allele Determinations for the 2800M Control DNA.

STR Locus	2800M
TH01	6, 9.3
D21S11	29, 31.2
D5S818	12, 12
D13S317	9, 11
D7S820	8, 11
D16S539	9, 13
CSF1PO	12, 12
Amelogenin	X, Y
vWA	16, 19
TPOX	11, 11

11.2. The Internal Lane Standard 600

The Internal Lane Standard 600 contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length (Figure 35). Each fragment is labeled with carboxy-X-rhodamine (CXR) and can be detected separately (as a fourth color) in the presence of *GenePrint*[®] 10-amplified material. The ILS 600 is designed for use in each CE injection to increase precision in analyses when using the *GenePrint*[®] 10 System. Protocols to prepare and use this internal lane standard are provided in Section 5.

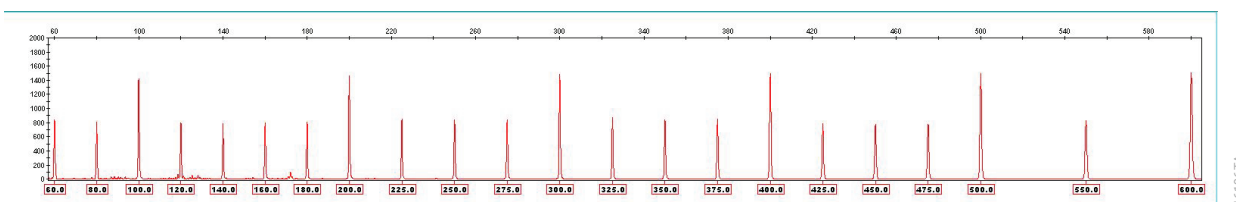


Figure 35. Internal Lane Standard 600. An electropherogram showing the Internal Lane Standard 600 fragments.

11.3. Direct Amplification of DNA from Storage Card Punches

This section contains protocols for depositing human tissue culture cells onto a storage card and direct amplification of DNA from storage card punches using the *GenePrint*[®] 10 System and GeneAmp[®] PCR System 9700.

Materials to Be Supplied by the User

- GeneAmp[®] PCR System 9700 with a gold-plated silver or silver sample block (Applied Biosystems)
- microcentrifuge
- MicroAmp[®] optical 96-well reaction plates (Applied Biosystems)
- aerosol-resistant pipette tips
- PunchSolution[™] Kit (Cat.# DC9271) for nonlytic card punches
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system
- phosphate-buffered saline (1X)
- storage cards (e.g., FTA[®], Fitzco, S&S 903)

Lytic cards (e.g., FTA[®], Fitzco) do not require pretreatment. Pretreat nonlytic sample types (e.g., S&S 903) with the PunchSolution[™] Kit (Cat.# DC9271) to lyse the samples before adding the PCR amplification mix. For more information, see the *PunchSolution*[™] Kit *Technical Manual* #TMD038. Failure to pretreat these samples may result in incomplete profiles.

Punch Card Sample Creation

1. Prepare cell suspensions in phosphate-buffered saline (1X) at a density of 10^6 cells/ml, based on cell counting.
2. While wearing gloves, spot a 20 μ l aliquot of the cell suspension on a storage card. The use of indicating paper ensures that you obtain a punch from the portion of the card that contains deposited cells.
3. Allow the sample to air dry at room temperature.
4. Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate. Automated punchers also can be used to create sample disks. Refer to the user's guide for your instrument for assistance with generating 1.2mm disks, technical advice and troubleshooting information.

Note: Static may be problematic when adding a punch to a well. For lytic card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonlytic card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

Amplification Setup

1. Thaw the *GenePrint*® 10 5X Master Mix and *GenePrint*® 10 5X Primer Pair Mix completely.
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 negative control reactions but not positive control reactions; a separate PCR amplification mix will be prepared in Step 5 for the positive control reaction. 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.

4. Prepare a PCR amplification mix for the direct-amplification samples and negative controls by adding the final volume of each reagent listed in Table 6 to a clean tube. Add Water, Amplification Grade, to the tube first, then add *GenePrint*[®] 10 5X Master Mix and *GenePrint*[®] 10 5X Primer Pair Mix. For lytic card punches, the template DNA will be added at Step 7.

Table 6. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches.

PCR Amplification Mix Component ¹	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	15µl	×		=	
<i>GenePrint</i> [®] 10 5X Master Mix	5µl	×		=	
<i>GenePrint</i> [®] 10 5X Primer Pair Mix	5µl	×		=	
total reaction volume	25µl				

5. For the positive control reaction, prepare a PCR amplification mix for amplification of the 2800M Control DNA by adding the final volume of each reagent listed in Table 7 to a clean tube. Add Water, Amplification Grade, to the tube first, then add *GenePrint*[®] 10 5X Master Mix and *GenePrint*[®] 10 5X Primer Pair Mix. For the positive control reaction, the template DNA will be added at Step 8.

Table 7. PCR Amplification Mix for Amplification of 2800M Control DNA.

PCR Amplification Mix Component ¹	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	10µl	×		=	
<i>GenePrint</i> [®] 10 5X Master Mix	5µl	×		=	
<i>GenePrint</i> [®] 10 5X Primer Pair Mix	5µl	×		=	
2800M Control DNA, 10ng/µl ¹	5µl				
total reaction volume	25µl				

¹The volume of 2800M Control DNA may require optimization. We recommend 2.5–5µl of 2800M Control DNA per positive control reaction, depending on the number of amplification cycles. If you are using a higher number of PCR cycles, use less 2800M Control DNA and adjust the volume of Water, Amplification Grade, so that the final reaction volume is 25µl.

6. Vortex the PCR amplification mix for 5–10 seconds. For direct-amplification samples and negative control reactions, pipet 25µl of the PCR amplification mix prepared in Step 4 into the appropriate reaction wells. For the positive control, pipet 20µl of the PCR amplification mix prepared in Step 5 into the appropriate reaction well.



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

7. For lytic storage cards, add one 1.2mm punch from a card containing human tissue directly to the appropriate wells of the reaction plate. For nonlytic card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

Note: It is also acceptable to add the lytic card punch first, and then add the PCR amplification mix.

8. For the positive amplification control, vortex the tube of 2800M Control DNA, and then add 5µl of the 2800M Control DNA to a reaction well containing 20µl of PCR amplification mix.

Notes:

1. Do not include blank storage card punches in the positive control reactions.
 2. Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences. Typically, 50ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.
9. Reserve a well containing PCR amplification mix as a negative amplification control.

Note: An additional negative control with a blank punch may be performed to detect contamination from the storage card or punch device.
 10. Seal the plate, and briefly centrifuge the plate to bring storage card punches to the bottom of the wells.

Thermal Cycling

This manual contains a protocol for use of the *GenePrint*[®] 10 System with the GeneAmp[®] PCR System 9700 with a gold-plated silver or silver sample block. We have not tested other thermal cyclers. For information about other thermal cyclers, please contact Promega Technical Services by e-mail: genetic@promega.com

Amplification and detection instrumentation may vary. You will need to optimize protocols, including cycle number (25–27 cycles), injection time and loading volume for your laboratory instrumentation. Testing at Promega shows that 26 cycles works well for a variety of sample types.

1. Place the MicroAmp[®] plate in the thermal cycler.
2. Select and run the recommended protocol. Be sure that 'Max' mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp[®] PCR System 9700 is provided below. The estimated total cycling time is 1.5 hours.

Note: 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

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

PCR Optimization

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Be sure to pretreat nonlytic samples with the PunchSolution™ Kit (Cat.# DC9271).
3. Prepare three identical reaction plates with punches from the same samples.
4. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25, 26 and 27 cycles).
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

11.4. Composition of Buffers and Solutions

Phosphate-buffered saline (1X)

8g NaCl
0.2g KCl
1.44g Na₂HPO₄ • 2H₂O
0.24g KH₂HPO₄

Dissolve chemicals in 800ml of deionized water. Adjust to pH 7.4 with HCl. Bring the final volume to 1 liter with deionized water. Sterilize by autoclaving.

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

1.21g Tris base
0.037g EDTA (Na₂EDTA • 2H₂O)

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 (Na₂EDTA • 2H₂O)
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.

11.5. Related Products

Sample Preparation Systems

PRODUCT	SIZE	CAT.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361
Wizard® Genomic DNA Purification Kit	100 isolations × 300µl	A1120
	500 isolations × 300µl	A1125
	100 isolations × 10ml	A1620
PunchSolution™ Kit*	100 preps	DC9271

*Not for Medical Diagnostic Use.

Maxwell® Automated Nucleic Acid Purification

PRODUCT	SIZE	CAT.#
Maxwell® RSC Cultured Cells DNA Kit	48 preps	AS1620

Accessory Components

PRODUCT	SIZE	CAT.#
PowerPlex® 4C Matrix Standard*	5 preps	DG4800
Internal Lane Standard 600	150µl	DG1071
2800M Control DNA (10ng/µl)*	25µl	DD7101
Water, Amplification Grade*	6,250µl (5 × 1,250µl)	DW0991

*Not for Medical Diagnostic Use.

Spectrum Compact CE System Accessories and Consumables

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

Not for Medical Diagnostic Use.

Summary of Change

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The following change was made to the 9/21 revision of this document:

Updated character rules table in Section 5.3.

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