TECHNICAL MANUAL

GenePrint® 24 System

Instructions for Use of Products
B1870 and B1874

Revised 6/20
TM465

Promega

GenePrint® 24 System

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

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1. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–9). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using fluorescence detection following electrophoretic separation.

The *GenePrint*® 24 System[a,b] is a 24-locus multiplex system designed to generate a multi-locus human DNA profile from a variety of human-derived biological sources. This five-color system allows co-amplification and fluorescent detection of the following autosomal STR loci: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D10S1248, D22S1045, D2S441, D1S1656, D12S391, D2S1338, D19S433, Penta D and Penta E plus Amelogenin for gender determination. In addition, the male-specific DYS391 locus is included to identify null Y allele results for Amelogenin.

The *GenePrint*® 24 System provides all materials necessary to amplify the STR loci listed above from human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the *GenePrint*® 24 5X Master Mix. This manual contains protocols for use of the *GenePrint*® 24 System with the GeneAmp® PCR System 9700 and Veriti® 96-well standard thermal cyclers, in addition to protocols to separate and detect amplified products on the Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. A protocol to operate the fluorescence-detection instrument should be obtained from the instrument manufacturer.

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

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

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

Information about other Promega fluorescent STR systems is available at: **www.promega.com**

2. Product Components and Storage Conditions

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

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

Pre-amplification Components Box

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

Post-amplification Components Box

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

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

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

Pre-amplification Components Box

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

Post-amplification Components Box

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

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

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

The proper panels and bin text files with stutter ratios and size standard .xml file for use with GeneMapper® 5.0 software can be downloaded at: **www.promega.com/resources/software-firmware/str-analysis/geneprint-systems-software-panels-and-bin-files/**

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3. Before You Begin

3.A. 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. Always wear gloves and use 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.B. Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument. The matrix standards are provided separately (*GenePrint*® 5C Matrix Standard, Cat.# B1930).

For protocols and additional information about performing the spectral calibration on these instruments using POP-7™ Polymer, see the GenePrint® *5C Matrix Standard Technical Manual* #TM475.

Note: This technical manual assumes that the Applied Biosystems® 3130, 3130*xl*, 3500 or 3500xL Genetic Analyzer has a passing spatial calibration.

For the Applied Biosystems® 3500 and 3500xL Genetic Analyzers, an initial Applied Biosystems G5 instrument validation and performance check must be performed prior to installing the Promega 5C spectral calibration.

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

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

The *GenePrint*® 24 System is optimized for the GeneAmp® PCR System 9700 thermal cycler with a gold-plated silver or silver sample block and the Veriti® 96-Well Thermal Cycler, 0.2ml.

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.

We routinely amplify 2.5–5.0ng of template DNA in a 12.5µl reaction volume using 26–27 cycles and the protocol described below.

Materials to Be Supplied by the User

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

Amplification Setup

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

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

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples and that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label it appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label them appropriately.

4. Protocol for DNA Amplification Using the *GenePrint*® 24 System (continued)

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

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

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

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

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

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

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

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

6. Add template DNA for each sample to the respective well containing PCR amplification mix.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 1.0ng/µl. Add 2.5ng or 5.0ng of diluted DNA to a reaction well containing PCR amplification mix.

8. For the negative amplification control, pipet Water, Amplification Grade, or TE^{-4} buffer instead of template DNA into a reaction well containing PCR amplification mix.

9. Seal or cap the plate, or close the tubes.

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

Thermal Cycling

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

Instrument	Average Peak Height (RFU)[1]
Applied Biosystems® 3500 and 3500xL Genetic Analyzer	4,000–12,000RFU
Applied Biosystems® 3130 and 3130*xl* Genetic Analyzer	2,000–5,000RFU

[1]Target peak heights should be determined by each laboratory. CE instrument sensitivities vary by as much as twofold within instrument type and fourfold or more between the Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers, and the Applied Biosystems® 3500 and 3500xL Genetic Analyzers.

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

   Notes:

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

Thermal Cycling Protocol
96°C for 1 minute, then:
94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 26–27 cycles, then:
60°C for 10 minutes
4°C soak

4. Protocol for DNA Amplification Using the *GenePrint*® 24 System (continued)

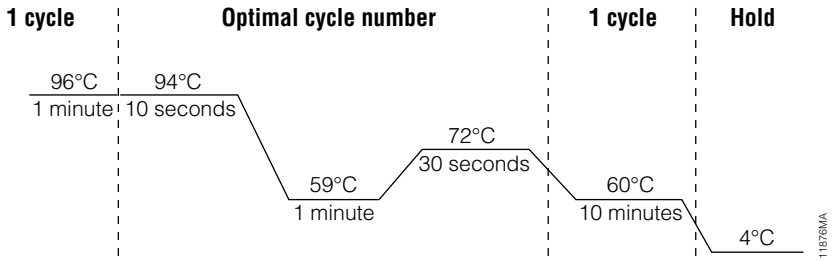

Figure 1. The thermal cycling protocol for the GeneAmp® PCR System 9700 with gold-plated silver and silver blocks and Veriti® 96-well standard thermal cyclers.

3. After completion of the thermal cycling protocol, immediately proceed with capillary electrophoresis. Alternatively, store amplified samples at –20°C in a light-protected box.

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

5. Sample Preparation and Instrument Setup

A matrix must be generated for each individual capillary electrophoresis (CE) instrument prior to analyzing samples amplified with the *GenePrint*® 24 System. For protocols and additional information about performing spectral calibration using POP-7™ Polymer, see the GenePrint® *5C Matrix Standard Technical Manual*, #TM475.

5.A. Amplicon Preparation Prior to Capillary Electrophoresis

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or a freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- MicroAmp® optical 96-well plate (or equivalent) and septa (Applied Biosystems)
- 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 can 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.

1. Prepare a loading cocktail by combining and mixing Internal Lane Standard (ILS) and Hi-Di™ formamide as follows:

   [(0.5µl ILS) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be adjusted to change the intensity of the size standard peaks based on laboratory preferences.

2. Vortex for 10–15 seconds to mix.

3. Pipet 10µl of formamide/internal lane standard mix into each well.

4. Add 1µl of amplified sample (or 1µl of Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

   Notes:

   1. 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 in the run module, select "Instrument Protocol" from the Library menu in the data collection software. 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.
   2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your laboratory's optimization.

5.A. Amplicon Preparation Prior to Capillary Electrophoresis (continued)

5. Centrifuge the plate briefly to remove air bubbles from the wells.

6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice, a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

   Note: Do not use a thermal cycler block to snap cool the samples. Amplified fragments can reanneal and cause additional peaks during analysis.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer

Materials to Be Supplied by the User

- 3500/3500xL capillary array, 50cm
- plate retainer and base set (standard)
- POP-7™ Polymer for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container

Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for the instrument maintenance schedule and instructions to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 2). To ensure that you are viewing the most up-to-date information, press the Refresh button. Ensure that the Consumables Information and Maintenance Notifications are acceptable.

   Set the oven temperature to 60°C, and then select **Start Pre-Heat**.

   Note: The instrument manufacturer recommends that you preheat the oven for at least 30 minutes before you start a run if the instrument is at ambient temperature.

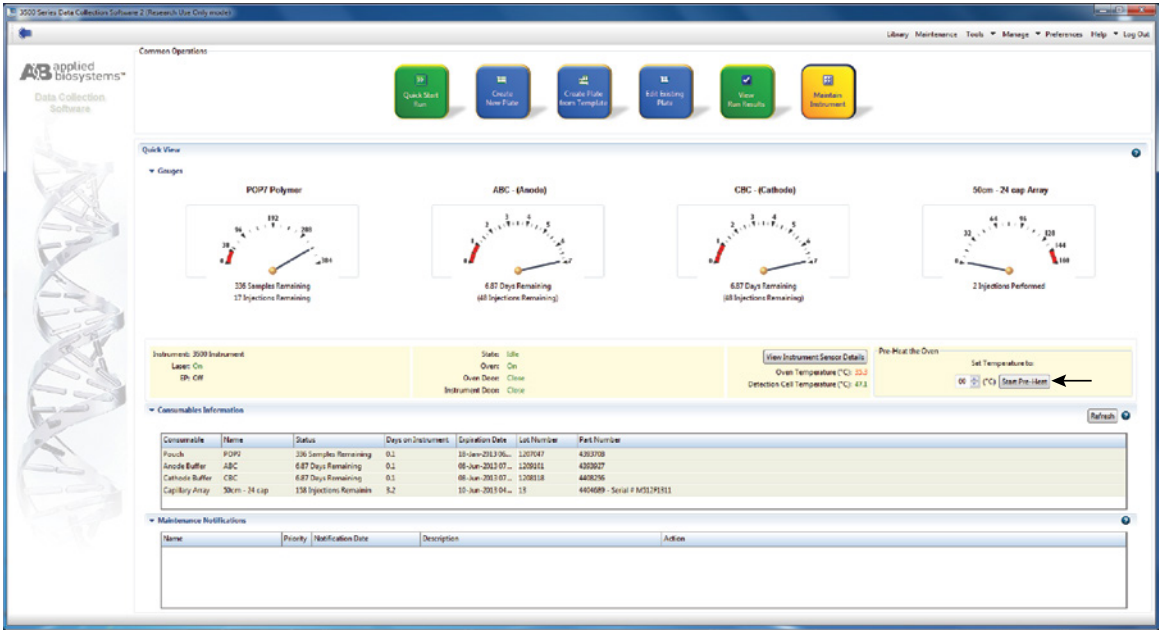

Figure 2. The Dashboard.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

Prior to the first analysis using the *GenePrint*® 24 System, you must create an Instrument Protocol, Size Standard, QC Protocol, Assay, File Name Convention and Results Group as described in Steps 2–7.

2. Create an Instrument Protocol as follows:

   a. Navigate to the Library, select **Instrument Protocols** and choose **Fragment** as the Application Type (Figure 3).

   b. From the list of protocols, choose the default protocol that is most similar to the one you will be using and duplicate it. For example, if you are using an Applied Biosystems® 3500xL Genetic Analyzer with a 50cm array and POP-7™ Polymer, choose **FragmentAnalysis50_POP7xl**, and select **Duplicate**.

   Do not choose **Create**. Alternatively, a previously created Instrument Protocol can be used.

   c. Edit the copy of this Instrument Protocol, and fill in the drop-down menus as appropriate for the chosen configuration. Make sure you choose **Fragment** as the Application Type, and select the dye set created in Section 3.B. The Run Module will be **FragmentAnalysis50_POP7xl**.

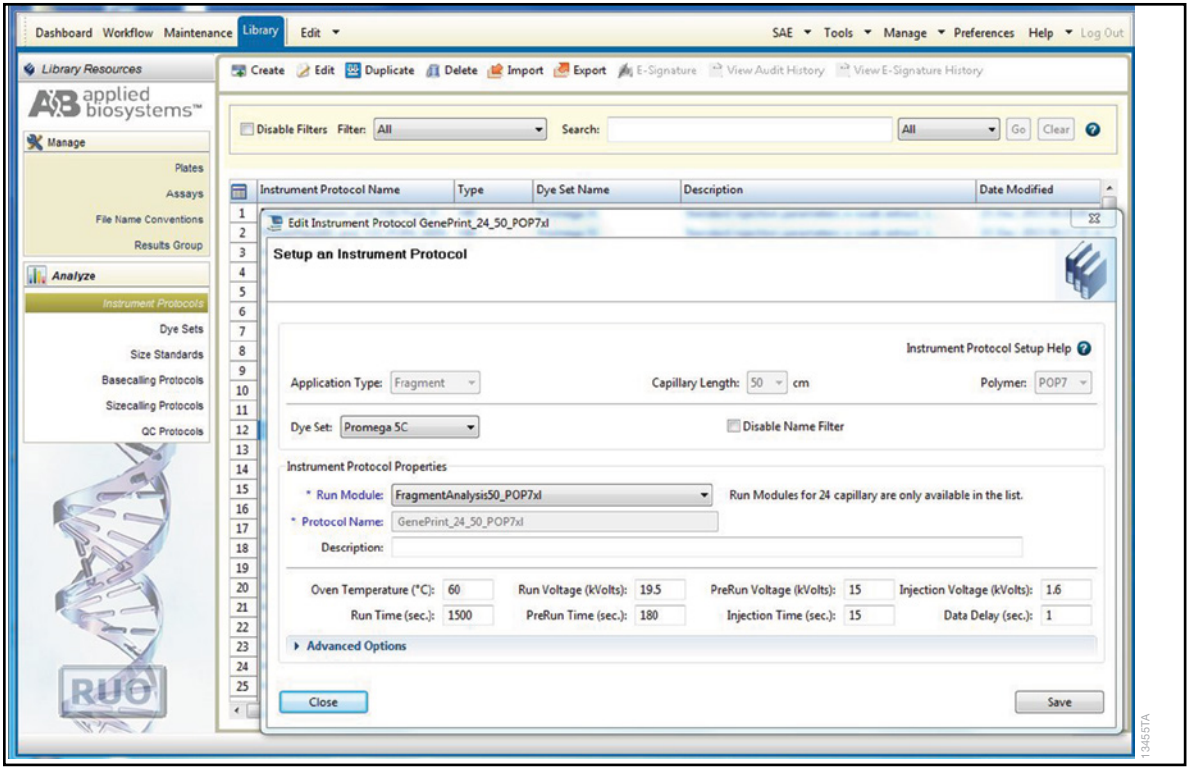

Figure 3. The 'Setup an Instrument Protocol' window.

d. Assign the Instrument Protocol a meaningful name to clarify its use for future runs.

The recommended settings are:

Application Type	Fragment
Capillary Length	50cm
Polymer	POP-7™
Dye Set	Promega 5C
Run Module	FragmentAnalysis_50_POP7xl
Injection Time[1]	15 seconds
Injection Voltage	1.6kV
Run Voltage	19.5kV
Run Time	1,500 seconds

[1]Injection time may be modified to increase or decrease peak heights.

When creating an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 5C spectral calibration.

Default parameters for oven temperature, pre-run time, pre-run voltage and data delay have been successfully tested. Run time and other instrument settings should be optimized in your laboratory.

3. Create a new Size Standard. To create a new Size Standard for the QC protocol, navigate to the Library. Select **Size Standards**, and then select **Create**. Alternatively, a previously created Size Standard can be used.

   Assign the Size Standard a descriptive name such as "WEN ILS 500". Choose **Orange** as the Dye Color. The fragments in the size standard are 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 4.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

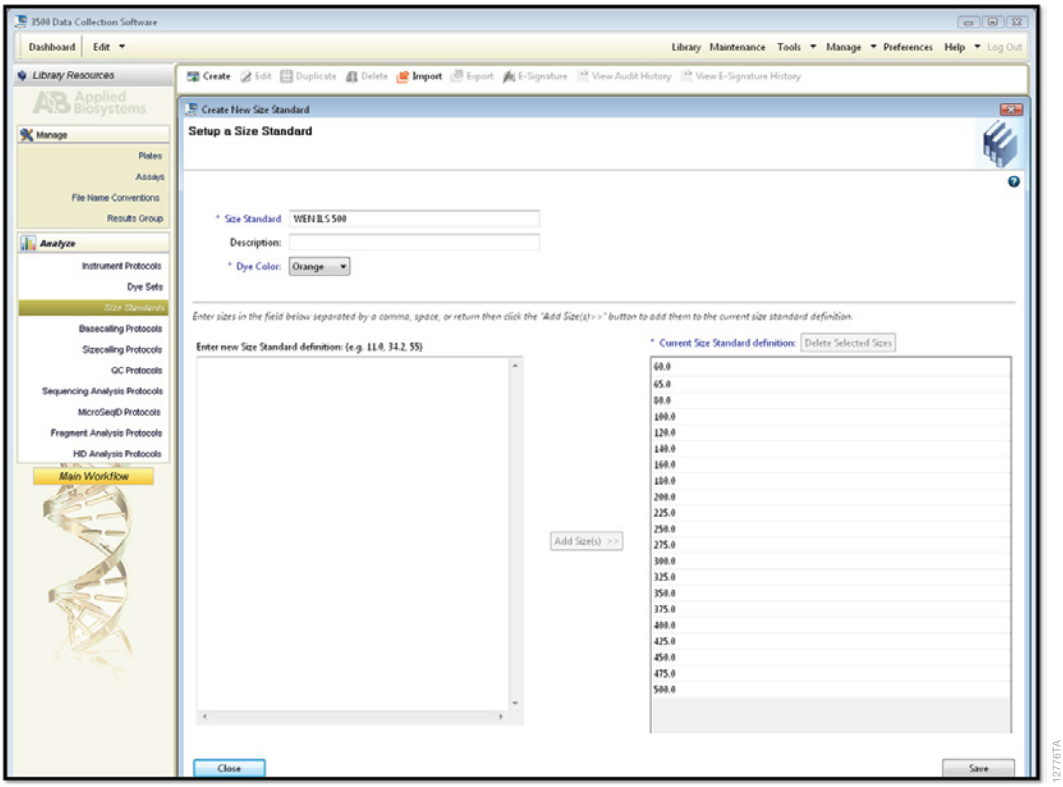

Figure 4. The 'Setup a Size Standard' window.

4. Create a new Sizecalling Protocol. To create a new Sizecalling Protocol, navigate to the Library. Select **Sizecalling Protocols**, and then select **Create**. Alternatively, a previously created Sizecalling Protocol can be used.

   Assign a descriptive protocol name such as "WEN ILS 500". Select the size standard created in Step 3. The settings for the Sizecalling Protocol should be based on the optimized conditions for the *GenePrint*® 24 System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 5 shows one option for these settings.

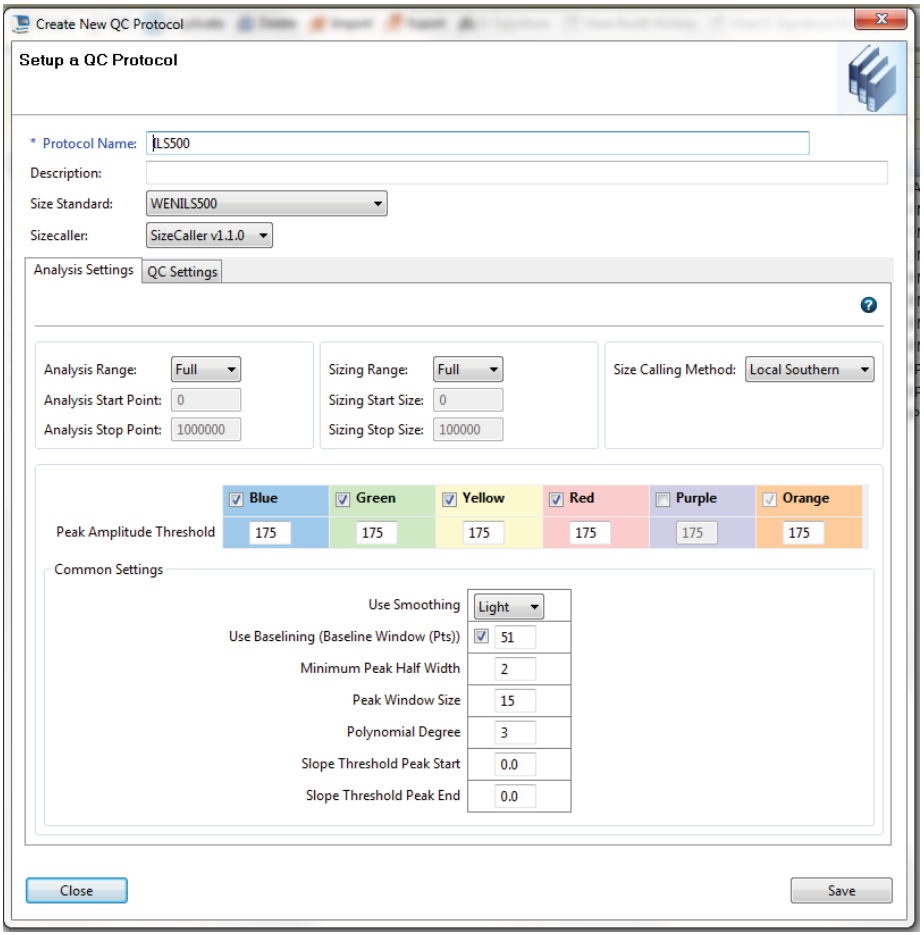

Figure 5. The 'Setup a Sizecalling Protocol' window.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

5. Create a new Assay. To create a new Assay, navigate to the Library. Select **Assays**, and then select **Create**. Alternatively, a previously created Assay can be used.

   In the Setup an Assay window (Figure 6), select the application type **Fragment**. Choose the Instrument Protocol created in Step 2 and the Sizecalling Protocol created in Step 4. Assign a descriptive assay name. An Assay is required for all named samples on a plate.

   Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.

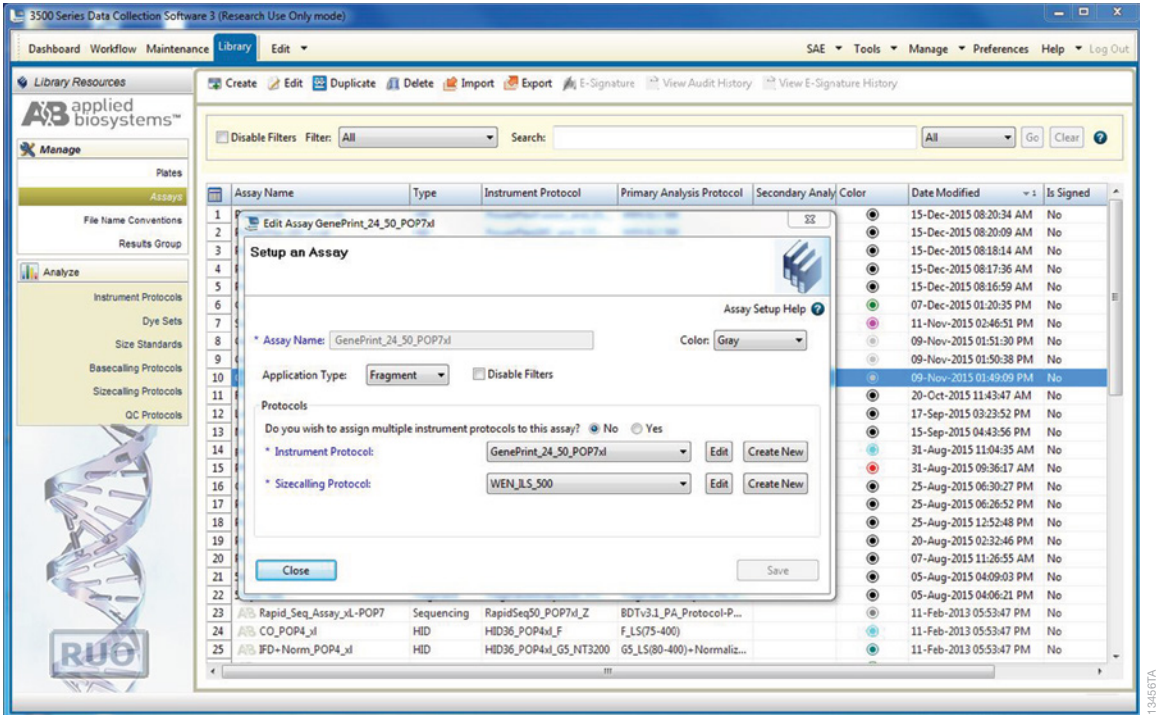

Figure 6. The 'Setup an Assay' window.

6. Create a new File Name Convention. To create a new File Name Convention (Figure 7), navigate to the Library. Select **File Name Conventions**, and then select **Create**. Alternatively, a previously created File Name Convention can be used.

   Select the File Name Attributes according to your laboratory practices, and save the File Name Convention with a descriptive name.

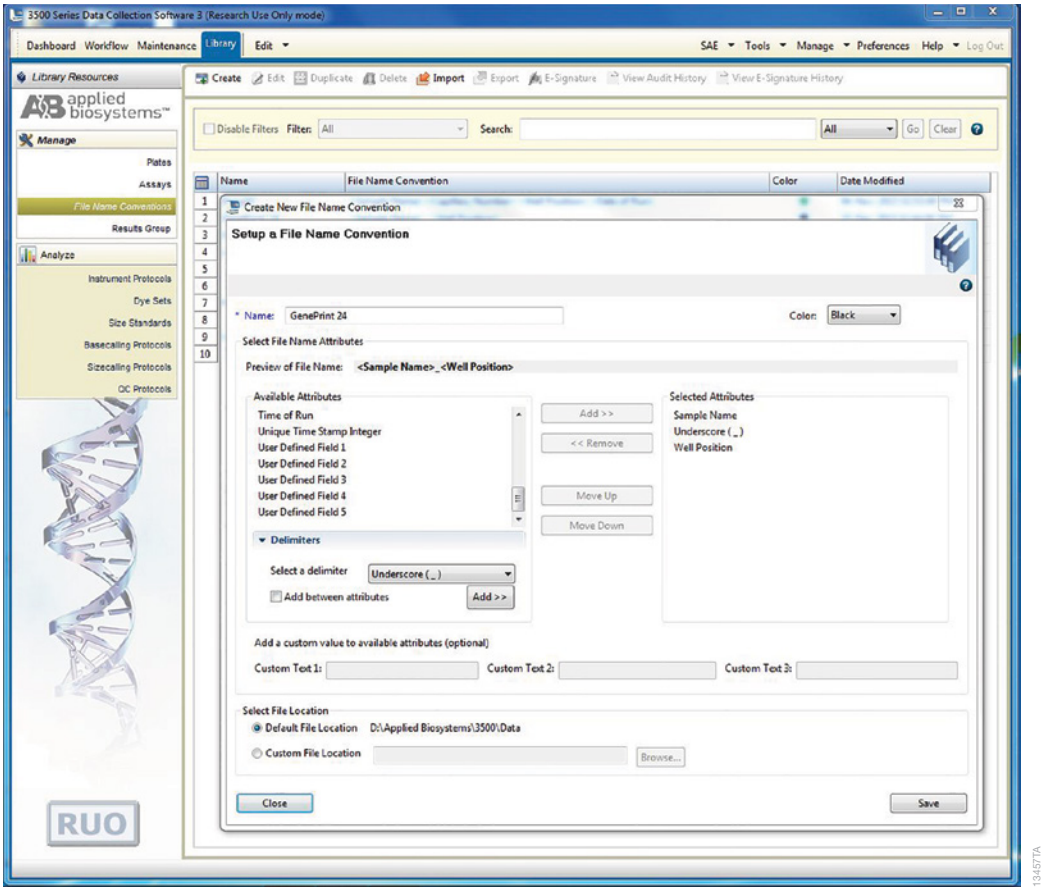

Figure 7. The 'Setup a File Name Convention' window.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

7. Create a new Results Group. To create a new Results Group (Figure 8), navigate to the Library. Select **Results Group**, and then select **Create**. Alternatively, a previously created Results Group can be used.

   Select the Results Group Attributes according to your laboratory practices. Save the Results Group with a descriptive name.

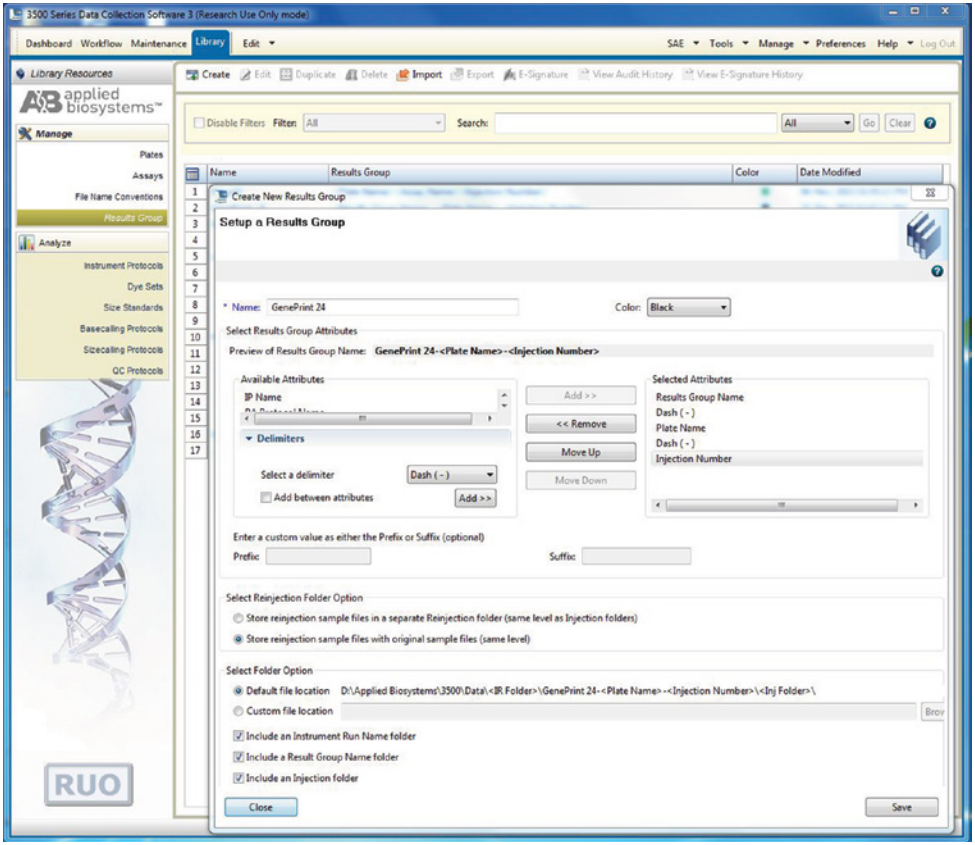

Figure 8. The 'Setup a Results Group' window.

8. Create a new Plate, and begin the run.

   a. To create a New Plate, navigate to the Library, and from the Manage menu, select **Plates** and then **Create**.

   b. Assign a descriptive plate name. Select the plate type **Fragment** from the drop-down menu (Figure 9).

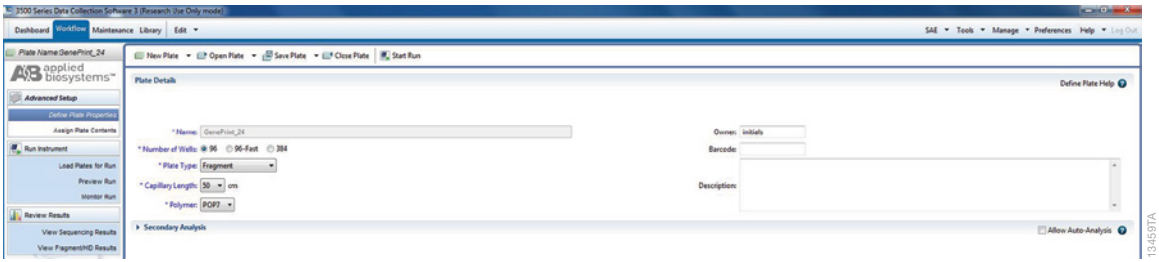

Figure 9. Defining plate properties.

   c. Select **Assign Plate Contents**.

   d. Assign sample names to wells.

   e. In the lower left portion of the screen (Figure 10), under Assays, use the Add from Library option to select the Assay created in Step 5 or one previously created. Click on the **Add to Plate** button, and close the window.

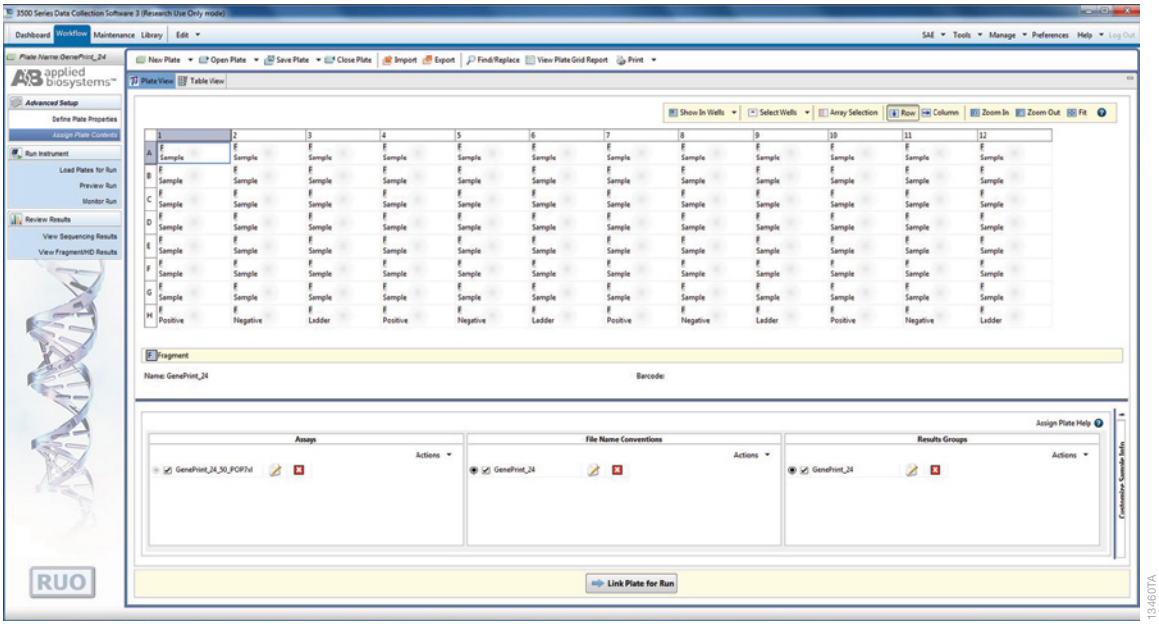

Figure 10. Assigning plate contents.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

f. Under File Name Conventions, use the 'Add from Library' option to select the File Name Convention created in Step 6 or one previously created. Click on the **Add to Plate** button, and close the window.

g. Under Results Groups, use the 'Add from Library' option to select the Results Group created in Step 7 or one previously created. Click on the **Add to Plate** button, and close the window.

h. Highlight the sample wells, and then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.

i. Select **Link Plate for Run**.

j. The 'Load Plate' window will appear. Select **Yes**.

k. In the Run Information window (Figure 11), assign a Run Name. Select **Start Run** (not shown).

Each injection will take approximately 40 minutes.

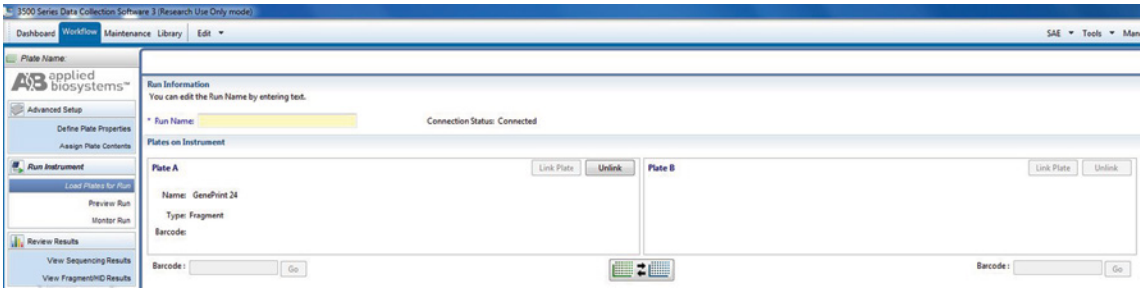

Figure 11. Assigning a run name.

5.C. Detection of Amplified Fragments Using the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.X or 4.0

Materials to Be Supplied by the User

- 3100 or 3130 capillary array, 50cm
- plate retainer and base set (standard)
- POP-7™ Polymer for the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer
- 10X genetic analyzer buffer with EDTA

Refer to the instrument user's manual for instructions on cleaning, installing the capillary array, performing a spatial calibration and adding polymer.

Analyze samples as described in the user's manual for the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.X or 4.0, with the following exceptions.

1. In the Module Manager, select **New**. Select **Regular** in the 'Type' drop-down list, and select the appropriate module for your instrument configuration such as **FragmentAnalysis50_POP7** in the 'Template' drop-down list. Use the default injection parameters, optimizing these parameters as needed for specific laboratory requirements. Give a descriptive name to your run module, and select **OK**.

   Note: Instrument sensitivities can vary. The injection time and voltage may be adjusted in the Module Manager. A suggested range for the injection time is 3–22 seconds and for the injection voltage is 1–3kV.

2. In the Protocol Manager, select **New**. Type a name for your protocol. Select **Regular** in the 'Type' drop-down list, and select the run module you created in the previous step in the 'Run Module' drop-down list. Lastly, select **G5** in the dye-set drop-down list. Select **OK**.

3. In the Plate Manager, create a new plate record as described in the instrument user's manual. In the dialog box that appears, select **GeneMapper—Generic** in the 'Application' drop-down list, and select the appropriate plate type (96-well). Add entries in the owner and operator windows, and select **OK**.

4. In the GeneMapper plate record, enter sample names in the appropriate cells. Scroll to the right. In the 'Results Group 1' column, select the desired results group. In the 'Instrument Protocol 1' column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select **OK**.

   Note: To create a new results group, select **New** in the drop-down menu in the 'Results Group' column. Select the 'General' tab, and enter a name. Select the 'Analysis' tab, and select **GeneMapper—Generic** in the 'Analysis type' drop-down list.

5. Place samples in the instrument and close the instrument doors.

6. In the spectral viewer, select **dye set G5**, and confirm that the active dye set is the file generated for the *GenePrint*® 5C chemistry.

It is critical to select the correct G5 spectral calibration for the *GenePrint*® 5C chemistry.

If the *GenePrint*® 5C chemistry is not the active dye set, locate the *GenePrint*® 5C spectral calibration in the List of Calibrations for Dye Set G5, and select **Set**. If a Promega 5C spectral calibration has not been performed, refer to the *GenePrint*® 5C Matrix Standard Technical Manual, #TM475, for information on how to perform the spectral calibration.

5.C. Detection of Amplified Fragments Using the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.X or 4.0 (continued)

7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.
8. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.
9. When the plate record is linked to the plate, the plate graphic changes from yellow to green, and the green Run Instrument arrow becomes enabled.
10. Click on the green **Run Instrument** arrow on the toolbar to start the sample run.
11. Monitor electrophoresis by observing the run, view, array or capillaries viewer window in the data collection software. Each injection will take approximately 40 minutes.

6. Data Analysis Using GeneMapper® Software

Note: 3130 and 3130xl data can be analyzed by GeneMapper® software version 4.0 and later; 3500 data requires version 4.1 or 5.0.

6.A. Getting Started

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

1. To obtain the analysis files for the *GenePrint*® 24 System, go to: **www.promega.com/resources/software-firmware/str-analysis/geneprint-systems-software-panels-and-bin-files/**
2. Select the *GenePrint*® 24 System and the version of GeneMapper® software that you are using. Select the POP-7™ Polymer type. Enter your contact information, and select **Submit**. An e-mail will be sent to the e-mail address provided with a link for the file download. There will be seven files included in the download: a WEN ILS/size standard file, a *GenePrint*® 24 panels file, a *GenePrint*® 24 bins file, a Table Settings file, a Plot Settings file and the *GenePrint*® 24 Analysis Method file.
3. Save all files to a known location on your computer.

6.B. 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 Section 6.A. Select the file and then **Import**.
6. In the navigation pane, highlight the *GenePrint*®24 panels folder that you just imported in Step 5.
7. Select **File** and then **Import Bin Set**.
8. Navigate to the bins text file downloaded in Section 6.A. Select the file and then **Import**.
9. In the navigation pane, highlight the *GenePrint*®24 panels folder that you just imported in Step 5 to view the marker information if desired.
10. At the bottom of the 'Panel Manager' window, select **Apply**. This will save the panels and bins files. Select **OK** to close the window.

6.C. Importing the WEN ILS 500 Size Standard

There are two options when creating an internal lane standard/size standard. Use this protocol or the alternative protocol in Section 6.D.

Importing a WEN ILS 500.xml file

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

6.D. Creating a Size Standard with GeneMapper® Software

1. Select **Tools** and then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **New**.
4. In the 'Size Standard Editor' window (Figure 12), select **Basic or Advanced** and then **OK**.
5. Enter a detailed name, such as "WEN_ILS_500" (Figure 12).

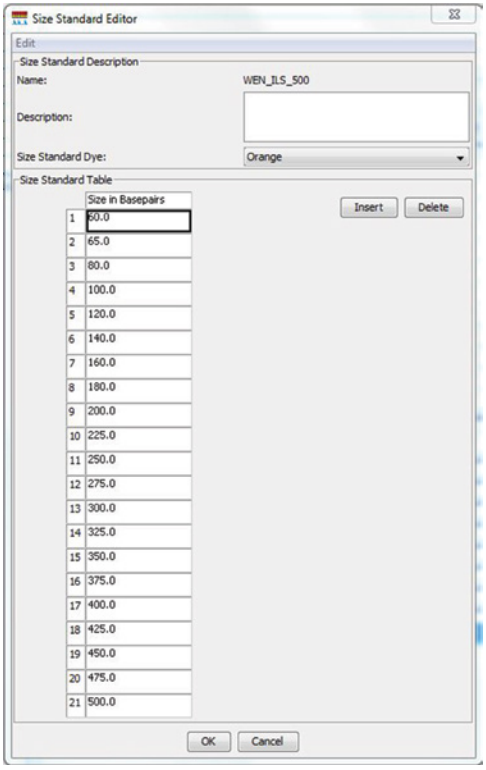

Figure 12. The GeneMapper® Software, Version 5.0, Size Standard Editor.

6. Choose **Orange** for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Figure 12 above.
8. Select **OK**.

6.E. Importing Table and Plot Settings Files

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

These files will now be available to choose in the drop-down menus in the Samples Tab view (for Table Setting, Figure 13) and Samples Plot view (Figure 14).

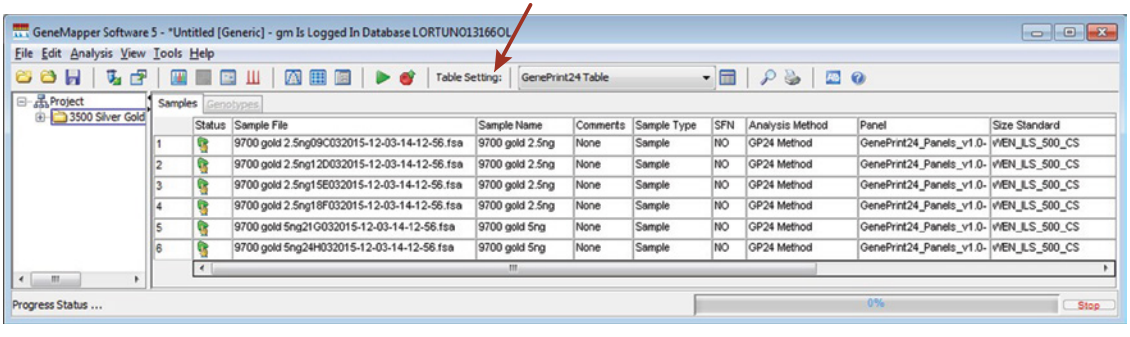

Figure 13. Drop-down menu location for the *GenePrint*® 24 Table Setting in the Samples Tab View.

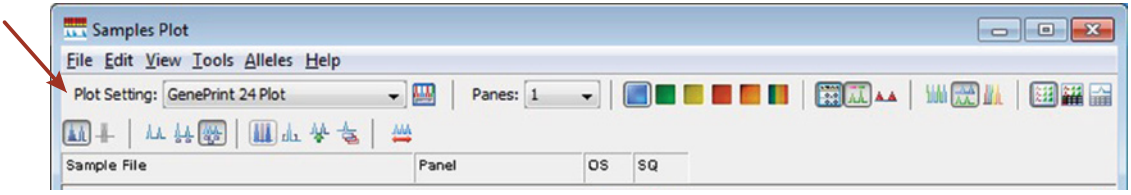

Figure 14. Drop-down menu location for the *GenePrint*® 24 Plot Setting in the Samples Plot View.

6.F. Importing the *GenePrint*® 24 Analysis Method

1. Select **Tools** and then **GeneMapper Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **Import**, and then navigate to the location of the *GenePrint*®24 Analysis Method file that you downloaded in Section 6.A. Import the file.
4. Note that this method will have "HID" as the analysis type (Figure 15).

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.

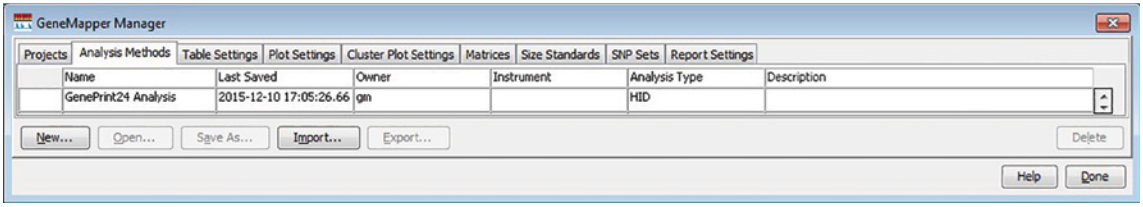

Figure 15. The *GenePrint*® 24 Analysis Method will have "HID" as the analysis type.

5. To verify the correct bin settings, locate the new *GenePrint*® 24 Analysis Method at the bottom of the list of methods. Select the analysis method, and choose **Open**. Select the 'Allele' tab, and choose **GenePrint_24_bins** from the drop-down menu at the top if it does not already appear. Verify that all settings appear as shown in Figure 16.

6.F. Importing the *GenePrint*® 24 Analysis Method (continued)

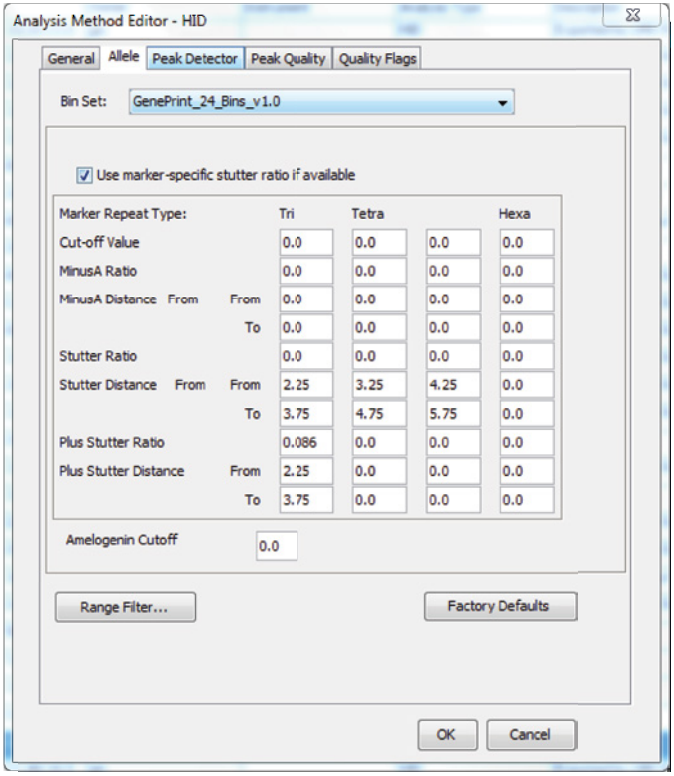

Figure 16. 'Allele' Tab settings.

6. To verify or set the correct peak detection settings, choose the 'Peak Detector' tab. Enter the peak amplitude threshold values for your laboratory, and leave all other settings as shown in Figure 17.

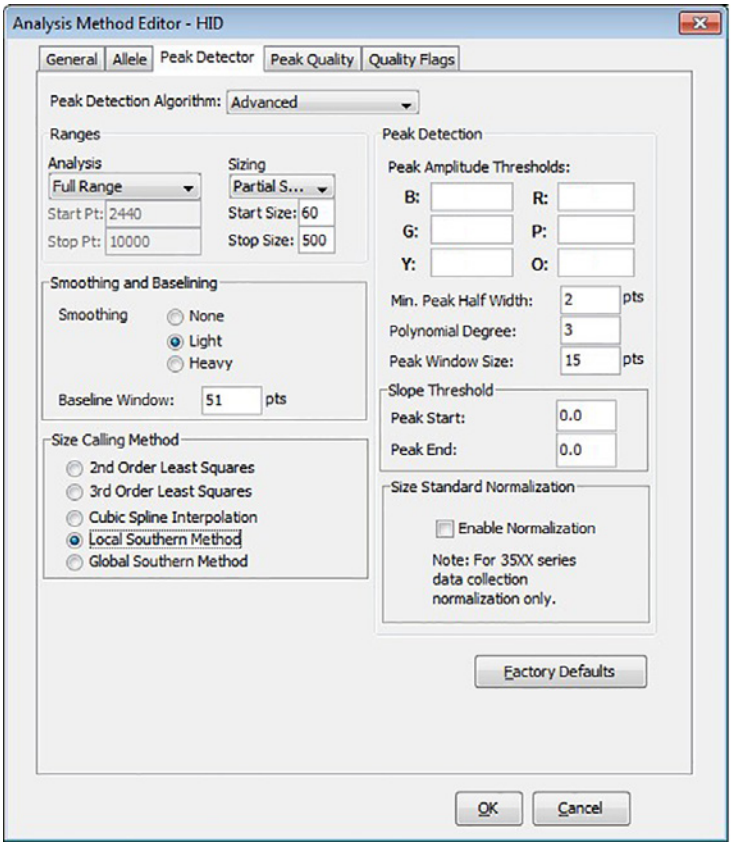

Figure 17. 'Peak Detector' tab settings.

7. Analysis of *GenePrint*® 24 Data in GeneMapper® Software

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

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

   Note: If you choose a Microsatellite project you will not be able to select your HID analysis method when analyzing the data, and correct genotyping across all loci will not occur.

2. 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.

3. In the top center of the window, choose **GenePrint 24 Table** from the drop-down menu.

4. In the 'Sample Type' column for the allelic ladder file in each folder of data, select **Allelic Ladder**. You also may use **Positive Control** for the positive control sample and **Negative Control** for the negative control sample in the 'Sample Type' column.

5. In the 'Analysis Method' column, choose the **GenePrint® 24 Analysis Method** from the drop-down menu.

6. In the 'Panel' column select the *GenePrint*® 24 panels file by opening the folder and double-clicking on the file.

7. In the 'Size Standard' column select the WEN_ILS_500 file.

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 first by analyzing the allelic ladder files. Analysis will pause for a few seconds about every 10 samples to save the analyzed data to the database. Data are now ready for review. See Figure 18.

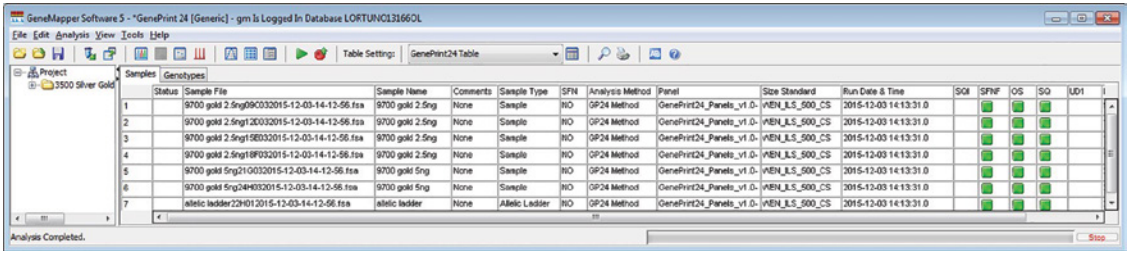

Figure 18. The analyzed project.

8. Results

Representative results of the *GenePrint*® 24 System are shown in Figure 19. The *GenePrint*® 24 Allelic Ladder Mix is shown in Figure 20.

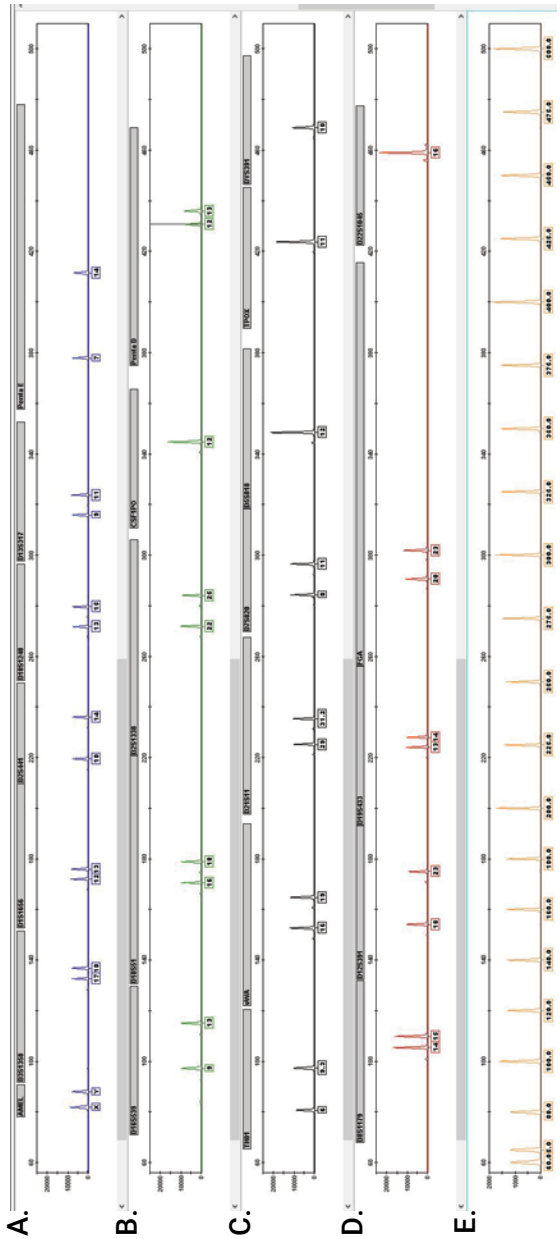

Figure 19. The *GenePrint*® 24 System. The 2800M Control DNA (2.5ng) was amplified using the *GenePrint*® 24 System, 26 cycles and an Applied Biosystems 9700 thermal cycler. Amplification products were mixed with WEN Internal Lane Standard 500 and analyzed with an Applied Biosystems® 3500 Genetic Analyzer using a 1.6kV, 15-second injection. Results were analyzed using GeneMapper® software, version 5.0. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D16S539, D18S51, D2S1338, CSF1PO and Penta D. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loci: TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D8S1179, D12S391, D19S433, FGA and D22S1045. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500.

8. Results (continued)

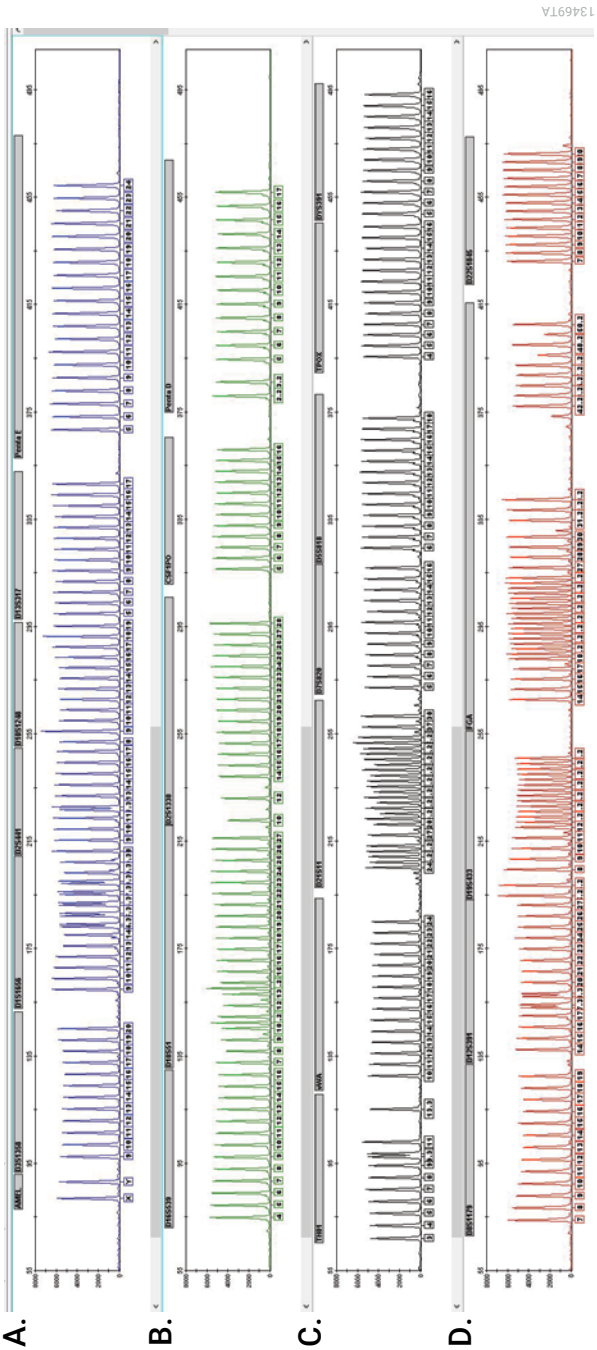

Figure 20. The *GenePrint*® 24 Allelic Ladder Mix. The *GenePrint*® 24 Allelic Ladder Mix was analyzed with an Applied Biosystems® 3500 Genetic Analyzer using a 1.6kV, 15-second injection. The sample file was analyzed with the GeneMapper® software, version 5.0, and *GenePrint*® 24 panels and bins text files. **Panel A.** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-ET-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products often are observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. A trinucleotide repeat locus, like D22S1045, will have more pronounced stutter in both n–3 and n+3 positions than a typical tetranucleotide repeat locus. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

The mean stutter percentage plus three standard deviations at each locus is used in the *GenePrint*® 24 stutter text file for locus-specific filtering in the GeneMapper® software.

In addition to stutter peaks, the following low-level artifact peaks (Table 2) and DNA-independent (with or without human genomic DNA) artifact peaks (Table 3) may be observed with the *GenePrint*® 24 System.

Table 2. DNA-Dependent Artifacts Observed in Amplification Reactions with Human Genomic DNA

Locus or Dye Label	Artifact Size
Fluorescein	~88–112 bases[1]
JOE	~77–80 bases[2]
	~218 bases[2]
	~251 bases[2]
CXR	~175–183 bases[3]
Amelogenin	n–1[4]
D1S1656	n–2, n+2[4]
D2S441	n–1[4]
D13S317	n–2, n+2[4]
D18S51	n–2, n+2[4]
D21S11	n–2, n+2[4]
D7S820	n–2, n+2[4]
D5S818	~n–9 to n–8[5]; n–2, n+2[4]
D12S391	n–2, n–3, n+2[4]
D19S433	n–2, n+2[4]

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

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

[3]Low level peaks in this size range may be observed with 27 cycles of amplification.

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

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

8. Results (continued)

Table 3. DNA-Independent Artifacts Observed in Amplification Reactions with or without Human Genomic DNA

Dye Label	Artifact Size[1]
Fluorescein	~60–68 bases
	~69–77 bases
	~79–88 bases[2]
	~91–97 bases
	~98–107 bases
JOE	~63–71 bases[2]
	~82–92 bases[2]
	~97–101
TMR-ET	~57–59 bases
	~60–64 bases

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

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

9. 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**

9.A. Amplification and Fragment Detection

This section provides troubleshooting information about amplification and detection. For questions about data analysis using GeneMapper® software, see Section 9.C.

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.

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	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.
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, 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 can 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.

9.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	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.
	Incorrect G5 spectral calibration was active when analyzing samples with the Applied Biosystems® 3130 or 3130xl Genetic Analyzer. Re-run samples, and confirm that the GenePrint® 5C spectral calibration is set for G5. See instructions for instrument preparation in Section 5.C.
	CE-related artifacts (contaminants). Contaminants in the water used with the instrument or to dilute the 10X genetic analyzer buffer can generate peaks in the fluorescein and JOE channels. Use autoclaved deionized water; change vials and wash buffer reservoir.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples. • Perform a new spectral calibration, and re-run the samples. • Confirm correct spectral calibration was used for the sample run. • Instrument sensitivities can vary. Optimize the injection conditions. See Section 5. • Reboot the Applied Biosystems® 3500 or 3500xL Genetic Analyzer and the instrument's computer. Repeat the spectral calibration. Do not allow borrowing when running the spectral calibration on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer.
	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 product at –20°C.
	An incorrect internal lane standard was used. Use the size standard provided in the kit.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.

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.
	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 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.B. Amplification of Extracted DNA

The following information is specific to amplification of purified DNA. For information about general amplification and detection, see Section 9.A.

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. As DNA sample volume increases relative to the total amplification reaction volume, this may be more likely.
	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 also affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA), TE^{-4} buffer with 20µg/ml glycogen or nuclease-free water.

9.B. Amplification of Extracted DNA (continued)

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	The reaction volume was too low. This system is optimized for a final reaction volume of 12.5µl for extracted DNA. Decreasing the reaction volume may result in suboptimal performance.
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 Section 8 for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Amplification of greater than the recommended amount of template can result in imbalance, with the 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 samples can lead to allele dropout or imbalance.
	Imbalance may be seen more often when using the maximum template volume or reduced amplification reaction volume.
	The reaction volume was too low. This system is optimized for a final reaction volume of 12.5µl. Decreasing the reaction volume may result in suboptimal performance.

9.C. GeneMapper® Software

This section provides troubleshooting information about data analysis using GeneMapper® software. For questions about amplification and detection, see Sections 9.A and 9.B.

Symptoms	Causes and Comments
Alleles not called	To analyze samples with GeneMapper® software, the analysis method and size standard must have "Basic or Advanced" as the analysis type. If they are different, an error is obtained.
	To analyze samples with GeneMapper® software, at least one allelic ladder must be defined per folder of sample files being analyzed in the project.
	Run was too short and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	An insufficient number of WEN ILS 500 fragments was defined. Be sure to define at least two WEN ILS 500 fragments smaller than the smallest sample peak and at least two WEN ILS 500 fragments larger than the largest sample peak.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section 7.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID 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.

9.C. GeneMapper® Software (continued)

Symptoms	Causes and Comments
Off-ladder alleles (continued)	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 POP-7™ may change the 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 in the analysis method or use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto Adjust Sizes**.
	Run was too short and larger peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Error message: "Either panel, size standard, or analysis method is invalid"	The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). We recommend advanced mode analysis methods and size standards. Be sure both files are set to the same mode, either Classic mode or Basic or Advanced mode.
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.
	No size standard was selected. In the size standard column, be sure to select the appropriate size standard.
	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 sizes will be called.

Symptoms	Causes and Comments
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 to 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.
	The bins text file assigned to the analysis method was deleted. In the GeneMapper® Manager, select the 'Analysis Methods' tab, and reopen the *GenePrint*® 24 analysis method. Select the 'Allele' tab, and select the *GenePrint*® 24 bins file.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration, and re-run the samples.
	Use of "Classic" mode analysis on samples can result in baselines with more noise than those analyzed using the "Basic or Advanced" mode analysis method. We recommend advanced mode analysis methods and size standards.
	Confirm that the correct spectral calibration was used to run the samples.
Error message after attempting to import Panels and Bins text files: "Unable to save panel data: java.SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated"	There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order.
Allelic ladder peaks labeled off-ladder	GeneMapper® Software was not used, or microsatellite analysis settings were used instead of HID analysis settings.

10. Appendix

10.A. *GenePrint*® 24 System Locus Information

The loci amplified using the *GenePrint*® 24 System are shown in Tables 4 and 5. Table 6 lists the *GenePrint*® 24 System alleles amplified from 2800M Control DNA.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage and terminal nucleotide addition (11,12). Repeat slippage, sometimes called "n–4 peaks", "stutter" or "shadow peaks", is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

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

Table 4. *GenePrint*® 24 System Locus-Specific Information.

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

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

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

[3]Amelogenin is not an STR.

NA = Not applicable

Table 5. The *GenePrint*® 24 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
Amelogenin[4]	Fluorescein	89, 95	X, Y
D3S1358	Fluorescein	103–147	9–20
D1S1656	Fluorescein	161–208	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	Fluorescein	214–250	8–11, 11.3, 12–17
D10S1248	Fluorescein	255–299	8–19
D13S317	Fluorescein	302–350	5–17
Penta E	Fluorescein	371–466	5–24
D16S539	JOE	84–132	4–16
D18S51	JOE	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE	224–296	10, 12, 14–28
CSF1PO	JOE	318–362	5–16
Penta D	JOE	377–450	2.2, 3.2, 5–17
TH01	TMR-ET	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR-ET	127–183	10–24
D21S11	TMR-ET	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
D7S820	TMR-ET	269–313	5–16
D5S818	TMR-ET	321–369	6–18
TPOX	TMR-ET	393–441	4–16
DYS391	TMR-ET	442–486	5–16
D8S1179	CXR-ET	76–124	7–19
D12S391	CXR-ET	133–185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
FGA	CXR-ET	265–411	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	CXR-ET	425–464	7–20

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

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

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

[4]Amelogenin is not an STR.

Table 6. The *GenePrint*® 24 System Allele Determinations for the 2800M Control DNA.

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

10.B.The WEN Internal Lane Standard 500

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

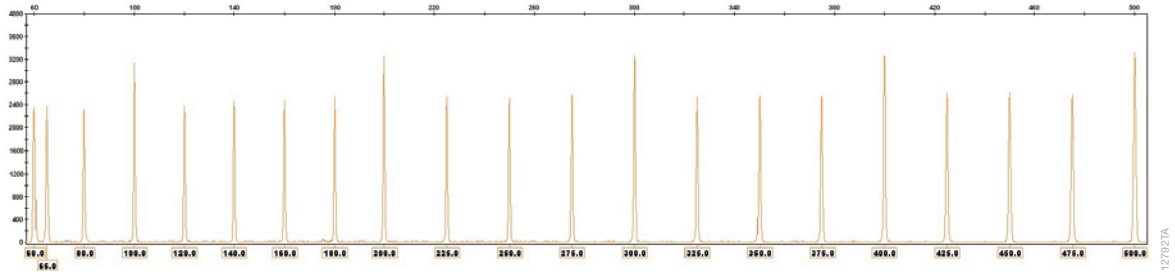

Figure 21. WEN Internal Lane Standard 500. An electropherogram showing the WEN Internal Lane Standard 500 fragments.

10.C. Composition of Buffers and Solutions

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

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

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

TE^{-4} buffer with 20µg/ml glycogen

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

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

10.D. References

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10.E. Related Products

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

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

10.F. Summary of Changes

The following changes were made to the 6/20 revision of this document:

1. Made text edits throughout the document.
2. Rearranged Section 9, Troubleshooting.
3. Updated Section 8.