



TECHNICAL MANUAL

GenePrint[®] 10 System for Use on the Applied Biosystems[®] Genetic Analyzers

Instructions for Use of Product
B9510

GenePrint[®] 10 System

for Use on the Applied Biosystems[®] Genetic Analyzers

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

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: <http://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 can 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: <http://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 is compatible with the Applied Biosystems[®] 3130, 3130*xl*, 3500 and 3500*xL* Genetic Analyzers. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation.

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 9.A. The 2800M Control DNA has a known genotype and can be used to verify genotyping accuracy.

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/resources/software-firmware/str-analysis/genepprint-systems-software-panels-and-bin-files/ This site provides instructions and applications to set the report parameters in the GeneMapper[®] software to make genotyping easier and more accurate.



2. Product Components and Storage Conditions

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

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
- 1.25ml Water, Amplification Grade
- 25µl 2800M Control DNA, 10ng/µl

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 $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$. For daily use, the *GenePrint*[®] 10 System components can be stored for up to 1 week at $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$. Make sure that the 2800M Control DNA is stored at $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$ 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

Matrix standards are required for initial setup of the color separation matrix (see Section 3.B). The matrix standard is sold separately and are available for the Applied Biosystems[®] 3130, 3130xl, 3500 and 3500xL Genetic Analyzers (PowerPlex[®] 4C Matrix Standard, Cat.# DG4800).

3. Before You Begin

3.A. Precautions

The quality of purified DNA or direct-amplification samples, small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification, as well as electrophoresis and fluorescence detection.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Take extreme care 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 (5X Master Mix, 5X 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 (e.g., ART[®] tips).

Some of the 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, 3130xl, 3500 and 3500xL Genetic Analyzers. A spectral calibration must be performed for each individual instrument. Very high peak heights may not be perfectly separated spectrally, and an allele peak in one color channel can bleed into another color channel. Use of a poor or incorrect matrix will allow this as well.

For protocols and additional information about spectral calibration, see the *PowerPlex[®] 4C Matrix Standard Technical Bulletin #TMD048*. This manual is available online at: www.promega.com/protocols/

4. Protocol for DNA Amplification Using the *GenePrint*[®] 10 System

The *GenePrint*[®] 10 System is optimized for the GeneAmp[®] PCR System 9700 thermal cycler. An amplification protocol for the ProFlex[®] PCR System is also provided for extracted DNA.

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 7.

Materials to Be Supplied by the User

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

The *GenePrint*[®] 10 System was 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 new MicroAmp[®] plate for reaction assembly, and label it appropriately.

- Add the final volume of each reagent listed in Table 1 to a new 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	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 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 1.0µl 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.



4. Protocol for DNA Amplification Using the *GenePrint*[®] 10 System (continued)

Thermal Cycling

This manual contains a protocol for use of the *GenePrint*[®] 10 System with the GeneAmp[®] PCR System 9700 thermal cycler and ProFlex[®] PCR System. 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 time and loading volume for your laboratory instrumentation. Testing at Promega shows that 30 cycles work well for 10ng of purified DNA template.

1. Place the MicroAmp[®] plate in the thermal cycler.
2. Select and run the recommended protocol. The estimated total cycling time is 1.5 hours.

Notes:

- a. When using the ProFlex[®] PCR System, use Max mode as the ramp speed.
- b. When using the GeneAmp[®] PCR System 9700, the program must be run with Max mode as the ramp speed. When using the GeneAmp[®] PCR System 9700 thermal cycler, this requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. 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 30 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 protected from light.

Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

5. Instrument Setup and Sample Preparation

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

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- 96-well retainer and base set (standard) (Applied Biosystems Cat.# 4410228)
- POP-4® polymer for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container
- MicroAmp® optical 96-well plate and septa, or equivalent (Applied Biosystems)
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)



The quality of formamide is critical. Use Hi-Di™ 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.

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

Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and 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 increased or decreased to adjust the intensity of the size standard peaks. If the peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0 μl of ILS 600 and 9.0 μl of Hi-Di™ formamide. If the peak heights are too high, we recommend altering the loading cocktail to contain 0.25 μl of ILS 600 and 9.75 μl of Hi-Di™ formamide.

2. Vortex for 10–15 seconds to mix.
3. Pipet 10 μl of formamide/internal lane standard mix into each well.
4. Add 1 μl of amplified sample (or 1 μl of Allelic Ladder Mix). Cover wells with appropriate septa.

Note: Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be adjusted. 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.
5. Centrifuge plate briefly to remove air bubbles from the wells.
6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice, in an ice-water bath or freezer plate block for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

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 1). Ensure that the Consumables Information and Maintenance Notifications are acceptable.

Set the oven temperature to 60°C, then select **Start Pre-Heat**. When the Oven Temperature and Detection Cell Temperature turn green, proceed with the first injection.

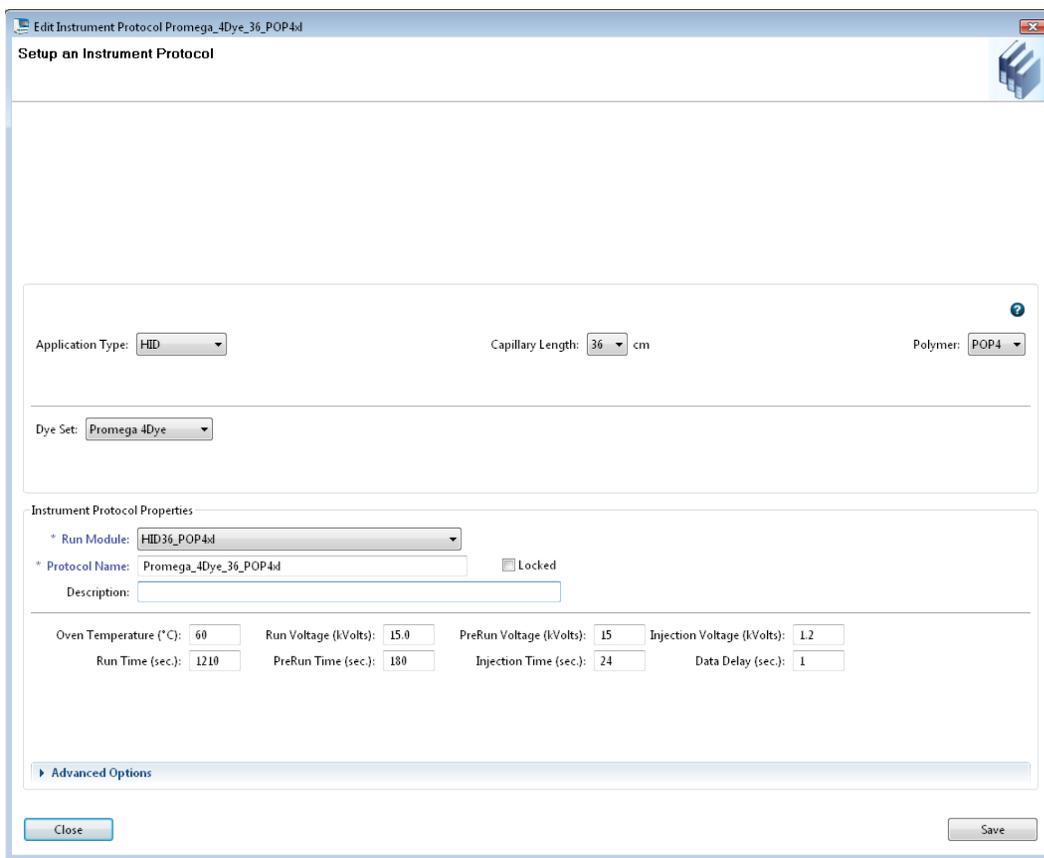


Figure 1. The Dashboard.

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

- To create a new Instrument Protocol, navigate to the Library, select **Instrument Protocol**, then select **Create**. Alternatively, a previously created instrument protocol can be used.

Figure 2 shows the settings used at Promega for the Applied Biosystems® 3500xL Genetic Analyzer for the application type, dye set, capillary length, polymer, run module and appropriate protocol information. The only setting that was changed from the default settings is dye set.



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Figure 2. The ‘Create New Instrument Protocol’ window.

The recommended settings are:

Application Type	HID
Capillary Length	36cm
Polymer	POP-4®
Dye Set	Promega 4Dye
Run Module	HID36_POP4(xl)
Injection Time ¹	24 seconds
Injection Voltage	1.2kV
Run Time	1,210–1,500 seconds

¹Injection time may be modified (2–24 seconds) to increase or decrease peak heights.

When creating a new Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 4-dye spectral calibration.



Run time and other instrument settings should be optimized in your laboratory.

When optimizing injection conditions in your laboratory, you may choose to create specific Instrument Protocols for each condition tested. If a single Instrument Protocol is used, follow the instructions in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide* to edit a library entry.

Assign a descriptive protocol name.

Note: For more detailed information refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

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

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

Assign the size standard the name “Promega_ILS600” or another appropriate name. Choose **Red** as the dye color. The fragments in the size standard are 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 3.

Note: Definition and detection of the 600bp fragment is optional.

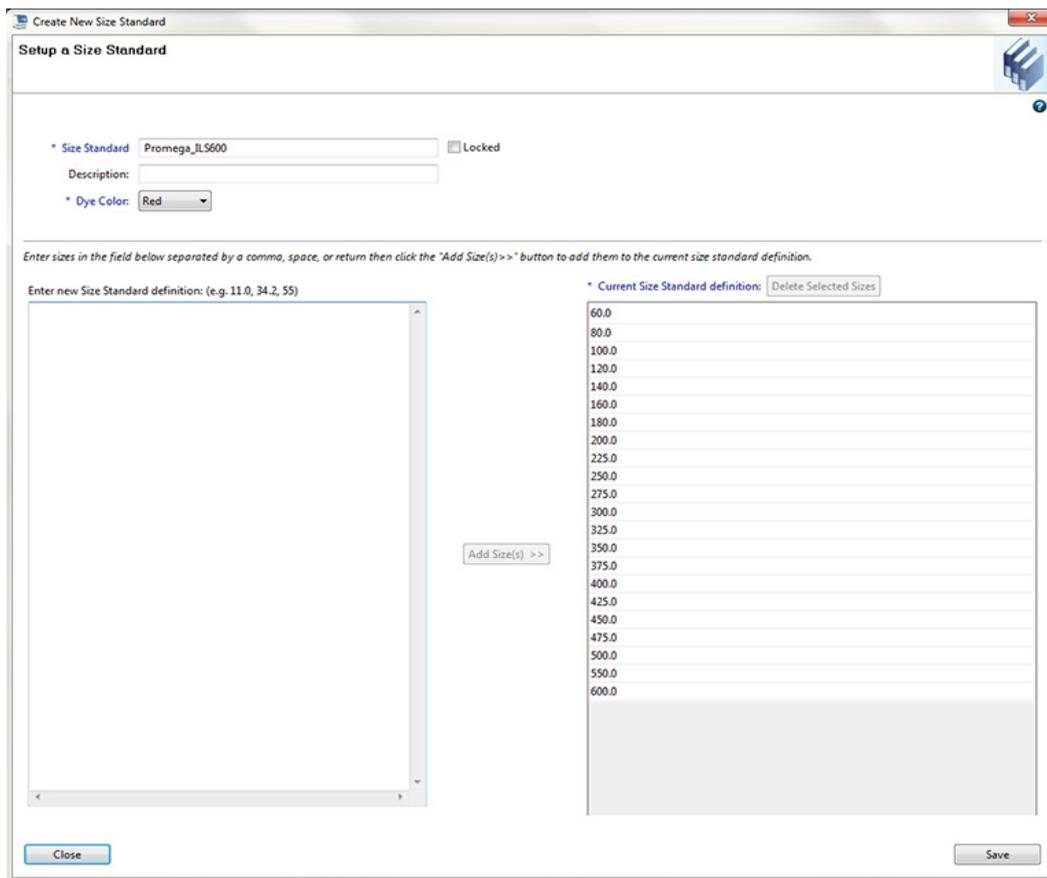


Figure 3. The ‘Create New Size Standard’ window.

- To create a new QC Protocol, navigate to the Library. Select **QC Protocols**, then select **Create**. Alternatively, a previously created QC Protocol may be used.

Assign a descriptive protocol name. Select the size standard created in Step 3. The settings for the QC protocol should be based on the internally optimized conditions for the *GenePrint*[®] 10 System on the Applied Biosystems[®] 3500 or 3500xL Genetic Analyzer. Figure 4 shows one option for these settings.

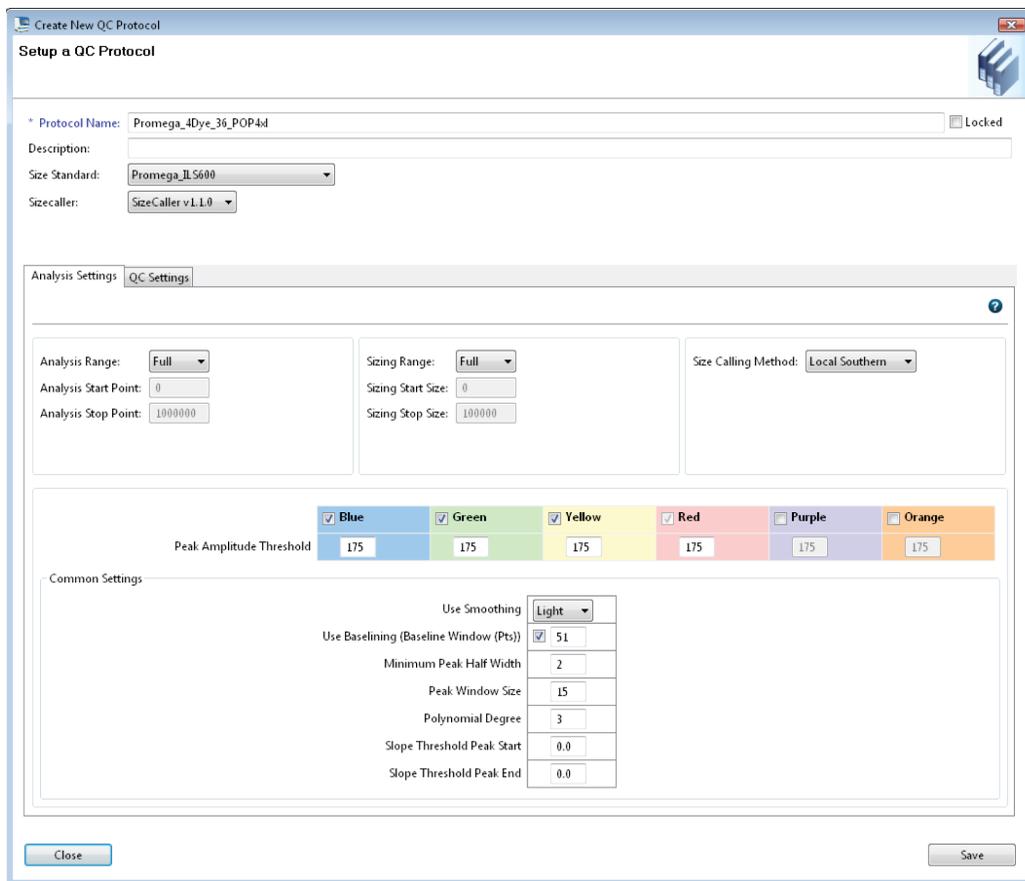


Figure 4. The 'Create New QC Protocol' window.

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

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

In the ‘Create New Assay’ window (Figure 5), select the Instrument Protocol created in Step 2 and the QC Protocol created in Step 4. Assign a descriptive assay name. Select the application type **HID**. An Assay is required for all named samples on a plate.

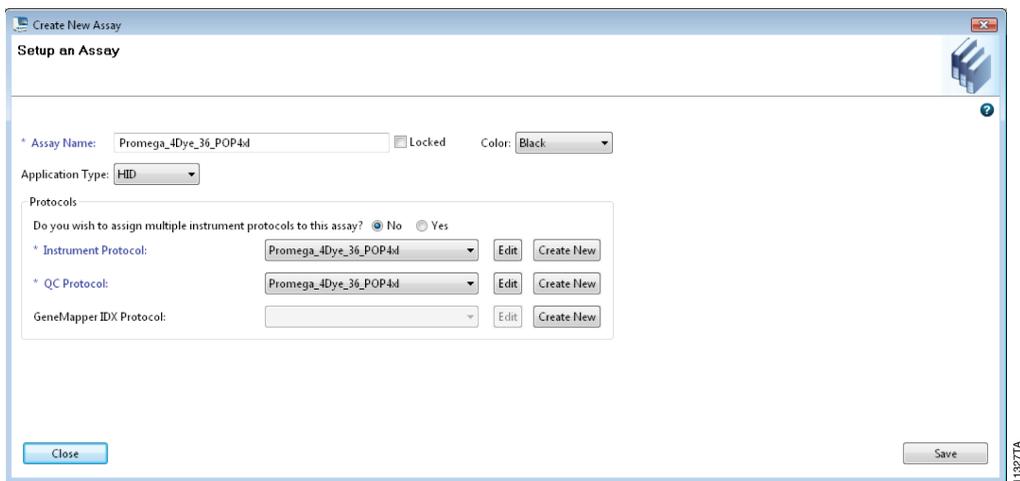


Figure 5. The ‘Create New Assay’ window.

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

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

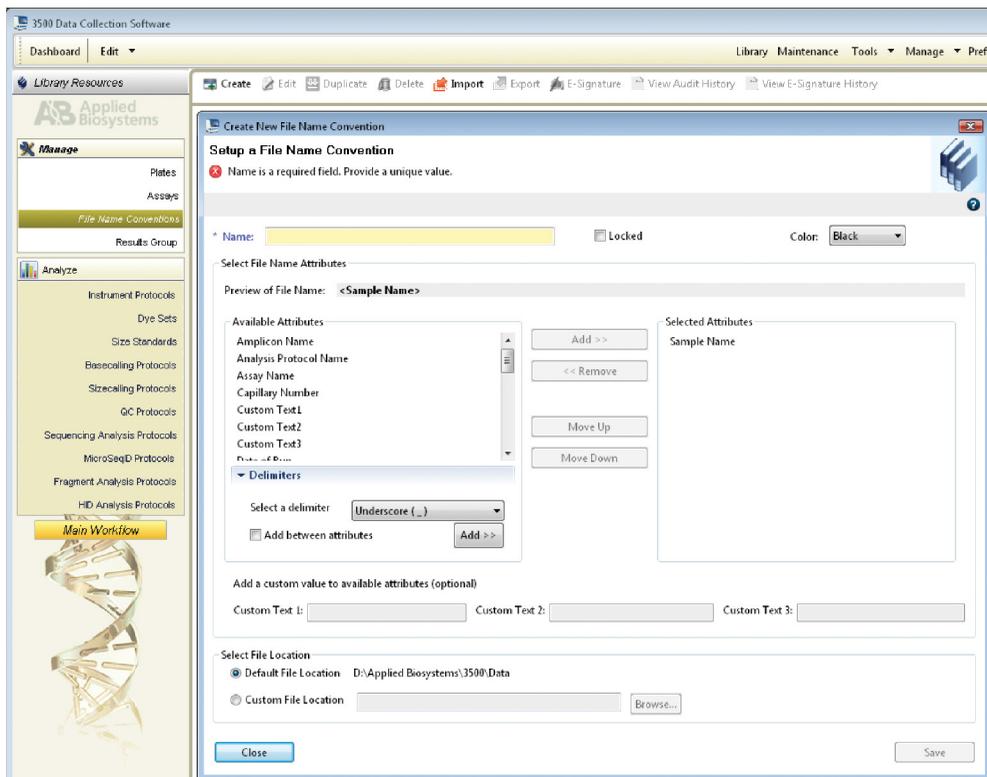
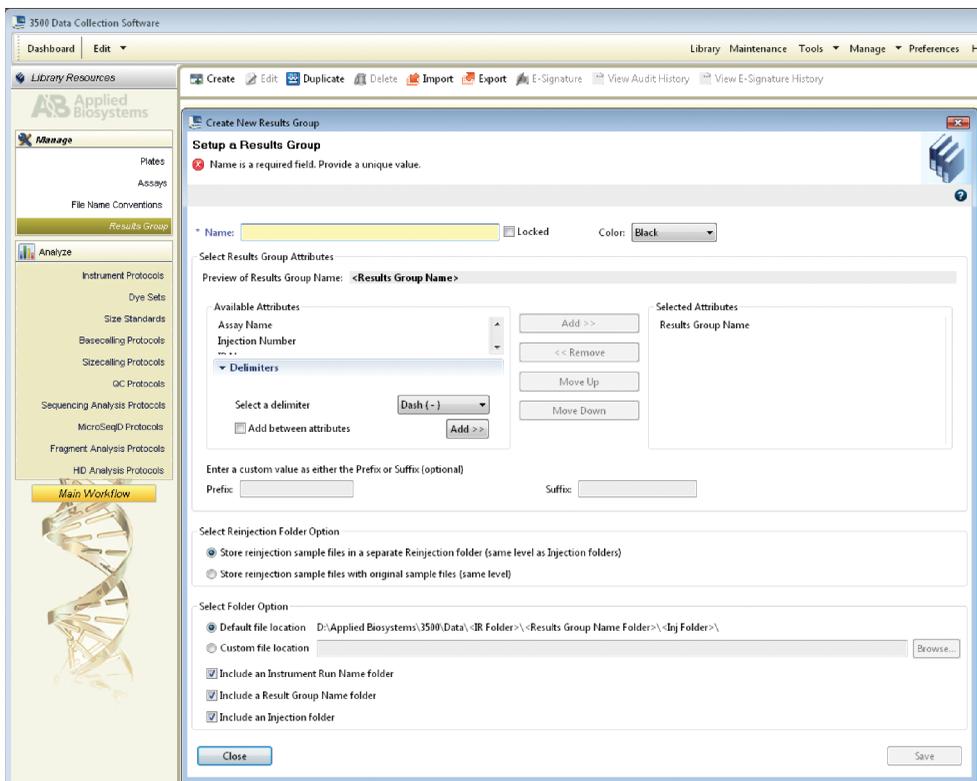


Figure 6. The ‘Create New File Name Convention’ window.

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

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

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



3500 Data Collection Software

Dashboard Edit Library Maintenance Tools Manage Preferences H

Library Resources

Applied Biosystems

Manage

Plates Assays File Name Conventions Results Group

Analyze

Instrument Protocols Dye Sets Size Standards Basecalling Protocols Sequencing Analysis Protocols MicroSeqD Protocols Fragment Analysis Protocols HD Analysis Protocols Main Workflow

Create New Results Group

Setup a Results Group

Name is a required field. Provide a unique value.

Name: Locked Color: Black

Select Results Group Attributes

Preview of Results Group Name: <Results Group Name>

Available Attributes

Assay Name Injection Number ...

Delimiters

Select a delimiter Dash (-) Add between attributes Add >>

Selected Attributes

Results Group Name

Add >> << Remove Move Up Move Down

Enter a custom value as either the Prefix or Suffix (optional)

Prefix: Suffix:

Select Reinjection Folder Option

Store reinjection sample files in a separate Reinjection folder (same level as Injection folders)

Store reinjection sample files with original sample files (same level)

Select Folder Option

Default file location D:\Applied Biosystems\3500.Data\<IR Folder>\<Results Group Name Folder>\<Inj Folder>

Custom file location Browse...

Include an Instrument Run Name folder

Include a Result Group Name folder

Include an Injection folder

Close Save

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Figure 7. The ‘Create New Results Group’ window.

8. To create a New Plate, navigate to the Library, and from the Manage menu, select **Plates**, then **Create**.
9. Assign a descriptive plate name. Select the plate type **HID** from the drop-down menu (Figure 8).

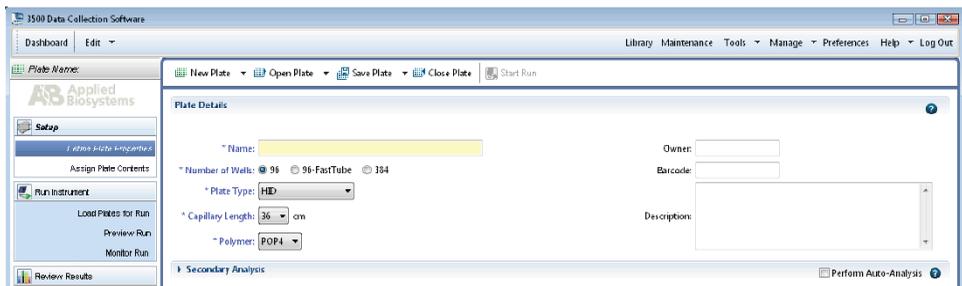


Figure 8. Defining plate properties.

10. Select **Assign Plate Contents** (Figure 9).

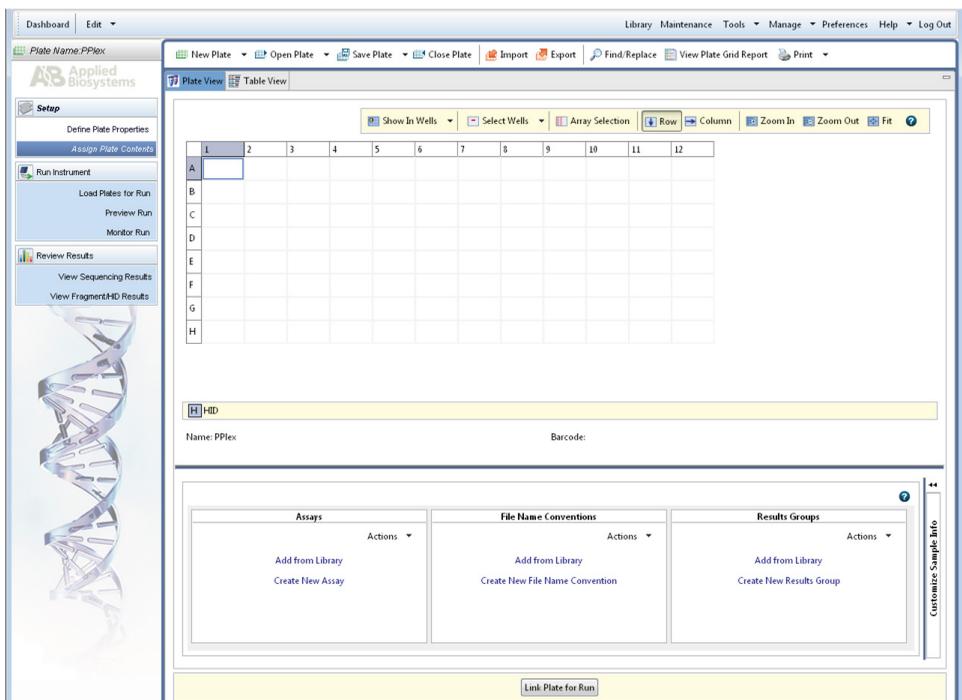


Figure 9. Assigning plate contents.

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

11. Assign sample names to wells.
12. In the lower left portion of the screen, under Assays, use the Add from Library option to select the Assay created in Step 5 or one previously created. Choose the **Add to Plate** button, and close the window.
13. Under File Name Convention, use the Add from Library option to select the File Name Convention created in Step 6 or one previously created. Choose the **Add to Plate** button, and close the window.
14. Under Results Groups, use the Add from Library option to select the Results Group created in Step 7 or one previously created. Choose the **Add to Plate** button, and close the window.
15. Highlight the sample wells, then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.
16. Select **Link Plate for Run**.
17. The 'Load Plate' window will appear. Select **Yes**.
18. In the Run Information window (Figure 10), assign a Run Name. Select **Start Run** (not shown).

Note: Each injection will take approximately 45 minutes.

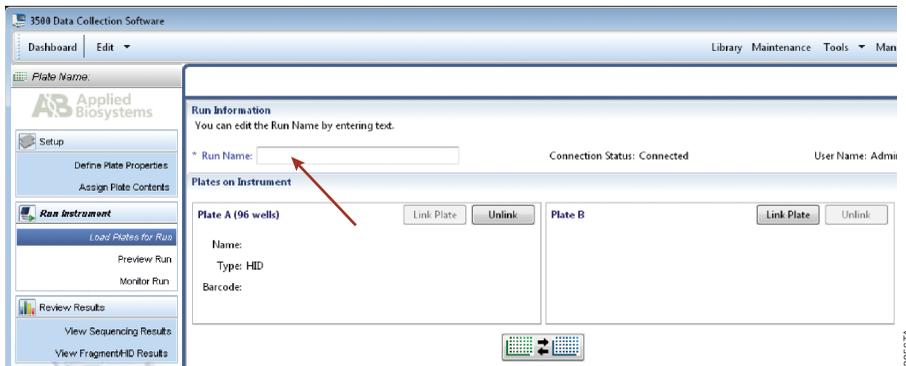


Figure 10. Assigning a run name.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3130 or 3130xl Genetic Analyzer with Data Collection Software, Version 3.0 or 4.0

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3130 capillary array, 36cm
- POP-4® polymer for the Applied Biosystems® 3130/3130xl Genetic Analyzer
- 10X genetic analyzer buffer with EDTA
- 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 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.

Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and 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 increased or decreased to adjust the intensity of the size standard peaks.

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). Cover wells with appropriate septa.

Note: Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time in the run module (see Instrument Preparation below). Alternatively, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles to achieve the desired signal intensity.

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, in an ice-water bath or freezer plate block for 3 minutes. Denature samples just prior to loading the instrument.

5.B. Detection of Amplified Fragments Using the Applied Biosystems® 3130 or 3130xl Genetic Analyzer with Data Collection Software, Version 3.0 or 4.0 (continued)

Instrument Preparation

Refer to the instrument users 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 3130xl Genetic Analyzer with Data Collection Software with the following exceptions.

1. In the Module Manager, select **New**. Select **Regular** in the 'Type' drop-down list, and select **HIDFragmentAnalysis36_POP4** in the 'Template' drop-down list. Confirm that the injection time is 5 seconds, the injection voltage is 3kV and the run time is 1,800 seconds. Give a descriptive name to your run module, and select **OK**.
Note: Instrument sensitivities can vary. The injection time may be adjusted in the Module Manager. A suggested range for the injection time is 3–22 seconds.
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 **Dye Set F** 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 fields, and select **OK**.
Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.
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 F**, and confirm that the active dye set is the file generated for the *GenePrint*® 10 chemistry.
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, select 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 will change from yellow to green, and the green **Run Instrument** arrow becomes enabled.

10. Select 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 45 minutes.
Note: If peaks are low or absent, the sample can be reinjected with an increased injection time. If the ILS 600 is also affected, check the laser power.

6. Data Analysis

To facilitate analysis of data generated 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: HID run files generated using the Applied Biosystems[®] 3500 or 3500xL Genetic Analyzer cannot be analyzed using GeneMapper[®] Software. You must analyze these files with GeneMapper[®] ID-X software.

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

6.A. Importing *GenePrint*[®] 10 Panels and Bins Text Files with GeneMapper[®] Software, Version 4.0 and Later

1. Open the GeneMapper[®] software, version 4.0.
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. 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. Select the file, and then **Import**.
9. At the bottom of the 'Panel Manager' window, select **OK**. This will save the files and close the window.



6.B. Importing the ILS 600 Size Standard into GeneMapper® Software, Version 4.0 and Later

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **Import**.
4. Browse to the location of the ILS_600.xml file.
5. Highlight the file, and then select **Import**.
6. Select **Done** to save changes and exit the GeneMapper Manager.

6.C. Importing the GenePrint® 10 GeneMapper® Analysis Method into GeneMapper® Software, Version 4.0 or Later

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **Import**.
4. Browse to the location of the GenePrint 10 Analysis Method.xml file.
5. Highlight the file, then select **Import**.
6. Select **Done** to save changes and exit the GeneMapper Manager.

6.D. Processing Data with GeneMapper® Software, Version 4.0 and Later

1. Select **File**, then **New Project**.
2. Select **Generic** as the Project Type in the 'New Project' window, then select **OK**.
3. Browse to the location of the run files. Highlight the desired files, then select **Add to list** followed by **Add**.
4. In the 'Sample Type' column, use the drop-down menu to select **Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated **Ladder** in the 'Sample Type' column for proper genotyping.
5. In the 'Analysis Method' column, select the *GenePrint*® 10 GeneMapper® 4.0 analysis method imported in Section 6.C.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.A.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.B.
8. Select **Analyze** (green arrow button) to start the data analysis.

6.E. Importing *GenePrint*[®] 10 Panels and Bins Text Files with GeneMapper[®] ID Software, Versions 3.2 and 3.2.1

1. Open the GeneMapper[®] ID software, version 3.2 or 3.2.1
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 imported above. Select the file, then **Import**.
6. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 5.
7. Select **File**, then **Import Bin Set**.
8. Navigate to the bins text file saved to your computer above. Select the file, then **Import**.
9. At the bottom of the 'Panel Manager' window, select **OK**. The 'Panel Manager' window will close automatically.

6.F. Importing the ILS 600 Size Standard into GeneMapper[®] ID Software, Versions 3.2 and 3.2.1

1. Select **Tools**, then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **Import**.
4. Browse to the location of the ILS_600.xml file.
5. Highlight the file, then select **Import**.
6. Select **Done** to save changes and exit the GeneMapper Manager.

6.G. Importing the *GenePrint*[®] 10 GeneMapper[®] ID Analysis Method into GeneMapper[®] ID Software, Versions 3.2 and 3.2.1

1. Select **Tools**, then **GeneMapper Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **Import**.
4. Browse to the location of the GenePrint 10 GMID 3.2 Analysis Method.xml file.
5. Highlight the file, then select **Import**.
6. Select **Done** to save changes and exit the GeneMapper Manager.

6.H. Processing Data with GeneMapper® ID Software, Versions 3.2 and 3.2.1

1. Select **File**, then **New Project**.
2. Select **Edit**, then **Add Samples to Project**.
3. Browse to location of the run files. Highlight desired files, then select **Add to List** followed by **Add**.
4. In the 'Sample Type' column, use the drop-down menu to select **Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated **Ladder** in the 'Sample Type' column for proper genotyping.
5. In the 'Analysis Method' column, select the GenePrint 10 GMID 3.2 Analysis Method.xml file imported in Section 6.G.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.E.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.F.
8. Select **Analyze** (green arrow button) to start data analysis.

6.I. Importing GenePrint® 10 Panels, Bins and Stutter Text Files with GeneMapper® ID-X Software, Versions 1.2 and Later

1. Open the GeneMapper® ID-X 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. In the 'Choose Security Group' window select the applicable security group from the drop-down menu, then select **OK**.
6. Navigate to the panels text file saved to your computer above. Select the file, then **Import**.
7. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 4.
8. Select **File**, then **Import Bin Set**.
9. Navigate to the bins text file saved to your computer above. Select the file, then **Import**.
10. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 4.
11. Select **File**, then **Import Marker Stutter**. A warning box will appear asking if you want to overwrite current values. Select **Yes**.
12. Navigate to the stutter text file saved to your computer above. Select the file, then **Import**.
13. At the bottom of the 'Panel Manager' window, select **OK**. This will save the panels, bins and stutter text files, then close the window automatically.

6.J. Importing the ILS 600 Size Standard into GeneMapper® ID-X Software, Versions 1.2 and Later

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Size Standard' tab.
3. Select **Import**.
4. In the 'Choose Security Group' window select the applicable security group from the drop-down menu, then select **OK**.
5. Browse to the location of the ILS_600.xml file.
7. Highlight the file, then select **Import**.
8. Select **Done** to save changes and exit the GeneMapper ID-X Manager.

6.K. Importing the GenePrint® 10 GeneMapper® ID-X Analysis Method into GeneMapper® ID-X Software, Versions 1.2 and Later

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **Import**.
4. In the 'Choose Security Group' window, select the applicable security group from the drop-down menu, then select **OK**.
5. Browse to the location of the GenePrint 10 GMIDX Analysis Method.xml file.
6. Highlight the file, then select **Import**.
7. Select **Done** to save changes and exit the GeneMapper ID-X Manager.

6.L. Processing Data with GeneMapper® ID-X Software, Versions 1.2 and Later

1. Select **File**, then **New Project**.
2. Select **Edit**, then **Add Samples to Project**.
3. Browse to the location of the run files. Highlight desired files, then select **Add to list** followed by **Add**.
4. In the 'Sample Type' column, use the drop-down menu to select **Allelic Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as **Allelic Ladder** in the 'Sample Type' column for proper genotyping.
5. In the 'Analysis Method' column, select the *GenePrint*® 10 GeneMapper® ID-X analysis method imported in Section 6.K.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.I.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.J.

6.L. Processing Data with GeneMapper® ID-X Software, Versions 1.2 and Later (continued)

8. Select **Analyze** (green arrow button) to start data analysis.

Note: By default, the software displays the ‘Analysis Requirements Summary’ and ‘Allelic Ladder Analysis Summary’ windows after quality review by the software. Ensure that all requirements are met as each window appears. If you do not have the ‘Analysis Requirements Summary’ window activated, you may need to do additional manual troubleshooting.

9. If all analysis requirements are met, the ‘Save Project’ window will open (Figure 11).

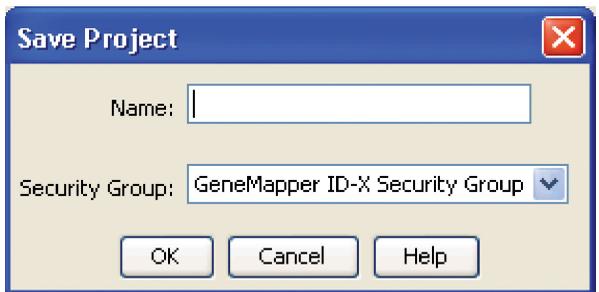


Figure 11. The ‘Save Project’ window.

10. Enter the project name. Choose the applicable security group from the drop-down menu, then select **OK**.

6.M. Controls

1. Examine the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.
2. Examine the results for the 2800M Control DNA. Compare the 2800M Control DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 2800M DNA allele designations for each locus are listed in Table 2.

Table 2. Expected Allele Designations for the 2800M Control DNA.

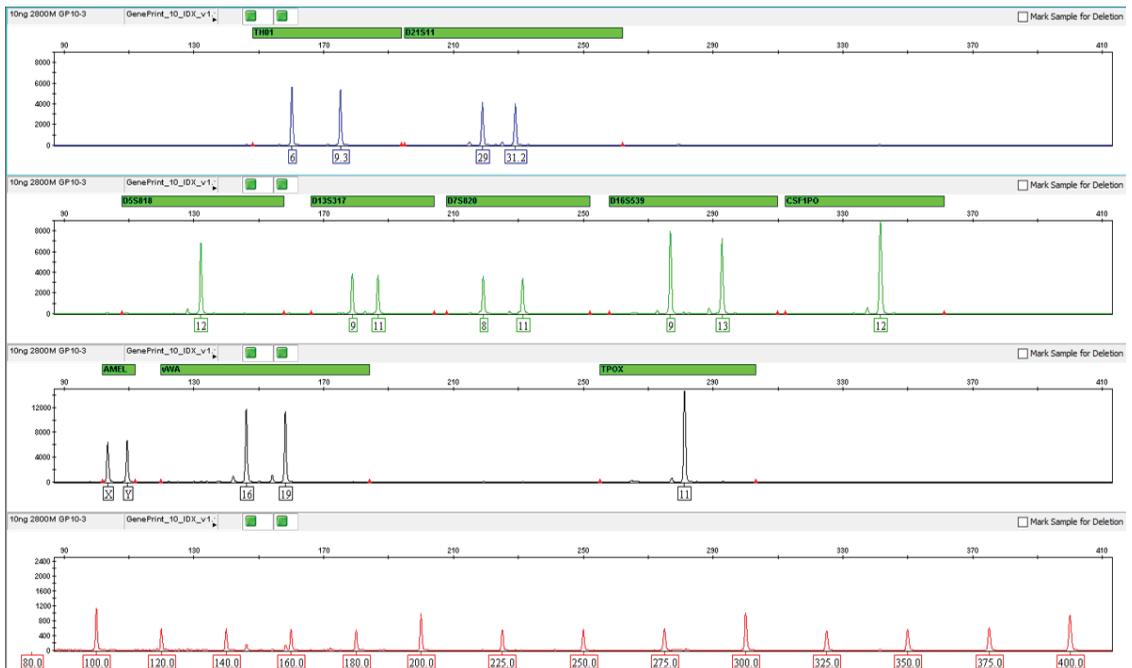
STR Locus	Alleles
TH01	6, 9.3
D21S11	29, 31.2
D5S818	12, 12
D13S317	9, 11
D7S820	8, 11
D16S539	9, 13
CSF1PO	12, 12
Amelogenin	X, Y
vWA	16, 19
TPOX	11, 11

6.N. Results

Representative results of the *GenePrint*[®] 10 System are shown in Figure 12. The *GenePrint*[®] 10 Allelic Ladder Mix is shown in Figure 13.

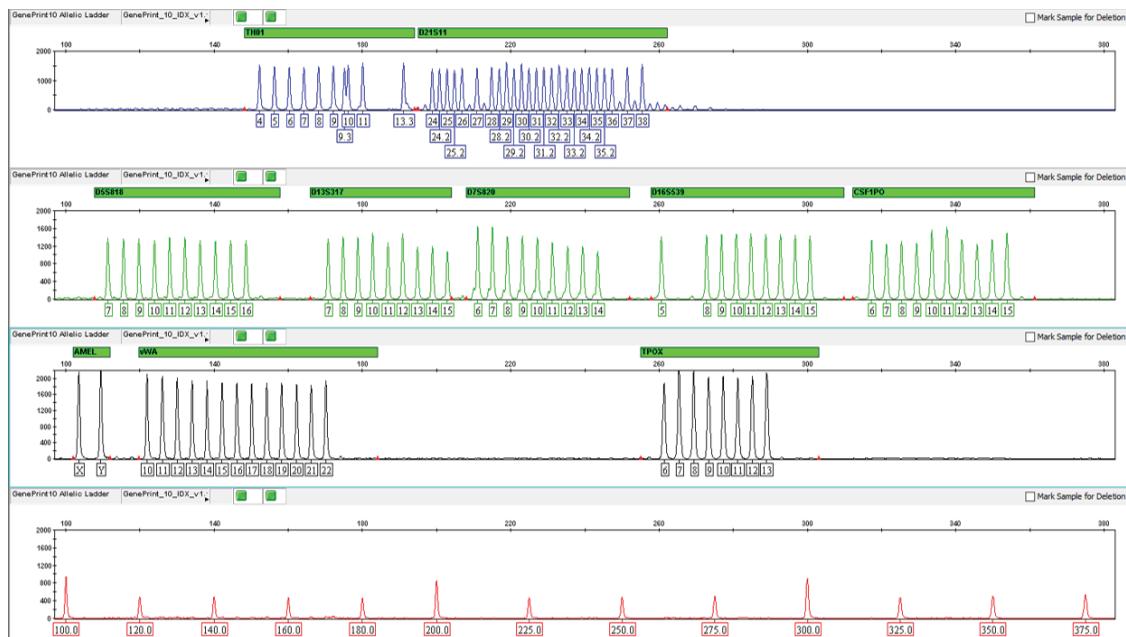


Locus-to-locus peak height imbalance will likely occur with cell line DNA. Normal genomic DNA has equal copies of each locus, and amplification will result in relatively even locus-to-locus balance. Cell line DNA can have mutations that affect the locus-to-locus allele peak height balance. Additionally, cell lines occasionally have tri-allelic patterns at a locus. 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.



13497A

Figure 12. Representative data for the *GenePrint*[®] 10 System. A single-source DNA template (10ng) was amplified using the *GenePrint*[®] 10 System. Amplification products were mixed with Internal Lane Standard 600 and analyzed using an Applied Biosystems[®] 3500xL Genetic Analyzer and a 1.2kV, 10-second injection. The results were analyzed using GeneMapper[®] ID-X software, version 1.4, and the appropriate panels, bins and stutter files.



113481A

Figure 13. The GenePrint® 10 Allelic Ladder. The GenePrint® 10 Allelic Ladder was analyzed using an Applied Biosystems® 3500xL Genetic Analyzer and a 1.2kV, 10-second injection. The sample file was analyzed using GeneMapper® ID-X software, version 1.4, and the appropriate panels, bins and stutter files.

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 can 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, 6–12 bases smaller than D16S539 alleles, or as low-level artifacts in vWA. 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.

6.N. Results (continued)

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. Reinject the samples to confirm.

7. 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

7.A. Amplification and Fragment Detection

This section provides information about general amplification and detection. For questions about amplification of extracted DNA, see Section 7.B. For questions about direct amplification, see Section 7.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.

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 protocols. 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 the laser power.

Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.

Faint or absent peaks for the positive control reaction

Improper storage of the 2800M Control DNA. Store the 2800M Control DNA at +2°C to +10°C. Make sure that the 2800M Control DNA is stored at +2°C to +10°C for at least 24 hours before use; do not refreeze. 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.

7.A. Amplification and Fragment Detection (continued)

Symptoms

Extra peaks visible in one or all color channels
(continued)

Causes and Comments

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 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 the 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.

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 10-minute extension step at 60°C after thermal cycling (Section 4 or 9.B).
- Decrease the number of cycles.
- Plasticware can alter heat transfer during amplification and prevent full adenylation. Increase the final extension time.

CE-related artifacts (“spikes”). Minor voltage changes or urea crystals passing by the laser can cause “spikes” or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Reinject samples to confirm.

CE-related artifacts (contaminants). Contaminants in the water used with the instrument or to dilute the 10X genetic analyzer buffer may generate peaks in the fluorescein and JOE channels. Use autoclaved deionized water; change vials and wash buffer reservoir.

Symptoms

Extra peaks visible in one or all color channels
(continued)

Causes and Comments

Incorrect spectral was active. Rerun samples, and confirm that the spectral is set for the dye set being used. See instructions on instrument preparation in Section 5.

Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples.

- Perform a new spectral calibration and rerun the samples.
- Confirm that the correct spectral was used for the sample run.
- Instrument sensitivities can vary. Optimize the injection conditions. Refer to the instrument user manual.
- 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 products at -20°C.

An incorrect internal lane standard was used. Use the size standard provided in the kit.

The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than 1 week.

Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.

Allelic ladder not running
the same as samples

Allelic ladder mix and primer pair mix were not compatible. Ensure that the allelic ladder mix is from the same kit as the primer pair mix.

Be sure the allelic ladder and samples are from the same instrument run.

Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE capillary over time. Use a different injection of allelic ladder to determine sizes.

7.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Allelic ladder not running the same as samples (continued)	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 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.

7.B. Amplification of Extracted DNA

The following information is specific to amplification of extracted DNA. For information about general amplification and detection, see Section 7.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 affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20 μ g/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25 μ l for extracted DNA. Decreasing the reaction volume may result in suboptimal performance.

Symptoms

Extra peaks visible in one or all color channels

Causes and Comments

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 6.N, Results, 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 the 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 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 can result in suboptimal performance.

7.C. 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 7.A.

Symptoms

Faint or absent allele peaks

Causes and Comments

For direct amplification reactions that require adding AmpSolution™ Reagent, its omission can inhibit 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 the storage card. Take punches from a different portion of the card. Increasing cycle number can improve low 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 the amplification reaction when using nonlytic card punches. Ensure that the heat block was set at 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.

Symptoms
Causes and Comments

Faint or absent allele peaks (continued)

Inactive PunchSolution™ Reagent was used to pretreat nonlytic storage card punches. Thaw the PunchSolution™ Reagent at +2°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.

Faint or absent peaks for the positive control reaction

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 the 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.

Extra peaks visible in one or all color channels

Punch was contaminated with DNA from another sample. Perform punches on a blank card between samples to minimize potential for carryover between samples.

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.

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.

7.C. Direct Amplification of DNA from Storage Card Punches (continued)

Symptoms

Extra peaks visible in one or all color channels (continued)

Causes and Comments

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 Section 6.N, Results, for additional information on stutter and artifacts.

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 10-minute extension step at 60°C after thermal cycling.
- Decrease cycle number.
- Increase the final extension time.

Peak height imbalance

Excessive amount of DNA. Amplification of high amounts of template can result in an imbalance, with smaller loci showing more product than larger loci.

- Be sure to use the recommended number of punches. Follow the manufacturer's recommendations when depositing sample onto the card.
- Decrease cycle number.

The cycle number was too high. Decrease the cycle number by one cycle and repeat the amplification.

For direct amplification reactions that require adding AmpSolution™ Reagent, its omission can inhibit the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.

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 can result in suboptimal performance due to inhibitors present in lytic cards and PunchSolution™ Reagent.

Symptoms

Peak height imbalance (continued)

Causes and Comments

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.

Active PunchSolution™ Reagent carried over into the amplification reaction with nonlytic card punches. Larger loci are most susceptible to carryover and will drop out before the smaller loci.

- 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 25µl or 12.5µl amplification reaction. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.
-

Inactive PunchSolution™ Reagent was used to pretreat nonlytic storage card punches. Thaw the PunchSolution™ Reagent at +2°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.

Extreme variability in sample-to-sample peak heights

There can be significant individual-to-individual variability in the number of cells onto a storage card, resulting in peak height variability. The PunchSolution™ Kit increases the recovery of amplifiable DNA from nonlytic storage card punches but does not normalize the amount of DNA present.

7.D. GeneMapper® Software

Symptoms

Alleles not called

Causes and Comments

To analyze samples with GeneMapper® software, at least one allelic ladder must be defined per folder of sample files being analyzed in the project.

An insufficient number of ILS 600 fragments was defined. Be sure to define at least two ILS 600 fragments smaller than the smallest sample peak or allelic ladder peak and at least two ILS 600 fragments larger than the largest sample peak or allelic ladder peak.

Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run.

- Create a new size standard using the internal lane standard fragments present in the sample.
- Rerun samples using a longer run time.

A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Off-ladder alleles

An allelic ladder from a different run than the samples was used. Reanalyze 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 reanalyze as described in Section 6.D.

Panel text file selected for analysis was incorrect for the STR system used. Assign correct panel 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 internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.

Symptoms
Causes and Comments

Off-ladder alleles (continued)	Incorrect analysis method used. Only the <i>GenePrint</i> [®] 10 System GeneMapper [®] 4.0 analysis method is capable of calibrating the bins based on sizes of alleles in the allelic ladder analyzed using the specific configuration of instrument, polymer and capillary length. Creating an analysis method within GeneMapper [®] software, version 4.0, does not enable this functionality and will result in off-ladder allele calls.
Size standard not called correctly	<p>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.</p> <p>Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select Edit and select Delete Size Label. Select Auto Adjust Sizes.</p> <p>Run was too short, and larger peaks in ILS were not captured. Not all 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. • Rerun samples using a longer run time.
Peaks in size standard missing	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Significantly raised baseline	<p>Poor spectral calibration. Perform a new spectral calibration, and rerun the samples.</p> <p>Incorrect spectral calibration was active. Confirm that the correct spectral was used to run the samples.</p>
No alleles called, but no error message appears	<p>Panels text file was not selected for sample in the project. Be sure to select the appropriate panels and size standard for all samples in the project.</p> <p>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 ladder peaks to be missing. This will cause your sizing quality to be flagged as “red”, and no allele sizes will be called.</p>

7.D. GeneMapper® Software (continued)

Symptoms

Error message:

“Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit”

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”.

Causes and Comments

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.

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 reimport files in a different order.

7.E. GeneMapper® ID Software

Symptoms

Alleles not called

Causes and Comments

To analyze samples with GeneMapper® ID software, the analysis parameters and size standard must both have “Basic or Advanced” as the analysis type. If they are different, an error is obtained.

To analyze samples with GeneMapper® ID 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.
- Rerun samples using a longer run time.

A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Off-ladder alleles

An allelic ladder from a different run than the samples was used. Reanalyze samples with an allelic ladder from the same run.

The GeneMapper® ID 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 reanalyze as described in Section 6.H.

Symptoms

Causes and Comments

Off-ladder alleles (continued)

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.

A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Size standard not called correctly

Starting data point was incorrect for the partial range chosen in Section 6.H. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.

Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto Adjust Sizes**.

Peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run.

- Create a new size standard using the internal lane standard fragments present in the sample.
- Rerun samples using a longer run time.

Peaks in size standard missing

If peaks are low-quality, redefine the size standard for the sample to skip these peaks.

An incorrect size standard was used.

No alleles called, but no error message appears

Panels text file was not selected for sample. In the 'Panel' column, select the appropriate panels text file for the STR system that was used.

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 ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.

7.E. GeneMapper® ID Software (continued)

Symptoms

Error message:

“Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit”

Causes and Comments

The bins text file assigned to the analysis method was deleted. In the GeneMapper Manager, select the ‘Analysis Methods’ tab, and open the analysis method of interest. Select the ‘Allele’ tab, and select an appropriate bins text file.

The wrong bins text file was chosen in the analysis method ‘Allele’ tab. Be sure to choose the appropriate bins text file, as shown in Figure 21.

Significantly raised baseline

Poor spectral calibration. Perform a new spectral calibration, and rerun the samples.

Rerun and optimize the matrix. Make sure that the matrix applied was generated on the same instrument.

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 reimport files in a different order.

7.F. GeneMapper® ID-X Software

Symptoms

Stutter peaks not filtered

Causes and Comments

Stutter file was not imported into the Panel Manager when the panels and bin text files were imported.

Be sure that the “Use marker-specific stutter ratio and distance if available” box is checked. If the “Use marker-specific stutter ratio and distance if available” box is not checked, stutter distance must be defined in the Analysis Method ‘Allele’ tab.

Samples in the project not analyzed

The ‘Analysis Requirements Summary’ window was not active, and there was an analysis requirement that was not met. Turn on ‘Analysis Requirements Summary’ in the ‘Project Options’ menu under File, and correct the necessary analysis requirements to continue analysis.

Edits in label edit viewer cannot be viewed

To view edits made to a project, the project must first be saved. Close the plot view window, go back to the main GeneMapper® ID-X page and save the project. Display the plot window again, then view the label edit table.

Symptoms

Causes and Comments

Marker header bar for some loci are gray

When an edit is made to a locus, the quality flags and marker header bar automatically change to gray. To change the GQ and marker header bar for a locus to green, override the GQ in the plot window.

Alleles not called

To analyze samples with GeneMapper® *ID-X* 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.
- Rerun samples using a longer run time.

A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Off-ladder alleles

An allelic ladder from a different run than the samples was used. Reanalyze samples with an allelic ladder from the same run.

The GeneMapper® *ID-X* 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 6.L.

The allelic ladder was not identified as an allelic ladder in the 'Sample Type' column.

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 POP-4® may change migration of the fragments. Alleles may migrate outside of the panel range established using POP-4®.

7.F. GeneMapper® ID-X Software (continued)

Symptoms

Causes and Comments

Size standard not called correctly

Starting data point was incorrect for the partial range chosen for the analysis. Adjust the starting data point or use a full range for the analysis. Alternatively, use a full range for the analysis.

Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto Adjust Sizes**.

An incorrect internal lane standard was used.

Run was too short, and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run.

- Create a new size standard using the internal lane standard fragments present in the sample.
- Rerun samples using a longer run time.

Peaks in size standard missing

If peaks are low-quality, redefine the size standard for the sample to skip these peaks.

An incorrect internal lane standard was used.

Significantly raised baseline

Poor spectral calibration. Perform a new spectral calibration, and rerun the samples.

Incorrect spectral calibration was active. Confirm that the correct spectral was used to run the samples.

8. References

1. Chatterjee, R. (2007) Cell biology. Cases of mistaken identity. *Science* **315**, 928–31.
2. Ruiz Bravo, N. and Gottesman, M. (2007) Notice regarding authentication of cultured cell lines. This can be viewed online at: <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html>
3. Yoshino, K. *et al.* (2006) Essential role for gene profiling analysis in the authentication of human cell lines. *Human Cell* **19**, 43–8.
4. Szibor, R. *et al.* (2003) Cell line DNA typing in forensic genetics—the necessity of reliable standards. *Forensic Sci. Int.* **138**, 37–43.
5. Dirks, W.G. *et al.* (2005) Short tandem repeat DNA typing provides an international reference standard for authentication of human cell lines. *ALTEX* **22**, 103–9.
6. Masters, J.R. *et al.* (2001) Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc. Natl. Acad. Sci. USA* **98**, 8012–7.
7. Capes-Davis, A. *et al.* (2010) Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int. J. Cancer* **127**, 1–8.
8. ANSI/ATCC ASN-0002-2011. Authentication of human cell lines: Standardization of STR profiling. ANSI eStandards Store, 2012.
9. Kline, M.C. *et al.* (2005) Results from the NIST 2004 DNA quantitation study. *J. Forensic Sci.* **50**, 570–8.
10. Levinson, G. and Gutman, G.A. (1987) Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* **4**, 203–21.
11. Schlötterer, C. and Tautz, D. (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* **20**, 211–5.
12. Smith, J.R. *et al.* (1995) Approach to genotyping errors caused by nontemplated nucleotide addition by *Taq* DNA polymerase. *Genome Res.* **5**, 312–7.
13. Magnuson, V.L. *et al.* (1996) Substrate nucleotide-determined non-templated addition of adenine by *Taq* DNA polymerase: Implications for PCR-based genotyping and cloning. *BioTechniques* **21**, 700–9.
14. Walsh, P.S., Fildes, N.J. and Reynolds, R. (1996) Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res.* **24**, 2807–12.
15. Möller, A., Meyer, E. and Brinkmann, B. (1994) Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. *Int. J. Leg. Med.* **106**, 319–23.
16. Brinkmann, B., Möller A. and Wiegand, P. (1995) Structure of new mutations in 2 STR systems. *Int. J. Leg. Med.* **107**, 201–3.
17. Griffiths, R.A. *et al.* (1998) New reference allelic ladders to improve allelic designation in a multiplex STR system. *Int. J. Legal Med.* **111**, 267–72.
18. Bär, W. *et al.* (1997) DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int. J. Legal Med.* **110**, 175–6.
19. Gill, P. *et al.* (1997) Considerations from the European DNA Profiling Group (EDNAP) concerning STR nomenclature. *Forensic Sci. Int.* **87**, 185–92.

9. Appendix

9.A. 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 Components
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 ³
D7S820	JOE	215–247	6–14
D16S539	JOE	264–304	5, 8–15
CSF1PO	JOE	321–357	6–15
Amelogenin ⁴	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 analysis.

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 affects 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).

⁴Amelogenin is not an STR.

9.B. 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 thermal cycler.

Materials to Be Supplied by the User

- GeneAmp[®] PCR System 9700 thermal cycler with a gold-plated silver or silver sample block (Applied Biosystems)
- microcentrifuge
- MicroAmp[®] optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips
- PunchSolution™ Kit (Cat. # DC9271) for nonlytic storage 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[®], S&S 903)

9.B. Direct Amplification of DNA from Storage Card Punches (continued)

Note: Samples on nonlytic storage cards must be pretreated with the PunchSolution™ Kit (Cat.# DC9271) to lyse samples before adding PCR amplification mix. For more information, see the *PunchSolution™ Kit Technical Manual* #TMD038. Failure to pretreat these samples may result in incomplete profiles.

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 the storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm disk. Use the plunger to eject one 1.2mm sample 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 storage card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonlytic storage 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, 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 new MicroAmp® plate for reaction assembly, and label 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 5 to a new tube. Add Water, Amplification Grade, to the tube first, then add *GenePrint*[®] 10 5X Master Mix and *GenePrint*[®] 10 5X Primer Pair Mix. For nonlytic storage card punches, the template DNA will be added at Step 7.

Table 5. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches.

PCR Amplification Mix Component	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 6 to a new 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 storage card punches, the template DNA will be added at Step 7.

Table 6. PCR Amplification Mix for Amplification of 2800M Control DNA.

PCR Amplification Mix Component	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.

9.B. Direct Amplification of DNA from Storage Card Punches (continued)

6. Vortex the PCR amplification mixes 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 mixes 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 culture cells directly to the appropriate wells of the reaction plate. For nonlytic storage card punches, add the PCR amplification mix to the PunchSolution™ Reagent-pretreated punch.

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

8. For the positive amplification control, vortex the tube of 2800M Control DNA, then add 5µl of 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 control DNA may be required, depending on cycling conditions and laboratory preferences. See Step 5.
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 thermal cycler. 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. The estimated total cycle time is 1.5 hours.

Note: When using the GeneAmp® PCR System 9700 thermal cycler, 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. 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 protected from light.

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 samples 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 (25–27 cycles).
5. Following amplification, use your laboratory’s optimized separation and detection protocols to determine the optimal cycle number.

9.C. The Internal Lane Standard 600

The Internal Lane Standard (ILS) 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 14). Each fragment is labeled with carboxy-X-rhodamine (CXR) and may be detected separately (as a fourth color) in the presence of *GenePrint*[®] 10 System-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.

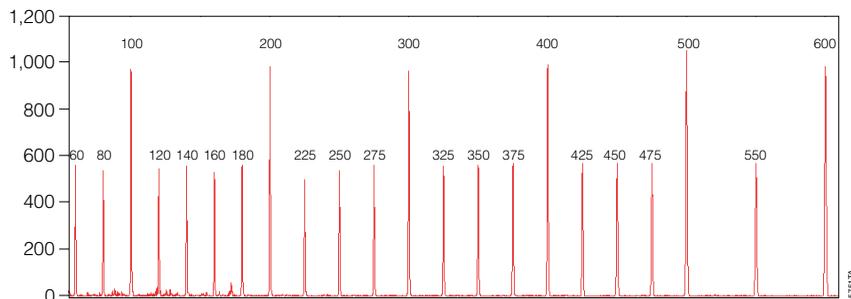


Figure 14. An electropherogram showing the fragments of the Internal Lane Standard 600.

9.D. 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.

9.E. Related Products

Accessory Components

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

*Not for Medical Diagnostic Use.

10. Summary of Changes

The following changes were made to the 7/22 revision of this document:

1. Added instructions for use of the ProFlex® PCR System.
2. Removed mention of ABI PRISM® Genetic Analyzers.
3. Updated Sections 6.N and 9.E.
4. Revised Section 7.
5. Removed disclaimers.
6. Incorporated other general updates.
7. Updated the cover page.

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