TECHNICAL MANUAL

# *GenePrint*® 10 System

Instructions for Use of Product
**B9510**


Revised 6/16
TM392

# *GenePrint*® 10 System

**All technical literature is available at: www.promega.com/protocols/**
**Visit the web site to verify that you are using the most current version of this Technical Manual.**
**E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com**


1. Description ..... 2
2. Product Components and Storage Conditions ..... 5
3. Before You Begin ..... 6
   - 3.A. Precautions ..... 6
   - 3.B. Spectral Calibration ..... 6
4. Protocol for DNA Amplification Using the *GenePrint*® 10 System ..... 7
5. Instrument Setup and Sample Preparation ..... 10
   - 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer ..... 10
   - 5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 ..... 22
6. Data Analysis ..... 24
   - 6.A. Importing *GenePrint*® 10 Panels and Bins Text Files with GeneMapper® Software, Version 4.0 ..... 25
   - 6.B. Importing the ILS 600 Size Standard into GeneMapper® Software, Version 4.0 ..... 25
   - 6.C. Importing the *GenePrint*® 10 GeneMapper® Analysis Method into GeneMapper® Software, Version 4.0 ..... 26
   - 6.D. Processing Data with GeneMapper® Software, Version 4.0 ..... 26
   - 6.E. Importing *GenePrint*® 10 Panels and Bins Text Files with GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 ..... 26
   - 6.F. Importing the ILS 600 Size Standard into GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 ..... 27
   - 6.G. Importing the *GenePrint*® 10 GeneMapper® *ID* Analysis Method into GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 ..... 27
   - 6.H. Processing Data with GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 ..... 27
   - 6.I. Importing *GenePrint*® 10 Panels, Bins and Stutter Text Files with GeneMapper® *ID*-X Software, Versions 1.2 and Later ..... 28
   - 6.J. Importing the ILS 600 Size Standard into GeneMapper® *ID*-X Software, Versions 1.2 and Later ..... 28

6.K. Importing the *GenePrint*® 10 GeneMapper® *ID*-X Analysis Method into GeneMapper® *ID*-X Software, Versions 1.2 and Later ..... 29
6.L. Processing Single-Source Data with GeneMapper® *ID*-X Software, Versions 1.2 and Later ..... 29
6.M. Controls ..... 30
6.N. Results ..... 31

7. Troubleshooting ..... 34
7.A. Amplification and Fragment Detection ..... 34
7.B. Amplification of Extracted DNA ..... 37
7.C. Direct Amplification of DNA from Storage Card Punches ..... 38
7.D. GeneMapper® Software ..... 41
7.E. GeneMapper® *ID* Software ..... 43
7.F. GeneMapper® *ID*-X Software ..... 45

8. References ..... 47

9. Appendix ..... 48
9.A. Additional STR Locus Information ..... 48
9.B. Direct Amplification of DNA from Storage Card Punches ..... 50
9.C. The Internal Lane Standard 600 ..... 55
9.D. Composition of Buffers and Solutions ..... 55
9.E. Related Products ..... 56

10. Summary of Changes ..... 57

## 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://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac_/Cross_Contaminations_v6_8.pdf**

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://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac_**

The *GenePrint*® 10 System[a–c] 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 $2.92 \times 10^9$.

The *GenePrint*® 10 System is compatible with the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL 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.

Figure 1 outlines the *GenePrint*® 10 System protocols in this manual for amplifying STR loci and detecting amplified products. These protocols were tested at Promega. The alleles are resolved using capillary electrophoresis (CE), and the resulting CE data are analyzed using genotyping software and the parameters given on the Promega web site at:
**www.promega.com/resources/software-firmware/geneprint-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.

## 1. Description (continued)

| Amplification Setup | | |
|---|---|---|
| *Sections 4 and 9.B.* | | |
| **Thermal Cycling** | | |
| *Sections 4 and 9.B.* | GeneAmp® PCR System 9700 | |
| **Instrument Setup and Sample Preparation** | | |
| *Section 5.* | Applied Biosystems® 3500 or 3500xL Genetic Analyzer | ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0 |
| | Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 | |
| **Data Analysis** | | |
| *Section 6.* | GeneMapper® Software, Version 4.0 | GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 |
| | GeneMapper® *ID*-X Software, Versions 1.2 and Later | |

**Figure 1. An overview of the *GenePrint*® 10 System protocol.**

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| ***GenePrint*® 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 2–10°C. For daily use, the *GenePrint*® 10 System components can be stored for up to 1 week at 2–10°C. Make sure that the 2800M Control DNA is stored at 2–10°C for at least 24 hours before use. The *GenePrint*® 10 5X Primer Pair Mix, *GenePrint*® 10 Allelic Ladder Mix and Internal Lane Standard 600 (ILS 600) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

### Available Separately

Matrix standards are required for initial setup of the color separation matrix (see Section 3.B). The matrix standards are sold separately and are available for the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 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 (*GenePrint*® 10 5X Master Mix, *GenePrint*® 10 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 (*GenePrint*® 10 Allelic Ladder Mix and Internal Lane Standard 600). 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 ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 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.

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 silver or gold-plated silver sample block (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.E)

The *GenePrint*® 10 System was optimized to amplify 10ng of template DNA in a 25µl 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. Increase the number of reactions by 10–15% to compensate for pipetting error (e.g., for 96 reactions, add 10 to 15 additional reactions). While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately.

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

4. Add the final volume of each reagent listed in Table 1 to a sterile 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 25.0µl | × | | = | |
| *GenePrint*® 10 5X Master Mix | 5.0µl | × | | = | |
| *GenePrint*® 10 5X Primer Pair Mix | 5.0µl | × | | = | |
| template DNA (10ng)[1,2] | up to 15µl | | | | |
| **total reaction volume** | 25µl | | | | |

[1]Store DNA templates in nuclease-free water, $TE^{-4}$ 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.

[2]Apparent DNA concentrations can differ, depending on the DNA quantification method used (9). The amount of DNA template recommended here is based on DNA concentrations determined by measuring absorbance at 260nm. We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

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

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

6. Add template DNA (10ng) for each sample to the respective well containing PCR amplification mix.
7. 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.
8. For the negative amplification control, pipet Water, Amplification Grade, or $TE^{-4}$ buffer instead of template DNA into a reaction well containing PCR amplification mix.
9. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

## Thermal Cycling

This manual contains a protocol for use of the *GenePrint*® 10 System with the GeneAmp® PCR System 9700 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 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. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below. The estimated total cycling time is 1.5 hours.

| **Thermal Cycling Protocol**[1] |
|---|
| 96°C for 1 minute, then: |
| 94°C for 10 seconds<br>59°C for 1 minute<br>72°C for 30 seconds<br>for 30 cycles, then: |
| 60°C for 10 minutes |
| 4°C soak |

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with Max mode as the ramp speed. (This requires a silver or gold-plated 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.

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

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

## 5. Instrument Setup and Sample Preparation

### 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer

**Materials to Be Supplied by the User**

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- 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 in a pouch for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container
- MicroAmp® optical 96-well plate and septa, or equivalent
- 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.

## Sample Preparation

1. Thaw the Internal Lane Standard 600.

   **Note:** Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing the Internal Lane Standard 600 and Hi-Di™ formamide as follows:

   [(0.5µl ILS 600) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# 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 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If the peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of Hi-Di™ formamide.

3. Vortex for 10–15 seconds to mix.

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

5. Add 1µl of amplified sample (or 1µl of *GenePrint*® 10 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.

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

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

## 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer (continued)

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

   Set the oven temperature to 60°C, then select "Start Pre-Heat" at least 30 minutes prior to the first injection to preheat the oven.


**Figure 2. The Dashboard.**

2. To create a new Instrument Protocol, navigate to the Library, select "Instrument Protocol", then select "Create". Alternatively, a previously created Instrument Protocol may be used.

   Figure 3 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.

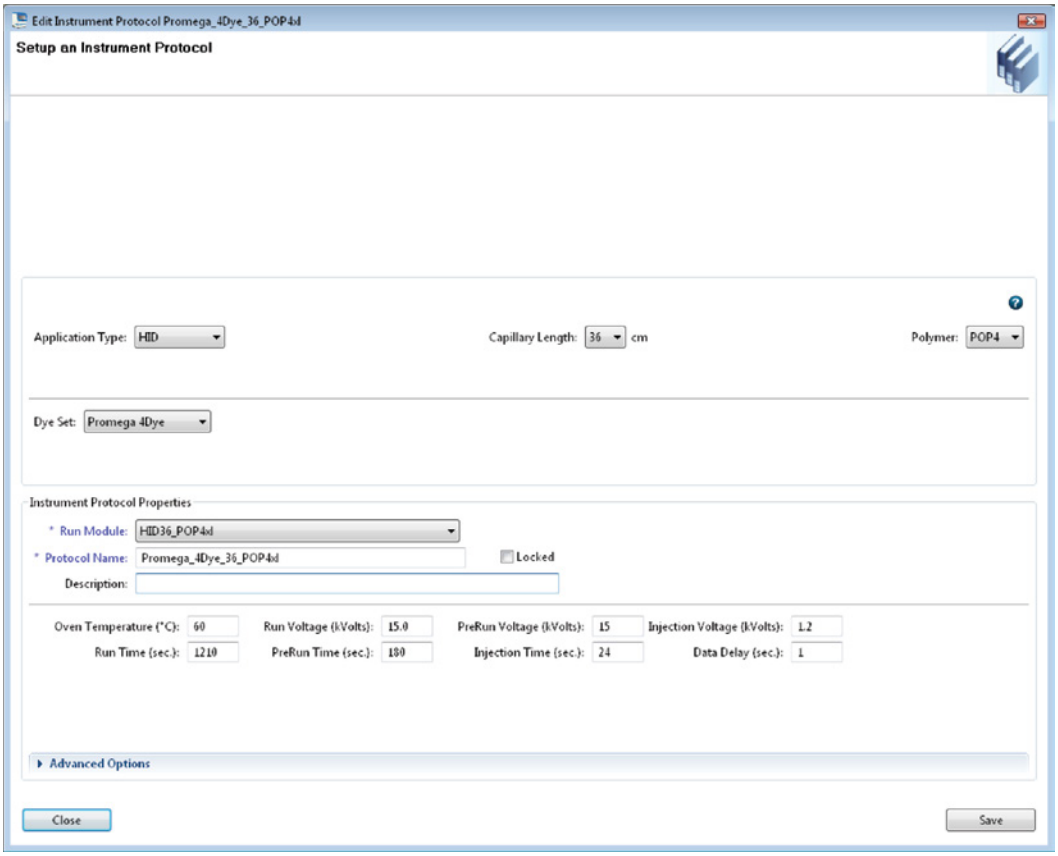


**Figure 3. The Edit Instrument Protocol window.**

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 |

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

**5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer (continued)**

When creating an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 4-dye spectral calibration. We recommend using a run time of 1,210–1,500 seconds and the default injection conditions.

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*.

3. 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 4.

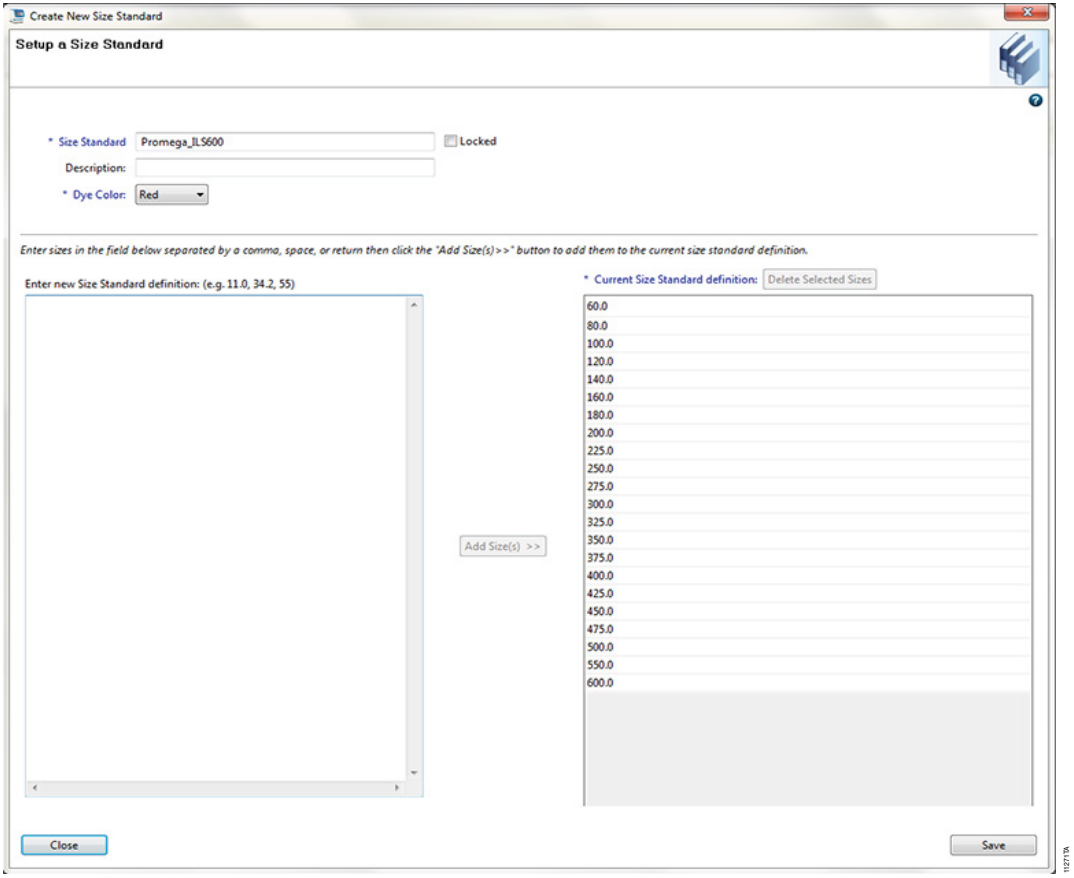


**Figure 4. The Edit Size Standard window.**

## 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer (continued)

4. 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 5 shows one option for these settings.

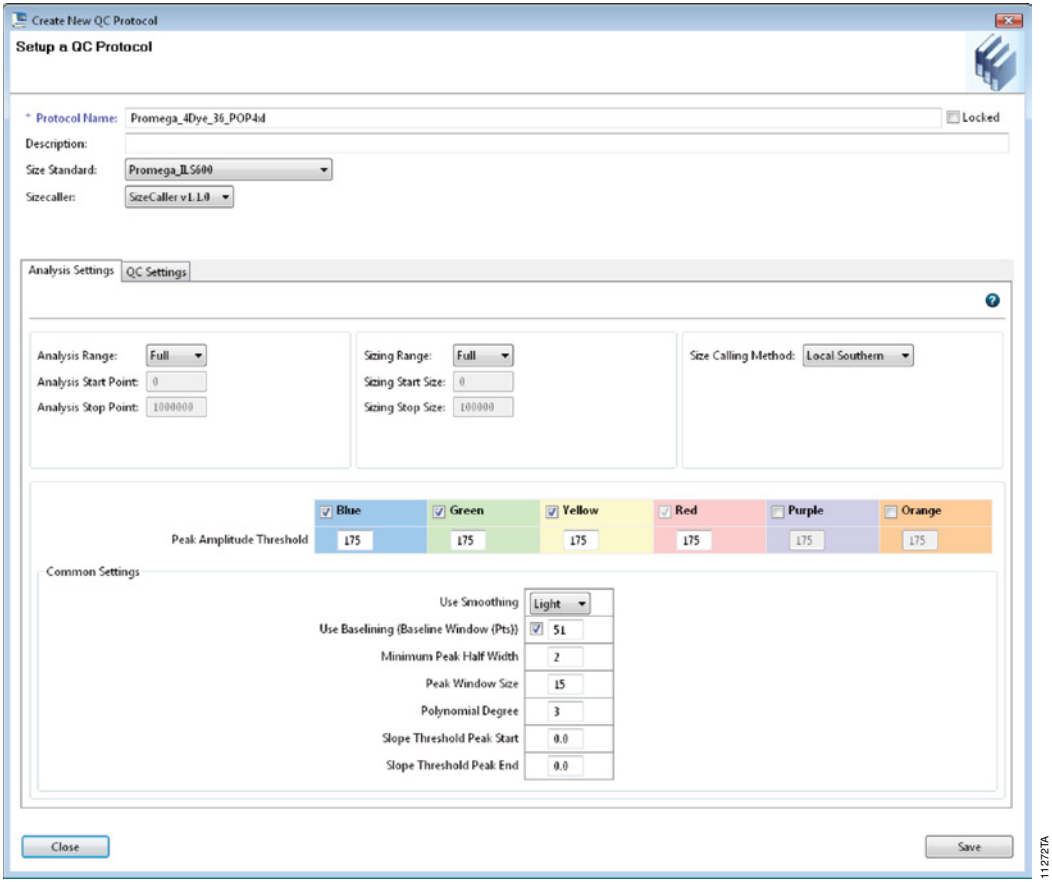


**Figure 5. The Create New QC Protocol window.**

5. 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 6), 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.

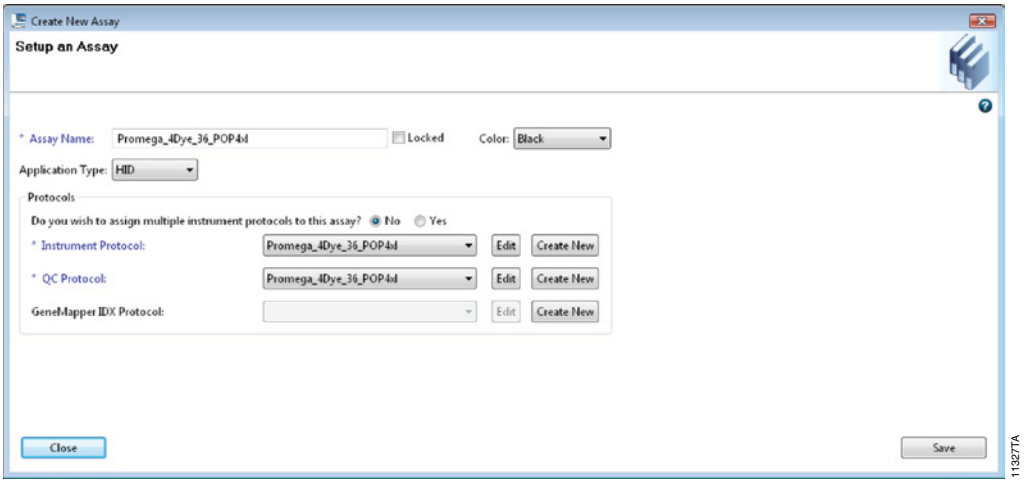


**Figure 6. The Create New Assay window.**

## 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer (continued)

6. To create a new File Name Convention (Figure 7), 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.

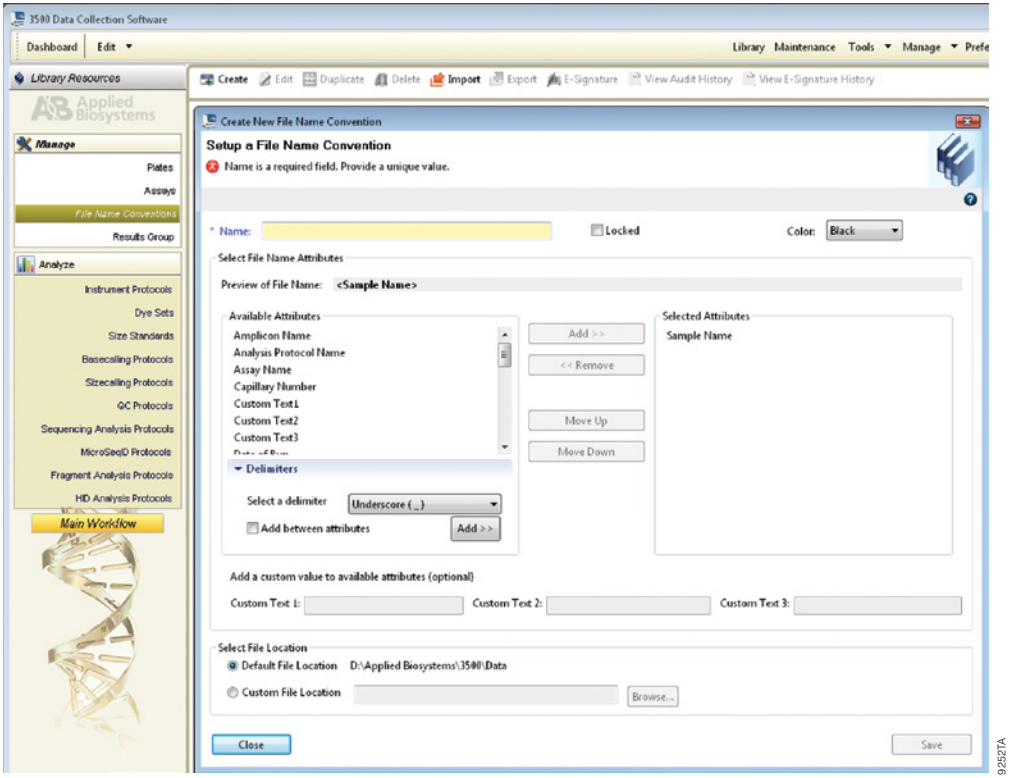


**Figure 7. The Create New File Name Convention window.**

7. To create a new Results Group (Figure 8), 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.

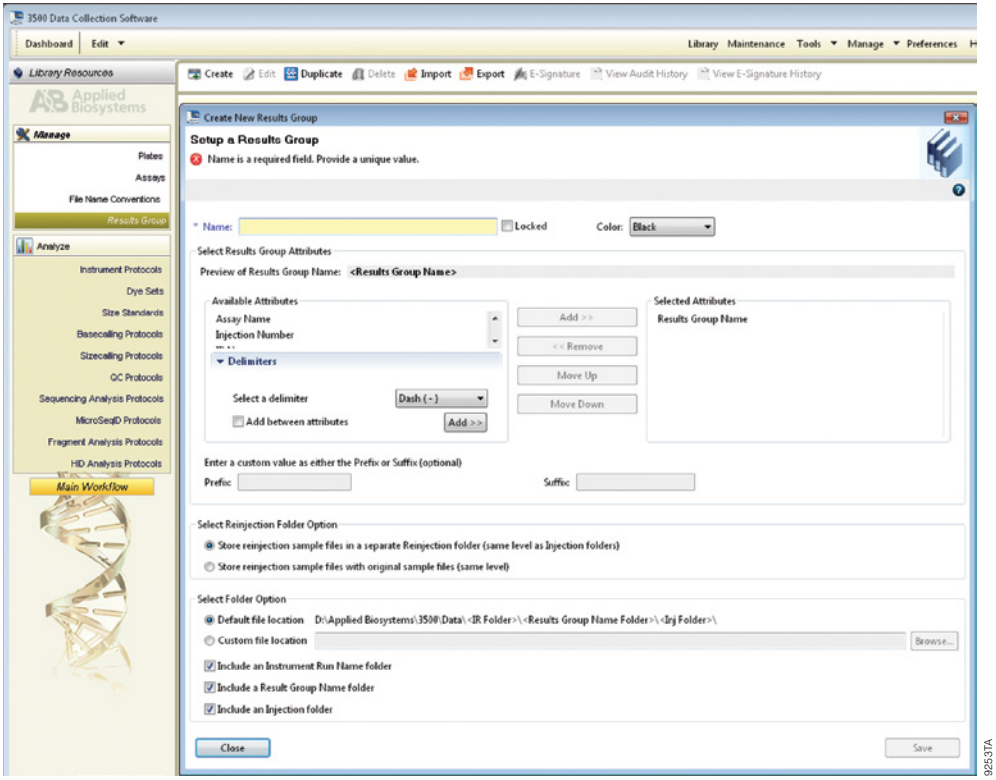


**Figure 8. The Create New Results Group window.**

## 5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® Polymer (continued)

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 9).

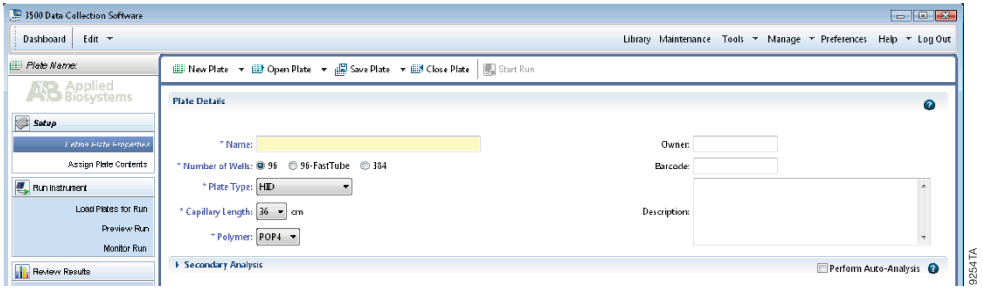


**Figure 9. Defining plate properties.**

10. Select “Assign Plate Contents” (Figure 10).

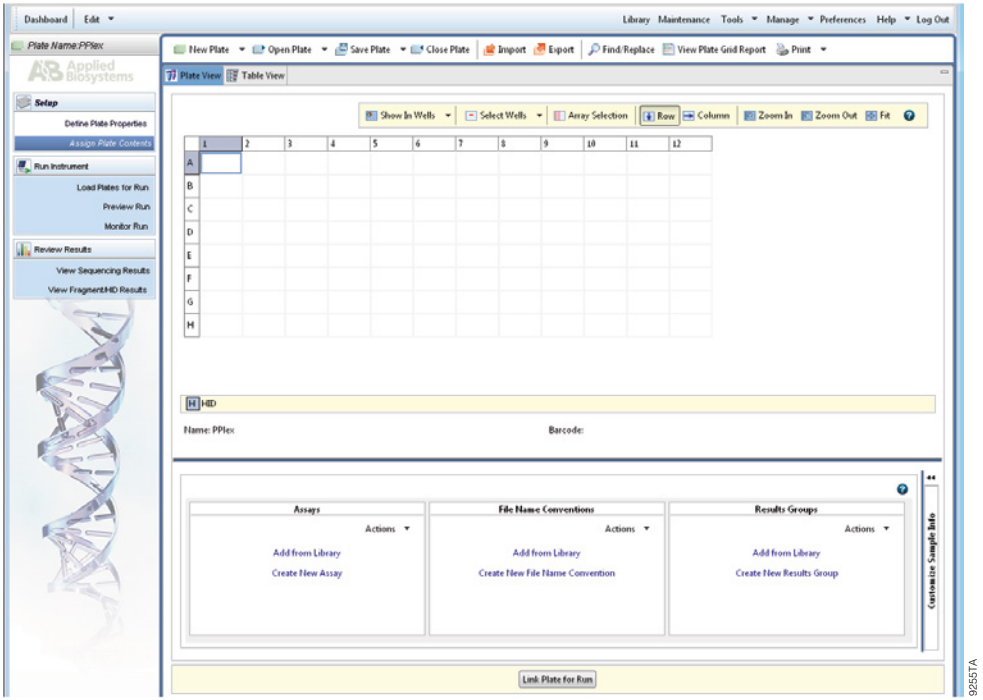


**Figure 10. Assigning plate contents.**

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. Click on 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. Click on 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. Click on 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 11), assign a Run Name. Select “Start Run” (not shown).

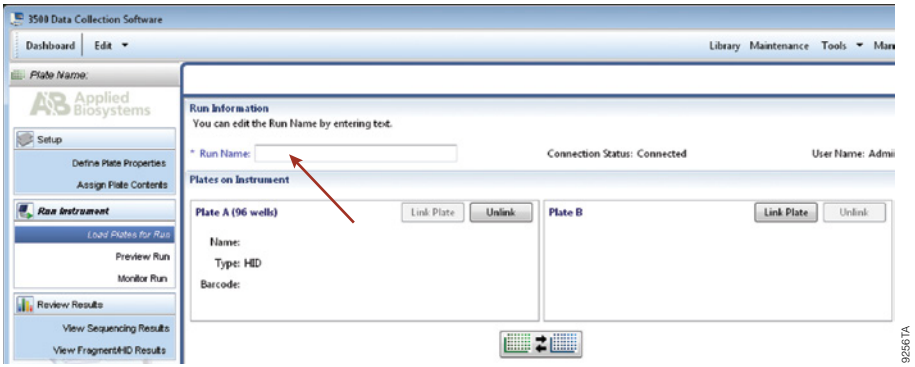


**Figure 11. Assigning a run name.**

## 5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0

### Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4®) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa, or equivalent
- 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.

### Sample Preparation

1. Thaw the Internal Lane Standard 600.

   **Note:** Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing the Internal Lane Standard 600 and Hi-Di™ formamide as follows:

   [(0.5µl ILS 600) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# 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 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If the peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of Hi-Di™ formamide.

3. Vortex for 10–15 seconds to mix.

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

5. Add 1µl of amplified sample (or 1µl of *GenePrint*® 10 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.

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

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

**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 ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0, 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 windows, 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.

**5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 (continued)**

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, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.
9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green Run Instrument arrow becomes enabled.
10. Click on the green Run Instrument arrow on the toolbar to start the sample run.
11. Monitor electrophoresis by observing the run, view, array or capillaries viewer window in the data collection software. Each injection will take approximately 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

Due to the structure of the .hid files generated on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer with POP-4® polymer and a 36cm array (HID application), data generated on this electrophoresis platform with this configuration can only be analyzed using GeneMapper® *ID*-X software, version 1.2 or later. Data generated on the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with POP-4® polymer or Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with POP-4® polymer can be analyzed using GeneMapper® 4.0 software or GeneMapper® *ID* software, version 3.2 or 3.2.1. See Table 2.

**Table 2. Capillary Electrophoresis Instrument and Data Analysis Compatibility.**

| Capillary Electrophoresis Instrument | Polymer | Array Length | Data Analysis Software |
|---|---|---|---|
| ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer (4 or 16 capillaries) | POP-4® | 36cm | GeneMapper® software, version 4.0, or GeneMapper® *ID* software, version 3.2 or 3.2.1 |
| Applied Biosystems® 3130 and 3130*xl* Genetic Analyzer (4 or 16 capillaries) | POP-4® | 36cm | GeneMapper® software, version 4.0, or GeneMapper® *ID* software, version 3.2 or 3.2.1 |
| Applied Biosystems® 3500 and 3500xL Genetic Analyzer (8 or 24 capillaries) | POP-4® | 36cm | GeneMapper® *ID*-X software, version 1.2 or later |

To facilitate analysis of data generated with the *GenePrint*® 10 System, we have created the following files for use with GeneMapper® software, version 4.0, GeneMapper® *ID* software, version 3.2 or 3.2.1, and GeneMapper® *ID*-X software, versions 1.2 and later.

- panels and bins text files
- ILS_600.xml size standard file
- *GenePrint*® 10 analysis method file
- stutter text file, for GeneMapper® *ID*-X software only

Each set of files can be downloaded as a zipped file at: **www.promega.com/resources/software-firmware/geneprint-systems-software-panels-and-bin-files/**. Three separate zipped files are provided: one for GeneMapper® software, version 4.0, one for GeneMapper® *ID* software, versions 3.2 and 3.2.1, and one for GeneMapper® *ID*-X software, versions 1.2 and later.

Instructions for importing these files into each software are given below, along with instructions on how to use these files to analyze *GenePrint*® 10 data.

**Note:** The analysis methods contain a 20% main peak filter.

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

1. Save the GenePrint_10_Panels_v1.0.txt and GenePrint_10_Bins_v1.0.txt files to a known location on your computer.
2. Open the GeneMapper® software, version 4.0.
3. Select “Tools”, then “Panel Manager”.
4. Highlight the Panel Manager icon in the upper left navigation pane.
5. Select “File”, then “Import Panels”.
6. Navigate to the panels text file imported above. Select the file, then “Import”.
7. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 6.
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. At the bottom of the Panel Manager window, select “OK”. The Panel Manager window will close automatically.

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

1. Save the ILS_600.xml file to a known location on your computer.
2. Select “Tools”, then “GeneMapper Manager”.
3. Select the Size Standard tab.
4. Select “Import”.
5. Browse to the location of the ILS_600.xml file.
6. Highlight the file, then select “Import”.
7. 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

Promega scientists have created an analysis method for GeneMapper® software, version 4.0: GenePrint 10 GM 4.0 Analysis Method.xml

1. Save the analysis method .xml file to a known location on your computer.
2. Select "Tools", then "GeneMapper Manager".
3. Select the Analysis Methods tab.
4. Select "Import".
5. Browse to the location of the GenePrint 10 GM 4.0 Analysis Method.xml file.
6. Highlight the file, then select "Import".
7. Select "Done" to save changes and exit the GeneMapper Manager.

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

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. Save the GenePrint_10_Panels_v1.0.txt and GenePrint_10_Bins_v1.0.txt files to a known location on your computer.
2. Open the GeneMapper® ID software, version 3.2 or 3.2.1
3. Select "Tools", then "Panel Manager".
4. Highlight the Panel Manager icon in the upper left navigation pane.

5 Select "File", then "Import Panels".

6. Navigate to the panels text file imported above. Select the file, then "Import".
7. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 6.
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. 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. Save the ILS_600.xml file to a known location on your computer.
2. Select "Tools", then "GeneMapper Manager".
3. Select the Size Standard tab.
4. Select "Import".
5. Browse to the location of the ILS_600.xml file.
6. Highlight the file, then select "Import".
7. 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. Save the GenePrint 10 GMID 3.2 Analysis Method.xml file to a known location on your computer.
   **Note:** This analysis method is compatible with GeneMapper® *ID* software, versions 3.2 and 3.2.1.
2. Select "Tools", then "GeneMapper Manager".
3. Select the Analysis Methods tab.
4. Select "Import".
5. Browse to the location of the GenePrint 10 GMID 3.2 Analysis Method.xml file.
6. Highlight the file, then select "Import".
7. 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.

### 6.H. Processing Data with GeneMapper® *ID* Software, Versions 3.2 and 3.2.1 (continued)

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. Save the GenePrint_10_Panels_IDX_v1.0.txt, GenePrint_10_Bins_IDX_v1.0.txt and GenePrint_10_Stutter_IDX_v1.0.txt files to a known location on your computer.
2. Open the GeneMapper® *ID*-X software.
3. Select "Tools", then "Panel Manager".
4. Highlight the Panel Manager icon in the upper left navigation pane.
5. Select "File", then "Import Panels".
6. In the "Choose Security Group" window select the applicable security group from the drop-down menu, then select "OK".
7. Navigate to the panels text file saved to your computer above. Select the file, then "Import".
8. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 5.
9. Select "File", then "Import Bin Set".
10. Navigate to the bins text file saved to your computer above. Select the file, then "Import".
11. In the navigation pane, highlight the GenePrint 10 panels folder that you just imported in Step 5.
12. Select "File", then "Import Marker Stutter". A warning box will appear asking if you want to overwrite current values. Select "Yes".
13. Navigate to the stutter text file saved to your computer above. Select the file, then "Import".
14. 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. Save the ILS_600.xml file to a known location on your computer.
2. Select "Tools", then "GeneMapper ID-X Manager".
3. Select the Size Standard tab.
4. Select "Import".

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

### 6.L. Processing Single-Source 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 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.
8. Select "Analyze" (green arrow button) to start data analysis.

   **Note:** By default, the software displays the Analysis Requirement Summary, Allelic Ladder Analysis Summary and 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 window activated, you may need to do additional manual troubleshooting.

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

9. If all analysis requirements are met, the Save Project window will open (Figure 12).

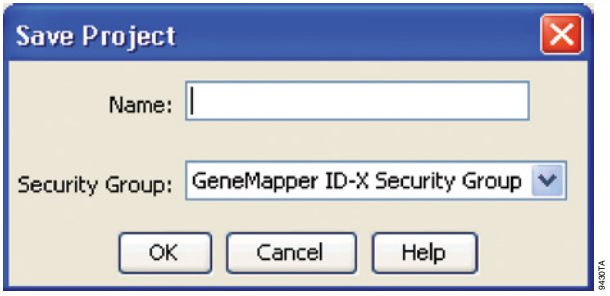


**Figure 12. The Save Project window.**

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 3.

**Table 3. 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 13. The *GenePrint*® 10 Allelic Ladder Mix is shown in Figure 14.

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

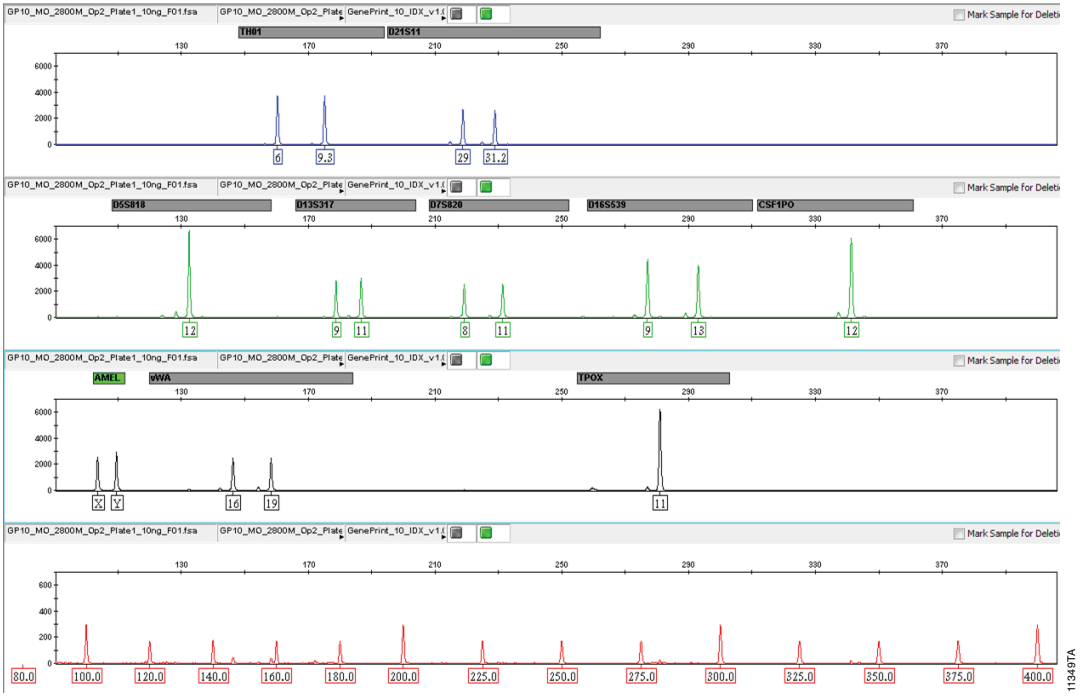


**Figure 13. Representative data for the *GenePrint*® 10 System.** A single-source DNA template (10ng) was amplified using the *GenePrint*® 10 System. Amplification products were mixed with Internal Lane Standard 600 and analyzed using an Applied Biosystems® 3130 Genetic Analyzer and a 3.0kV, 4-second injection. The results were analyzed using GeneMapper® *ID*-X software, version 1.2, and the appropriate panels, bins and stutter files.

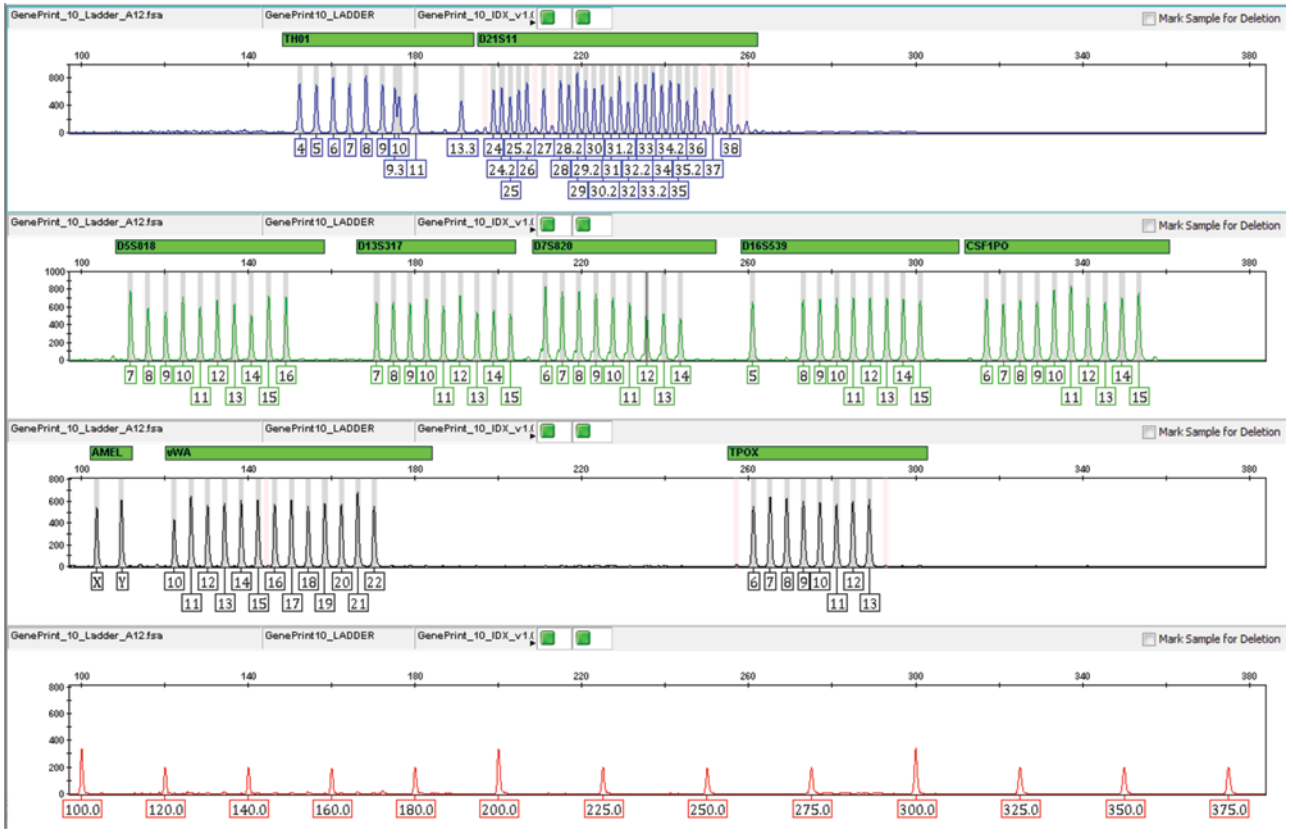


**Figure 14. The *GenePrint*® 10 Allelic Ladder.** The *GenePrint*® 10 Allelic Ladder was analyzed using an Applied Biosystems® 3130 Genetic Analyzer and a 3kV, 4-second injection. The sample file was analyzed using GeneMapper® *ID*-X software, version 1.2, and the appropriate panels, bins and stutter 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. 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.

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

Terminal nucleotide addition (12,13) occurs when a nonproofreading thermostable DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 10 minutes (14) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

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

CE-related artifacts are occasionally seen in one or all color channels. Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject the samples to confirm.

## 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 *GenePrint*® 10 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 15 seconds before dispensing into the PCR amplification mix. |
| | An air bubble formed at the bottom of the reaction well. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling. |
| | The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance. |
| | Improper storage of the 2800M Control DNA. |
| | Thermal cycler or plate problems. Review the thermal cycling protocol in Section 4 or 9.B. We have not tested other reaction plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary. |
| | Primer concentration was too low. Use the recommended primer concentration. Vortex the *GenePrint*® 10 5X Primer Pair Mix for 15 seconds before use. |
| | Samples were not denatured completely. Heat-denature samples for the recommended time, then cool on crushed ice or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing. |
| | Poor capillary electrophoresis injection (ILS 600 peaks also affected). Re-inject the sample. Check the syringe pump system for leakage. Check the laser power. |
| | Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples. |

| Symptoms | Causes and Comments |
|---|---|
| Extra peaks visible in one or all color channels | Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly. |
| | Samples were not denatured completely. Heat denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing. |
| | 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.<br>• Be sure to perform the 10-minute extension step at 60°C after thermal cycling (Section 4 or 9.B).<br>• Decrease the number of cycles.<br>• Plasticware can alter heat transfer during amplification and prevent full adenylation. Increase the final extension time. |
| | Artifacts. The signal strength of certain 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. |
| | Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of "shadow" peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distance as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing. |
| | CE-related artifacts ("spikes"). Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm. |

### 7.A. Amplification and Fragment Detection (continued)

| Symptoms | Causes and Comments |
|---|---|
| Extra peaks visible in one or all color channels (continued) | Incorrect spectral was active. Re-run 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.<br>• Perform a new spectral calibration and re-run the samples.<br>• Instrument sensitivities can vary. Optimize the injection conditions. See Section 5. |
| | 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. |
| | Repeat sample preparation using fresh formamide. Long-term storage of amplified samples in formamide can result in degradation. |
| | The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one 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. |
| | Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples. |
| | 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 column over time. Use a different injection of allelic ladder to determine sizes. |
| | Poor injection of allelic ladder. Include more than one ladder per instrument run. |

| Symptoms | Causes and Comments |
|---|---|
| Peak height imbalance | 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. |
| | Miscellaneous balance problems. Thaw the 5X Primer Pair Mix and 5X Master Mix completely, and vortex for 15 seconds before use. Note that the 5X Master Mix will take longer to thaw than the 5X Primer Pair Mix. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely. |
| | PCR amplification mix prepared in Section 4 or 9.B was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction 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. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample. |
| | Insufficient template. Use the recommended amount of template DNA if available. |
| | 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), $TE^{-4}$ buffer with 20µg/ml glycogen or nuclease-free water. |
| Extra peaks visible in one or all color channels | Artifacts of STR amplification. Amplification of excess amounts of purified DNA can result in a higher number of artifact peaks. Use the recommended amount of template DNA. See Section 6.N for additional information on stutter and artifacts. |

### 7.B. Amplification of Extracted DNA (continued)

| Symptoms | Causes and Comments |
|---|---|
| Peak height imbalance | Excessive amount of DNA. Amplification of >10ng of template per 25µl reaction can result in an imbalance, with smaller loci showing more product than larger loci. Decrease the number of cycles. |
| | Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield. Repurify template DNA if possible. |
| | 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. |

### 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 | Causes and Comments |
|---|---|
| Faint or absent allele peaks | The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and PunchSolution™ Reagent. Decreasing the reaction volume may result in suboptimal performance. |
| | Poor sample deposition. Collection of cells was variable. Increase cycle number. |
| | Poor sample transfer to storage card or variable sampling from storage card. Take punches from a different portion of the card. Increasing cycle number can improve low peak heights. |
| | DNA was not accessible on nonlytic material. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins. |

| Symptoms | Causes and Comments |
|---|---|
| Faint or absent allele peaks (continued) | Too much sample in the reaction. Use one 1.2mm storage card punch per 25µl reaction. Follow the manufacturer's recommendations when depositing sample onto the storage card. With storage cards, reducing the reaction volumes below 25µl may result in amplification failure. |
| | Active PunchSolution™ Reagent carried over into the amplification reaction when using nonFTA card punches. Ensure that the heat block was set at 70°C and samples were incubated for 30 minutes. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. We have not tested longer incubation times. |
| | Inactive PunchSolution™ Reagent. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Avoid multiple freeze-thaw cycles, 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. We recommend 5µl (50ng) of 2800M Control DNA per 25µl amplification reaction. Do not include a blank punch in the positive control reaction. Presence of a blank punch may inhibit amplification of 2800M Control DNA. |
| | Improper storage of the 2800M Control DNA. |
| | The appropriate PCR amplification mix was not used for the positive control reaction with the 2800M Control DNA. Prepare the appropriate PCR amplification mix as described in Table 7 of Section 9.B, and add 5µl (50ng) of 2800M Control DNA to a well containing PCR amplification mix. |

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

| Symptoms | Causes and Comments |
|---|---|
| Extra peaks visible in one or all color channels | Punch may be contaminated. Take punches from blank paper between samples. |
| | Artifacts of STR amplification. Direct amplification of >20ng of template can result in a higher number of artifact peaks. Use the recommended punch size and number. See Section 6.N 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.<br>• Be sure to perform the 10-minute extension step at 60°C after thermal cycling (Section 9.B).<br>• Decrease cycle number.<br>• Increase the final extension time. |
| Peak height imbalance | Excessive amount of DNA. Amplification of >20ng of template can result in an imbalance, with smaller loci showing more product than larger loci.<br>• Use one 1.2mm punch from a storage card per 25µl reaction. Follow the manufacturer's recommendations when depositing sample onto the card.<br>• Decrease cycle number. |
| | The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and PunchSolution™ Reagent. Decreasing the reaction volume can result in suboptimal performance. |
| | DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins. |
| Extreme variability in sample-to-sample peak heights | There can be significant individual-to-individual variability in the deposition of cells onto a storage card, resulting in peak height variability. The PunchSolution™ Kit increases the recovery of amplifiable DNA from samples but does not normalize the amount of DNA present. |

## 7.D. GeneMapper® Software

| Symptoms | Causes and Comments |
|---|---|
| Alleles not called | 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.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| | A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis. |
| Off-ladder alleles | An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run. |
| | The GeneMapper® software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze as described in Section 6.D. |
| | Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file for the *GenePrint*® 10 System. |
| | 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. |

### 7.D. GeneMapper® Software (continued)

| Symptoms | Causes and Comments |
|---|---|
| Off-ladder alleles (continued) | Incorrect analysis method used. Only the *GenePrint*® 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 | 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". |
| | 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.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| Peaks in size standard missing | If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the red channel to include peaks. |
| | If peaks are low-quality, redefine the size standard for the sample to skip these peaks. |
| 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 *GenePrint*® 10 System. |
| | 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. |
| Significantly raised baseline | Poor spectral calibration. Perform a new spectral calibration, and re-run the samples. |
| | Re-run and optimize the matrix. Make sure that the matrix applied was generated on the same instrument. |

| Symptoms | Causes and Comments |
|---|---|
| Error message after attempting to import panels and bins text files: "Unable to save panel data: java.SQLEException:ORA-00001: unique constraint (IFA.CKP_NNN) violated". | There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order. |
| Samples in the project not analyzed | The *GenePrint*® 10 GeneMapper® 4.0 analysis method can be imported into GeneMapper® software, version 4.1. However, while the method can be imported, it is not possible to use this analysis method in GeneMapper® software, version 4.1, as it is not an available method in the Analysis Method column of the Project window. |

### 7.E. GeneMapper® *ID* Software

| Symptoms | Causes and Comments |
|---|---|
| Alleles not called | 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. |
| | 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.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| | A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis. |
| Off-ladder alleles | An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run. |
| | The GeneMapper® *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 re-analyze as described in Section 6.H. |

### 7.E. GeneMapper® *ID* Software (continued)

| 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 for the *GenePrint*® 10 System. |
| | 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. |
| Size standard not called correctly | 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". |
| | 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.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| Peaks in size standard missing | If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the red channel to include peaks. |
| | If peaks are low-quality, redefine the size standard for the sample to skip these peaks. |
| 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 *GenePrint*® 10 System. |
| | 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. |

| Symptoms | Causes and Comments |
|---|---|
| Significantly raised baseline | Poor spectral calibration. Perform a new spectral calibration, and re-run the samples. |
| | Re-run 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.SQLEException:ORA-00001: unique constraint (IFA.CKP_NNN) violated". | There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order. |

### 7.F. GeneMapper® *ID*-X Software

| Symptoms | Causes and Comments |
|---|---|
| Stutter peaks not filtered | 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. |
| Samples in the project not analyzed | The Analysis Requirement Summary window was not active, and there was an analysis requirement that was not met. Turn on Analysis Requirement Summary in the Options menu, 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. |
| 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. |

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

| Symptoms | Causes and Comments |
|---|---|
| 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. |
| | 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. In this instance, the allelic ladder would have failed the allelic ladder quality check. |
| | 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.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| | A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis. |
| Off-ladder alleles | An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run. |
| | The GeneMapper® *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. |
| | Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file for the *GenePrint*® 10 System. |
| | 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. |

| Symptoms | Causes and Comments |
|---|---|
| Size standard not called correctly | Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes". |
| | Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run.<br>• Create a new size standard using the internal lane standard fragments present in the sample.<br>• Re-run samples using a longer run time. |
| Peaks in size standard missing | If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the red channel to include peaks. |
| | If peaks are low-quality, redefine the size standard for the sample to skip these peaks. |
| Significantly raised baseline | Poor spectral calibration. Perform a new spectral calibration, and re-run the samples. |
| | Re-run and optimize the matrix. Make sure that the matrix applied was generated on the same instrument. |

## 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.

## 8. References (continued)


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 4. *GenePrint*® 10 System allelic ladder information can be found in Table 5.

**Table 4. The *GenePrint*® 10 System Locus-Specific Information.**

| STR Locus | Label | Chromosomal Location | GenBank® Locus and Locus Definition | Repeat Sequence[1] 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 |

[1]The 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".

[2]Amelogenin is not an STR.

TMR = carboxy-tetramethylrhodamine
FL = fluorescein
JOE = 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxyfluorescein
NA = not applicable

**Table 5. 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 |
| D7S820 | JOE | 215–247 | 6–14[3] |
| D16S539 | JOE | 264–304 | 5, 8–15 |
| CSF1PO | JOE | 321–357 | 6–15 |
| Amelogenin | TMR | 106, 112 | X, Y |
| vWA | TMR | 123–171 | 10–22 |
| TPOX | TMR | 262–290 | 6–13 |

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

[2]When using an internal lane standard, such as the 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.

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

### 9.B. Direct Amplification of DNA from Storage Card Punches

**Materials to Be Supplied by the User**

- GeneAmp® PCR System 9700 thermal cycler with a silver or gold-plated silver sample block (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.E)
- PunchSolution™ Kit (Cat.# DC9271) for nonFTA card punches
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system
- phosphate-buffered saline (1X)
- storage cards (e.g., FTA®, Fitzco, S&S 903)

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.

**Note:** Samples on nonFTA 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 20µl aliquot of the cell suspension on an FTA® or nonFTA (e.g., Fitzco or S&S 903) 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 FTA® card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonFTA 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. Increase the number of reactions by 10–15% to compensate for pipetting error (e.g., for 96 reactions, add 10 to 15 additional reactions). While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.
3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately.

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

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

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

| PCR Amplification Mix Component | Volume Per Reaction | × | Number of Reactions | = | Final Volume |
|---|---|---|---|---|---|
| Water, Amplification Grade | 15.0µl | × | | = | |
| *GenePrint*® 10 5X Master Mix | 5.0µl | × | | = | |
| *GenePrint*® 10 5X Primer Pair Mix | 5.0µl | × | | = | |
| **total reaction volume** | 25µl | | | | |

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

**Table 7. 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 | × | | = | |
| *GenePrint*® 10 5X Master Mix | 5.0µl | × | | = | |
| *GenePrint*® 10 5X Primer Pair Mix | 5.0µl | × | | = | |
| 2800M Control DNA, 10ng/µl[1] | 5.0µl | | | | |
| **total reaction volume** | 25µl | | | | |

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

6. Vortex the PCR amplification 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 FTA® 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 nonFTA card punches, add the PCR amplification mix to the PunchSolution™ Reagent-pretreated punch.

   **Note:** It also is acceptable to add the FTA® 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.

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

2. Select and run the recommended protocol. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below. The estimated total cycle time is 1.5 hours.

| Thermal Cycling Protocol[1] |
|---|
| 96°C for 1 minute, then: |
| 94°C for 10 seconds<br>59°C for 1 minute<br>72°C for 30 seconds<br>for 26 cycles, then: |
| 60°C for 10 minutes |
| 4°C soak |

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with Max mode as the ramp speed. (This requires a silver or gold-plated 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.

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

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

**PCR Optimization**

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

1. Choose several samples that represent typical samples you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Be sure to pretreat nonFTA 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 15). 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.

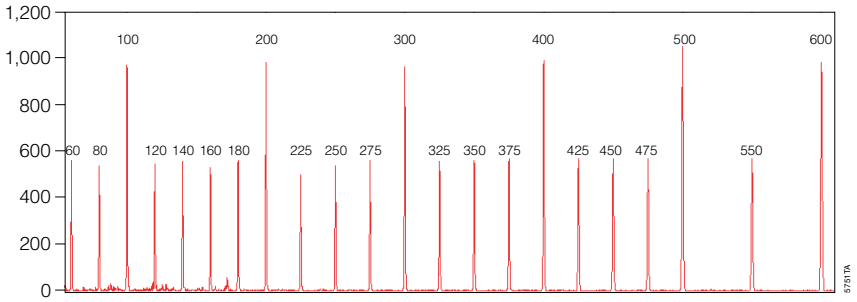


**Figure 15. An electropherogram showing the fragments of the Internal Lane Standard 600.**

## 9.D. Composition of Buffers and Solutions

### Phosphate-buffered saline (1X)

| | |
|---|---|
| 8g | NaCl |
| 0.2g | KCl |
| 1.44g | $Na_2HPO_4 \cdot 2H_2O$ |
| 0.24g | $KH_2HPO_4$ |

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 ($Na_2EDTA \cdot 2H_2O$) |

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

### $TE^{-4}$ buffer with 20µg/ml glycogen

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

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

## 9.E. Related Products

### Sample Preparation Systems

| Product | Size | Cat.# |
|---|---|---|
| Wizard® SV Genomic DNA Purification System | 50 preps | A2360 |
| | 250 preps | A2361 |
| Wizard® Genomic DNA Purification Kit | 100 isolations × 300µl | A1120 |
| | 500 isolations × 300µl | A1125 |
| | 100 isolations × 10ml | A1620 |
| MagneSil® Genomic, Fixed Tissue System | 100 samples | MD1490 |
| DNA IQ™ System | 100 reactions | DC6701 |
| | 400 reactions | DC6700 |
| PunchSolution™ Kit | 100 preps | DC9271 |
| Slicprep™ 96 Device | 10 pack | V1391 |

### Maxwell® Automated Nucleic Acid Purification

| Product | Size | Cat.# |
|---|---|---|
| Maxwell® 16 Tissue DNA Purification Kit | 48 preps | AS1030 |

For more information about other Maxwell® nucleic acid purification kits, visit: **www.promega.com/maxwell16/**

### 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.

### Cell Viability and Cytotoxicity Assays

| Product | Size | Cat.# |
|---|---|---|
| CytoTox-Glo™ Cytotoxicity Assay | 10ml | G9290 |
| MultiTox-Fluor Multiplex Cytotoxicity Assay | 10ml | G9200 |
| CytoTox-Fluor™ Cytotoxicity Assay | 10ml | G9260 |
| CellTiter-Glo® Luminescent Cell Viability Assay | 10ml | G7570 |

Additional Sizes Available.

**Apoptosis Assays**

| Product | Size | Cat.# |
|---|---|---|
| Apo-ONE® Homogeneous Caspase-3/7 Assay (fluorescent) | 10ml | G7790 |
| Caspase-Glo® 3/7 Assay | 10ml | G8091 |
| Caspase-Glo® 8 Assay | 10ml | G8201 |
| Caspase-Glo® 9 Assay | 10ml | G8211 |

Additional Sizes Available.

## 10. Summary of Changes

The following change was made to the 6/16 revision of this document:

1. Description of quantification method for 2800M Control DNA was removed.
2. Other general updates were incorporated.

Promega

[a]Allele sequences for one or more of the loci vWA, FGA, D8S1179, D21S11 and D18S51 in allelic ladder mixtures is licensed under U.S. Pat. Nos. 7,087,380 and 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

[b]Australian Pat. No. 724531, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. No. 3602142 and other patents pending.



Apo-ONE, Caspase-Glo, CellTiter-Glo, *GenePrint*, MagneSil, Maxwell, PowerPlex and Wizard are registered trademarks of Promega Corporation. CytoTox-Fluor, CytoTox-Glo, DNA IQ, PunchSolution and Slicprep are trademarks of Promega Corporation.

ABI PRISM and MicroAmp are registered trademarks of Applera Corporation. Applied Biosystems, GeneMapper and POP-4 are registered trademarks of Applied Biosystems. ART is a registered trademark of Molecular Bio-Products, Inc. FTA is a registered trademark of Flinders Technologies, Pty, Ltd., and is licensed to Whatman. GeneAmp is a registered trademark of Roche Molecular Systems, Inc. GenBank is a registered trademark of U.S. Dept. of Health and Human Services. Hi-Di is a trademark of Applera Corporation.

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

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

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


Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA · Toll Free in USA 800-356-9526 · 608-274-4330 · Fax 608-277-2516
TM392 · Revised 6/16