PowerPlex® ESI 17 Fast System for Use on the Applied Biosystems® Genetic Analyzers

Instructions for Use of Products DC1720 and DC1721
# PowerPlex® ESI 17 Fast System

for Use on the Applied Biosystems® Genetic Analyzers

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

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1. Description

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

The PowerPlex® ESI 17 Fast System(a–e) is used for human identification applications including forensic analysis, relationship testing and research use. This system allows co-amplification and four-color fluorescent detection of seventeen loci (sixteen STR loci and Amelogenin), including D22S1045, D2S1338, D19S433, D3S1358, Amelogenin, D2S441, D10S1248, D1S1656, D18S51, D16S539, D12S391, D21S11, vWA, TH01, SE33, FGA and D8S1179.

The PowerPlex® ESI 17 Fast System is an update to the PowerPlex® ESI 17 Pro System (Cat.# DC7780, DC7781). All autosomal primer pairs are identical in both systems. A new 5X Master Mix was optimized specifically for this system and allows fast cycling.

The PowerPlex® ESI 17 Fast System is designed with six of the original seven European Standard Set (ESS) loci (D3S1358, D18S51, TH01, vWA, D8S1179 and the more common FGA alleles) along with D16S539 and
D19S433 as smaller amplicons (<250bp), while the loci recommended by the European Network of Forensic Science Institutes (ENFSI) and European DNA Profiling Group (EDNAP) (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) are present as larger amplicons. A complementary system, the PowerPlex® ESX 17 Fast System, amplifies the same seventeen loci present in the PowerPlex® ESI 17 Fast System but with the new ENFSI/EDNAP loci designed as mini-STRs (<125bp; D2S441, D10S1248 and D22S1045) or midi-STRs (125–185bp; D1S1656 and D12S391). Therefore, these two STR systems can be used to complement each other when analyzing degraded or challenging samples to maximize recovery of allelic information from as many loci as possible and allow confirmation of results obtained with the other system.

The PowerPlex® ESI 17 Fast System and all system components are manufactured in accordance with ISO 18385:2016. All necessary materials are provided to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the PowerPlex® ESI/ESX Fast 5X Master Mix. This manual contains a protocol for use of the PowerPlex® ESI 17 Fast System with the GeneAmp® PCR System 9700 and Veriti® 96-Well Thermal Cycler in addition to protocols to separate amplified products and detect separated material on the ABI PRISM® 310, 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xl Genetic Analyzers (Figure 1). Protocols to operate the fluorescence-detection instruments should be obtained from the instrument manufacturer. Amplification and detection instrumentation may vary. You may need to optimize protocols including amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

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

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Section 5. Applied Biosystems® 3500 or 3500xl Genetic Analyzer

Section 5.A

Applied Biosystems® 3130 or 3130xl Genetic Analyzer

Section 5.B

Data Analysis

Section 6. GeneMapper® ID-X Software

GeneMapper® ID Software

Figure 1. An overview of the PowerPlex® ESI 17 Fast System protocol.
2. Product Components and Storage Conditions

**PRODUCT** | **SIZE** | **CAT.#**
--- | --- | ---
PowerPlex® ESI 17 Fast System | 100 reactions | DC1721

Not For Medical Diagnostic Use. This system contains sufficient reagents for 100 reactions of 25µl each. Includes:

**Pre-amplification Components Box**
- 500µl PowerPlex® ESI/ESX Fast 5X Master Mix
- 250µl PowerPlex® ESI 17 Fast 10X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 5 × 1,250µl Water, Amplification Grade

**Post-amplification Components Box**
- 50µl PowerPlex® ESI 17 Fast Allelic Ladder Mix
- 200µl WEN Internal Lane Standard 500 ESS

**PRODUCT** | **SIZE** | **CAT.#**
--- | --- | ---
PowerPlex® ESI 17 Fast System | 400 reactions | DC1720

Not For Medical Diagnostic Use. This system contains sufficient reagents for 400 reactions of 25µl each. Includes:

**Pre-amplification Components Box**
- 4 × 500µl PowerPlex® ESI/ESX Fast 5X Master Mix
- 4 × 250µl PowerPlex® ESI 17 Fast 10X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 10 × 1,250µl Water, Amplification Grade

**Post-amplification Components Box**
- 4 × 50µl PowerPlex® ESI 17 Fast Allelic Ladder Mix
- 2 × 200µl WEN Internal Lane Standard 500 ESS

The PowerPlex® ESI 17 Fast Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening. The Water, Amplification Grade, is provided in a separate, sealed bag for shipping. This component should be moved to the pre-amplification box after opening.

**Storage Conditions:** Upon receipt, store all components except the 2800M Control DNA at −30°C to −10°C in a nonfrost-free freezer. Store the 2800M Control DNA at 2–10°C. Make sure that the 2800M Control DNA is stored at 2–10°C for at least 24 hours before use. After the first use, store the WEN Internal Lane Standard (WEN ILS) 500 ESS at 2–10°C, protected from light; do not refreeze. The PowerPlex® ESI 17 Fast 10X Primer Pair Mix, PowerPlex® ESI 17 Fast Allelic Ladder Mix and WEN ILS 500 ESS 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.

**Optional:** The PowerPlex® ESI 17 Fast System components may be stored for up to 1 year at 2–10°C without loss of activity.
Available Separately

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>SIZE</th>
<th>CAT.#</th>
</tr>
</thead>
<tbody>
<tr>
<td>PunchSolution™ Kit</td>
<td>100 preps</td>
<td>DC9271</td>
</tr>
<tr>
<td>SwabSolution™ Kit</td>
<td>100 preps</td>
<td>DC8271</td>
</tr>
<tr>
<td>5X AmpSolution™ Reagent</td>
<td>100 preps</td>
<td>DM1231</td>
</tr>
</tbody>
</table>

The PunchSolution™ Kit is required to process non-FTA punches prior to direct amplification. The SwabSolution™ Kit is required to process swabs prior to direct amplification. The 5X AmpSolution™ Reagent is required for direct amplification of DNA from storage card punches and swab extracts. Both the PunchSolution™ Kit and SwabSolution™ Kit contain the 5X AmpSolution™ Reagent.

The proper panels, bins and stutter text files and size standard .xml files for use with GeneMapper® ID and ID-X software can be downloaded at: [www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/](http://www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/)

Matrix standards are required for initial setup of the color separation matrix. Matrix standards are provided separately and are available for the ABI PRISM® 310 Genetic Analyzer (PowerPlex® 5C Matrix Standards, 310, Cat.# DG5640) and ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers (PowerPlex® 5C Matrix Standard, Cat.# DG4850).

3. Before You Begin

3.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (10,11). Guidelines for the validation process are published in the Internal Validation Guide of Autosomal STR Systems for Forensic Laboratories (12).

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 and fluorescence detection. Additional research and validation are required if any modifications to the recommended protocols are made.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X 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 (PowerPlex® ESI 17 Fast Allelic Ladder Mix and WEN Internal Lane Standard 500 ESS). 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.
3.A. Precautions (continued)

Some reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

3.B. Matrix Standardization or Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the ABI PRISM® 310, 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument.

The PowerPlex® 5C Matrix Standards, 310 (Cat.# DG5640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer. The PowerPlex® 5C Matrix Standard (Cat.# DG4850) cannot be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

The PowerPlex® 5C Matrix Standard (Cat.# DG4850) is required for spectral calibration on the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. The PowerPlex® 5C Matrix Standards, 310 (Cat.# DG5640), cannot be used to generate a matrix on these instruments.

For protocols and additional information about matrix generation and spectral calibration, see the PowerPlex® 5C Matrix Standards, 310, Technical Manual #TMD050 and PowerPlex® 5C Matrix Standard Technical Manual #TMD049. These manuals are available online at: www.promega.com/protocols/

4. Protocols for DNA Amplification Using the PowerPlex® ESI 17 Fast System

The PowerPlex® ESI 17 Fast System was developed for amplification of extracted DNA and direct-amplification samples. Slight protocol variations are recommended for optimal performance with each template source. Protocols for amplification in a 25µl reaction volume using extracted DNA (Section 4.A), FTA® and nonFTA storage card punches (Section 4.B) and swabs (Section 4.C) are included in the following amplification sections. Protocols for amplification in a 12.5µl reaction volume using FTA® and nonFTA storage card punches and swabs are included in Sections 9.D and 9.E, respectively.

The PowerPlex® ESI 17 Fast System is compatible with the GeneAmp® PCR System 9700 thermal cycler with a silver or gold-plated silver sample block and the Veriti® 96-Well Thermal Cycler.

Note: It may be possible to use thermal cyclers other than those listed in this technical manual. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. Use of thermal cyclers with an aluminum block is NOT recommended with the PowerPlex® ESI 17 Fast System.

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre- 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.
4.A. Amplification of Extracted DNA in a 25µl Reaction Volume

The PowerPlex® ESI 17 Fast System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different. Testing at Promega shows that 30 cycles works well for 0.5ng of purified DNA templates. Developmental validation of the kit showed routine generation of full profiles using 30 cycles of amplification with lower amounts of DNA template down to 62.5pg. Partial profiles were typically observed for DNA template of 32pg and below (13). In-house optimization and validation should be performed to establish the performance of the kit in your laboratory (12).

Materials to Be Supplied by the User

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

Amplification Setup

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and Amplification-Grade Water completely.
   
   **Note:** Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

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

3. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.
4.A. Amplification of Extracted DNA in a 25µl Reaction Volume (continued)

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

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.

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

<table>
<thead>
<tr>
<th>Table 1. PCR Amplification Mix for Amplification of Extracted DNA.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCR Amplification Mix Component</strong></td>
</tr>
<tr>
<td>Water, Amplification Grade</td>
</tr>
<tr>
<td>PowerPlex® ESI/ESX Fast 5X Master Mix</td>
</tr>
<tr>
<td>PowerPlex® ESI 17 Fast 10X Primer Pair Mix</td>
</tr>
<tr>
<td>template DNA (0.5ng)</td>
</tr>
<tr>
<td>total reaction volume</td>
</tr>
</tbody>
</table>

1Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESI/ESX Fast 5X Master Mix and PowerPlex® ESI 17 Fast 10X Primer Pair Mix. The template DNA will be added at Step 6.

2Store DNA templates in nuclease-free water, TE⁻¹ buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE⁻¹ 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.

3Apparent DNA concentrations can differ, depending on the DNA quantification method used (14). 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.

4The PowerPlex® ESI 17 Fast System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.
6. Add template DNA for each sample to the respective well containing PCR amplification mix. 
  **Note:** The PowerPlex® ESI 17 Fast System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 0.5ng in the desired template volume. Add 0.5ng of diluted DNA to a reaction well containing PCR amplification mix.

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

9. Seal or cap the plate, or close the tubes. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

**Thermal Cycling**

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 30 cycles work well for 0.5ng of purified DNA templates. In-house validation should be performed.

1. Place the reaction plate or tubes in the thermal cycler.

2. Select and run the recommended protocol.

**Notes:**

1. When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%.

2. When using the GeneAmp® PCR System 9700, 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. When the Select Method Options screen appears, select “Max” for the ramp speed and enter the reaction volume.

<table>
<thead>
<tr>
<th><strong>Thermal Cycling Protocol</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>96°C for 1 minute, then:</td>
</tr>
<tr>
<td>96°C for 5 seconds</td>
</tr>
<tr>
<td>60°C for 35 seconds</td>
</tr>
<tr>
<td>72°C for 5 seconds</td>
</tr>
<tr>
<td>for 30 cycles, then:</td>
</tr>
<tr>
<td>60°C for 2 minutes</td>
</tr>
<tr>
<td>4°C soak</td>
</tr>
</tbody>
</table>

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

**Note:** Long-term storage of amplified samples at 4°C or higher may produce artifacts.
4.B. Direct Amplification of DNA from Storage Card Punches in a 25µl Reaction Volume

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block or Veriti® 96-Well Thermal Cycler (Applied Biosystems)
- centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- PunchSolution™ Kit (Cat.# DC9271) for nonFTA card punches
- 5X AmpSolution™ Reagent (Cat.# DM1231, also supplied with the PunchSolution™ Kit)
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system

This section contains a protocol for direct amplification of DNA from storage card punches in a 25µl reaction volume using the PowerPlex® ESI 17 Fast System and GeneAmp® PCR System 9700 or Veriti® 96-Well Thermal Cycler. A protocol for direct amplification of DNA from storage card punches in a 12.5µl reaction volume is provided in Section 9.D.

When using the protocol detailed below, add the number of 1.2mm storage card punches indicated below to each 25µl amplification reaction.

Note: You will need to optimize and validate the number of storage card punches per reaction in your laboratory. See the PCR Optimization recommendations at the end of this section.

FTA®-based sample types include:

- Buccal cells collected on FTA® cards with Whatman EasiCollect™ or Fitzco Sampact™ devices (one or two punches per 25µl amplification reaction)
- Buccal cells collected with swabs transferred to FTA® or Indicating FTA® cards (one or two punches per 25µl amplification reaction)
- Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards (one punch per 25µl amplification reaction)

NonFTA sample types include:

- Buccal samples on Bode Buccal DNA Collector™ devices (one punch per 25µl amplification reaction)
- Blood and buccal samples on nonFTA card punches (e.g., S&S 903) (one punch per 25µl amplification reaction)

Pretreat nonFTA sample types with the PunchSolution™ Kit (Cat.# DC9271) to lyse nonFTA samples before adding the PCR amplification mix. For more information, see the PunchSolution™ Kit Technical Manual #TMD038. Failure to pretreat these samples may result in incomplete profiles.

Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate.
Automated punchers also can be used to create sample disks. Refer to the user’s guide for your instrument for assistance with generating 1.2mm disks, technical advice and troubleshooting information.

**Note:** Static may be problematic when adding a punch to a well. For 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 PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and Amplification-Grade Water completely.
   
   **Note:** Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.
   
   **Note:** The 5X AmpSolution™ Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

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

4. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.
4.B. Direct Amplification of DNA from Storage Card Punches in a 25µl Reaction Volume (continued)

5. Add the final volume of each reagent listed in Table 2 to a clean tube.

Table 2. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches in a 25µl Reaction Volume.

<table>
<thead>
<tr>
<th>PCR Amplification Mix Component¹</th>
<th>Volume Per Reaction</th>
<th>×</th>
<th>Number of Reactions</th>
<th>=</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, Amplification Grade</td>
<td>12.5µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI/ESX Fast 5X Master Mix</td>
<td>5.0µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI 17 Fast 10X Primer Pair Mix</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5X AmpSolution™ Reagent</td>
<td>5.0µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total reaction volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25µl</td>
</tr>
</tbody>
</table>

¹Add Water, Amplification Grade, to the tube first, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. For FTA® card punches, the template DNA will be added at Step 7.

6. Vortex the PCR amplification mix for 5–10 seconds, then pipet 25µl of PCR amplification mix into each reaction well.

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

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

7. For FTA® storage cards, add one or two 1.2mm punches from a card containing a buccal sample or one 1.2mm punch from a card containing whole blood to the appropriate wells of the reaction plate. For nonFTA card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

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

8. For the positive amplification control, add 1µl (10ng) of the 2800M Control DNA to a reaction well containing 25µl of PCR amplification mix.

Notes:

1. Do not include blank storage card punches in the positive control reactions.

2. Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences. Typically, 10ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.
9. Reserve a well containing PCR amplification mix as a negative amplification control.  
   **Note:** An additional negative control with a blank punch may be performed to detect contamination from 
   the storage card or punch device.

10. Seal or cap the plate, or close the tubes. Briefly centrifuge the plate to bring storage card punches to the 
    bottom of the wells and remove any air bubbles.

### Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the number 
of storage card punches, cycle number, injection conditions and loading volume for your laboratory instrumen-
tation. Testing at Promega shows that 26 cycles works well for a variety of sample types. Cycle number will need to 
be optimized in each laboratory for each sample type that is amplified.

1. Place the reaction plate or tubes in the thermal cycler.

2. Select and run the recommended protocol.

   **Notes:**
   1. When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%.
   2. When using the GeneAmp® PCR System 9700, 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. When the Select Method Options screen appears, select “Max” for the 
      ramp speed and enter the reaction volume.

<table>
<thead>
<tr>
<th>Thermal Cycling Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>96°C for 1 minute, then:</td>
</tr>
<tr>
<td>96°C for 5 seconds</td>
</tr>
<tr>
<td>60°C for 35 seconds</td>
</tr>
<tr>
<td>72°C for 5 seconds</td>
</tr>
<tr>
<td>for 26 cycles, then:</td>
</tr>
<tr>
<td>60°C for 2 minutes</td>
</tr>
<tr>
<td>4°C soak</td>
</tr>
</tbody>
</table>

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.
4.B. Direct Amplification of DNA from Storage Card Punches in a 25µl Reaction Volume (continued)

**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, number of punches and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.

2. Depending on your preferred protocol, place one or two 1.2mm storage card punches containing a buccal sample or one 1.2mm punch of a storage card containing whole blood into each well of a reaction plate. 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, 26 and 27 cycles).

5. Following amplification, use your laboratory’s validated separation and detection protocols to determine the optimal cycle number for the sample type and number of storage card punches.

4.C. Direct Amplification of DNA from Swabs in a 25µl Reaction Volume

**Materials to Be Supplied by the User**

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block or Veriti® 96-Well Thermal Cycler (Applied Biosystems)
- centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- SwabSolution™ Kit (Cat.# DC8271)

This section contains a protocol for amplifying DNA from swab extracts in a 25µl reaction volume using the PowerPlex® ESI 17 Fast System and GeneAmp® PCR System 9700 or Veriti® 96-Well Thermal Cycler. A protocol for direct amplification of DNA from swab extracts in a 12.5µl reaction volume is provided in Section 9.E.

Pretreat OmniSwab™ (GE Healthcare) or cotton swabs using the SwabSolution™ Kit (Cat.# DC8271) as described in the SwabSolution™ Kit Technical Manual #TMD037 to generate a swab extract.

**Amplification Setup**

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and Amplification-Grade Water completely.

   **Note:** Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

**Note:** The 5X AmpSolution™ Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

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

4. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

5. Add the final volume of each reagent listed in Table 3 to a clean tube.

### Table 3. PCR Amplification Mix for Direct Amplification of DNA from Swabs in a 25µl Reaction Volume.

<table>
<thead>
<tr>
<th>PCR Amplification Mix Component¹</th>
<th>Volume Per Reaction</th>
<th>×</th>
<th>Number of Reactions</th>
<th>=</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, Amplification Grade</td>
<td>10.5µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI/ESX Fast 5X Master Mix</td>
<td>5.0µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI 17 Fast 10X Primer Pair Mix</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5X AmpSolution™ Reagent</td>
<td>5.0µl</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>swab extract</td>
<td>2.0µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total reaction volume</td>
<td>25µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Add Water, Amplification Grade, to the tube first, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. The swab extract will be added at Step 7.

6. Vortex the PCR amplification mix for 5–10 seconds, then pipet 23µl of PCR amplification mix into each reaction well.

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

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

7. Pipet 2.0µl of swab extract for each sample into the appropriate well of the reaction plate.
4.C. Direct Amplification of DNA from Swabs in a 25µl Reaction Volume (continued)

8. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 5ng/µl. Add 2µl (10ng) to a reaction well containing 23µl of PCR amplification mix.
   **Note:** Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences.

9. For the negative amplification control, pipet 2µl of Amplification-Grade Water or TE–4 buffer instead of swab extract into a reaction well containing PCR amplification mix.
   **Note:** Additional negative controls can be included. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed as a blank without a swab.

10. Seal or cap the plate, or close the tubes. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

**Thermal Cycling**

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 26 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified (see below).

1. Place the reaction plate or tubes in the thermal cycler.
2. Select and run the recommended protocol.

**Notes:**

1. When using the Veriti® 96-Well Thermal Cycler, set the ramping rate to 100%.
2. When using the GeneAmp® PCR System 9700, 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. When the Select Method Options screen appears, select “Max” for the ramp speed and enter the reaction volume.

**Thermal Cycling Protocol**

<table>
<thead>
<tr>
<th>96°C for 1 minute, then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>96°C for 5 seconds</td>
</tr>
<tr>
<td>60°C for 35 seconds</td>
</tr>
<tr>
<td>72°C for 5 seconds</td>
</tr>
<tr>
<td>for 26 cycles, then:</td>
</tr>
<tr>
<td>60°C for 2 minutes</td>
</tr>
<tr>
<td>4°C soak</td>
</tr>
</tbody>
</table>

3. After completion of the thermal cycling protocol, proceed with fragment analysis or store amplified samples at –20°C in a light-protected box.
   **Note:** Long-term storage of amplified samples at 4°C or higher may produce artifacts.
**PCR Optimization**

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.

2. Prepare three identical reaction plates with aliquots of the same swab extracts.

3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25, 26 and 27 cycles).
   **Note:** This recommendation is for 2µl of swab extract. Additional cycle number testing may be required.

4. Following amplification, use your laboratory’s validated separation and detection protocols to determine the optimal cycle number for the sample type.

---

**5. Instrument Setup and Sample Preparation**

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

**Materials to Be Supplied by the User**

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- 96-well retainer & 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
- 96-well plate and septa (e.g., Plate, Barcoded, Semi-Skirted, 96-Well [Cat.# V7845] and Septa Mat, 96-Well [Cat. # CE2696] or MicroAmp® optical 96-well plate [or equivalent] and septa [Applied Biosystems])
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.
5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

Sample Preparation

1. Prepare a loading cocktail by combining and mixing WEN Internal Lane Standard 500 ESS and formamide as follows:

\[
[(0.5 \mu l \text{ WEN ILS 500 ESS}) \times (# \text{ samples})] + [(9.5 \mu l \text{ formamide}) \times (# \text{ samples})]
\]

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

2. Vortex for 10–15 seconds to mix.

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

4. Add 1μl of amplified sample (or 1μl of PowerPlex® ESI 17 Fast Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

**Notes:**

1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. To modify the injection time or injection voltage in the run module, select “Instrument Protocol” from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.

2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

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

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

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

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 2). Press the Refresh button. 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.
5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)


2.a. 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.

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

Run time and other instrument settings should be optimized and validated 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*. 
Figure 3. The Create New Instrument Protocol window.

The recommended settings are:

<table>
<thead>
<tr>
<th>Setting</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application Type</td>
<td>HID</td>
</tr>
<tr>
<td>Capillary Length</td>
<td>36cm</td>
</tr>
<tr>
<td>Polymer</td>
<td>POP-4®</td>
</tr>
<tr>
<td>Dye Set</td>
<td>Promega G5</td>
</tr>
<tr>
<td>Run Module</td>
<td>HID36_POP4-xl</td>
</tr>
<tr>
<td>Injection Time¹</td>
<td>15 seconds for the Applied Biosystems® 3500 Genetic Analyzer</td>
</tr>
<tr>
<td></td>
<td>24 seconds for the Applied Biosystems® 3500xL Genetic Analyzer</td>
</tr>
<tr>
<td>Injection Voltage</td>
<td>1.2kV</td>
</tr>
<tr>
<td>Run Voltage</td>
<td>15kV</td>
</tr>
<tr>
<td>Run Time</td>
<td>1,210 seconds</td>
</tr>
</tbody>
</table>

¹Injection time may be modified to increase or decrease peak heights.
5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

2.b. 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 “ILS500” or another appropriate name. Choose “Orange” as the Dye Color. The fragments in the size standard are 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 4.

Figure 4. The Create New Size Standard window.
2.c. 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 2.b. The settings for the QC protocol should be based on the internally validated conditions for the PowerPlex® ESI 17 Fast 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.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued))

2.d. 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.a and the QC Protocol created in Step 2.c. 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.
2.e. 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.
5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

2.f. 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.

![Create New Results Group window](image)

Figure 8. The Create New Results Group window.
3. To create a New Plate, navigate to the Library, and from the Manage menu, select “Plates”, then “Create”.

4. Assign a descriptive plate name. Select the plate type “HID” from the drop-down menu (Figure 9).

![Figure 9. Assigning plate contents.](image)

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

![Figure 10. Assigning plate contents.](image)
5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

6. Assign sample names to wells.

7. In the lower left portion of the screen, under “Assays”, use the Add from Library option to select the Assay created in Step 2.d or one previously created. Click on the Add to Plate button, and close the window.

8. Under “File Name Convention”, use the Add from Library option to select the File Name Convention created in Step 2.e or one previously created. Click on the Add to Plate button, and close the window.

9. Under “Results Groups”, use the Add from Library option to select the Results Group created in Step 2.f or one previously created. Click on the Add to Plate button, and close the window.

10. Highlight the sample wells, then select the boxes in the Assays, File Name Conventions and Results Groups.

11. Select “Link Plate for Run”.

12. The Load Plate window will appear. Select “Yes”.

13. 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 3130xl Genetic Analyzer with Data Collection Software, Version 3.0 or 4.0

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4® polymer) for the Applied Biosystems® 3100 or 3130 Genetic Analyzer
- 10X genetic analyzer buffer with EDTA
- 96-well plate and septa (e.g., Plate, Barcoded, Semi-Skirted, 96-Well [Cat.# V7845] and Septa Mat, 96-Well [Cat.# CE2696] or MicroAmp® optical 96-well plate [or equivalent] and septa [Applied Biosystems])
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Sample Preparation

1. Prepare a loading cocktail by combining and mixing WEN Internal Lane Standard 500 ESS and formamide as follows:
   
   \[(0.5\mu l \text{ WEN ILS 500 ESS}) \times (# \text{ samples})\] + \[(9.5\mu l \text{ formamide}) \times (# \text{ samples})\]

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

2. Vortex for 10–15 seconds to mix.

3. Pipet 10µl of formamide/internal lane standard mix into each well.
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 3130xl Genetic Analyzer with Data Collection Software, Version 3.0 or 4.0 (continued)

4. Add 1µl of amplified sample (or 1µl of PowerPlex® ESI 17 Fast Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

**Notes:**

1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module (see Instrument Preparation below).

2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

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

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

**Instrument Preparation**

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

Analyze samples as described in the user's manual for the ABI PRISM® 3100 or 3100-Avant Genetic Analyzer, and the Applied Biosystems® 3130 or 3130xl Genetic Analyzer, 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 1,500 seconds. Give a descriptive name to your run module, and select “OK”.

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

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

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

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

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


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

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

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

⚠️ It is critical to select the correct G5 spectral for the PowerPlex® 5-dye chemistry.

If the PowerPlex® 5-dye chemistry is not the active dye set, locate the PowerPlex® 5-dye spectral in the List of Calibrations for Dye Set G5, and select “Set”.

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 40 minutes.

### 5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer and POP-6™ Polymer

**Materials to Be Supplied by the User**

- 95°C dry heating block, water bath or thermal cycler
- crushed ice, ice-water bath or a freezer plate block
- 310 capillaries, 47cm × 50µm
- performance optimized polymer 6 (POP-6™ polymer)
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa
- aerosol-resistant pipette tips
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

⚠️ Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take the necessary precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.
5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer and POP-6™ Polymer (continued)

Sample Preparation

1. Prepare a loading cocktail by combining the WEN Internal Lane Standard 500 ESS and formamide as follows:

\[(0.5\mu l \text{ WEN ILS 500 ESS}) \times (# \text{ samples})\] + \[(24.5\mu l \text{ formamide}) \times (# \text{ samples})\]

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

2. Vortex for 10–15 seconds to mix.

3. Combine 25.0μl of prepared loading cocktail and 1.0μl of amplified sample (or 1μl of PowerPlex® ESI 17 Fast Allelic Ladder Mix).

**Notes:**

1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module (see Instrument Preparation below).

2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your internal validation.

4. Centrifuge tubes briefly to remove air bubbles from the wells.

5. Denature samples and ladder by heating at 95°C for 3 minutes, and immediately chill on crushed ice, a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading.

6. Place tubes in the appropriate autosampler tray.

7. Place the autosampler tray in the instrument, and close the instrument doors.

Instrument Preparation

Refer to the instrument user’s manual for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.

1. Open the ABI PRISM® 310 Data Collection Software.

2. To preheat the ABI PRISM® 310 Genetic Analyzer to 60°C, select “Manual Control” in the Window menu. In the Function menu, select “Temperature Set”. Set Value to “60.0”, then select “Execute”.

3. To make and save a module for use with POP-6™ polymer, choose the GS STR POP4 (1mL) G5v2.md5 module using the Module drop-down menu.
4. Click on the folded page icon (Figure 12).

![Figure 12. The Manual Control screen.](image)

5. Change the Collection Time to “50” and Syringe Pump Time to “360” (Figure 13). Select “Save Copy In”.

![Figure 13. Changing the collection time and syringe pump time.](image)

6. Save the new module in the Modules folder. Change the file name to “GS STR POP6 (1mL) G5v2.md5”, and select “Save” (Figure 14).

![Figure 14. The Save screen.](image)

7. In the File menu, select “New” to open the Create New menu. Open a GeneScan® sample sheet (either “48-Tube” or “96-Tube”).

8. In the upper right corner of the sample sheet, change “4 Dyes” to “5 Dyes”. Enter the appropriate sample information in the Sample Name field.
5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer and POP-6™ Polymer (continued)

9. To save the sample sheet, select “Save As” in the File menu. Assign a name to the file, and save in the Sample Sheet folder. Close the file.

10. In the File menu, select “New” to open the Create New menu.

11. Open the GeneScan® injection list.

12. Select the sample sheet (i.e., the .gss file) that was created in Step 9.

13. Choose the GS STR POP6 (1mL) G5v2.md5 module created in Step 6 using the drop-down menu. The settings should be:

   Inj. Secs: 3
   Inj. kV: 15.0
   Run kV: 15.0
   Run °C: 60
   Run Time (minutes): 50

   You may need to optimize the injection time, depending on instrument sensitivity. We recommend injection times of 2–5 seconds for amplification reactions that contain 0.5ng of template DNA.

   Note: Migration of fragments may vary slightly over the course of a long ABI PRISM® 310 Genetic Analyzer run. This may be due to changes in temperature or changes in the column. When analyzing many samples, injections of allelic ladder at different times throughout the run can aid in accurately genotyping samples.

14. Select the matrix file that was made with the GS STR POP6 (1mL) G5v2.md5 module.

15. After loading the sample tray and closing the doors, select “Run” to start the capillary electrophoresis system.

16. Monitor the electrophoresis by observing the raw data and status windows. Each sample will take approximately 60 minutes for syringe pumping, sample injection and electrophoresis.

   Note: The files that are created will be .fsa files. After the run is finished, save or transfer the .fsa files to a secure location where they can be opened in an analysis project.

6. Data Analysis

The instructions in this section are for use with GeneMapper® ID-X software, version 1.2, or GeneMapper® ID software, version 3.2. Due to potential differences between software versions, some of the instructions may not apply to all software versions.

To facilitate analysis of data generated with the PowerPlex® ESI 17 Fast System, we have created panels, bins and stutter text files to allow automatic assignment of genotypes using GeneMapper® ID-X software. We recommend that users receive training from Applied Biosystems on the GeneMapper® ID-X software to familiarize themselves with proper operation of the software.

**Note:** The panels, bins and stutter text files mentioned here are compatible with earlier versions of the GeneMapper® ID-X software.

**Getting Started**

1. To obtain the proper panels, bins and stutter text files for the PowerPlex® ESI 17 Fast System go to: www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/
2. Select the PowerPlex® System that you are using, and select “GeneMapper ID-X”. Enter your contact information, and select “Submit”.
3. Save the PowerPlex_ESI_Fast_Panels_IDX_vX.x.txt, PowerPlex_ESI_Fast_Bins_IDX_vX.x.txt and PowerPlex_ESI_Fast_Stutter_IDX_vX.x.txt files, where “X.x” refers to the most recent version of the panels, bins and stutter text files, to a known location on your computer.
4. Save the WEN_ILS_500_IDX.xml file to a known location on your computer.

**Importing Panels, Bins and Stutter Text Files**

1. Open the GeneMapper® ID-X software.
2. Select “Tools”, then “Panel Manager”.
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select “File”, then “Import Panels”.
5. Navigate to the panels text file downloaded in the Getting Started section above. Select the file, then “Import”.
6. In the navigation pane, highlight the PowerPlex ESI Fast panels folder that you just imported in Step 5.
7. Select “File”, then “Import Bin Set”.
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, then “Import”.
9. In the navigation pane, highlight the PowerPlex ESI Fast panels folder that you just imported in Step 5.
10. Select “File”, then “Import Marker Stutter”. A warning box will appear asking if you want to overwrite current values. Select “Yes”.
11. Navigate to the stutter text file downloaded in the Getting Started section above. Select the file, then “Import”.
12. At the bottom of the Panel Manager window, select “OK”. This will save the panels, bins and stutter text files, then close the window.
6.B. Importing the WEN ILS 500 ESS Size Standard into GeneMapper® ID-X Software, Version 1.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.C. The WEN_ILS_500_IDX.xml file can be used for the WEN ILS 500 ESS size standard supplied with the PowerPlex® ESI 17 Fast System.

1. Select “Tools”, then “GeneMapper ID-X Manager”.
2. Select the Size Standard tab.
3. Select “Import”.
4. Navigate to the location of the WEN_ILS_500_IDX.xml file on your computer.
5. Highlight the file, then select “Import”.
6. Select “Done” to save changes and close the GeneMapper® ID-X Manager.


1. Select “Tools”, then “GeneMapper ID-X Manager”.
2. Select the Size Standard tab.
3. Select “New”.
4. In the Size Standard Editor window (Figure 15), select “GeneMapper ID-X Security Group” as the Security Group. This allows access for all users of the software. Other security groups may be used.
Figure 15. The GeneMapper® ID-X Size Standard Editor.

5. Enter a detailed name, such as “WEN_ILS_500_IDX.”


7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 27.

8. Select “OK”.

5.

6.

7.

8.

These instructions are intended as a guide to start analyzing data in GeneMapper® ID-X Software. They are not intended as a comprehensive guide for using GeneMapper® ID-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select “Tools”, then “GeneMapper ID-X Manager”.
2. Select the Analysis Methods tab.
4. In the Analysis Method Editor window, select “GeneMapper ID-X Security Group” as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as “PowerPlex ESI 17 Fast”.
6. Select the Allele tab (Figure 16).

![GeneMapper® ID-X](image)

**Figure 16. The GeneMapper® ID-X Allele tab.**

7. Select the bins text file that was imported in Section 6.A.

8. Ensure that the “Use marker-specific stutter ratio and distance if available” box is checked.

9. We recommend the values shown in Figure 16 for proper filtering of stutter peaks when using the PowerPlex® ESI 17 Fast System. You may need to optimize these settings. In-house validation should be performed.
6.D. Creating a Casework Analysis Method with GeneMapper® ID-X Software, Version 1.2 (continued)

10. Select the Peak Detector tab (Figure 17). You will need to optimize these settings. In-house validation should be performed.

Figure 17. The GeneMapper® ID-X Peak Detector tab.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.
3. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

4. The peak window size shown in Figure 17 is not the default setting. It has been changed to 11 from the default value of 15. This ensures that the software can resolve alleles in the ladder that are 1 base apart for the larger loci such as D1S1656, D12S391 and D2S441.

11. Select the Peak Quality tab. You may change the settings for peak quality.

   **Note:** For Steps 11 and 12, see the GeneMapper® ID-X user’s manual for more information.

12. Select the SQ & GQ Settings tab. You may change these settings.

13. Select “Save” to save the new analysis method.

14. Select “Done” to exit the GeneMapper® ID-X Manager.

**Processing Data for Casework Samples**

1. Select “File”, then “New Project”.

2. Select “Edit”, then “Add Samples to Project”.

3. Browse to the location of the run files. Highlight desired files, then select “Add to list” followed by “Add”.

4. In the Sample Type column, use the drop-down menu to select “Allelic Ladder”, “Sample”, “Positive Control” or “Negative Control” as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as “Allelic Ladder” in the Sample Type column for proper genotyping.

5. In the Analysis Method column, select the analysis method created previously in this section.

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 or created in Section 6.C.

8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.

9. 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 Requirement Summary window activated, you may need to do additional manual troubleshooting.
(continued)

10. If all analysis requirements are met, the Save Project window will open (Figure 18).

![Save Project Window](image)

**Figure 18. The Save Project window.**

11. Enter the project name.

12. Choose the applicable security group from the drop-down menu, then select “OK”.

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory’s data analysis protocols.

6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® ID-X Software, Version 1.2

These instructions are intended as a guide to start analyzing data in GeneMapper® ID-X software. They are not intended as a comprehensive guide for using the GeneMapper® ID-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select “Tools”, then “GeneMapper ID-X Manager”.

2. Select the Analysis Methods tab.


4. In the Analysis Method Editor window, select “GeneMapper ID-X Security Group” as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as “PowerPlex ESI 17 Fast 20% Filter”.
6. Select the Allele tab (Figure 19).
7. Select the bins text file that was imported in Section 6.A.
8. We recommend the values shown in Figure 19 for proper filtering of stutter peaks when using the PowerPlex® ESI 17 Fast System. You may need to optimize these settings. In-house validation should be performed.

Figure 19. The GeneMapper® ID-X Allele tab with settings for using a 20% peak filter.
6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® ID-X Software, Version 1.2 (continued)

9. Select the Peak Detector tab (Figure 17). You will need to optimize these settings. In-house validation should be performed.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

3. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

4. The peak window size shown in Figure 17 is not the default setting. It has been changed to 11 from the default value of 15. This ensures that the software can resolve alleles in the ladder that are 1 base apart for the larger loci such as D1S1656, D12S391 and D2S441.

10. Select the Peak Quality tab. You may change the settings for peak quality.

Note: For Steps 10 and 11, see the GeneMapper® ID-X user’s manual for more information.

11. Select the SQ & GQ Settings tab. You may change these settings.

12. Select “Save” to save the new analysis method.

13. Select “Done” to exit the GeneMapper® ID-X Manager.

Processing Data for Databasing or Paternity Samples

1. Select “File”, then “New Project”.

2. Select “Edit”, then “Add Samples to Project”.

3. Browse to the location of 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.

In the Analysis Method column, select the analysis method created previously in this section.

5. In the Panel column, select the panels text file that was imported in Section 6.A.

6. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.
7. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.

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 Requirement Summary window activated, you may need to do additional manual troubleshooting.

9. If all analysis requirements are met, the Save Project window will open (Figure 18).

10. Enter the project name.

11. Choose the applicable security group from the drop-down menu, then select “OK”.

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory’s data analysis protocols.

6.F. Importing PowerPlex® ESI Fast Panels and Bins Text Files into GeneMapper® ID Software, Version 3.2

To facilitate analysis of data generated with the PowerPlex® ESI 17 Fast System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® ID software, version 3.2. We recommend that users of GeneMapper® ID software, version 3.2, complete the Applied Biosystems GeneMapper® ID Software Human Identification Analysis Tutorial to familiarize themselves with proper operation of the software. For GeneMapper® ID software, version 3.1, users we recommend upgrading to version 3.2.

Run files generated using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer cannot be analyzed using GeneMapper® ID software. You must analyze these files with GeneMapper® ID-X software.

**Getting Started**

1. To obtain the panels and bins text files for the PowerPlex® ESI 17 Fast System go to:  
   www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/

2. Select the PowerPlex® System that you are using, and select “GeneMapper ID”. Enter your contact information, and select “Submit”.

3. Save the PowerPlex_ESI_Fast_Panels_vX.x.txt and PowerPlex_ESI_Fast_Bins_vX.x.txt files, where “X.x” refers to the most recent version of the panels and bins text files, to a known location on your computer.

4. Save the WEN_ILS_500_IDX.xml file to a known location on your computer."
6.F. Importing PowerPlex® ESI Fast Panels and Bins Text Files into GeneMapper® ID Software, Version 3.2 (continued)

Importing Panels and Bins Text Files

These instructions loosely follow the Applied Biosystems GeneMapper® ID software tutorial, pages 1–4.

1. Open the GeneMapper® ID software, version 3.2.
2. Select “Tools”, then “Panel Manager”.
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select “File”, then “Import Panels”.
5. Navigate to the panels text file downloaded in the Getting Started section above. Select the file, then “Import”.
6. In the navigation pane, highlight the PowerPlex ESI Fast panels folder that you just imported in Step 5.
7. Select “File”, then “Import Bin Set”.
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, then “Import”.
9. At the bottom of the Panel Manager window, select “OK”. This will save the panels and bins text files and close the window automatically.

6.G. Importing the WEN ILS 500 ESS Size Standard into GeneMapper® ID Software, Version 3.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.H.

The WEN_ILS_500.xml file can be used for the WEN ILS 500 ESS size standard supplied with the PowerPlex® ESI 17 Fast System. The WEN_ILS_500.xml file is available for download at:
www.promega.com/resources/software-firmware/genemapper-id-software-panels-and-bin-sets/

1. Select “Tools”, then “GeneMapper Manager”.
2. Select the Size Standard tab.
3. Select “Import”.
4. Browse to the location of the WEN_ILS_500.xml file.
5. Highlight the file, then select “Import”.
6. Select “Done” to save changes and exit the GeneMapper® Manager.

1. Select “Tools”, then “GeneMapper Manager”.

2. Select the Size Standard tab.

3. Select “New”.

4. Select “Basic or Advanced” (Figure 20). The type of analysis method selected must match the type of analysis method created earlier. Select “OK”.

![Figure 20. The Select Dye and Analysis Method window.](image-url)

5. Enter a detailed name, such as “WEN_ILS_500”, in the Size Standard Editor (Figure 21).

![Size Standard Editor](image)

Figure 21. The Size Standard Editor.


7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 27.

8. Select “OK”.
6.1. Creating a Casework Analysis Method with GeneMapper® ID Software, Version 3.2

These instructions loosely follow the Applied Biosystems GeneMapper® ID software tutorial, pages 5–11.

1. Select “Tools”, then “GeneMapper Manager”.
2. Select the Analysis Methods tab.
4. Select “HID”, and select “OK”.
   **Note:** If you do not see the HID option, you do not have the GeneMapper® ID software. Contact Applied Biosystems.
5. Enter a descriptive name for the analysis method, such as “PowerPlexESI 17 Fast”.
6. Select the Allele tab (Figure 22).

![Analysis Method Editor - HID](image)

**Figure 22. The GeneMapper® ID Allele tab.**
6.I. Creating a Casework Analysis Method with GeneMapper® ID Software, Version 3.2 (continued)

7. Select the bins text file that was imported in Section 6.F.

8. Ensure that the “Use marker-specific stutter ratio if available” box is checked.

9. Enter the values shown in Figure 22 for proper filtering of stutter peaks when using the PowerPlex® ESI 17 Fast System. For an explanation of the proper usage and effects of these settings, refer to the Applied Biosystems user bulletin titled “Installation Procedures and New Features for GeneMapper ID Software 3.2”.

   **Note:** Some of these settings have been optimized and are different from the recommended settings in the user bulletin. You will need to optimize these settings. In-house validation should be performed.

10. Select the Peak Detector tab (Figure 23). You will need to optimize these settings. In-house validation should be performed.

   **Notes:**

   1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

   2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

   3. The peak window size shown in Figure 23 is not the default setting. It has been changed to 11 from the default value of 15. This ensures that the software can resolve alleles in the ladder that are 1 base apart for the larger loci, such as D1S1656, D12S391 and D2S441.
Figure 23. The GeneMapper® ID Peak Detector tab.
6.I. Creating a Casework Analysis Method with GeneMapper® ID Software, Version 3.2 (continued)

11. Select the Peak Quality tab. You may change the settings for peak quality.  
   **Note:** For Steps 11 and 12, see the GeneMapper® ID user’s manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select “OK” to save your settings.

**Processing Data for Casework Samples**

1. Select “File”, then “New Project”.
2. Select “Edit”, then “Add Samples to Project”.
3. Browse to the location of the run files. Highlight desired files, then select “Add to list” followed by “Add”.
4. In the Sample Type column, use the drop-down menu to select “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 “Ladder” in the Sample Type column for proper genotyping.
5. In the Analysis Method column, select the analysis method created previously in this section.
6. In the Panel column, select the panels text file that was imported in Section 6.F.
7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.
8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.
9. Select “Analyze” (green arrow button) to start data analysis.

6.J. Creating a Databasing or Paternity Analysis Method with GeneMapper® ID Software, Version 3.2

1. Select “Tools”, then “GeneMapper Manager”.
2. Select the Analysis Methods tab.
4. Select “HID”, and select “OK”.  
   **Note:** If you do not see the HID option, you do not have the GeneMapper® ID software. Contact Applied Biosystems.
5. Enter a descriptive name for the analysis method, such as “PowerPlexESI 17 Fast_20%filter”.
6. Select the Allele tab (Figure 24).
7. Select the bins text file that was imported in Section 6.F.
8. Ensure that the “Use marker-specific stutter ratio if available” box is checked.
9. Enter the values shown in Figure 24 for proper filtering of peaks when using the PowerPlex® ESI 17 Fast System. For an explanation of the proper usage and effect of these settings, refer to the Applied Biosystems user bulletin titled “Installation Procedures and New Features for GeneMapper ID Software 3.2”.

Figure 24. The GeneMapper® ID Allele tab with settings for using a 20% peak filter.
6.J. Creating a Databasing or Paternity Analysis Method with GeneMapper® ID Software, Version 3.2 (continued)

10. Select the Peak Detector tab (Figure 23). You will need to optimize these settings. In-house validation should be performed.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.

2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

3. The peak window size shown in Figure 23 is not the default setting. It has been changed to 11 from the default value of 15. This ensures that the software can resolve alleles in the ladder that are 1 base apart for the larger loci such as D1S1656, D12S391 and D2S441.

11. Select the Peak Quality tab. You may change the settings for peak quality.

Note: For Steps 11 and 12, see the GeneMapper® ID user's manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select “OK” to save your settings.

Processing Data for Databasing or Paternity Samples

1. Select “File”, then “New Project”.

2. Select “Edit”, then “Add Samples to Project”.

3. Browse to the location of the run files. Highlight desired files, then select “Add to list” followed by “Add”.

4. In the Sample Type column, use the drop-down menu to select “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 “Ladder” in the Sample Type column for proper genotyping.

5. In the Analysis Method column, select the analysis method created previously in this section.

6. In the Panel column, select the panels text file that was imported in Section 6.F.

7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.

8. If analyzing data from an ABI PRISM® 310 Genetic Analyzer, ensure that the appropriate matrix file is selected in the Matrix column.

9. Select “Analyze” (green arrow button) to start the data analysis.
6.K. Controls

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.

2. Observe the results for the 2800M Control DNA. The expected 2800M DNA allele designations for each locus are listed in Table 6 (Section 9.A).

6.L. Results

Representative results of the PowerPlex® ESI 17 Fast System are shown in Figure 25. The PowerPlex® ESI 17 Fast Allelic Ladder Mix is shown in Figure 26.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis (16,17). Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci. Increased stutter often is associated with D22S1045, as it is a trinucleotide repeat marker.

The mean stutter plus three standard deviations observed at each locus is used in the PowerPlex® ESI Fast panels text file for locus-specific filtering in the GeneMapper® ID software, version 3.2, and in the stutter text file for locus-specific filtering in GeneMapper® ID-X software.
Figure 25. **The PowerPlex® ESI 17 Fast System.** The 2800M Control DNA (0.5 ng) was amplified using the PowerPlex® ESI 17 Fast System. Amplification products were mixed with WEN Internal Lane Standard 500 ESS and analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. Results were analyzed using GeneMapper® ID-X software, version 1.4. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, D19S433, D2S1338 and D22S1045. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D16S539, D18S51, D1S1656, D10S1248 and D2S441. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loci: TH01, vWA, D21S11 and D12S391. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D8S1179, FGA and SE33. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500 ESS.
Figure 26. The PowerPlex® ESI 17 Fast Allelic Ladder Mix. The PowerPlex® ESI 17 Fast Allelic Ladder Mix was analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. The sample file was analyzed with the GeneMapper® ID-X software, version 1.4, and PowerPlex® ESI Fast panels and bins text files. Panel A. The fluorescein-labeled allelic ladder components and their allele designations. Panel B. The JOE-labeled allelic ladder components and their allele designations. Panel C. The TMR-ET-labeled allelic ladder components and their allele designations. Panel D. The CXR-ET-labeled allelic ladder components and their allele designations.
6.L. Results (continued)

In addition to stutter peaks, the following low-level artifact peaks may be observed with the PowerPlex® ESI 17 Fast System loci.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Artifact’éttes</th>
</tr>
</thead>
</table>
| Fluorescein | 51–64 bases$^1$
| | 65–68 bases and 69–72 bases$^2$
| | 84–91 bases$^3$
| | Amelogenin n–1$^4$
| JOE   | D1S1656 n–2; n+2$^5$
| | D2S441 n–2; n+2$^5$
| TMR   | 59–60 bases$^6$
| | 60–62 bases$^7$
| | D21S11 n–2; n+2$^5$
| CXR   | FGA n–2; n+2$^5$
| | SE33 n–2; n+2$^5$
| | D8S1179 60–69 bases$^8$; 69–71 bases$^1$; 78–82 bases$^5$; 94–96 bases$^1$
| | SE33 n–13 to n–14 and n–17 to n–18$^9$

$^1$Artifact is present in no-template amplification reactions as well as those containing DNA but is not present in the no-amplification controls.

$^2$These variably sized peaks may represent double-stranded DNA derived from the Amelogenin amplicon (double-stranded DNA is known to migrate faster than single-stranded DNA on capillary electrophoresis instruments). This artifact is only seen with high peak heights for the X and Y alleles.

$^3$Low-level, DNA-dependent artifact is noticeable with very high template amounts and allele peak heights. These peaks may be above or below analysis threshold, depending on sensitivity of the capillary electrophoresis instrument.

$^4$The n–1 artifact is more noticeable with high template amounts and allele peak heights.

$^5$Two bases below and above the true allele peak, respectively.

$^6$Peak is found in no-template and template-containing amplification reactions as well as no-amplification controls.

$^7$Peak is found in no-template amplification reactions as well as those containing DNA and can vary in height.

$^8$Low-level peak found in no-template as well as template-containing amplification reactions. Artifacts more likely to be seen if amplification reaction mix remains at ambient temperature for extended periods prior to thermal cycling. This artifact is not observed in no-amplification controls. Peak may be above or below analysis threshold depending on sensitivity of the capillary electrophoresis instrument.

$^9$At high heterozygous peak heights (≥4,000RFU on an Applied Biosystems® 3130 or 3130x/ Genetic Analyzer), it is possible to see peaks that migrate approximately 13–14 bases and 17–18 bases in front of the main allele. These peaks are of low intensity (50–100RFU on an Applied Biosystems® 3130 or 3130x/ Genetic Analyzer) compared to the main allele and may represent DNA secondary structure.
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 Sections 7.C and 7.D.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faint or absent allele peaks</td>
<td>The PowerPlex® ESI/ESX Fast 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 15 seconds before dispensing into the PCR amplification mix.</td>
</tr>
<tr>
<td></td>
<td>An air bubble formed at the bottom of the reaction tube. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling.</td>
</tr>
<tr>
<td></td>
<td>Thermal cycler, plate or tube problems. Review the thermal cycling protocols in Section 4 or 9. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.</td>
</tr>
<tr>
<td></td>
<td>Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerPlex® ESI 17 Fast 10X Primer Pair Mix for 15 seconds before use.</td>
</tr>
<tr>
<td></td>
<td>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 the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.</td>
</tr>
<tr>
<td></td>
<td>Poor capillary electrophoresis injection (WEN ILS 500 ESS peaks also affected). Re-inject the sample. If using an ABI PRISM® 310 Genetic Analyzer, check the syringe pump system for leakage.</td>
</tr>
<tr>
<td></td>
<td>Poor capillary electrophoresis injection (WEN ILS 500 ESS peaks also affected). Check the laser power.</td>
</tr>
<tr>
<td></td>
<td>Poor-quality formamide was used. Use only the recommended formamide when analyzing samples.</td>
</tr>
<tr>
<td>Faint or absent peaks for the positive control reaction</td>
<td>Improper storage of the 2800M Control DNA.</td>
</tr>
</tbody>
</table>
7.A. Amplification and Fragment Detection (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra peaks visible in one or all color</td>
<td>Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.</td>
</tr>
<tr>
<td>channels</td>
<td>Samples were not denatured completely. Heat-denature samples for the recommended time, and then cool on crushed ice, a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.</td>
</tr>
<tr>
<td></td>
<td>Do not store the PCR amplification mix for a prolonged period. Add the mix to the wells of the reaction plate as soon as the mix is prepared. Add the punches as soon as possible to each well and follow immediately by thermal cycling.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>• Be sure to perform the 2-minute extension step at 60°C after thermal cycling (Section 4 or 9).</td>
</tr>
<tr>
<td></td>
<td>• Decrease the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation.</td>
</tr>
<tr>
<td></td>
<td>• Decrease cycle number.</td>
</tr>
<tr>
<td></td>
<td>• Increase the final extension time.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Causes and Comments</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Extra peaks visible in one channel (continued)</td>
<td>CE-related artifacts (&quot;spikes&quot;). Minor voltage or all color changes or urea crystals passing by the laser can cause &quot;spikes&quot; 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.</td>
</tr>
<tr>
<td>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. Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions on instrument preparation in Section 5.</td>
<td></td>
</tr>
<tr>
<td>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. For the ABI PRISM® 310 Genetic Analyzer, generate a new matrix, and apply it to the samples. For the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130 and 3130xl, 3500 and 3500xL Genetic Analyzers, perform a new spectral calibration, and re-run the samples. Instrument sensitivities can vary. Optimize the injection conditions. See Section 5.</td>
<td></td>
</tr>
<tr>
<td>Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in artifacts.</td>
<td></td>
</tr>
<tr>
<td>The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.</td>
<td></td>
</tr>
<tr>
<td>Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.</td>
<td></td>
</tr>
<tr>
<td>Allelic ladder not running the same as samples</td>
<td>Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.</td>
</tr>
<tr>
<td>Poor-quality formamide. Use only the recommended formamide when analyzing samples.</td>
<td></td>
</tr>
</tbody>
</table>
### 7.A. Amplification and Fragment Detection (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allelic ladder not running the same as samples (continued)</td>
<td>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.</td>
</tr>
<tr>
<td>Peak height imbalance</td>
<td>Miscellaneous balance problems. Thaw the 10X Primer Pair Mix and 5X Master Mix completely, and vortex for 15 seconds before use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely. PCR amplification mix prepared in Section 4 or 9 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.</td>
</tr>
<tr>
<td>Split peak or n–1 at SE33 with data generated on an ABI PRISM® 310 Genetic Analyzer with POP-6™ polymer</td>
<td>Ensure that the WEN ILS 500 ESS was used when analyzing PowerPlex® ESI 17 Fast amplifications. Use of the WEN ILS 500 will result in splitting of the main peak at SE33, which appears as a peak approximately 1 base shorter than the main allele. Current CE instrumentation cannot adequately maintain a denaturing environment at the exposed cathode end of the capillary, and re-annealing of the unlabeled SE33 primer to the labeled SE33 amplicon strand can occur after electrokinetic injection. The resulting partially double-stranded amplicon migrates faster than the single-stranded amplicon, generating the split peak (15). The WEN ILS 500 ESS contains an oligo that competes with the labeled amplicon strand for annealing to the unlabeled primer, thereby eliminating the split peak. The WEN ILS 500 does not contain this oligo. This split peak is detected only on an ABI PRISM® 310 Genetic Analyzer with POP-6™ polymer and not on Applied Biosystems® 3130 and 3500 Genetic Analyzers with POP-4® polymer. On these instruments with POP-4® polymer, re-annealing does occur, but a split peak is not observed. However, there is broadening of the allele peak at the SE33 locus.</td>
</tr>
</tbody>
</table>
### Symptoms
- Artifact peaks (one or two peaks) in the D8S1179 allele-calling range with data generated using an ABI PRISM® 310 Genetic Analyzer and POP-6™ polymer

### Causes and Comments
These peaks are due to the unlabeled SE33 amplicon strand annealing after electrokinetic injection to the CXR-ET-labeled SE33 primer as described above for the split peak. This annealing retards migration of the CXR-ET-labeled SE33 primer relative to its unhybridized form and results in a small amount of the CXR-ET-labeled SE33 primer migrating in the D8S1179 allele-calling range. The number of peaks that appear depends on the SE33 genotype. If two alleles with a significant size difference are present, then two peaks are observed. If there is only one allele or the two alleles are similar in size, then one peak may be observed. The WEN ILS 500 ESS contains an oligo that prevents annealing of the CXR-ET-labeled SE33 primer and unlabeled amplicon strand, whereas the WEN ILS 500 does not.

### 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
- Faint or absent allele peaks

### Causes and Comments
- Impure template DNA. Because a small amount of template is 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⁺, Mg²⁺ 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.
- 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.
7.B. Amplification of Extracted DNA (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra peaks visible in one or all</td>
<td>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.L for additional information about stutter and artifacts. You will need to optimize the amount of template DNA if you are using reduced reaction volumes.</td>
</tr>
<tr>
<td>color channels</td>
<td></td>
</tr>
<tr>
<td>Peak height imbalance</td>
<td>Amplification of excessive amount of template DNA can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles.</td>
</tr>
<tr>
<td></td>
<td>Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.</td>
</tr>
<tr>
<td></td>
<td>Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.</td>
</tr>
<tr>
<td></td>
<td>Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.</td>
</tr>
<tr>
<td></td>
<td>The reaction volume was too low. This system is optimized for a final reaction volume of 25μl to overcome inhibitors present in DNA samples. Decreasing the reaction volume can result in suboptimal performance.</td>
</tr>
</tbody>
</table>

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 information about general amplification and detection, see Section 7.A.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faint or absent allele peaks</td>
<td>DNA was not accessible on nonlytic material. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.</td>
</tr>
<tr>
<td></td>
<td>Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td><strong>Causes and Comments</strong></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Faint or absent allele peaks (continued)</td>
<td>Poor sample transfer to storage card or variable sampling from the storage card. Take punches from a different portion of the card. Increasing cycle number can improve low peak heights.</td>
</tr>
<tr>
<td></td>
<td>Too much sample in the reaction. Use the recommended number of storage card punches. Follow the manufacturer’s recommendations when depositing sample onto the storage card.</td>
</tr>
<tr>
<td></td>
<td>Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.</td>
</tr>
<tr>
<td></td>
<td>Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.</td>
</tr>
<tr>
<td></td>
<td>Active PunchSolution™ Reagent carried over into the amplification reaction when using nonFTA card punches. Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. We have not tested longer incubation times.</td>
</tr>
<tr>
<td></td>
<td>Inactive PunchSolution™ Reagent. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.</td>
</tr>
<tr>
<td>Faint or absent peaks for the positive control reaction</td>
<td>If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with storage card punches, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. We recommend 10ng of 2800M Control DNA per 25μl amplification reaction and 5ng of 2800M Control DNA per 12.5μl reaction. This mass of DNA should be reduced if the cycle number is increased and decreased if the cycle number is increased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively. Optimize the amount of 2800M Control DNA for your thermal cycling conditions and laboratory preferences.</td>
</tr>
</tbody>
</table>
### 7.C. Direct Amplification of DNA from Storage Card Punches (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faint or absent peaks for the positive control reaction (continued)</td>
<td>Do not include a blank punch in the positive control reaction. Presence of a blank punch may inhibit amplification of 2800M Control DNA.</td>
</tr>
<tr>
<td>Extra peaks visible in one or all color channels</td>
<td>Punch was contaminated. Take punches from blank paper between samples.</td>
</tr>
<tr>
<td></td>
<td>Amplification of processed punches with high amounts of DNA can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. Be sure to use the recommended number of punches. Use of a larger punch size or a smaller reaction volume may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number.</td>
</tr>
<tr>
<td></td>
<td>Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain the DNA in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as “shadow” peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two “shadow” peaks that differ in size from one another by approximately the same distance as the single-stranded alleles.</td>
</tr>
<tr>
<td></td>
<td>Artifacts of STR amplification. Direct amplification of excessive amounts of template DNA can result in a higher number of artifact peaks. Use the recommended punch size and number of punches. Optimize the cycle number. See Section 6.L for additional information on stutter and artifacts.</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Causes and Comments</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Peak height imbalance</td>
<td>Amplification of excessive amounts of template DNA can result in an imbalance, with smaller loci showing more product than larger loci.</td>
</tr>
<tr>
<td></td>
<td>• Use the recommended number of punches. Follow the manufacturer’s recommendations when depositing sample onto the storage card.</td>
</tr>
<tr>
<td></td>
<td>• Decrease cycle number.</td>
</tr>
<tr>
<td></td>
<td>Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Active PunchSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to carryover and will drop out before the smaller loci.</td>
</tr>
<tr>
<td></td>
<td>• Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent.</td>
</tr>
<tr>
<td></td>
<td>• We recommend treating one 1.2mm nonFTA card punch with 10µl of PunchSolution™ Reagent and using one punch per 25µl or 12.5µl amplification reaction. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.</td>
</tr>
<tr>
<td></td>
<td>Inactive PunchSolution™ Reagent. Thaw PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.</td>
</tr>
</tbody>
</table>
## 7.C. Direct Amplification of DNA from Storage Card Punches (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme variability in sample-to-sample peak heights</td>
<td>There can be significant individual-to-individual variability in the number of cells on a punch, resulting in peak height variability between samples. The PunchSolution™ Kit increases the recovery of amplifiable DNA from samples but does not normalize the amount of DNA present.</td>
</tr>
</tbody>
</table>

## 7.D. Direct Amplification of DNA from Swabs

The following information is specific to amplification of DNA from swabs after pretreatment using the SwabSolution™ Kit. For information about general amplification and detection, see Section 7.A.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faint or absent allele peaks</td>
<td>Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.</td>
</tr>
<tr>
<td></td>
<td>Inactive SwabSolution™ Reagent. Thaw SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.</td>
</tr>
<tr>
<td></td>
<td>Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block is heating to 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer. We have tested 60-minute incubation times and observed no difference in performance compared to a 30-minute incubation.</td>
</tr>
<tr>
<td>DNA was not accessible on nonlytic material.</td>
<td>Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.</td>
</tr>
<tr>
<td>Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>Causes and Comments</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Faint or absent peaks for the</td>
<td>If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with swab extracts, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. We recommend 10ng of 2800M Control DNA per 25μl amplification reaction and 5ng of 2800M Control DNA per 12.5μl reaction. This mass of DNA should be reduced if the cycle number is increased and decreased if the cycle number is increased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively. Optimize the amount of 2800M Control DNA for your thermal cycling conditions and laboratory preferences.</td>
</tr>
<tr>
<td>positive control reaction</td>
<td></td>
</tr>
<tr>
<td>Extra peaks visible in one or</td>
<td>Swab extract was contaminated. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed and incubated as a blank without a swab. Artifacts of STR amplification. Amplification of swab extracts with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturated signal on the CE instrument. If signal is saturated, repeat the amplification with less swab extract or a reduced cycle number. Amplification of excess template for a given cycle number resulted in overloading of the capillary upon electrokinetic injection. In addition to signal saturation, excess DNA in the capillary is difficult to maintain in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as “shadow” peaks migrating in front of the main peaks. If this occurs at a heterozygous locus it is possible to observe the presence of two “shadow” peaks that differ in size by approximately the same distance as the single-stranded alleles.</td>
</tr>
<tr>
<td>all color channels</td>
<td></td>
</tr>
<tr>
<td>Peak height imbalance</td>
<td>Excess DNA in the amplification reaction can result in locus-to-locus imbalance within a dye channel such that the peak heights at the smaller loci are greater than those at the larger loci (ski-slope effect). Use less swab extract, or reduce the cycle number.</td>
</tr>
</tbody>
</table>
### 7.D. Direct Amplification of DNA from Swabs (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak height imbalance (continued)</td>
<td>Active SwabSolution™ Reagent carried over from swab extracts into the amplification reaction. Larger loci are most susceptible to reagent carryover and will drop out before the smaller loci. Ensure that the heat block is heating to 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer.</td>
</tr>
<tr>
<td></td>
<td>Inactive SwabSolution™ Reagent. Thaw SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not re-freeze, as this may reduce activity.</td>
</tr>
<tr>
<td>DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.</td>
<td>DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.</td>
</tr>
<tr>
<td>Extreme variability in sample-to-sample peak heights</td>
<td>There can be significant individual-to-individual variability in cell deposition onto buccal swabs. This will appear as variability in peak heights between swab extracts. The extraction process maximizes recovery of amplifiable DNA from buccal swabs but does not normalize the amount of DNA present. If variability is extreme, quantify the DNA using a fluorescence-based double-stranded DNA quantification method or qPCR-based quantification method. The quantification values can be used to normalize input template amounts to minimize variation in signal intensity.</td>
</tr>
</tbody>
</table>
### 7.E. GeneMapper® ID-X Software

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stutter peaks not filtered</td>
<td>Stutter text file was not imported into the Panel Manager when the panels and bins text files were imported. Be sure that the “Use marker-specific stutter ratio and distance if available” box is checked. Stutter distance was not defined in the analysis method Allele tab.</td>
</tr>
<tr>
<td>Samples in the project not analyzed</td>
<td>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.</td>
</tr>
<tr>
<td>Edits in label edit viewer cannot be viewed</td>
<td>To view edits made to a project, the project first must be saved. Close the plot view window, return to the main GeneMapper® ID-X page and save the project. Display the plot window again, then view the label edit table.</td>
</tr>
<tr>
<td>Marker header bar for some loci are gray</td>
<td>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.</td>
</tr>
<tr>
<td>Alleles not called</td>
<td>Alleles were not resolved in D12S391 or D2S441. Change polymer and capillary array to resolve the 17.3 and 18 alleles and 18.3 and 19 alleles in the D12S391 allelic ladder and the 11.3 and 12 alleles in the D2S441 allelic ladder. We highly recommend the use of POP-6™ polymer for the ABI PRISM® 310 Genetic Analyzer. To analyze samples with GeneMapper® ID-X software, at least one allelic ladder must be defined. Peaks in ILS were not captured. Not all WEN ILS 500 ESS peaks defined in the size standard were detected during the run.</td>
</tr>
<tr>
<td></td>
<td>- Create a new size standard using the internal lane standard fragments present in the sample.</td>
</tr>
<tr>
<td></td>
<td>- Re-run samples using a longer run time.</td>
</tr>
<tr>
<td></td>
<td>A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.</td>
</tr>
</tbody>
</table>
### 7.E. GeneMapper® ID-X Software (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-ladder alleles</td>
<td>Alleles were not resolved in D12S391 or D2S441. Change polymer and capillary array to resolve the 17.3 and 18 alleles and 18.3 and 19 alleles in the D12S391 allelic ladder and the 11.3 and 12 alleles in the D2S441 allelic ladder. We highly recommend the use of POP-6™ polymer for the ABI PRISM® 310 Genetic Analyzer.</td>
</tr>
<tr>
<td></td>
<td>An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.</td>
</tr>
<tr>
<td></td>
<td>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.D or 6.E.</td>
</tr>
<tr>
<td></td>
<td>Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.</td>
</tr>
<tr>
<td></td>
<td>The allelic ladder was not identified as an allelic ladder in the Sample Type column.</td>
</tr>
<tr>
<td></td>
<td>The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.</td>
</tr>
<tr>
<td></td>
<td>A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.</td>
</tr>
<tr>
<td>Size standard not called</td>
<td>Starting data point was incorrect for the partial range chosen in Section 6.D or 6.E. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.</td>
</tr>
<tr>
<td>correctly</td>
<td>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”.</td>
</tr>
</tbody>
</table>
### Symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
</table>
| Size standard not called correctly (continued) | Peaks in ILS were not captured. Not all WEN ILS 500 ESS peaks defined in the size standard were detected during the run.  
  - Create a new size standard using the internal lane standard fragments present in the sample.  
  - Re-run samples using a longer run time. |
| Peaks in size standard missing | An incorrect size standard was used.  
  If peaks are low-quality, redefine the size standard for the sample to skip these peaks. |
| Significantly raised baseline |  
  - Poor spectral calibration for the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. Perform a new spectral calibration, and re-run the samples.  
  - Poor matrix for the ABI PRISM® 310 Genetic Analyzer. Re-run and optimize the matrix. Make sure that the matrix applied was generated on the same instrument.  
  Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions for instrument preparation in Section 5. |

### 7.F. GeneMapper® ID Software

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
</table>
| Alleles not called | Alleles were not resolved in D12S391 or D2S441. Change polymer and capillary array to resolve the 17.3 and 18 alleles and 18.3 and 19 alleles in the D12S391 allelic ladder and the 11.3 and 12 alleles in the D2S441 allelic ladder.  
  We highly recommend the use of POP-6™ polymer for the ABI PRISM® 310 Genetic Analyzer.  
  To analyze samples with GeneMapper® ID software, the analysis parameters and size standard must both have “Basic or Advanced” as the analysis type. If they are different, an error is obtained.  
  To analyze samples with GeneMapper® ID software, at least one allelic ladder must be defined. |
### 7.F. GeneMapper® ID Software (continued)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
</table>
| Alleles not called (continued) | Peaks in ILS were not captured. Not all WEN ILS 500 ESS peaks defined in the size standard were detected during the run.  
• Create a new size standard using the internal lane standard fragments present in the sample.  
• Re-run samples using a longer run time.  |
| A low-quality allelic ladder was used during analysis.  
Ensure that only high-quality allelic ladders are used for analysis.  |
| Off-ladder alleles | Alleles were not resolved in D12S391 or D2S441. Change polymer and capillary array to resolve the 17.3 and 18 alleles and 18.3 and 19 alleles in the D12S391 allelic ladder and the 11.3 and 12 alleles in the D2S441 allelic ladder.  
We highly recommend the use of POP-6™ polymer for the ABI PRISM® 310 Genetic Analyzer.  
Alleles were not resolved. Reduce the Peak Window Size.  
Change Smooth Options to “none”.  
An allelic ladder from a different run than the samples was used. Re-analyze samples using 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.I or 6.J.  
Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.  
The allelic ladder was not identified as an allelic ladder in the Sample Type column.  
The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.  
The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.  
A low-quality allelic ladder was used during analysis.  
Ensure that only high-quality allelic ladders are used for analysis.  |
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Causes and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size standard not called correctly</td>
<td>Starting data point was incorrect for the partial range chosen in Section 6.J or 6.I. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.</td>
</tr>
<tr>
<td></td>
<td>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”.</td>
</tr>
<tr>
<td></td>
<td>Peaks in ILS were not captured. Not all WEN ILS 500 ESS peaks defined in the size standard were detected during the run.</td>
</tr>
<tr>
<td></td>
<td>• Create a new size standard using the internal lane standard fragments present in the sample.</td>
</tr>
<tr>
<td></td>
<td>• Re-run samples using a longer run time.</td>
</tr>
<tr>
<td>Peaks in size standard missing</td>
<td>An incorrect size standard was used.</td>
</tr>
<tr>
<td></td>
<td>If peaks were low-quality, redefine the size standard for the sample to skip these peaks.</td>
</tr>
<tr>
<td>Error message: “Either panel, size standard, analysis method is invalid”</td>
<td>The size standard and analysis method were not in the same mode (“Classic” vs. “Basic or Advanced”). Be sure both or files are set to the same mode, either Classic or Basic or Advanced mode.</td>
</tr>
<tr>
<td>No alleles called, but no error message appears</td>
<td>Panels text file was not selected for sample. In the Panel column, select the appropriate panels text file for the STR system that was used.</td>
</tr>
<tr>
<td></td>
<td>No size standard was selected. In the Size Standard column, be sure to select the appropriate size standard.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Error message: “Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit”</td>
<td>The bins text file assigned to the analysis method was deleted. In the GeneMapper Manager, select the Analysis Methods tab, and open the analysis method of interest. Select the Allele tab, and select an appropriate bins text file. The wrong bins text file was chosen in the analysis method Allele tab. Be sure to choose the appropriate bins text file, as shown in Figure 22.</td>
</tr>
</tbody>
</table>
### Symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Causes and Comments</th>
</tr>
</thead>
</table>
| Significantly raised baseline | • Poor spectral calibration for the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. Perform a new spectral calibration, and re-run the samples.  
• Poor matrix for the ABI PRISM® 310 Genetic Analyzer. Re-run and optimize the matrix. Make sure that the matrix applied was generated on the same instrument.  
Use of Classic mode analysis method. Use of Classic mode analysis on samples can result in baselines with more noise than those analyzed using the Basic or Advanced mode analysis method. Advanced mode analysis methods and size standards are recommended.  
Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions on instrument preparation in Section 5. |
| Red bar appears during analysis of samples, and the following error message appears when data are displayed: “Some selected sample(s) do not contain analysis data. Those sample(s) will not be shown”. | If none of the samples had matrices applied when run on the ABI PRISM® 310 Genetic Analyzer, no data will be displayed. Apply a matrix file during analysis in the GeneMapper® ID software and re-analyze. |
| Error message after attempting to import panels and bins text files: “Unable to save panel data: java.SQLException: ORA-00001: unique constraint (IFA.CKP_NNN) violated”. | There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order. |
### Symptoms
Allelic ladder peaks labeled off-ladder

### Causes and Comments
GeneMapper® ID software was not used, or microsatellite analysis settings were used instead of HID analysis settings. GeneMapper® software does not use the same algorithms as GeneMapper® ID software and cannot correct for sizing differences using the allelic ladder. We recommend using GeneMapper® ID software to analyze PowerPlex® reactions. If using GeneMapper® ID software, version 3.2, be sure that the analysis method selected is an HID method. This can be verified by opening the analysis method using the GeneMapper Manager, then selecting the General tab. The analysis type cannot be changed. If the method is not HID, it should be deleted and a new analysis method created.

Alleles were incorrectly labeled in allelic ladder at one or all of the following loci: D1S1656, D2S441 and D12S391. The D1S1656, D2S441 and D12S391 allelic ladders contain alleles that differ in size by one base. Because these loci are large in the PowerPlex® ESI 17 Fast System, these alleles are sensitive to poor resolution, unlike alleles separated by more than one base. If there is poor resolution of alleles separated by one base, the GeneMapper® ID software is not able to distinguish these alleles and cannot call alleles correctly. Poor resolution is usually due to an old capillary, old capillary array or use of POP-4® polymer. Install a new array or polymer on your CE instrument, and repeat the injection of those samples. We highly recommend the use of POP-6™ polymer with the ABI PRISM® 310 Genetic Analyzer.

### References
8. References (continued)


9. **Appendix**

9.A. **Advantages of Using the Loci in the PowerPlex® ESI 17 Fast System**

The loci included in the PowerPlex® ESI 17 Fast System (Tables 4 and 5) were selected because they meet the recommendations of the European Network of Forensic Science Institutes (ENFSI). The PowerPlex® ESI 17 Fast System amplifies all ENFSI core loci plus SE33 in a single reaction. Table 6 lists the PowerPlex® ESI 17 Fast System alleles amplified from the 2800M Control DNA.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage and terminal nucleotide addition (16,17). Repeat slippage, 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 or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

Terminal nucleotide addition (18,19) occurs when a thermostable nonproofreading 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 (20) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.
9.A. Advantages of Using the Loci in the PowerPlex® ESI 17 Fast System (continued)

Table 4. The PowerPlex® ESI 17 Fast System Locus-Specific Information.

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>Label</th>
<th>Chromosomal Location(^1)</th>
<th>Repeat Sequence(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D22S1045</td>
<td>Fluorescein</td>
<td>22q12.3 (35.779Mb)</td>
<td>ATT</td>
</tr>
<tr>
<td>D2S1338</td>
<td>Fluorescein</td>
<td>2q35 (218.705Mb)</td>
<td>TGCC/TTC</td>
</tr>
<tr>
<td>D19S433</td>
<td>Fluorescein</td>
<td>19q12 (35.109Mb)</td>
<td>AAGG Complex</td>
</tr>
<tr>
<td>D3S1358</td>
<td>Fluorescein</td>
<td>3p21.31 (45.557Mb)</td>
<td>TCTA Complex</td>
</tr>
<tr>
<td>Amelogenin(^3)</td>
<td>Fluorescein</td>
<td>Xp22.1–22.3 and Y</td>
<td>NA</td>
</tr>
<tr>
<td>D2S441</td>
<td>JOE</td>
<td>2p14 (68.214Mb)</td>
<td>TCTA</td>
</tr>
<tr>
<td>D10S1248</td>
<td>JOE</td>
<td>10q26.3 (130.567Mb)</td>
<td>GGAA</td>
</tr>
<tr>
<td>D1S1656</td>
<td>JOE</td>
<td>1q42 (228.972Mb)</td>
<td>TAGA Complex</td>
</tr>
<tr>
<td>D18S51</td>
<td>JOE</td>
<td>18q21.33 (59.1Mb)</td>
<td>AGAA (21)</td>
</tr>
<tr>
<td>D16S539</td>
<td>JOE</td>
<td>16q24.1 (84.944Mb)</td>
<td>GATA</td>
</tr>
<tr>
<td>D12S391</td>
<td>TMR-ET</td>
<td>12p12 (12.341Mb)</td>
<td>AGAT/AGAC Complex</td>
</tr>
<tr>
<td>D21S11</td>
<td>TMR-ET</td>
<td>21q21.1 (19.476Mb)</td>
<td>AAAGA</td>
</tr>
<tr>
<td>vWA</td>
<td>TMR-ET</td>
<td>12p13.31 (5.963Mb)</td>
<td>TCTA Complex (21)</td>
</tr>
<tr>
<td>TH01</td>
<td>TMR-ET</td>
<td>11p15.5 (2.149Mb)</td>
<td>AATG (21)</td>
</tr>
<tr>
<td>SE33</td>
<td>CXR-ET</td>
<td>6q14 (89.043Mb)</td>
<td>AAAG Complex</td>
</tr>
<tr>
<td>FGA</td>
<td>CXR-ET</td>
<td>4q28 (155.866Mb)</td>
<td>TTTC Complex (21)</td>
</tr>
<tr>
<td>D8S1179</td>
<td>CXR-ET</td>
<td>8q24.13 (125.976Mb)</td>
<td>TCTA Complex (21)</td>
</tr>
</tbody>
</table>

\(^1\)Information about chromosomal location of these loci can be found in references 22 and 23 and at: [www.cstl.nist.gov/biotech/strbase/chrom.htm](http://www.cstl.nist.gov/biotech/strbase/chrom.htm)

\(^2\)The August 1997 report (24,25) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, “1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used”.

\(^3\)Amelogenin is not an STR.

NA = Not applicable.
### Table 5. The PowerPlex® ESI 17 Fast System Allelic Ladder Information.

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>Label</th>
<th>Size Range of Allelic Ladder Components&lt;sup&gt;1,2&lt;/sup&gt; (bases)</th>
<th>Repeat Numbers of Allelic Ladder Components&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>D22S1045</td>
<td>Fluorescein</td>
<td>306–345</td>
<td>7–20</td>
</tr>
<tr>
<td>D2S1338</td>
<td>Fluorescein</td>
<td>223–295</td>
<td>10, 12, 14–28</td>
</tr>
<tr>
<td>D3S1358</td>
<td>Fluorescein</td>
<td>103–147</td>
<td>9–20</td>
</tr>
<tr>
<td>Amelogenin&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Fluorescein</td>
<td>89, 95</td>
<td>X, Y</td>
</tr>
<tr>
<td>D2S441</td>
<td>JOE</td>
<td>347–383</td>
<td>8–11, 11.3, 12–17</td>
</tr>
<tr>
<td>D10S1248</td>
<td>JOE</td>
<td>286–330</td>
<td>8–19</td>
</tr>
<tr>
<td>D18S51</td>
<td>JOE</td>
<td>134–214</td>
<td>7–10, 10.2, 11–13, 13.2, 14–27</td>
</tr>
<tr>
<td>D16S539</td>
<td>JOE</td>
<td>84–132</td>
<td>4–16</td>
</tr>
<tr>
<td>vWA</td>
<td>TMR-ET</td>
<td>124–180</td>
<td>10–24</td>
</tr>
<tr>
<td>TH01</td>
<td>TMR-ET</td>
<td>72–115</td>
<td>3–9, 9.3, 10–11, 13.3</td>
</tr>
<tr>
<td>D8S1179</td>
<td>CXR-ET</td>
<td>76–124</td>
<td>7–19</td>
</tr>
</tbody>
</table>

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

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

<sup>3</sup>For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: [www.cstl.nist.gov/div831/strbase/](http://www.cstl.nist.gov/div831/strbase/)

<sup>4</sup>Amelogenin is not an STR.
9.A. Advantages of Using the Loci in the PowerPlex® ESI 17 Fast System (continued)

Table 6. The PowerPlex® ESI 17 Fast System Allele Determinations for the 2800M Control DNA.

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>2800M</th>
</tr>
</thead>
<tbody>
<tr>
<td>D22S1045</td>
<td>16, 16</td>
</tr>
<tr>
<td>D2S1338</td>
<td>22, 25</td>
</tr>
<tr>
<td>D19S433</td>
<td>13, 14</td>
</tr>
<tr>
<td>D3S1358</td>
<td>17, 18</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>X, Y</td>
</tr>
<tr>
<td>D2S441</td>
<td>10, 14</td>
</tr>
<tr>
<td>D10S1248</td>
<td>13, 15</td>
</tr>
<tr>
<td>D1S1656</td>
<td>12, 13</td>
</tr>
<tr>
<td>D18S51</td>
<td>16, 18</td>
</tr>
<tr>
<td>D16S539</td>
<td>9, 13</td>
</tr>
<tr>
<td>D12S391</td>
<td>18, 23</td>
</tr>
<tr>
<td>D21S11</td>
<td>29, 31.2</td>
</tr>
<tr>
<td>vWA</td>
<td>16, 19</td>
</tr>
<tr>
<td>TH01</td>
<td>6, 9.3</td>
</tr>
<tr>
<td>SE33</td>
<td>15, 16</td>
</tr>
<tr>
<td>FGA</td>
<td>20, 23</td>
</tr>
<tr>
<td>D8S1179</td>
<td>14, 15</td>
</tr>
</tbody>
</table>

9.B. DNA Extraction and Quantification Methods and Automation Support

Promega offers a wide variety of reagents and automated methods for sample preparation, DNA purification and DNA quantification prior to STR amplification.

For analysis of database, reference and other single-source samples, we recommend direct amplification of DNA from FTA® card punches or direct amplification of DNA from swabs and nonFTA punches following a preprocesing step with the SwabSolution™ Kit or PunchSolution™ Kit, respectively. The SwabSolution™ Kit contains reagents for rapid DNA preparation from buccal swabs prior to amplification. The procedure lyses cells contained on the swab head and releases into solution sufficient DNA for STR amplification. A small volume of the final swab extract is added to the PowerPlex® reaction. The PunchSolution™ Kit is used to process punches from nonFTA storage cards containing blood or buccal samples prior to direct amplification. When performing direct amplification with the PowerPlex® ESI 17 Fast System, make sure that the PCR amplification mix contains AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.

For casework or samples that require DNA purification, we recommend the DNA IQ™ System (Cat.# DC6700),
which is a DNA isolation system designed specifically for forensic samples (26). This system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With DNA-rich samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with PowerPlex® Systems to ensure a streamlined process.

For applications requiring human-specific DNA quantification, the PowerQuant® System (Cat.# PQ5002, PQ5008) and the Plexor® HY System (Cat.# DC1000) were developed (27, 28). These qPCR-based methods provide total human and male-specific DNA quantification in one reaction.

For information about automation of Promega chemistries on automated workstations using Identity Automation™ solutions, contact your local Promega Branch Office or Distributor (contact information available at: [www.promega.com/support/worldwide-contacts/](http://www.promega.com/support/worldwide-contacts/)), e-mail: genetic@promega.com or visit: [www.promega.com/idautomation/](http://www.promega.com/idautomation/)

9.C. The WEN Internal Lane Standard 500 ESS

The WEN Internal Lane Standard 500 ESS contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 27). Each fragment is labeled with WEN dye and can be detected separately (as a fifth color) in the presence of PowerPlex® ESI 17 Fast-amplified material. The WEN ILS 500 ESS is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® ESI 17 Fast System. Protocols to prepare and use this internal lane standard are provided in Section 5.

Low-level artifact peaks at approximately 132 and 176 bases may be observed with the WEN ILS 500 ESS in the orange channel. The peak height of these artifacts may vary from lot-to-lot and may be labeled by the software. These peaks are not used during sizing of the peaks present in the sample.

![Figure 27. WEN Internal Lane Standard 500 ESS. An electropherogram showing the WEN Internal Lane Standard 500 ESS fragments.](image-url)
9.D. Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume

Testing at Promega has shown successful direct amplification of DNA from storage card punches in a 12.5µl reaction volume (13). This section contains a protocol for direct amplification of DNA from storage card punches in a 12.5µl reaction volume using the PowerPlex® ESI 17 Fast System and GeneAmp® PCR System 9700. When using the protocol detailed below, add only one 1.2mm storage card punch to each 12.5µl amplification reaction.

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block (Applied Biosystems)
- centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- PunchSolution™ Kit (Cat.# DC9271) for nonFTA card punches
- 5X AmpSolution™ Reagent (Cat.# DM1231, also supplied with the PunchSolution™ Kit)
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system

Notes:

1. It may be possible to use thermal cyclers other than those listed in this technical manual. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. Use of thermal cyclers with an aluminum block is NOT recommended with the PowerPlex® ESI 17 Fast System.
2. You will need to optimize and validate the number of storage card punches per reaction in your laboratory. See the PCR Optimization recommendations at the end of this section.

FTA®-based sample types include:

- Buccal cells collected on FTA® cards with Whatman EasiCollect™ or Fitzco Sampact™ devices (one punch per 12.5µl amplification reaction)
- Buccal cells collected with swabs transferred to FTA® or Indicating FTA® cards (one punch per 12.5µl amplification reaction)
- Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards (one punch per 12.5µl amplification reaction)

NonFTA sample types include:

- Buccal samples on Bode Buccal DNA Collector™ devices (one punch per 12.5µl amplification reaction)
- Blood and buccal samples on nonFTA card punches (e.g., S&S 903) (one punch per 12.5µl amplification reaction)

Pretreat nonFTA sample types with the PunchSolution™ Kit (Cat.# DC9271) to lyse nonFTA samples before adding the PCR amplification mix. For more information, see the PunchSolution™ Kit Technical Manual #TMD038. Failure to pretreat these samples may result in incomplete profiles.
Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate.

Automated punchers also can be used to create sample disks. Refer to the user’s guide for your instrument for assistance with generating 1.2mm disks, technical advice and troubleshooting information.

**Note:** Static may be problematic when adding a punch to a well. For 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 PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and Amplification-Grade Water completely.
   **Note:** Centrifuge tubes briefly to bring contents to the bottom, and then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.
   **Note:** The 5X AmpSolution™ Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, and then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

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

4. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

5. Add the final volume of each reagent listed in Table 7 to a clean tube.
9.D. Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume (continued)

Table 7. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume.

<table>
<thead>
<tr>
<th>PCR Amplification Mix Component¹</th>
<th>Volume Per Reaction</th>
<th>Number of Reactions</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, Amplification Grade</td>
<td>6.25µl</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI/ESX Fast 5X Master Mix</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI 17 Fast 10X Primer Pair Mix</td>
<td>1.25µl</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>5X AmpSolution™ Reagent</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td><strong>total reaction volume</strong></td>
<td><strong>12.5µl</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Add Water, Amplification Grade, to the tube first, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. For FTA® card punches, the template DNA will be added at Step 7.

6. Vortex the PCR amplification mix for 5–10 seconds, and then pipet 12.5µl of PCR amplification mix into each reaction well.

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

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

7. For FTA® storage cards, add one 1.2mm punch from a card containing buccal cells or one 1.2mm punch from a card containing whole blood to the appropriate wells of the reaction plate. For nonFTA card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

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

8. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 5ng/µl. Add 1µl (5ng) of the 2800M Control DNA to a reaction well containing 12.5µl of PCR amplification mix.

**Notes:**
1. Do not include blank storage card punches in the positive control reactions.
2. Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences. Typically, 5ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.
9. Reserve a well containing PCR amplification mix as a negative amplification control.

**Note:** An additional negative control with a blank punch may be performed to detect contamination from the storage card or punch device.

10. Seal or cap the plate, or close the tubes. Briefly centrifuge the plate to bring storage card punches to the bottom of the wells and remove any air bubbles.

**Thermal Cycling**

Amplification and detection instrumentation may vary. You will need to optimize protocols, including cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 25 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type.

**Note:** It may be possible to use thermal cyclers other than those listed in this technical manual. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. Use of thermal cyclers with an aluminum block is NOT recommended with the PowerPlex® ESI 17 Fast System.

1. Place the reaction plate or tubes in the thermal cycler. Select and run the recommended protocol.

**Note:** The GeneAmp® PCR System 9700 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. When the Select Method Options screen appears, select “Max” for the ramp speed and enter the reaction volume.

<table>
<thead>
<tr>
<th>Thermal Cycling Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>96°C for 1 minute, then:</td>
</tr>
<tr>
<td>96°C for 5 seconds</td>
</tr>
<tr>
<td>60°C for 35 seconds</td>
</tr>
<tr>
<td>72°C for 5 seconds</td>
</tr>
<tr>
<td>for 25 cycles, then:</td>
</tr>
<tr>
<td>60°C for 2 minutes</td>
</tr>
<tr>
<td>4°C soak</td>
</tr>
</tbody>
</table>

2. 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.
9.D. **Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume**  
(continued)

**PCR Optimization**

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.

2. Depending on your preferred protocol, place one 1.2mm storage card punch containing a buccal sample or one 1.2mm punch of a storage card containing whole blood into each well of a reaction plate. 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 (24, 25 and 26 cycles).

5. Following amplification, use your laboratory’s validated separation and detection protocols to determine the optimal cycle number for the sample type.

9.E. **Direct Amplification of DNA from Swabs in a 12.5µl Reaction Volume**

Testing at Promega has shown successful direct amplification of DNA from swabs in a 12.5µl reaction volume (13). This section contains a protocol for amplifying DNA from swab extracts in a 12.5µl reaction volume using the PowerPlex® ESI 17 Fast System and GeneAmp® PCR System 9700.

**Materials to Be Supplied by the User**

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block (Applied Biosystems)
- Centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- Aerosol-resistant pipette tips
- SwabSolution™ Kit (Cat.# DC8271)

Pretreat OmniSwab™ (GE Healthcare) or cotton swabs using the SwabSolution™ Kit (Cat.# DC8271) as described in the SwabSolution™ Kit Technical Manual #TMD037 to generate a swab extract.

**Note:** It may be possible to use thermal cyclers other than those listed in this technical manual. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. Use of thermal cyclers with an aluminum block is NOT recommended with the PowerPlex® ESI 17 Fast System.
Amplification Setup

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and Amplification-Grade Water completely.
   
   **Note:** Centrifuge tubes briefly to bring contents to the bottom, and then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.
   
   **Note:** The 5X AmpSolution™ Reagent should be thawed completely, mixed by vortexing and stored at 2–10°C. The reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the buffer briefly at 37°C, and then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

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

4. Use a clean plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

5. Add the final volume of each reagent listed in Table 8 to a clean tube.

**Table 8. PCR Amplification Mix for Direct Amplification of DNA from Swabs in a 12.5µl Reaction Volume.**

<table>
<thead>
<tr>
<th>PCR Amplification Mix Component¹</th>
<th>Volume Per Reaction</th>
<th>×</th>
<th>Number of Reactions</th>
<th>=</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, Amplification Grade</td>
<td>4.25µl</td>
<td>×</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI/ESX Fast 5X Master Mix</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>PowerPlex® ESI 17 Fast 10X Primer Pair Mix</td>
<td>1.25µl</td>
<td>×</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>5X AmpSolution™ Reagent</td>
<td>2.5µl</td>
<td>×</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>swab extract</td>
<td>2.0µl</td>
<td></td>
<td></td>
<td></td>
<td>12.5µl</td>
</tr>
<tr>
<td>total reaction volume</td>
<td>12.5µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Add Water, Amplification Grade, to the tube first, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESI 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. The swab extract will be added at Step 7.
9.E. Direct Amplification of DNA from Swabs in a 12.5µl Reaction Volume (continued)

6. Vortex the PCR amplification mix for 5–10 seconds, and then pipet 10.5µl of PCR amplification mix into each reaction well.

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

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

7. Pipet 2.0µl of swab extract for each sample into the appropriate well of the reaction plate.

8. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 2.5ng/µl. Add 2µl (5ng) to a reaction well containing 10.5µl of PCR amplification mix.

Note: Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences.

9. For the negative amplification control, pipet 2µl of Amplification-Grade Water or TE–4 buffer instead of swab extract into a reaction well containing PCR amplification mix.

Note: Additional negative controls can be included. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed as a blank without a swab.

10. Seal or cap the plate, or close the tubes. Optional: Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 25 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type.

Note: It may be possible to use thermal cyclers other than those listed in this technical manual. Use of thermal cyclers not listed here may require optimization of cycling conditions and validation in your laboratory. Use of thermal cyclers with an aluminum block is NOT recommended with the PowerPlex® ESI 17 Fast System.

1. Place the reaction plate or tubes in the thermal cycler. Select and run the recommended protocol.

Note: The GeneAmp® PCR System 9700 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. When the Select Method Options screen appears, select “Max” for the ramp speed and enter the reaction volume.
Thermal Cycling Protocol

96°C for 1 minute, then:
96°C for 5 seconds
60°C for 35 seconds
72°C for 5 seconds
for 25 cycles, then:
60°C for 2 minutes
4°C soak

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

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

PCR Optimization

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.

2. Prepare three identical reaction plates with aliquots of the same swab extracts.

3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (24, 25 and 26 cycles).

Note: This recommendation is for 2µl of swab extract. Additional cycle number testing may be required.

4. Following amplification, use your laboratory’s validated separation and detection protocols to determine the optimal cycle number for the sample type.

9.F. Composition of Buffers and Solutions

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

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

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

**TE-4 buffer with 20µg/ml glycogen**

1.21g Tris base
0.037g EDTA (Na₂EDTA • 2H₂O)
20µg/ml glycogen

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

**Fluorescent STR Systems**

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>PowerPlex® ESX 17 Fast System</td>
<td>100 reactions</td>
<td>DC1711</td>
</tr>
<tr>
<td></td>
<td>400 reactions</td>
<td>DC1710</td>
</tr>
<tr>
<td>PowerPlex® ESX 16 Fast System</td>
<td>100 reactions</td>
<td>DC1611</td>
</tr>
<tr>
<td></td>
<td>400 reactions</td>
<td>DC1610</td>
</tr>
<tr>
<td>PowerPlex® ESI 16 Fast System</td>
<td>100 reactions</td>
<td>DC1621</td>
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<tr>
<td></td>
<td>400 reactions</td>
<td>DC1620</td>
</tr>
<tr>
<td>PowerPlex® Fusion 6C System</td>
<td>50 (or 100 direct-amp)</td>
<td>DC2705</td>
</tr>
<tr>
<td></td>
<td>reactions</td>
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<tr>
<td></td>
<td>200 (or 400 direct-amp)</td>
<td>DC2720</td>
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<td>PowerPlex® Fusion System</td>
<td>200 reactions</td>
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<td></td>
<td>800 reactions</td>
<td>DC2408</td>
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<tr>
<td>PowerPlex® Y23 System</td>
<td>50 reactions</td>
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<td>200 reactions</td>
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<td>PowerPlex® 21 System</td>
<td>200 reactions</td>
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<td>PowerPlex® 18D System</td>
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<td>800 reactions</td>
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<td>PowerPlex® CS7 System</td>
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<td>DC6613</td>
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<td>PowerPlex® 16 Monoplex System, Penta E (Fluorescein)</td>
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<td>DC6591</td>
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<td>PowerPlex® 16 Monoplex System, Penta D (JOE)</td>
<td>100 reactions</td>
<td>DC6651</td>
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<td>PowerPlex® ES Monoplex System, SE33 (JOE)</td>
<td>100 reactions</td>
<td>DC6751</td>
</tr>
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</table>

Not for Medical Diagnostic Use.

**Accessory Components**

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</tr>
</thead>
<tbody>
<tr>
<td>Plate, Barcoded, Semi-Skirted, 96-Well</td>
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<tr>
<td>Septa Mat, 96-Well</td>
<td>10 each</td>
<td>CE2696</td>
</tr>
<tr>
<td>PowerPlex® 5C Matrix Standards, 310</td>
<td>50µl (each dye)</td>
<td>DG5640</td>
</tr>
<tr>
<td>PowerPlex® 5C Matrix Standard</td>
<td>5 preps</td>
<td>DG4850</td>
</tr>
<tr>
<td>WEN Internal Lane Standard 500 ESS</td>
<td>200µl</td>
<td>DG5101</td>
</tr>
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</table>

Not for Medical Diagnostic Use.
Accessory Components (continued)

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<tr>
<th>Product</th>
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<tbody>
<tr>
<td>PunchSolution™ Kit</td>
<td>100 preps</td>
<td>DC9271</td>
</tr>
<tr>
<td>SwabSolution™ Kit</td>
<td>100 preps</td>
<td>DC8271</td>
</tr>
<tr>
<td>2800M Control DNA (10ng/µl)</td>
<td>25µl</td>
<td>DD7101</td>
</tr>
<tr>
<td>2800M Control DNA (0.25ng/µl)</td>
<td>500µl</td>
<td>DD7251</td>
</tr>
<tr>
<td>Water, Amplification Grade</td>
<td>6,250µl (5 × 1,250µl)</td>
<td>DW0991</td>
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<tr>
<td>5X AmpSolution™ Reagent</td>
<td>500µl</td>
<td>DM1231</td>
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</table>

Not for Medical Diagnostic Use.

Sample Preparation and Quantification Systems

<table>
<thead>
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<th>Size</th>
<th>Cat. #</th>
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<td>PowerQuant® System*</td>
<td>200 reactions</td>
<td>PQ5002</td>
</tr>
<tr>
<td></td>
<td>800 reactions</td>
<td>PQ5008</td>
</tr>
<tr>
<td>DNA IQ™ System</td>
<td>100 reactions</td>
<td>DC6701</td>
</tr>
<tr>
<td></td>
<td>400 reactions</td>
<td>DC6700</td>
</tr>
<tr>
<td>Differex™ System*</td>
<td>50 samples</td>
<td>DC6801</td>
</tr>
<tr>
<td></td>
<td>200 samples</td>
<td>DC6800</td>
</tr>
<tr>
<td>Tissue and Hair Extraction Kit (for use with DNA IQ™)</td>
<td>100 reactions</td>
<td>DC6740</td>
</tr>
<tr>
<td>Maxwell® FSC Instrument</td>
<td>1 each</td>
<td>AS4600</td>
</tr>
<tr>
<td>Maxwell® FSC DNA IQ™ Casework Kit</td>
<td>48 preps</td>
<td>AS1550</td>
</tr>
<tr>
<td>Casework Extraction Kit</td>
<td>100 preps</td>
<td>DC6745</td>
</tr>
<tr>
<td>Plexor® HY System*</td>
<td>200 reactions</td>
<td>DC1001</td>
</tr>
<tr>
<td></td>
<td>800 reactions</td>
<td>DC1000</td>
</tr>
<tr>
<td>Slicprep™ 96 Device</td>
<td>10 pack</td>
<td>V1391</td>
</tr>
</tbody>
</table>

*Not for Medical Diagnostic Use.

9.H. Summary of Changes

The following changes were made to the 4/17 revision of this document:

1. Added support for half-volume direct-amplification reactions and use of the Veriti® 96-Well Thermal Cycler.
2. Added caution against storing PCR amplification mix prior to thermal cycling.
3. Revised artifact table to include D8S1179 artifact information.
4. Revised document name to specify that this manual is for use on Applied Biosystems instruments.
5. Incorporated other general updates.


(c) U.S. Pat. No. 9,139,868 and other patents pending.

(d) 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, 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

(e) TMR-ET, CXR-ET and WEN dyes are proprietary.

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