

TECHNICAL MANUAL

PowerPlex[®] ESX 16 System

Instructions for use of Products
DC6710 and DC6711



PowerPlex® ESX 16 System



All technical literature is available on the Internet at: www.promega.com/protocols/
Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
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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® ESX 16 System^(a–d) 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 sixteen loci (fifteen STR loci and Amelogenin), including D18S51, D21S11, TH01, D3S1358, Amelogenin, D16S539, D2S1338, D1S1656, D10S1248, FGA, D8S1179, vWA, D22S1045, D19S433, D12S391 and D2S441.

The PowerPlex® ESX 16 System amplifies the loci recommended by the European Network of Forensic Science Institutes (ENFSI) and European DNA Profiling Group (EDNAP) as mini-STRs (<125bp; D2S441, D10S1248 and D22S1045) or midi-STRs (125–185bp; D1S1656 and D12S391). To complement this design and allow maximal recovery of allelic information from degraded samples, the PowerPlex® ESI 16 System, which amplifies the same sixteen loci present in the PowerPlex® ESX 16 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 new ENFSI/EDNAP loci are present as larger amplicons. 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® ESX 16 System is compatible with the ABI PRISM® 310, 3100 and 3100-Avant Genetic Analyzers and Applied Biosystems® 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. Amplification and detection instrumentation may vary.

You may need to optimize protocols including cycle number and injection conditions (or loading volume) for each laboratory instrument. In-house validation should be performed.

The PowerPlex® ESX 16 System provides all materials necessary to amplify STR regions of human genomic DNA, including hot-start *Taq* DNA polymerase, which is a component of the PowerPlex® ESX 5X Master Mix. This manual contains a protocol for use of the PowerPlex® ESX 16 System with the GeneAmp® PCR system 9700 and 2720 thermal cyclers in addition to protocols to separate amplified products and detect separated material (Figure 1). Protocols to operate the fluorescence-detection instruments should be obtained from the instrument manufacturer.

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

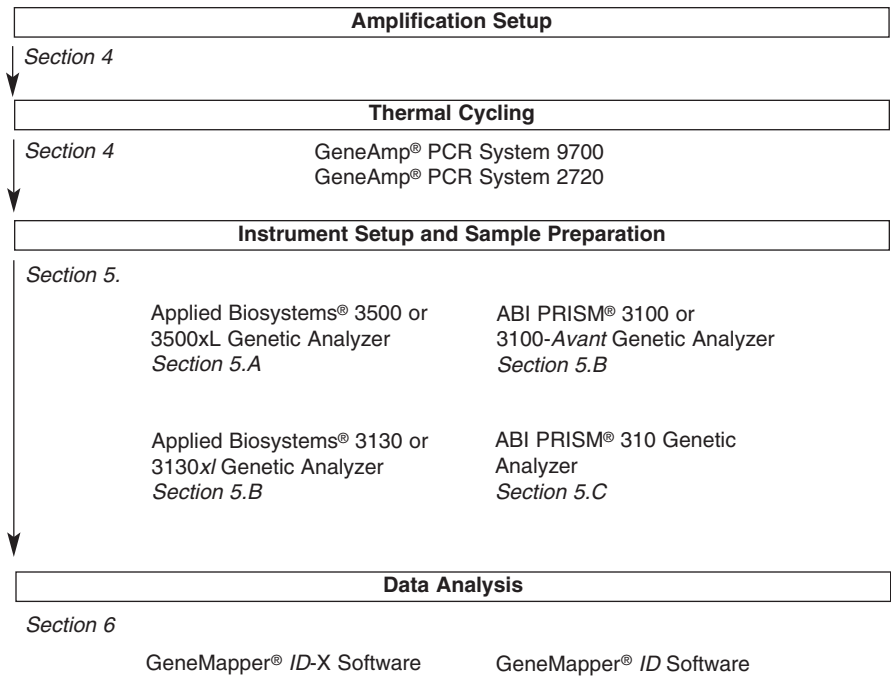


Figure 1. An overview of the PowerPlex® ESX 16 System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® ESX 16 System	100 reactions	DC6711

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

Pre-amplification Components Box (Blue Label)

500µl	PowerPlex® ESX 5X Master Mix
250µl	PowerPlex® ESX 16 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
5 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box (Beige Label)

50µl	PowerPlex® ESX 16 Allelic Ladder Mix
200µl	WEN Internal Lane Standard 500

Product	Size	Cat.#
PowerPlex® ESX 16 System	400 reactions	DC6710


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

Pre-amplification Components Box (Blue Label)

4 × 500µl	PowerPlex® ESX 5X Master Mix
4 × 250µl	PowerPlex® ESX 16 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
10 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box (Beige Label)

4 × 50µl	PowerPlex® ESX 16 Allelic Ladder Mix
2 × 200µl	WEN Internal Lane Standard 500

 The PowerPlex® ESX 16 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 500 (WEN ILS 500) at 2-10°C, protected from light; do not refreeze. The PowerPlex® ESX 16 Primer Pair Mix, PowerPlex® ESX 16 Allelic Ladder Mix and WEN ILS 500 are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Optional: The PowerPlex® ESX 16 System components may be stored for up to 1 year at 2-10°C without loss of activity.

Available Separately

The proper panels, bins and stutter text files for use with GeneMapper® ID and ID-X software are available for download at: 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. The 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, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and 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 sample DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 16 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® ESX 16 Allelic Ladder Mix and WEN Internal Lane Standard 500). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

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



3.B. Matrix Standardization or Spectral Calibration

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

The PowerPlex® 5C Matrix Standards, 310 (Cat.# DG5640), is required for matrix standardization on 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, 3130*xl*, 3500 and 3500*xL* 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 standardization, see the *PowerPlex® 5C Matrix Standards, 310, Technical Manual #TMD050*. For protocols and additional information about spectral calibration, see the *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® ESX 16 System

The PowerPlex® ESX 16 System is optimized for the GeneAmp® PCR System 9700 thermal cycler. An amplification protocol for the GeneAmp® PCR Systems 2720 thermal cycler also is provided for extracted DNA.

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

 Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 7.A.

4.A. Amplification of Extracted DNA

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 or 2720 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 16 10X Primer Pair Mix and Water, Amplification Grade, 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 MicroAmp® plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.
4. Add the final volume of each reagent listed in Table 1 into a sterile tube.

Table 1. PCR Amplification Mix for the PowerPlex® ESX 16 System.

PCR Amplification Mix Component ¹	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
PowerPlex® ESX 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 16 10X Primer Pair Mix	2.5µl	×		=	
template DNA (0.5ng) ^{2,3}	up to 17.5µl				
total reaction volume	25µl				

¹Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESX 5X Master Mix and PowerPlex® ESX 16 10X Primer Pair Mix. The template DNA will be added at Step 6.

²Store DNA templates in nuclease-free water or TE⁻⁴ buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA). 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 quantification method used (13). 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.

4.A. Amplification of Extracted DNA (continued)

5. Vortex the PCR amplification mix for 5–10 seconds, then pipet PCR amplification mix into each reaction well.
- ❗ Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.
6. Pipet the template DNA (0.5ng) for each sample into the respective well containing PCR amplification mix.
7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 0.5ng in the desired template DNA volume. Pipet 0.5ng of diluted DNA into a reaction well containing PCR amplification mix.
8. For the negative amplification control, pipet Water, Amplification Grade, or TE⁻⁴ buffer instead of template DNA into a reaction well containing PCR amplification mix.
9. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom and remove any air bubbles.

Thermal Cycling

This section contains a protocol for use of the PowerPlex® ESX 16 System with the GeneAmp® PCR System 9700 and 2720 thermal cyclers.

Amplification and detection instrumentation may vary. You may need to optimize protocols including cycle number and injection conditions for each laboratory instrument. 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 MicroAmp® plate or reaction tubes in the thermal cycler.
2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 and 2720 thermal cyclers is provided below.

Thermal Cycling Protocol ¹
96°C for 2 minutes, then:
94°C for 30 seconds
59°C for 2 minutes
72°C for 90 seconds
for 30 cycles, then:
60°C for 45 minutes
4°C soak

¹When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select “9600” for the ramp speed, and enter the reaction volume.

3. After completion of the thermal cycling protocol, proceed with fragment analysis or store 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

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips
- 5X AmpSolution™ Reagent (Cat.# DM1231)
- PunchSolution™ Kit (DC9271)
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat

This section contains a protocol for direct amplification of DNA from storage card punches using the PowerPlex® ESX 16 System and GeneAmp® PCR System 9700 thermal cycler.

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

Note: You will need to optimize and validate the number of storage card punches per reaction in your laboratory.

FTA®-based sample types include:

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

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

NonFTA sample types include: (one punch per 25µl amplification reaction)

- Buccal samples on Bode Buccal DNA Collector™ devices
- Blood and buccal samples on nonFTA card punches (e.g., S&S 903)

Pretreat these 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.

Sample Preparation

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 into a well. Adding PCR amplification mix to the well before adding the punch may help alleviate static problems.

Amplification Setup

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 16 10X Primer Pair Mix and Water, Amplification Grade, completely.

Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the PowerPlex® 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 MicroAmp® plate for reaction assembly, and label appropriately.

4. Add the final volume of each reagent listed in Table 2 into a sterile tube.

Table 2. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches.

PCR Amplification Mix Component ¹	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	12.5µl	×		=	
PowerPlex® ESX 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
total reaction volume	25µl				

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

5. 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: In this protocol, the PCR amplification mix is added to each well first, and the storage card punch is added in Step 6. It is also acceptable to add the storage card punch first, then add the PCR amplification mix.

6. Add one or two 1.2mm punches from a storage card containing a buccal sample or one 1.2mm punch from a storage card containing whole blood into the appropriate wells of the reaction plate.
7. 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 based 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.
8. 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.

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

9. Seal the plate, and briefly centrifuge the plate to bring storage card punches to the bottom of the wells and remove any air bubbles.

Note: Place the amplification plate in the thermal cycler, and start the thermal cycling program as soon as the PowerPlex® System PCR amplification mix is added to all wells. Prolonged storage of assembled reactions prior to cycling may result in poor performance (i.e., lower peak heights for large amplicons).

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including cycle number and injection conditions for each laboratory instrument. Testing at Promega shows that 24 cycles works well for a variety of sample types. Buccal samples may require more amplification cycles than blood samples. Cycle number will need to be optimized in each laboratory for each sample type that is amplified.

1. Place the MicroAmp® plate in the thermal cycler.
2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below.

Thermal Cycling Protocol¹

96°C for 2 minutes, then:

94°C for 30 seconds

59°C for 2 minutes

72°C for 90 seconds

for 24 cycles, then:

60°C for 45 minutes

4°C soak

¹When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "9600" for the ramp speed, and enter the reaction volume.

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

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

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- aerosol-resistant pipette tips
- SwabSolution™ Kit (Cat.# DC8271)

This section contains a protocol for amplifying swab extracts using the PowerPlex® ESX 16 System and GeneAmp® PCR System 9700 thermal cycler.

Sample Preparation

Pretreat cotton or OmniSwabs™ (GE Healthcare) swabs with the SwabSolution™ Kit (Cat.# DC8271) as described in the *SwabSolution™ Kit Technical Manual* TMD037 to generate a swab extract. Be sure to include a blank swab as a negative control when processing samples.



4.C. Direct Amplification of DNA from Swabs (continued)


Amplification Setup

1. Thaw the PowerPlex® ESX 5X Master Mix, PowerPlex® ESX 16 10X Primer Pair Mix and Water, Amplification Grade, 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 MicroAmp® plate for reaction assembly, and label appropriately.
4. Add the final volume of each reagent listed in Table 3 into a sterile tube.

Table 3. PCR Amplification Mix for Direct Amplification of DNA From Swabs.

PCR Amplification Mix Component ¹	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	10.5µl				
PowerPlex® ESX Master Mix	5.0µl				
PowerPlex® ESX 16 10X Primer Pair Mix	2.5µl				
5X AmpSolution™ Reagent	5.0µl				
swab extract	2.0µl				
total reaction volume	25µl				

¹Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESX 5X Master Mix and PowerPlex® ESX 16 10X Primer Pair Mix. The swab extract will be added at Step 6.

5. 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.
6. Pipet 2.0µl of swab extract for each sample into the appropriate well of the reaction plate.

7. For the positive amplification control, dilute 2800M Control DNA to 2.5ng/μl, and add 2μl to a reaction well containing 23μl of PCR amplification mix.
Note: Optimization of the amount of 2800M Control DNA may be required, based on thermal cycling conditions and laboratory preferences.
8. For the negative amplification control, pipet Water, Amplification Grade, instead of swab extract into a reaction well containing PCR amplification mix.
Note: As an additional negative control, assemble a reaction containing the swab extract prepared from a blank swab.
9. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including cycle number and injection conditions for each laboratory instrument. 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.

1. Place the MicroAmp® plate in the thermal cycler.
2. Select and run the recommended protocol. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below.
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.

Thermal Cycling Protocol¹

96°C for 2 minutes, then:

94°C for 30 seconds

59°C for 2 minutes

72°C for 90 seconds

for 26 cycles, then:

60°C for 45 minutes

4°C soak

¹When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with 9600 as the ramp speed. The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "9600" for the ramp speed, and enter the reaction volume.

4.C. Direct Amplification of DNA from Swabs (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. 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 and 3500xL Genetic Analyzers

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- 96-well retainer & 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
- conditioning reagent pouch for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- MicroAmp® optical 96-well plate (or equivalent) and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

- ❗ The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.
- ❗ Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

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

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® ESX 16 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 sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time in the run module, select “Instrument Protocol” from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.
2. Use a volume of allelic ladder that results in peak heights that are all consistently above the peak amplitude threshold determined as part of your 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 or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

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

Instrument Preparation

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

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

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

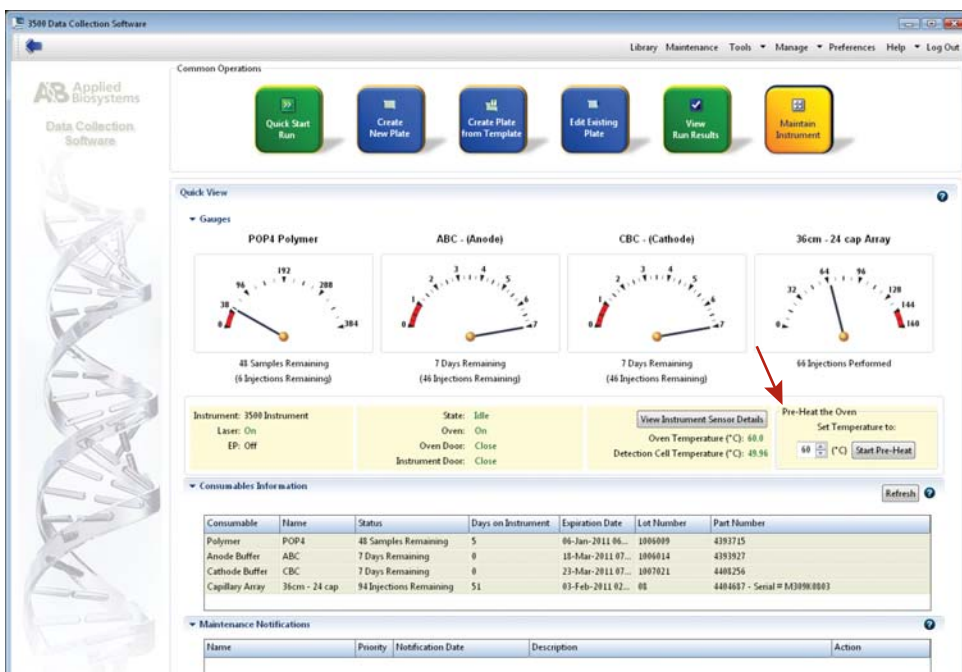


Figure 2. The Dashboard.

2. Prior to the first analysis using the PowerPlex® ESX 16 System, you must create an Instrument Protocol, Size Standard, QC Protocol, Assay, File Name Convention and Results Group.
 - 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 an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 5-dye spectral calibration. We recommend using a run time of 1,210 seconds and the default injection conditions.

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

Assign a descriptive protocol name.

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

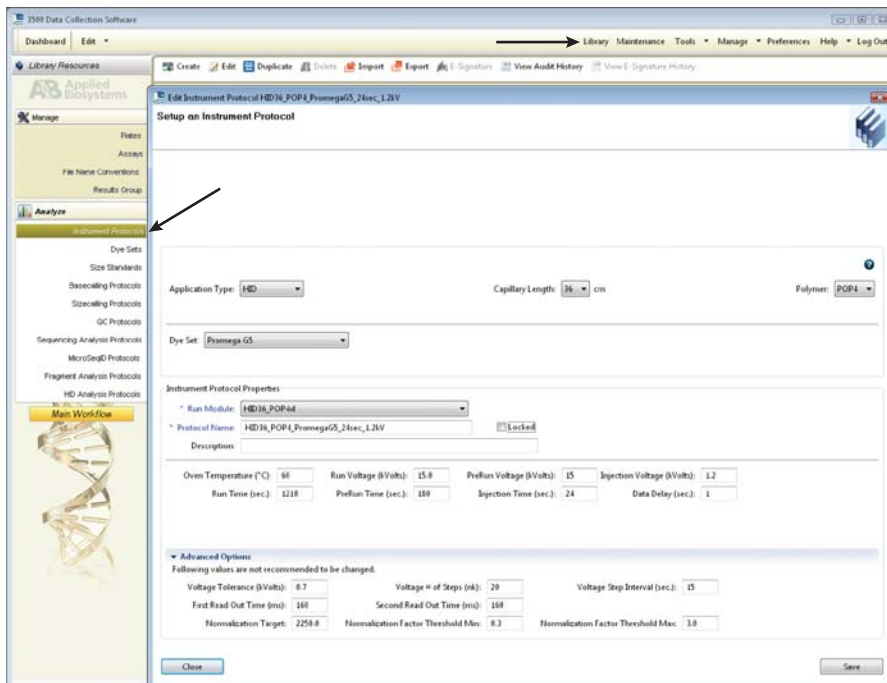


Figure 3. The Create New Instrument Protocol window.

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

- 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.

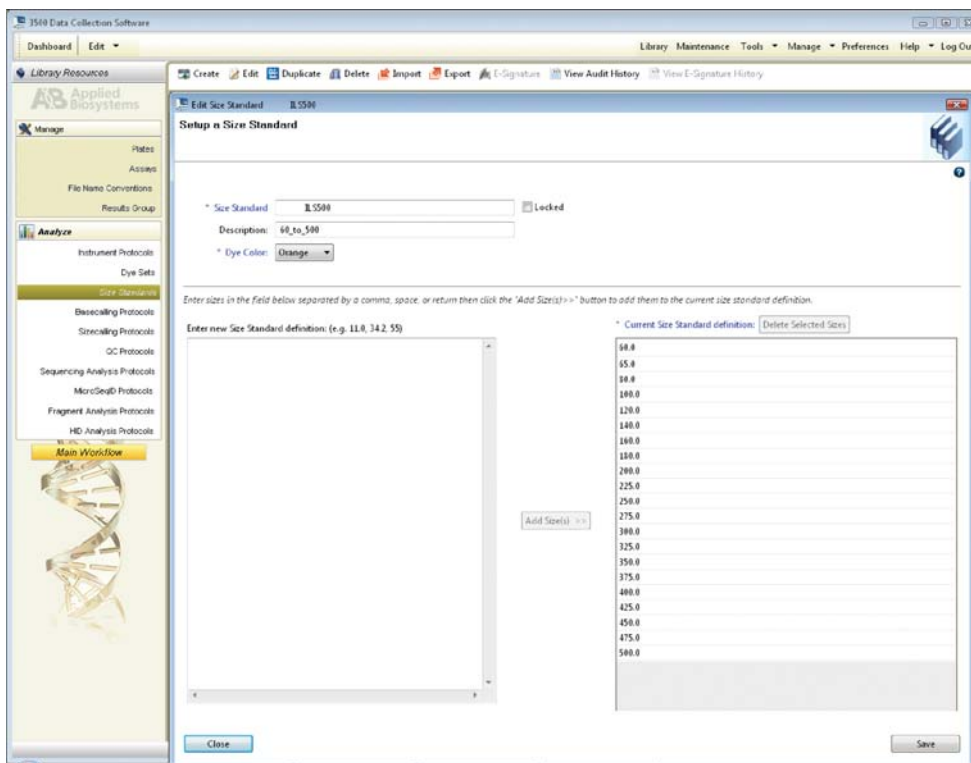


Figure 4. The Create New Size Standard window.

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



- 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® ESX 16 System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 5 shows one option for these settings.

Create New QC Protocol

Setup a QC Protocol

* Protocol Name: JLS500

Description:

Size Standard: WENILS500

Sizecaller: SizeCaller v1.1.0

Analysis Settings: QC Settings

Analysis Range: Full

Sizing Range: Full

Size Calling Method: Local Southern

Analysis Start Point: 0

Sizing Start Size: 0

Analysis Stop Point: 1000000

Sizing Stop Size: 100000

Color	Peak Amplitude Threshold
Blue	175
Green	175
Yellow	175
Red	175
Purple	175
Orange	175

Common Settings

Use Smoothing: Light

Use Baseline (Baseline Window (Pts)): 51

Minimum Peak Half Width: 2

Peak Window Size: 15

Polynomial Degree: 3

Slope Threshold Peak Start: 0.0

Slope Threshold Peak End: 0.0

Close Save

Figure 5. The Create New QC Protocol window.

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

- 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.

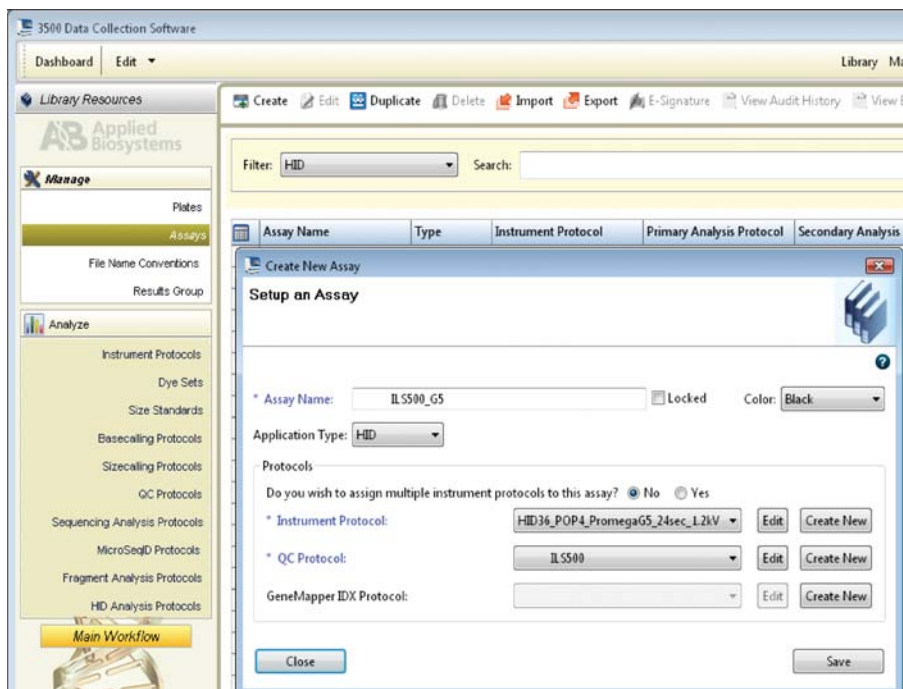


Figure 6. The Create New Assay window.

- 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 your laboratory practices, and save with a descriptive name.

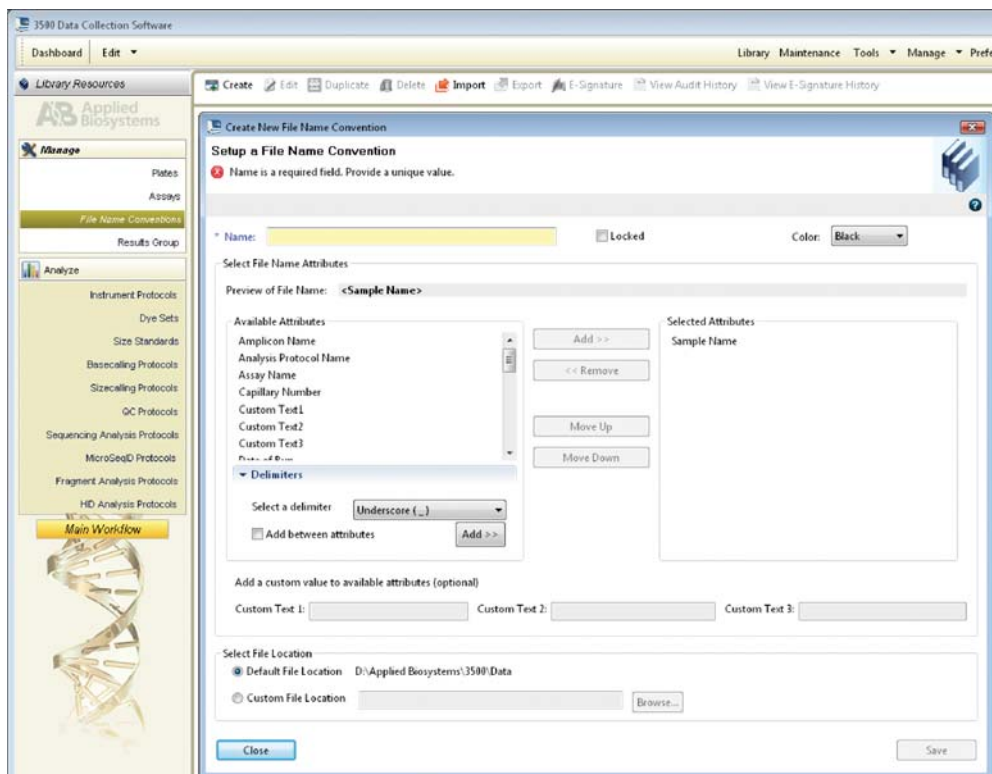


Figure 7. The Create New File Name Convention window.

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

- 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 your laboratory practices. Save with a descriptive name.

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

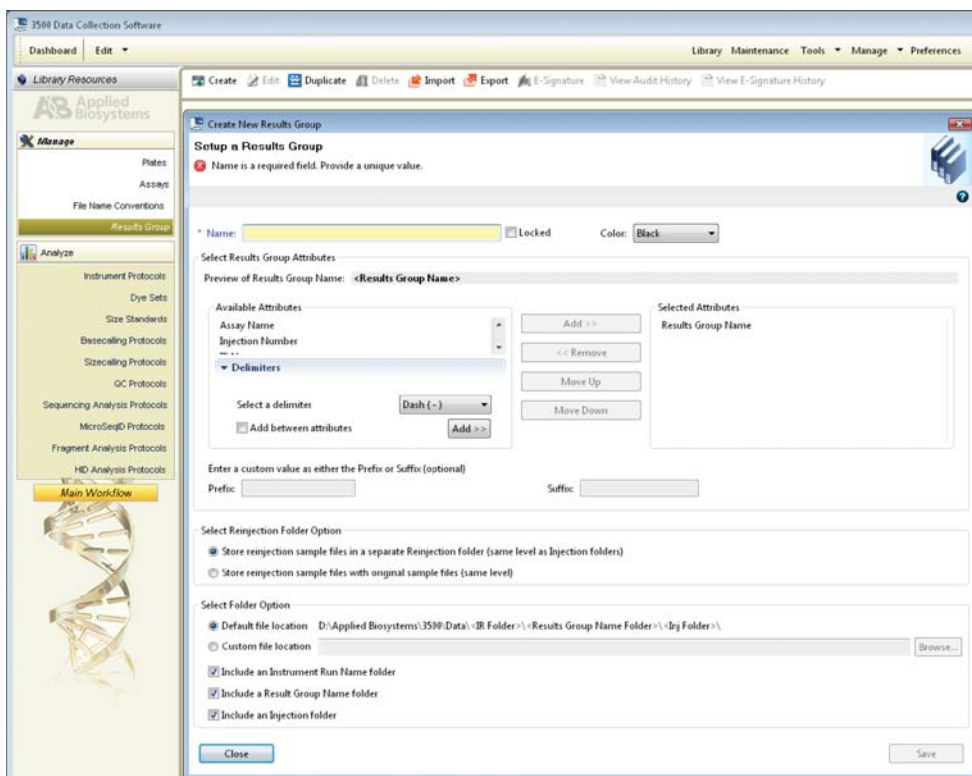


Figure 8. The Create New Results Group window.

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

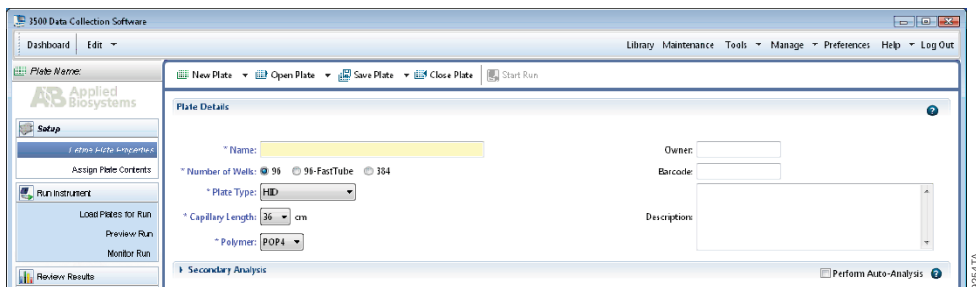


Figure 9. Defining plate properties.

- Select “Assign Plate Contents” (Figure 10).
- Assign sample names to wells.
- 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.

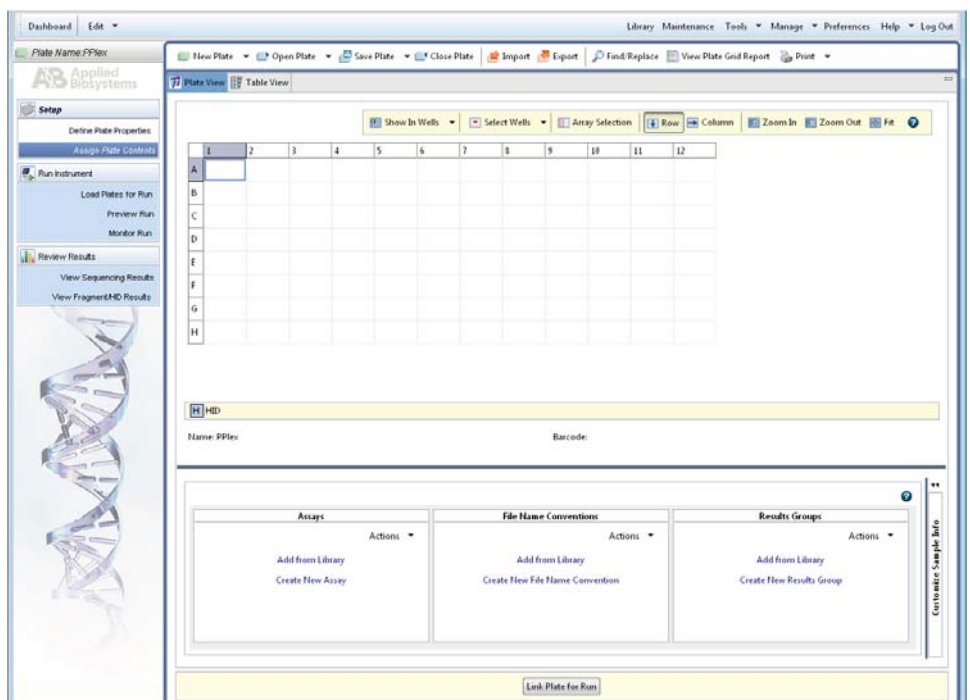


Figure 10. Assigning plate contents.

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

8. Under “File Name Conventions”, 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 that pertain to those samples.
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).

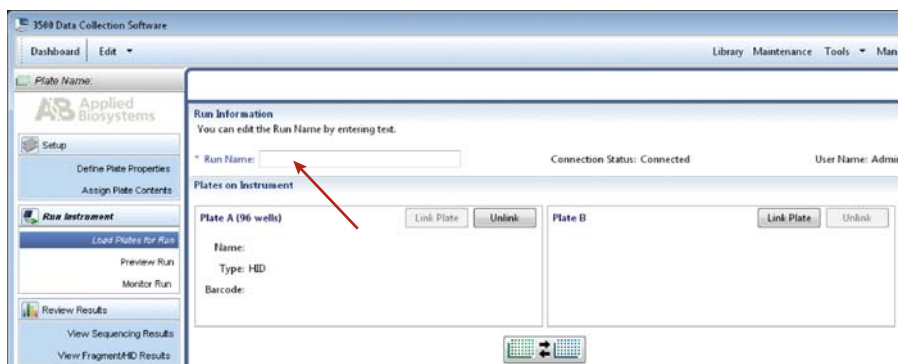


Figure 11. Assigning a run name.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and 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 or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4®) for the Applied Biosystems 3100 or 3130 Genetic Analyzer
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Sample Preparation

1. Prepare a loading cocktail by combining and mixing WEN Internal Lane Standard 500 and Hi-Di™ formamide as follows:

$$[(0.5\mu\text{l WEN ILS 500}) \times (\# \text{ samples})] + [(9.5\mu\text{l Hi-Di}^{\text{TM}} \text{ formamide}) \times (\# \text{ samples})]$$

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.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 or 4.0 (continued)

4. Add 1µl of amplified sample (or 1µl of PowerPlex® ESX 16 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 sample 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). 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 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 3130*xl* 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

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- 310 capillaries, 47cm × 50µm
- performance optimized polymer 4 (POP-4®)
- 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 Hi-Di™ formamide. Freeze formamide in aliquots at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.
- !** Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Prepare a loading cocktail by combining the WEN Internal Lane Standard 500 and Hi-Di™ formamide as follows:

$$[(0.5\mu\text{l WEN ILS 500}) \times (\# \text{ samples})] + [(24.5\mu\text{l Hi-Di}^{\text{TM}} \text{ formamide}) \times (\# \text{ samples})]$$

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® ESX 16 Allelic Ladder Mix).

Notes:

1. Instrument detection limits vary; therefore, injection time, injection voltage or the amount of sample 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). 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.

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 or in an ice-water bath for 3 minutes. Denature samples just prior to loading.
6. Place the 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. Prepare a GeneScan® sample sheet as described in the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Enter the appropriate sample information in the Sample Info column.
3. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the drop-down menu.
4. Select the "GS STR POP4 (1ml) G5" Module using the drop-down menu. Change the injection time to 3 seconds and the run time to 28 minutes. Keep the settings for the remaining parameters as shown below:

Inj. Secs:	3
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time:	28



You may need to optimize the injection time for individual instruments. Injection times of 2–5 seconds are suggested for samples 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.

5. Select the appropriate matrix file.

5.C. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer (continued)

6. To analyze data automatically, select the auto analyze checkbox and the appropriate analysis parameters and size standard. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for specific information about these options.
7. After loading the sample tray and closing the doors, select "Run" to start the capillary electrophoresis system.
8. Monitor electrophoresis by observing the raw data and status windows. Each sample will take approximately 35 minutes for syringe pumping, sample injection and sample electrophoresis.

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.

6.A. PowerPlex® ESX Panels, Bins and Stutter Text Files with GeneMapper® ID-X Software, Version 1.2

To facilitate analysis of data generated with the PowerPlex® ESX 16 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® ESX 16 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_ESX_Panels_IDX_vX.x.txt, PowerPlex_ESX_Bins_IDX_vX.x.txt and PowerPlex_ESX_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 panel file downloaded in the Getting Started section. Select the file, then “Import”.
6. In the navigation pane, highlight the PowerPlex ESX panels folder that you just imported in Step 5.
7. Select “File”, then “Import Bin Set”.
8. Navigate to the bins file downloaded in the Getting Started section. Select the file, then “Import”.
9. In the navigation pane, highlight the PowerPlex ESX 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 file downloaded in the Getting Started section. 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.

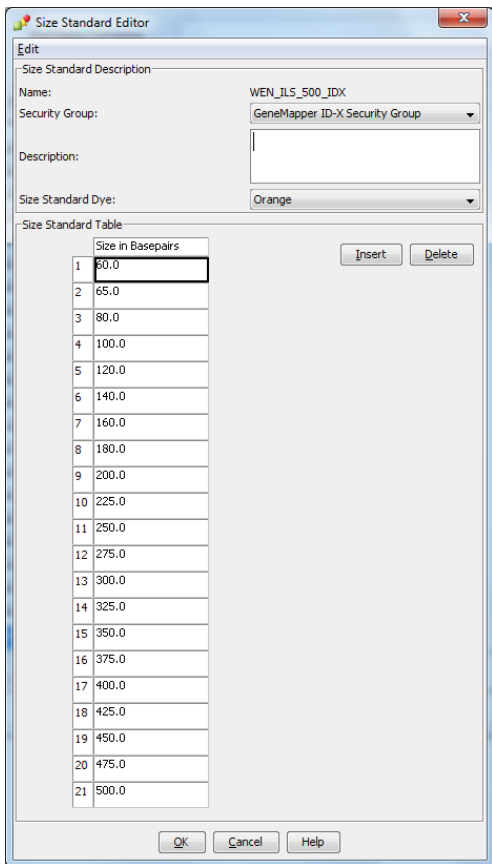
6.B. Creating a Size Standard with GeneMapper® ID-X Software, Version 1.2

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

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 12), select “GeneMapper ID-X Security Group” as the Security Group. This allows access to all users of the software. Other security groups may be used.
5. Enter a detailed name, such as “WEN_ILS_500_IDX”.

6.B. Creating a Size Standard with GeneMapper® ID-X Software, Version 1.2 (continued)

6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 25.
8. Select "OK".



Size Standard Editor

Edit

Size Standard Description

Name: WEN_ILS_500_IDX

Security Group: GeneMapper ID-X Security Group

Description:

Size Standard Dye: Orange

Size Standard Table

	Size in Basepairs
1	60.0
2	65.0
3	80.0
4	100.0
5	120.0
6	140.0
7	160.0
8	180.0
9	200.0
10	225.0
11	250.0
12	275.0
13	300.0
14	325.0
15	350.0
16	375.0
17	400.0
18	425.0
19	450.0
20	475.0
21	500.0

Insert Delete

OK Cancel Help

Figure 12. The GeneMapper® ID-X Size Standard Editor

6.C. Importing the WEN ILS 500 IDX Size Standard into GeneMapper® ID-X Software, Version 1.2

The WEN_ILS_500_IDX.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 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.

6.D. Creating a Casework Analysis Method with GeneMapper® ID-X Software, Version 1.2

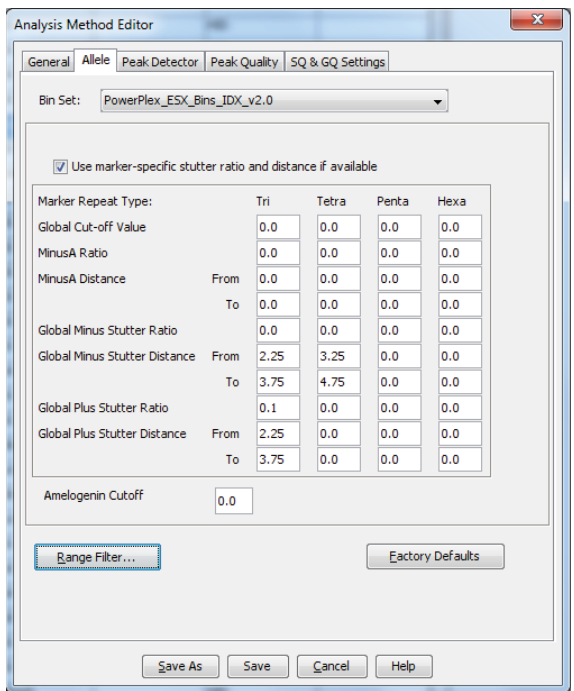
These instructions are intended as a guide to start analyzing data in GeneMapper® ID-X software. They are not intended as a comprehensive guide for using GeneMapper® ID-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select “Tools”, then “GeneMapper ID-X Manager”.
2. Select the Analysis Methods tab.
3. Select “New”, and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select “GeneMapper ID-X Security Group” as the Security Group. This allows access to all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as “PowerPlexESX 16”.
6. Select the Allele tab (Figure 13).
7. Select the bins file that was imported in Section 6.A.
8. Ensure that the “Use marker-specific stutter ratio and distance if available” box is checked.



6.D. Creating a Casework Analysis Method with GeneMapper® ID-X Software, Version 1.2 (continued)

9. We recommend the values shown in Figure 13 for proper filtering of stutter peaks when using the PowerPlex® ESX 16 System. You may need to optimize these settings. In-house validation should be performed.



Marker Repeat Type:	Tri	Tetra	Penta	Hexa
Global Cut-off Value	0.0	0.0	0.0	0.0
MinusA Ratio	0.0	0.0	0.0	0.0
MinusA Distance	From 0.0 To 0.0	From 0.0 To 0.0	From 0.0 To 0.0	From 0.0 To 0.0
Global Minus Stutter Ratio	0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From 2.25 To 3.75	From 3.25 To 4.75	From 0.0 To 0.0	From 0.0 To 0.0
Global Plus Stutter Ratio	0.1	0.0	0.0	0.0
Global Plus Stutter Distance	From 2.25 To 3.75	From 0.0 To 0.0	From 0.0 To 0.0	From 0.0 To 0.0
Amelogenin Cutoff	0.0			

Buttons: Range Filter..., Factory Defaults, Save As, Save, Cancel, Help

Figure 13. The GeneMapper® ID-X Allele tab.

10. Select the Peak Detector tab (Figure 14). 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.

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.

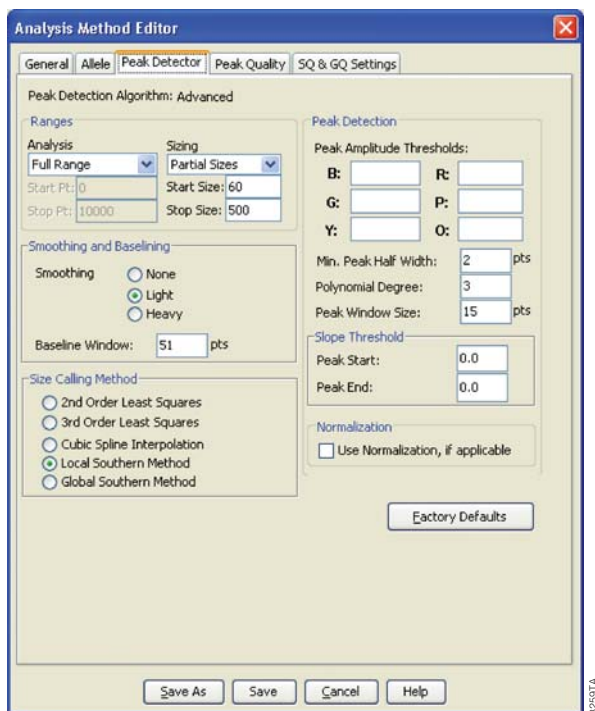


Figure 14. The GeneMapper® ID-X Peak Detector tab.

6.D. Creating a Casework Analysis Method with GeneMapper® ID-X Software, Version 1.2 (continued)

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 the 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 file that was imported in Section 6.A.
7. In the Size Standard column, select the size standard that was created in Section 6.B or imported 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.
10. If all analysis requirements are met, the Save Project window will open (Figure 15).
11. Enter the project name.
12. Choose the applicable security group from the drop-down menu, then select "OK".

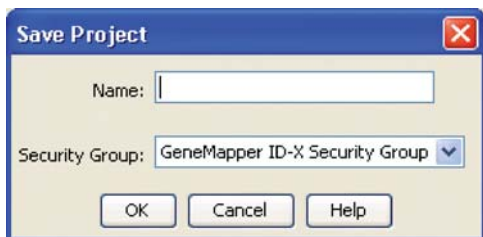


Figure 15. The Save Project window.

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.

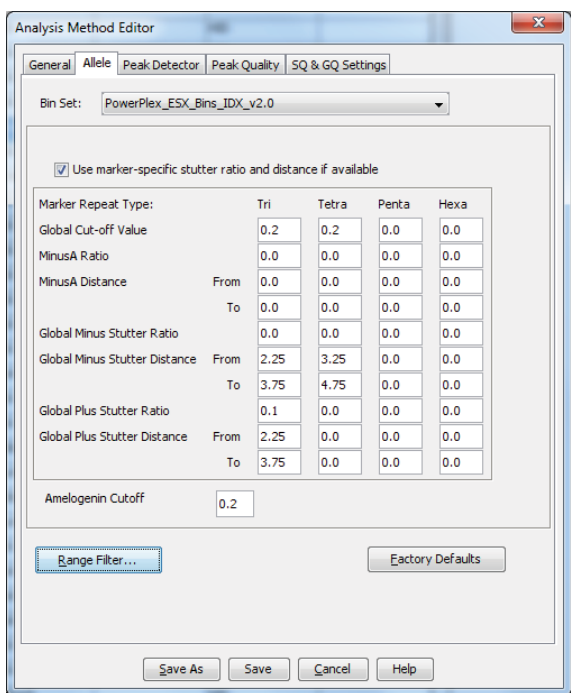
6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® ID-X Software, Version 1.2

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.
3. Select "New", and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select "GeneMapper ID-X Security Group" as the Security Group. This allows access to all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlexESX 16 20% Filter".
6. Select the Allele tab (Figure 16).
7. Select the bins file that was imported in Section 6.A.

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

8. We recommend the values shown in Figure 16 for proper filtering of stutter peaks when using the PowerPlex® ESX 16 System. You may need to optimize these settings. In-house validation should be performed.



Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.2	0.2	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	2.25	3.25	0.0	0.0
	To	3.75	4.75	0.0	0.0
Global Plus Stutter Ratio		0.1	0.0	0.0	0.0
Global Plus Stutter Distance	From	2.25	0.0	0.0	0.0
	To	3.75	0.0	0.0	0.0

Amelogenin Cutoff: 0.2

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

9. Select the Peak Detector tab (Figure 14). 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.

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 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 file that was imported in Section 6.A.
6. In the Size Standard column, select the size standard that was created in Section 6.B or imported 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 15).

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

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. PowerPlex® ESX Panels and Bins Text Files with GeneMapper® ID Software, Version 3.2

To facilitate analysis of data generated with the PowerPlex® ESX 16 System, we have created panels and bins 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.

For analysis using GeneMapper® ID software, version 3.2, you will need the proper panels and bins files: PowerPlex_ESX_Panels_vX.x.txt and PowerPlex_ESX_Bins_vX.x.txt files, where “X.x” refers to the most recent version of the panels and bins files.

Note: Run files generated using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer cannot be analyzed using GeneMapper® ID Software, version 3.2. You must analyze these files with GeneMapper® ID-X software, version 1.0 or higher.

Getting Started

1. To obtain the panels and bins files for the PowerPlex® ESX 16 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_ESX_Panels_vX.x.txt and PowerPlex_ESX_Bins_vX.x.txt files to a known location on your computer.
4. Save the WEN_ILS_500.xml file to a known location on your computer.

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 file downloaded in the Getting Started section above. Select the file, then “Import”.
6. In the navigation pane, highlight the PowerPlex ESX panels folder that you just imported in Step 5.
7. Select “File”, then “Import Bin Set”.
8. Navigate to the bins 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.

6.G. Creating a Size Standard with 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.

1. Select “Tools”, then “GeneMapper Manager”.
2. Select the Size Standard tab.
3. Select “New”.
4. Select “Basic or Advanced” (Figure 17). The type of analysis method selected must match the type of analysis method created earlier. Select “OK”.

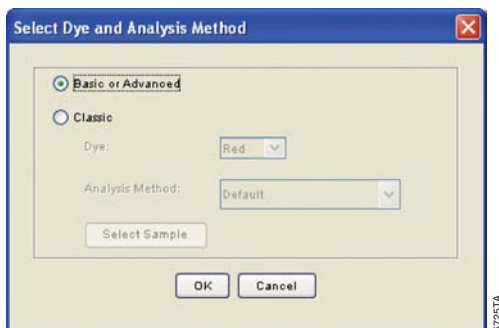


Figure 17. The Select Dye and Analysis Method window.

6.G. Creating a Size Standard with GeneMapper® ID Software, Version 3.2 (continued)

5. Enter a detailed name, such as “WEN_ILS_500”, in the Size Standard Editor (Figure 18).
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 25.
8. Select “OK”.

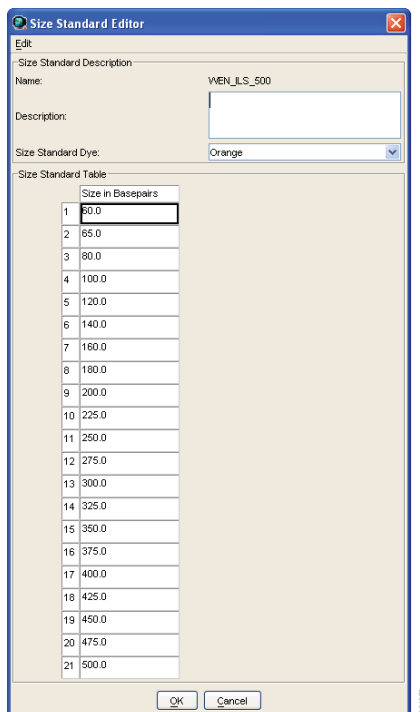


Figure 18. The Size Standard Editor.

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

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 on your computer.
5. Highlight the file, then select “Import”.
6. Select “Done” to save changes and exit the GeneMapper® Manager.

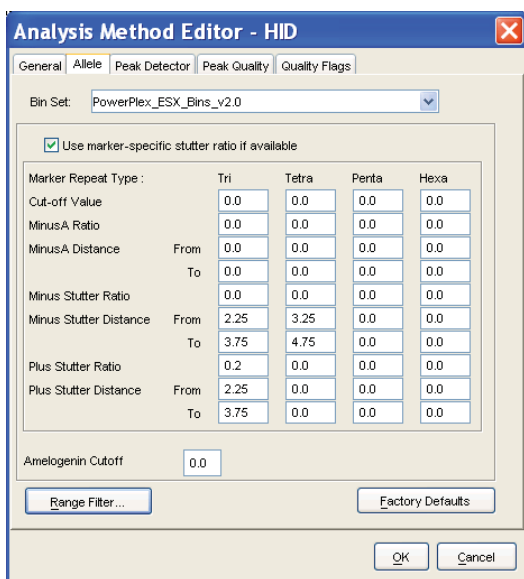
6.I. Creating a Casework Analysis Method with GeneMapper® ID Software, Version 3.2

These instructions are intended as a guide to start analyzing data in GeneMapper® ID software. They are not intended as a comprehensive guide for using GeneMapper® ID software. We recommend that users contact Applied Biosystems for training on the software. 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.
3. Select “New”, and a new analysis method dialog box will open.
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 “PowerPlexESX 16”.
6. Select the Allele tab (Figure 19).
7. Select the bins text file that was imported in Section 6.F.



Marker Repeat Type :		Tri	Tetra	Penta	Hexa
Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Minus Stutter Ratio		0.0	0.0	0.0	0.0
Minus Stutter Distance	From	2.25	3.25	0.0	0.0
	To	3.75	4.75	0.0	0.0
Plus Stutter Ratio		0.2	0.0	0.0	0.0
Plus Stutter Distance	From	2.25	0.0	0.0	0.0
	To	3.75	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Buttons: Range Filter..., Factory Defaults, OK, Cancel

Figure 19. The GeneMapper® ID Allele tab.

6.I. Creating a Casework Analysis Method with GeneMapper® ID Software, Version 3.2 (continued)

8. Ensure that the “Use marker-specific stutter ratio if available” box is checked.
9. Enter the values shown in Figure 19 for proper filtering of stutter peaks when using the PowerPlex® ESX 16 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 may need to optimize these settings. In-house validation should be performed.

10. Select the Peak Detector tab (Figure 20). 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.

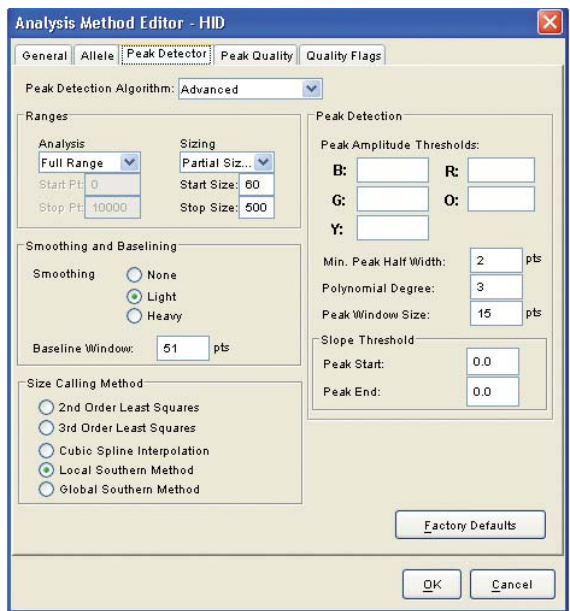


Figure 20. The GeneMapper® ID Peak Detector tab.

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

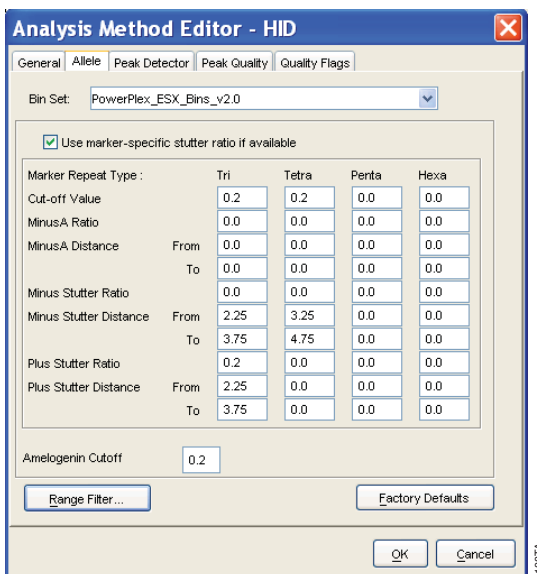
1. Select "File", then "New Project".
2. Select "Edit", then "Add Samples to Project".
3. Browse to location of the run files. Highlight desired files, then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "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 file that was imported in Section 6.F.
7. In the Size Standard column, select the size standard that was created in Section 6.G or imported 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.
3. Select "New", and a new analysis method dialog box will open.
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 "PowerPlexESX 16_20%filter".
6. Select the Allele tab (Figure 21).
7. Select the bins file that was imported in Section 6.F.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.

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

9. Enter the values shown in Figure 21 for proper filtering of peaks when using the PowerPlex® ESX 16 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*”.



Analysis Method Editor - HID

General | **Allele** | Peak Detector | Peak Quality | Quality Flags

Bin Set: PowerPlex_ESX_Bins_v2.0

☒ Use marker-specific stutter ratio if available

Marker Repeat Type :	Tri	Tetra	Penta	Hexa
Cut-off Value	0.2	0.2	0.0	0.0
MinusA Ratio	0.0	0.0	0.0	0.0
MinusA Distance	From 0.0	From 0.0	From 0.0	From 0.0
	To 0.0	To 0.0	To 0.0	To 0.0
Minus Stutter Ratio	0.0	0.0	0.0	0.0
Minus Stutter Distance	From 2.25	From 3.25	From 0.0	From 0.0
	To 3.75	To 4.75	To 0.0	To 0.0
Plus Stutter Ratio	0.2	0.0	0.0	0.0
Plus Stutter Distance	From 2.25	From 0.0	From 0.0	From 0.0
	To 3.75	To 0.0	To 0.0	To 0.0

Amelogenin Cutoff: 0.2

Range Filter... Factory Defaults

OK Cancel

Figure 21. The GeneMapper® ID Allele tab with settings for using a 20% peak filter.

10. Select the Peak Detector Tab (Figure 20).

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.
 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 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 file that was imported in Section 6.F.
7. In the Size Standard column, select the size standard that was created in Section 6.G or imported 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 7 (Section 9.A).

6.L. Results

Representative results of the PowerPlex® ESX 16 System are shown in Figure 23. The PowerPlex® ESX 16 Allelic Ladder Mix is shown in Figure 24.

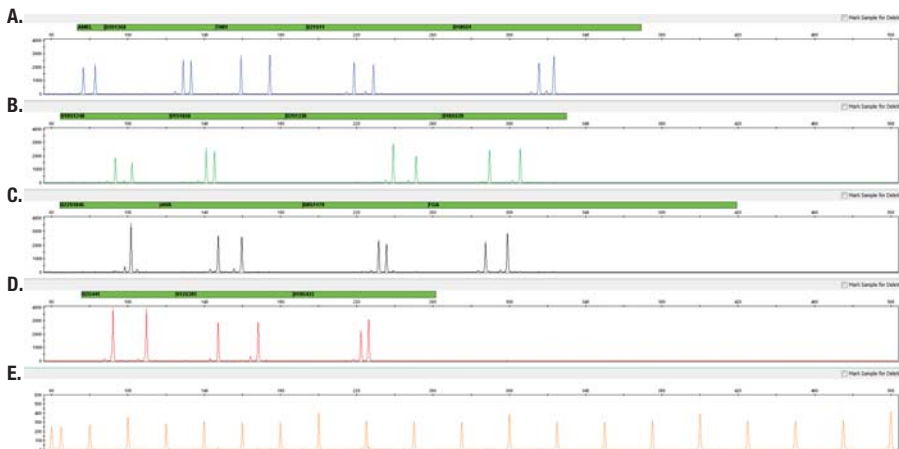


Figure 23. The PowerPlex® ESX 16 System. The 2800M Control DNA (0.5ng) was amplified using the PowerPlex® ESX 16 System. Amplification products were mixed with WEN Internal Lane Standard 500 and analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. Results were analyzed using GeneMapper® ID-X software, version 1.4. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, TH01, D21S11 and D18S51. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D10S1248, D1S1656, D2S1338 and D16S539. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loci: D22S1045, vWA, D8S1179 and FGA. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D2S441, D12S391 and D19S433. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the WEN Internal Lane Standard 500.

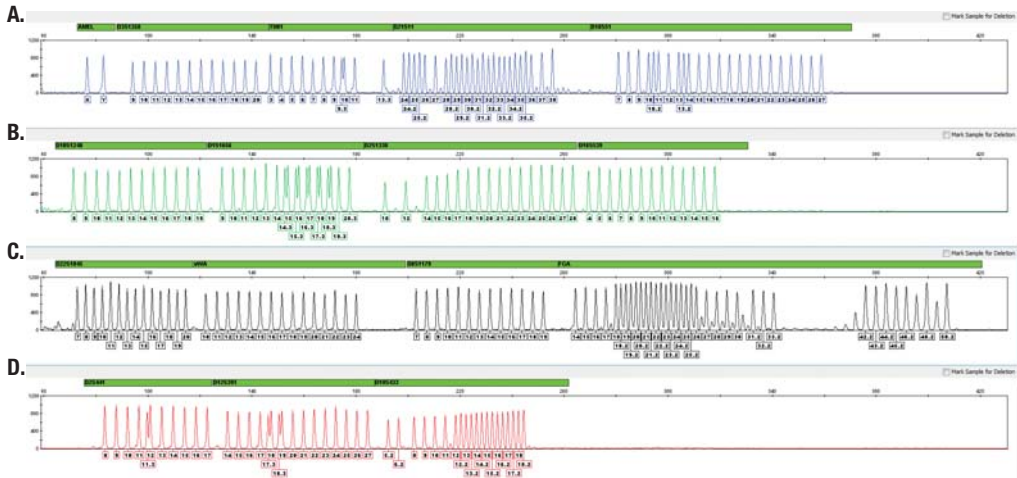


Figure 24. The PowerPlex® ESX 16 Allelic Ladder Mix. The PowerPlex® ESX 16 Allelic Ladder Mix was analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. The sample file was analyzed with the GeneMapper® ID-X software, version 1.4, and PowerPlex® ESX panels and bins text files. **Panel A.** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-ET-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter peaks are a common amplification artifact associated with STR analysis (14,15). Stutter products often are observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. 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 highest stutter observed at each locus is used in the PowerPlex® ESX panels text files for locus-specific filtering in the GeneMapper® ID software, version 3.2, and GeneMapper® ID-X software.

In addition to stutter peaks, the following low-level artifact peaks may be observed with the PowerPlex® ESX 16 System loci.

Table 4. Artifact Peaks Observed with the PowerPlex® ESX 16 System.

Dye	Artifact
Fluorescein	Amelogenin n-1 ¹ 57-65 bases 65-67 bases ^{2, 3} 70 bases ^{3,4} 71-73 bases ^{2, 3} (male samples only) 81 bases ^{3,4} (male samples only) D21S11 n-2; n+2 ⁵
JOE	D1S1656 n-2; n+2 ⁵
CXR	D2S441 n-2; n+2 ⁵

¹The n-1 artifact is more noticeable with high template amounts and allele peak heights.

²These artifacts are observed on ABI PRISM® 310 Analyzer only.

³These 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.

⁴These artifacts are observed on Applied Biosystems® 3130 Genetic Analyzers only.

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

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

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample.
	Insufficient template. Use the recommended amount of template DNA if available.
	The PowerPlex® ESX 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 5–10 seconds before dispensing into the PCR amplification mix.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K ⁺ , Na ⁺ , Mg ²⁺ or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE ⁻⁴ buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or nuclease-free water.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocol in Section 4. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerPlex® ESX 16 10X Primer Pair Mix for 15 seconds before use.
	Poor capillary electrophoresis injection (WEN ILS 500 peaks also affected). Re-inject the sample. For the ABI PRISM® 310 System, check the syringe pump system. Check the laser power.
	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.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.

7.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	<p>Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.</p> <p>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.</p> <p>Artifacts of STR amplification. Use the recommended amount of template DNA. You will need to optimize the amount of template DNA if you are using reduced reaction volumes. See Section 6.K for information on stutter and artifacts.</p> <p>Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3' A residue. Be sure to perform the 45-minute extension step at 60°C after thermal cycling (Section 4).</p> <p>CE-related artifacts (“spikes”). Minor voltage changes or urea crystals passing by the laser can cause “spikes” or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.</p> <p>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.</p> <p>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.</p> <ul style="list-style-type: none"> For the ABI PRISM® 310 Genetic Analyzer, generate a new matrix, and apply it to the samples. For the ABI PRISM® 3100 and 3100-<i>Avant</i> Genetic Analyzers and Applied Biosystems® 3130, 3130<i>xl</i>, 3500 and 3500<i>xL</i> Genetic Analyzers, perform a new spectral calibration and re-run the samples. Instrument sensitivities can vary. Optimize the injection conditions. See Section 5. <p>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 water; change vials and wash buffer reservoir. Repeat sample preparation using fresh formamide. Long-term storage of amplified samples in formamide can result in</p>

	artifacts.
Symptoms	Causes and Comments
Extra peaks visible in one or more color channels (continued)	<p>The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.</p> <p>Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.</p>
Allelic ladder not running the same as samples	<p>Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.</p> <p>Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples.</p> <p>Be sure the allelic ladder and samples are from the same instrument run.</p> <p>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.</p> <p>Poor injection of allelic ladder. Include more than one ladder per instrument run.</p>
Peak height imbalance	<p>Excessive amount of DNA. Amplification of >0.5ng of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template.</p> <p>Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield. Repurify template DNA.</p> <p>Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.</p> <p>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 after mixing. Calibrate thermal cyclers and pipettes routinely.</p> <p>PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.</p> <p>Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.</p>

7.B. Direct Amplification from Storage Card Punches

The following information is specific to direct amplification. For information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	<p>The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in storage cards. Decreasing the reaction volume may result in suboptimal performance, especially when amplifying DNA on storage punches directly.</p> <p>Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.</p> <p>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 also can improve low peak heights.</p> <p>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. With storage cards, reducing the reaction volumes below 25µl may result in amplification failure.</p> <p>Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.</p> <p>Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.</p> <p>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.</p> <p>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; avoid multiple freeze-thaw cycles, as this may reduce activity.</p>
Faint or absent peaks for the positive control reaction	<p>If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction.</p> <ul style="list-style-type: none"> Do not include a blank punch in the positive control reaction. Presence of a blank punch may inhibit amplification of 2800M Control DNA. Optimize the amount of 2800M Control DNA for your thermal cycling conditions and laboratory preferences.

Symptoms	Causes and Comments
Extra peaks visible in one or or all color channels	<p>Punch was contaminated. Take punches from blank paper samples.</p> <p>Amplification of processed punches with high amounts of DNA can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. Use the recommended number of 1.2mm punch per 25µl reaction. Use of a larger punch size or a smaller reaction volume may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number.</p>
	<p>Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain the DNA in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as “shadow” peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two “shadow” peaks that differ in size from one another by approximately the same distance as the single-stranded alleles.</p>
	<p>Artifacts of STR amplification. Direct amplification of >20ng of template can result in a higher number of artifact peaks. Use the recommended punch size and number of punches. Optimize the cycle number. Do not reduce the reaction volume below 25µl. See Section 6.K for additional information on stutter and artifacts.</p>
	<p>Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3’ A residue. Be sure to perform a 45-minute extension step at 60°C after thermal cycling (Section 4).</p>
Peak height imbalance	<p>Excessive amount of DNA. Amplification of >20ng of template can result in an imbalance with smaller loci showing more product than larger loci.</p> <ul style="list-style-type: none"> • Use the recommended number of 1.2mm punches. Follow the manufacturer’s recommendations when depositing sample onto the storage card. • Decrease number of cycles. <p>The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in storage cards. Decreasing the reaction volume can result in suboptimal performance.</p> <p>Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.</p>

7.B. Direct Amplification from Storage Card Punches (continued)

Symptoms	Causes and Comments
Peak height imbalance (continued)	<p>Active PunchSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to carryover and will drop out before the smaller loci.</p> <ul style="list-style-type: none"> • Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells were dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. • Using a smaller amplification reaction volume may compromise performance when using 10µl of PunchSolution™ Reagent. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required. <p>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; <u>avoid multiple freeze-thaw cycles, as this may reduce activity.</u> Carryover of excess PunchSolution™ Reagent into amplification reaction. We recommend treating one 1.2mm nonFTA card punch with 10µl of PunchSolution™ Reagent, and using one punch per 25µl amplification reaction. Use of a smaller amplification reaction volume may compromise performance if using 10µl of PunchSolution™ Reagent. Reducing the PunchSolution™ Reagent volume may improve results when using a reduced amplification reaction volume. Laboratory optimization and validation are required.</p>
Extreme variability in sample-to-sample peak heights	<p>There can be significant individual-to-individual variability in the deposition of cells onto 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.</p>

7.C. Amplification of DNA from Swabs

The following information is specific to amplification of DNA from swabs. For information about general amplification and detection, see Section 7.A.



Symptoms	Causes and Comments
Faint or absent allele peaks	<p>Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.</p> <p>Inactive SwabSolution™ Reagent. Thaw the 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; avoid multiple freeze-thaw cycles, as this may reduce activity.</p> <p>Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block reached 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. Only use 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.</p> <p>Make sure that the PCR amplification mix also contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.</p>
Faint or absent peaks for the positive control reaction	<p>If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with swab extracts, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. We recommend 5ng of 2800M Control DNA per 25µl amplification reaction. This mass of DNA should be reduced if the cycle number used 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.</p>
Extra peaks visible in one or all color channels	<p>Swab extract was contaminated. Include a blank swab as a negative control when processing samples.</p> <p>Artifacts of STR amplification. Amplification of swab extracts with high concentrations of DNA can result in artifact peaks due to overamplification, resulting in saturated signal on the CE instrument. We recommend 2µl of swab extract per 25µl reaction. Using more than 2µl in a 25µl reaction or using 2µl with a smaller reaction volume may result in overamplification and signal saturation. If signal is saturated, repeat the amplification with less swab extract or a reduced cycle number.</p>

7.C. Amplification of DNA from Swabs (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Amplification of excess template for a given cycle number resulted in overloading of the capillary upon electrokinetic injection. 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.
Peak height imbalance	<p>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.</p> <p>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 reached 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. Only use a heat block to maintain efficient heat transfer.</p> <p>Inactive SwabSolution™ Reagent. Thaw the 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; avoid multiple freeze-thaw cycles, as this may reduce activity.</p>
Extreme variability in sample-to-sample peak heights	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, quantitate the DNA using a fluorescence-based double-stranded DNA quantitation method or qPCR-based quantitation method (see TMD037 for instructions). The quantitation values can be used to normalize input template amounts to minimize variation in signal intensity.

7.D. GeneMapper® ID-X Software



Symptoms	Causes and Comments
Stutter peaks not filtered	<p>Stutter file was not imported into the Panel Manager when the panels and bins text files were imported.</p> <p>Stutter distance was not defined in the analysis method Allele tab.</p>
Samples in the project not analyzed	<p>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.</p>
Edits in label edit viewer cannot be viewed	<p>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.</p>
Marker header bar for some loci are gray	<p>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.</p>
Alleles not called	<p>To analyze samples with GeneMapper® ID-X software, at least one allelic ladder must be defined.</p> <p>Peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run.</p> <ul style="list-style-type: none"> • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time. <p>A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.</p>
Off-ladder alleles	<p>An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.</p> <p>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.</p> <p>Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels file that corresponds to the STR system used for amplification.</p> <p>The allelic ladder was not identified as an allelic ladder in the Sample Type column.</p> <p>The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.</p>

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

Symptoms	Causes and Comments
Off-ladder alleles (continued)	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Size standard not called correctly	<p>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.</p> <p>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”.</p> <p>Peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run.</p> <ul style="list-style-type: none"> Create a new size standard using the internal lane standard fragments present in the sample. Re-run samples using a longer run time.
Peaks in size standard missing	<p>An incorrect size standard was used</p> <p>If peaks are low-quality, redefine the size standard for the sample to skip these peaks.</p>
Significantly raised baseline	<ul style="list-style-type: none"> Poor spectral calibration for the ABI PRISM® 3100 and 3100-<i>Avant</i> Genetic Analyzers and Applied Biosystems® 3130, 3130<i>xl</i>, 3500 and 3500<i>xl</i> 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. <p>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.</p>

7.E. GeneMapper® ID Software

Symptoms	Causes and Comments
Alleles not called	<p>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.</p> <p>To analyze samples with GeneMapper® ID software, at least one allelic ladder must be defined.</p> <p>Peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run.</p> <ul style="list-style-type: none"> Create a new size standard using the internal lane standard fragments present in the sample. Re-run samples using a longer run time.

Symptoms	Causes and Comments
Off-ladder alleles	<p>An allelic ladder from a different run than the samples was used. Re-analyze samples using an allelic ladder from the same run.</p> <p>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.</p> <p>Panels text file selected for analysis was incorrect for the STR system used. Assign correct paneld text file that corresponds to the STR system used for amplification.</p> <p>The allelic ladder was not identified as an allelic ladder in the Sample Type column.</p> <p>The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.</p> <p>The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.</p>
Size standard not called correctly	<p>Starting data point was incorrect for the partial range chosen. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.</p> <p>Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".</p> <p>Peaks in ILS were not captured. Not all WEN ILS 500 peaks defined in the size standard were detected during the run.</p> <ul style="list-style-type: none"> • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	<p>An incorrect size standard was used.</p> <p>If peaks were low-quality, redefine the size standard for the sample to skip these peaks.</p>
Error message: "Either panel, size standard, or analysis method is invalid"	<p>The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). Be sure both files are set to the same mode, either Classic or Basic or Advanced mode.</p>
No alleles called, but no error message appears	<p>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.</p> <p>No size standard was selected. In the Size Standard column, be sure to select the appropriate size standard.</p> <p>Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.</p>

7.E. GeneMapper® ID Software (continued)

Symptoms	Causes and Comments
Error message: Analysis Method and the Panel must belong to the same Chemistry Kit"	<p>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</p> <p>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 19.</p>
Significantly raised baseline	<ul style="list-style-type: none"> Poor spectral calibration for the ABI PRISM® 3100 and 3100-Avant Genetic Analyzers, and Applied Biosystems® 3130, and 3130xl 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. <p>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.</p> <p>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.</p>
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".	<p>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.</p>
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".	<p>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.</p>
Allelic ladder peaks labeled off-ladder	<p>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. Promega recommends 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.</p>

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9. Appendix

9.A. Advantages of Using the Loci in the PowerPlex® ESX 16 System

The loci included in the PowerPlex® ESX 16 System (Tables 5 and 6) were selected because they meet the recommendations of the European Network of Forensic Science Institutes (ENFSI). The PowerPlex® ESX 16 System amplifies all ENFSI core loci in a single reaction. Table 7 lists the PowerPlex® ESX 16 System alleles revealed in 2800M Control DNA.

Terminal nucleotide addition (16,17) occurs when *Taq* 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 band 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 (18) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

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

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

The SwabSolution™ Kit (Cat.# DC8271) contains reagents for rapid DNA preparation from single-source buccal swab samples prior to PowerPlex® System analysis. 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 DNA IQ™ System (Cat.# DC6700) is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (24). 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™ System 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 and quantify 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 the PowerPlex® Systems to ensure a streamlined process.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) was developed (25).

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/), e-mail: genetic@promega.com or visit: www.promega.com/idautomation/



Table 5. The PowerPlex® ESX 16 System Locus-Specific Information.

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

¹Information about chromosomal location of these loci can be found in references 20 and 21 and at: www.cstl.nist.gov/biotech/strbase/chrom.htm

²The August 1997 report (22,23) 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”.

³Amelogenin is not an STR.

NA. Not applicable.

Table 6. The PowerPlex® ESX 16 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components ^{1,2} (bases)	Repeat Numbers of Allelic Ladder Components ³
D18S51	Fluorescein	286–366	7–10, 10.2, 11–13, 13.2, 14–27
D21S11	Fluorescein	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
TH01	Fluorescein	152–195	3–9, 9.3, 10–11, 13.3
D3S1358	Fluorescein	103–147	9–20
Amelogenin ⁴	Fluorescein	87, 93	X, Y
D16S539	JOE	273–321	4–16
D2S1338	JOE	197–269	10, 12, 14–28
D1S1656	JOE	137–184	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D10S1248	JOE	83–127	8–19
FGA	TMR-ET	264–410	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D8S1179	TMR-ET	203–251	7–19
vWA	TMR-ET	124–180	10–24
D22S1045	TMR-ET	79–118	7–20
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
D12S391	CXR-ET	130–182	14–17, 17.3, 18, 18.3, 19–27
D2S441	CXR-ET	88–124	8–11, 11.3, 12–17

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

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

³For 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/

⁴Amelogenin is not an STR.

Table 7. The PowerPlex® ESX 16 System Allele Determinations for the 2800M Control DNA.

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

9.C. The WEN Internal Lane Standard 500

The WEN Internal Lane Standard 500 contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 25). Each fragment is labeled with WEN dye and can be detected separately (as a fifth color) in the presence of PowerPlex® ESX 16-amplified material. The WEN ILS 500 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® ESX 16 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 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.

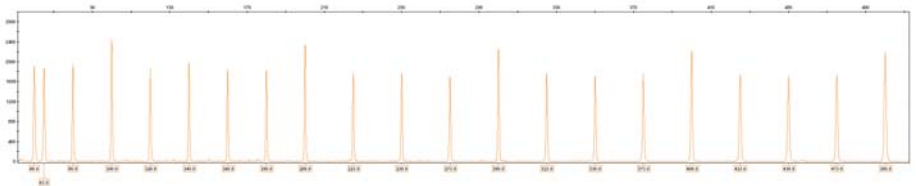


Figure 25. WEN Internal Lane Standard 500. An electropherogram showing the WEN Internal Lane Standard 500 fragments.

9.D. 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.E. Related Products

Fluorescent STR Systems

Product	Size	Cat.#
PowerPlex® ESX 17 System	100 reactions	DC6721
	400 reactions	DC6720
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 Pro System	100 reactions	DC7781
	400 reactions	DC7780
PowerPlex® 16 Monoplex System, Penta E (Fluorescein)	100 reactions	DC6591
PowerPlex® 16 Monoplex System, Penta D (JOE)	100 reactions	DC6651
PowerPlex® ES Monoplex System, SE33 (JOE)	100 reactions	DC6751
PowerPlex® 21 System	200 reactions	DC8902
	4 × 200 reactions	DC8942
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® S5 System	100 reactions	DC6951
	400 reactions	DC6950
PowerPlex® CS7 System, Custom	100 reactions	X6613
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320

Not for Medical Diagnostic Use.

9.E. Related Products (continued)

Accessory Components

Product	Size	Cat.#
PowerPlex® 5C Matrix Standards, 310	50µl (each dye)	DG5640
PowerPlex® 5C Matrix Standard	5 preps	DG4850
PunchSolution™ Kit	100 preparations	DC9271
SwabSolution™ Kit	100 preparations	DC8271
5X AmpSolution™ Reagent	500µl	DM1231
WEN Internal Lane Standard 500	200µl	DG5001
Water, Amplification Grade	6,250µl (5 × 1,250µl)	DW0991

Not for Medical Diagnostic Use.

Sample Preparation Systems

Product	Size	Cat.#
PowerQuant® System*	200 reactions	PQ5002
	800 reactions	PQ5008
DNA IQ™ System**	100 reactions	DC6701
	400 reactions	DC6700
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Tissue and Hair Extraction Kit (for use with DNA IQ™)**	100 reactions	DC6740
Maxwell® 16 Forensic Instrument*	1 each	AS3060
DNA IQ™ Reference Sample Kit for Maxwell® 16**	48 preps	AS1040
DNA IQ™ Casework Pro Kit for Maxwell® 16*	48 preps	AS1240
Plexor® HY System*	800 reactions	DC1000
	200 reactions	DC1001
Slicprep™ 96 Device	10 pack	V1391

*Not for Medical Diagnostic Use.

**For Research Use Only. Not for use in diagnostic procedures.

9.F. Summary of Changes

The following changes were made to the 5/16 revision of this document:

1. The kit component CC5 Internal Lane Standard 500 (CC5 ILS 500) was replaced with the WEN Internal Lane Standard 500 (WEN ILS 500). Instructions were updated throughout the document for use of this new internal lane standard.
2. The artifacts table in Section 6.L was updated.
3. Instructions for older versions of GeneScan® and Genotyper software were removed.
4. Other general updates were incorporated.



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

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